UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

School of Chemistry

Synthetic Studies towards Natural Products as Potential Treatment for Liver Fibrosis

by

Gary Robert William Pitt

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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Whether your liver is infected with a virus, injured by chemicals or under attack from your own immune system, the damage to your liver is likely to progress in a similar way. In the early stages of liver disease, your liver becomes inflamed. If left untreated, the inflamed liver will start to scar. As excess scar tissue grows, it replaces the healthy liver tissues. This process is called fibrosis. MBP039-06, a natural product isolated and patented by Mitsubishi Pharmaceuticals, and dithiosilvatin a member of the epipolydithiodiketopiperazine alkoliod family, have evidence to indicate them as potential treatments for liver fibrosis. We report the total synthesis of MBP039-06 and its absolute configuration, utilising the Lacey-Dieckmann condensation. Both enantiomers were prepared, and comparison of CD spectra with those of the naturally isolated material have enabled its absolute configuration to be established. In addition we report the synthesis of a small library of tetramic acid analogues. In our investigation we have shown MBP039-06 to be an effective inhibitor of HIF proyl hydroxylase. Inhibitors of this kind offer potential new therapies in ischaemic/ hypoxic disease. In the second part of the thesis we report our work completed towards the total synthesis of dithiosilvatin, concentrating on an unnatural amino acid approach to the synthesis. We have established routes to a range of *bis* protected dithiodiketopiperazines, an important intermediate in the synthesis of the epipolydithiodiketopiperazine core. In addition we have discovered and disclose the structure of two novel potent inhibitors of Lys9-specific histone methyltransferase G9a. Methyltransferases are misregulated in tumors and are involved in neurodegenerative diseases such as Alzheimer's.

Contents

Preface

The research described in this thesis was carried out under the supervision of Dr A. Ganesan at the University of Southampton between October 2004 and March 2008. No part of this thesis has been previously submitted for a degree.

Acknowledgements

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Thank you to Dr Junko Takashima of Mitsubishi Pharma Corporation, for supplying us with spectroscopic data of the natural product (MBP039-06).

Also, I would like to thank my past lab colleagues at Ferring Research for making it possible for me to juggle a full time job with my PhD studies. Many thanks to Christine, David and Ian for your assistance and proof-reading.

I would especially like to thank my wife Clair for putting up with my continuous mood swings. Without her continued reassurance and support I would have never completed this thesis.

Abbreviations

Å	angstrom
Ac	acetyl
AIBN	azo-*bis*-(isobutyronitrile)
app	apparent
aq	aqueous
br	broad
Bu	butyl
Boc	*t*-butoxycarbonyl
C	celsius
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CD	circular dichroism
celite	celite 521®
cat	catalytic
CHN	combustion analysis
cm	centimetre(s)
d	doublet
DKP	diketopiperazine
DMAP	4-(dimethylamino)pyridine
DCC	1,3-dicyclohexylcarbodiimide
dm	decimetre(s)
Dmb	2,4-dimethoxybenzyl
DME	1,2-dimethoxyethane
DMF	*N,N*-dimethylformamide
DTNB	3, 3'-dithio bis(6-nitrobenzoic acid)
DTT	1,4-dithiolthreitol
dr	diastereomeric ratio
E	entgegen (*trans*)
ee	enantiomeric excess
equiv	equivalent(s)
ES	electrospray

ECM	extracellular matrix
Et	ethyl
ETP	epidithiodiketopiperazine
Fmoc	9-fluorenylmethoxycarbonyl
FT	Fourier Transform
g	gaseous
g	gram(s)
h	hour(s)
HPLC	high performance liquid chromatography
HSC	hepatic stellate cells
HMPA	hexamethylphosphorictriamide
Hz	hertz
hυ	visible and ultraviolet radiation
IR	infrared
LHMDS	lithium hexamethyldisilazide
LCMS	liquid chromatography/mass spectrometry
LDA	lithium diisopropylamide
lit	literature
LRMS	low resonance mass spectrometry
m	moderate
m	multiplet
M	molar (mol dm^{-3})
Me	methyl
MHz	megahertz
min	minute(s)
mL	millilitre(s)
mm	millimetre(s)
mmol	millimole(s)
MMP	matrix metalloproteinase
mol	mole(s)
mp	melting pont
MS	mass spectrometry
MW	molecular weight
NAD	nicotinamide adenine dinucleotide

n	normal
NBS	*N*-bromosuccinimide
NOG	*N*-oxalylglycine
NMR	nuclear magnetic resonance
NMM	*N*-methylmorpholine
p	*para*
Pg	protecting group
Ph	phenyl
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
Pmb	*p*-methoxybenzyl
q	quartet
q	quaternary
rt	room temperature
RCM	ring closing metathesis
s	singlet
s	strong
sat	saturated
soln	solution
t	*tert*, *tertiary*
t	triplet
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
Tcboc	2,2,2-trichloro-1,1-dimethylethylcarbonyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TIMP	tissue inhibitors of metalloproteinase
TLC	thin layer chromatography
TMEDA	*N*,*N*,*N*',*N*'-tetramethylethylene diamine
TMS	trimethylsilyl
Tmob	2,4,6-trimethoxybenzyl
Ts	*p*-toluenesulfonate
UV	ultraviolet
w	weak

Chapter I: Total Synthesis and Evaluation of MBP039-06

Chapter I: Total Synthesis and Evaluation of MBP039-06

I.1 Introduction to Liver Fibrosis

I.1.1Overview

The healing process of all tissues involves the formation of new connective tissue matrix by resident and immigrant cells multiplying to various degrees. In many common clinically diverse diseases this fibroproliferative response itself can become detrimental. The resulting accumulation of fibrocellular scar tissue compromises the normal function of the affected tissue and in time becomes the main cause of morbidity and mortality in these conditions.

Liver fibrosis (cirrhosis) is one such disease which is generally preceded by hepatocyte necrosis. Considerable progress has been made in the past 20 years in our understanding of the molecular pathophysiology of liver fibrosis. Hepatic stellate cells (HSC) under normal conditions are reservoirs for fat and vitamin A in the liver. Activation of stellate cells causes the loss of vitamin A and the cells undergo a major phenotypical transformation to smooth muscle α-actin positive myofibroblasts (activated HSC), which produce a wide variety of collagenous and non-collagenous extracellular matrix. This is considered to be induced by a paracrine effect through the mediation of some factors released from necrotic hepatocytes themselves or Kupffer cells – (specialised leukocyte white blood cells) in the liver or endothelial cells. The activation of the HSC as a result of liver injury leads to a downregulation in interstitial collagenase / matrix metalloproteinase-1 (MMP-1) and increases in matrix type MMP (MT1-MMP) and gelatinase A (MMP-2). Crucially, with respect to fibrosis, the phenotypic change results in an increase in expression of the tissue inhibitors of metalloproteinase-1 and -2 (TIMP-1 and TIMP-2). The overall result of the altered expression of proteinases and inhibitors is degradation of the basement membrane and accumulation of collagen types I and III, resulting in advanced production of extracellular matrix and progressive fibrosis.

In summary, activated HSC might produce a fibrogenic environment within the liver through a combination of ECM overproduction, diminished MMP activation and inhibition of active MMPs by TIMPs.[1, 2]

I.1.2 The Causes of Liver Damage

In the past decades liver fibrosis consistently ranks among the ten most common causes of death in the United States. Liver fibrosis and cirrhosis results from the majority of chronic liver insults and represents a common and difficult clinical challenge. Liver insult may come in many forms including persistent viral and helminthic infections, bilary or hepatic obstruction, chemical, autoimmune and hereditary conditions. The causes of liver fibrosis vary world wide, reflecting both cultural and social differences.[1]

I.1.3 Viral Causes of Fibrosis

Fibrosis attributable to persistent viral infection is induced by the hepatitis viruses, in particular types B, C and D. Hepatitis B virus (HBV) is a member of the hepadnavirus family and has a semi-double stranded DNA genome. Hepatitis B is at present the most common cause of fibrosis worldwide. The World Health Organisation estimates there to be approximately 400 million chronic HBV carriers, equal to 5% of the world population. Twenty five percent of chronic disease sufferers are at risk of life threatening liver disease with its associated complications. In the UK the rate of infection with HBV is about 1 in 1000. There is an effective vaccine against the hepatitis B virus available, although it is only available to those in risk categories.

The hepatitis C virus (HCV) is an enveloped virus, sharing similarities with the Flaviviruses, with a positive-strand RNA genome. Hepatitis C is rapidly taking over as the most common cause of fibrosis, especially in the developed world. It is estimated that world wide 3% of the population are infected, 200 million chronically. HCV infection occurs primarily as a result of blood to blood contact, sexual intercourse carries only a slight risk of HCV transmission. It is believed that 0.1% of the general population and between 60-90% of intravenous drug users in the United Kingdom (UK) are infected. There is however a large number of patients that have been infected as a

result of transfusion with blood or blood products. In the UK screening was not performed for HCV until September 1991. Unlike HBV, hepatitis C has no available vaccine making the protection of risk groups impossible. Currently 40% to 60% of chronic liver disease is attributable to HCV.[1, 3]

I.1.4 Chemically Induced Fibrosis

Chemically induced fibrosis is at present the primary cause of liver disease in the developed world. The chemical induction of fibrosis represents a major cultural difference between developed and developing worlds, as a major cause in the West is chronic alcoholism. Alcoholism is believed to lead to liver damage at the site of its metabolism to acetaldehyde or ethanal. The metabolism of ethanol is performed by the nicotinamide adenine dinucleotide (NAD^+) dependent enzyme, alcohol dehydrogenase. Due to its toxicity ethanal has to be further metabolised. Metabolism of the toxic ethanal is performed by the enzyme aldehyde dehydrogenase, which converts it to ethanoic acid (acetic acid or acetate). This conversion is also dependent on NAD^+. The consumption of large quantities of alcohol leads to a temporary localized reduction of NAD^+ in the liver and thus leads to an accumulation of ethanal. It is believed that it is the accumulation of ethanal in the liver that leads to injury.[4]

I.1.5 Hereditary Conditions Leading to Fibrosis

Hereditary diseases also represent a significant number of the cases of liver fibrosis worldwide. These include Wilson's disease and hemochromatosis. Wilson's disease is a recessive autosomal disorder.[1, 5] It results from the normal absorption of copper but with an inability of the body to excrete excess copper in the bile leading to a toxic accumulation in the cytoplasm of the hepatocytes. The disease usually presents in late childhood as a viral-like hepatitis. This hepatitis progresses without liver transplantation to fibrosis and then cirrhosis.

Primary or familial hemochromatosis is believed to be due to an increased absorption of iron resulting in elevated levels of serum iron. There is an accumulation of the excess iron in hepatocytes. This disease is an autosomal recessive disorder, in 90% of cases caused by the inheritance of two copies of a mutated gene located on chromosome 6

linked to HLA-A3. The exact cause is not known but it is believed to result in increased intestinal absorption. The increased iron saturates the capacity for protein iron storage in the form of transferrin and hemosiderin. Typically, onset is at 30-40 years of age once iron-binding protein is saturated.[6]

Secondary hemochromatosis is the name given to any condition that results in iron overload, as previously described, such as increased consumption or absorbance.

I.1.6 Current Therapy

Currently, the only curative treatment of the end phase of cirrhosis is transplantation but this relies on the availability of compatible donor organs. Chronic hepatitis C virus is conventionally treated with human type 1 interferon alpha (IFN-α) and ribavirin but, it is at present only successful in 50% of all cases. Fibrosis, and particularly cirrhosis, is associated with significant morbidity and mortality therefore there is considerable activity to discover antifibrotic strategies that are applicable to liver fibrosis.[1]

Recent developments in the understanding of the process of hepatic fibrogenesis suggest a capacity for recovery from advanced cirrhosis and fibrosis is possible. This has been most clearly documented in autoimmune disease, but is paralleled by observations of haemochromatotic patients after venesection and patients with hepatitis B and C after successful interferon therapy.[7] These observations are highly encouraging and suggest that the liver has a capacity to remodel scar tissue which, if harnessed and manipulated, would offer a novel therapeutic approach to the treatment of liver fibrosis. The Southampton Liver Group (SLG) has shown that reversion of fibrosis is associated with the clearance of activated wound healing myofibroblasts from the injured liver by apoptosis. It is difficult, if not impossible to follow the cellular mechanisms mediating recovery in humans, as ethical considerations prevent serial biopsy samples from being taken from patients with liver disease and fibrosis which seems to be resolving clinically. Recovery from fibrosis has been studied extensively in rat models. In 'proof of principle' studies the SLG have demonstrated that experimental stimulation of the hepatic myofibroblast apoptosis leads to dramatic acceleration of the reversion of rat liver fibroblasts.[1, 2]

I.2 Aims of the Thesis

I.2.1 Our Aims

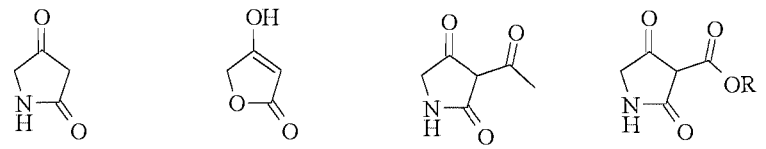
In today's environment more traditional approaches to finding novel chemical structures as potential therapies to diseases, such as liver fibrosis are being investigated. This can range from investigating traditional herbal remedies and de-convoluting the active substances, to screening natural products. We chose to investigate two classes of natural products, which had been reported as potential therapies in the treatment of liver fibrosis. The first example was from the tetramic acid structural class, this natural product MBP039-06 (figure 1.4), had been isolated and patented by Mitsubishi Pharmaceuticals as a potential therapeutic for the treatment of liver cirrhosis and fibrosis.[8, 9] We intended to develop a total synthesis of the natural product and investigate its antifibrotic properties.

The second was a member of the epipolydithiodiketopiperazine alkaloid family, which includes gliotoxin. Numerous biological studies have been completed on gliotoxin investigating its apoptosis inducing properties. There is strong evidence that this biological property could be used as a potential treatment of liver fibrosis.[10] We targeted dithiosilvatin (figure 2.3), another member of this family to investigate as a total synthesis had not yet been reported. Both of these natural products could then be used as tools to further understand the processes involved in the disease.

I.3 Tetramic Acid Natural Products

I.3.1 Introduction

The pyrrolidine-2,4-dione ring system (tetramic acid) has been known since the beginning of the 20th century. The pyrrolidine-2,4-dione (**1.01**) has a $_{P}K_a$ of 6.4 as opposed to 3.7 for the tetronic acid (**1.02**). Consequently, it is not highly enolised and exists in the 2,4-diketo form (figure 1.1). The addition of a simple acyl group (**1.03**) or alkoxycxarbonyl (**1.04**) to this ring system however, can lead to derivatives that are very strongly acidic giving pK_a values of 3.0-3.5 and 2.3-2.7 respectively, hence the name tetramic acids.



1.01 **1.02** **1.03** **1.04**

Figure 1.1 Examles of the pyrrolidine-2,4-dione ring system (tetramic acid)

The 3-acyl tetramic acids of which tenuazonic acid (**1.03**) is an example can exist in four different tautomeric forms due to enolisation (Figure 1.02).[11] The equilibrium between A and B or C and D is too fast to measure. However, the interconversion between A and C or B and D is much slower. Initially it was believed that the major tautomer was A. Further studies on the ratios of tautomers by Steyn and Wessels have shown that the exo-enol form, D is the major tautomer as it is energetically more favoured and thermodynamically more stable. The ratios of individual tautomers A to D were also calculated in their study and are given as 5:15:0:80 for simple 3-acyltetramic acids.[12, 13]

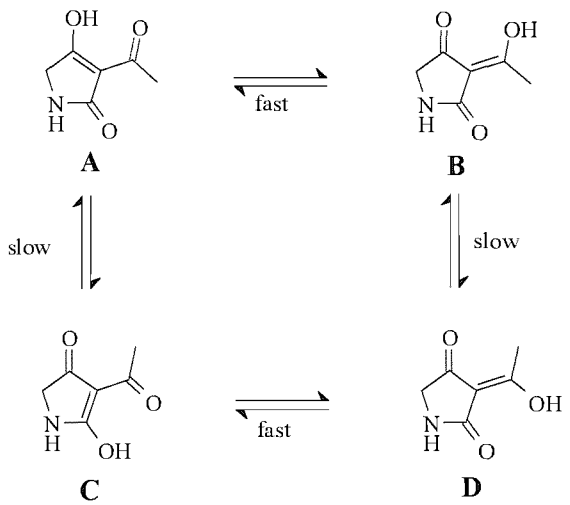


Figure 1.2 Four different tautomeric forms of tenuazonic acid due to enolisation

This important class of nitrogen heterocycles is a key structural motif in many natural products of terrestrial and marine origin. These natural products include a wide range of biologically and pharmaceutical active substances. They are well known for their potent antibiotic, antiviral and antifungal activities as well as their cytotoxic activity.[11, 14] In many examples this activity has been explained by their ability to chelate to biologically important metal ions.[15] Natural products containing the pyrrolidine-2,4-dione heterocycle are extremely varied. The 3-acyl derivatives have a particularly high

incidence in biological active due to this ability to chelate to metal ions and to mimic phosphate groups in binding sites in kinases. The *Lactobacillus reuteri* metabolite reutericyclin (figure 1.03) is one example. It inhibits *Helicobacter pylori* which causes stomach ulcers. Melophlins are a class of *N*-methyl-3-acyltetramic acids recently isolated from the marine sponge *Melophulus sarassinorum*. Melophlin A (figure 1.3) displayed cytotoxic activity against HL60 cells at 0.2 μg mL^{-1} and arrested NIH3T3 cells in the G1 phase of the cell cycle at 1- 5 μg/mL.[16]

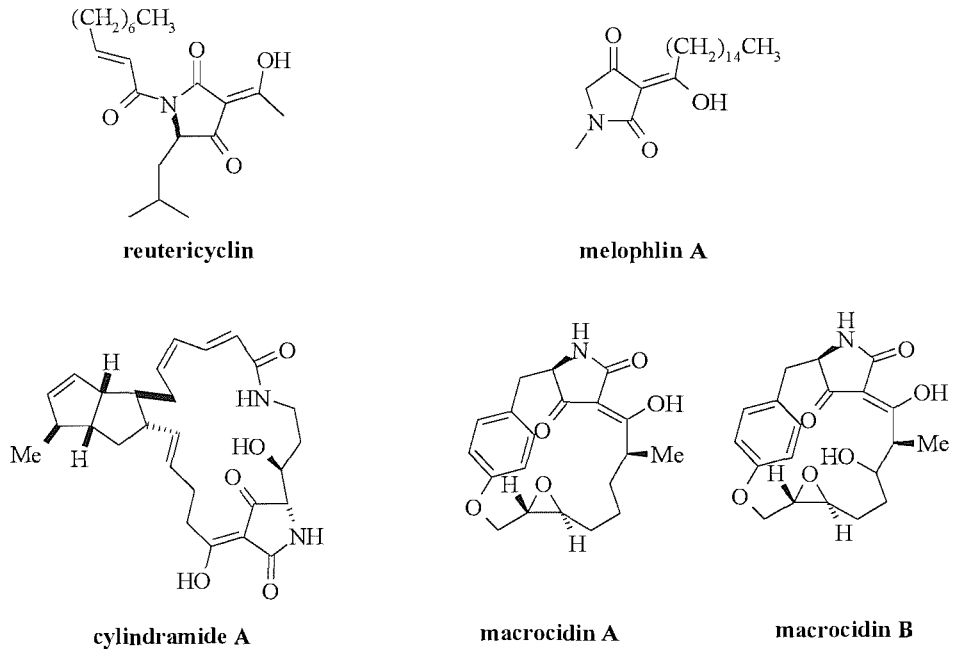
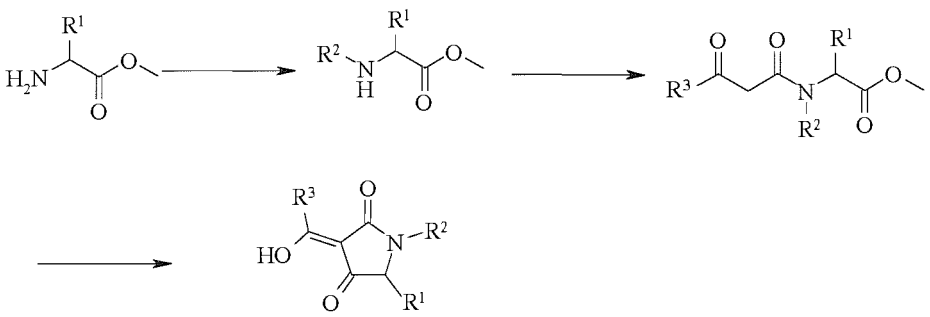


Figure 1.3 Typical natural tetramic acid derivatives reutericyclin, melophlin A and cylindramide A.

Cylindramide A (figure 1.3) is an example of a macrocyclic tetramic acid isolated from the sponge *Halichondria cylindrata* in 1993. Cylindramide A was found to be cyotoxic to B16 melonoma cells with an IC_{50} of 0.8 μg mL^{-1} and is structurally related to a number of tetramic acids containing macrolactams. Macrocidins A and B, are the first representatives of a new family of cyclic tetramic acids, isolated from liquid cultures of *Phoma macrostoma,* obtained from Canada thistle growing in several geographically diverse regions. Biological testing observed purified samples had significant herbicidal activity on broadleaf weeds.

I.3.2 Solution and Solid Phase Synthesis Methods

The most widely used protocol for tetramic acid synthesis is *via* a Claisen-type cyclisation often referred to as the Lacey-Dieckmann condensation. A general solution phase approach which is widely used is shown below (scheme 1.1). Starting from any amino acid we can obtain further functionalisation of the amine *via* a reductive alkylation. The product can be acylated to give a 1,3-dicarbonyl (*β*-ketoamide). The resulting 1,3-dicarbonyl undergoes a Claisen-type cyclisation with bases such as sodium methoxide to give the tetramic acid. The Lacey-Dieckmann cyclisation has been applied to the synthesis of numerous tetramic acid natural products.[17]

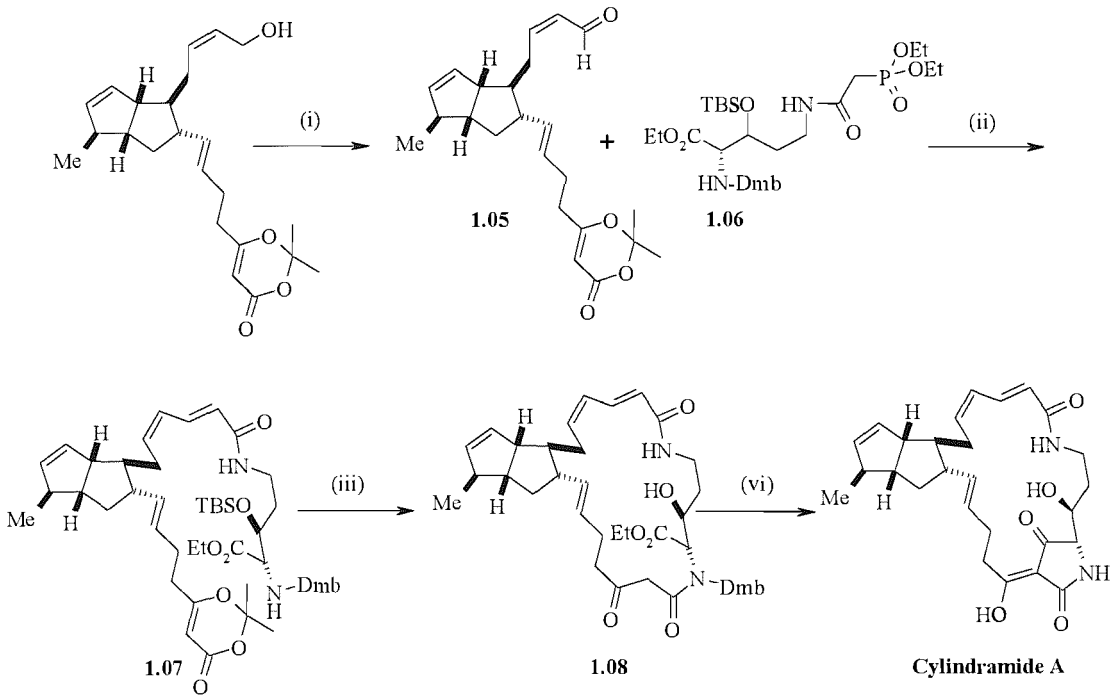


Scheme 1.1 Route to tetramic acids *via* Lacey-Dieckmann condensation.

If the starting amino acid is chiral, one issue is racemisation under basic conditions. This can be minimised by avoiding very strong bases and long reaction times. The Lacey-Dieckmann method is very flexible and can be used to prepare 3-acyltetramic acids with a large range of subtituents. However it is not so effective in the synthesis of natural products with complex side chains. Problems regarding yields and decomposition of substrates when employing the Lacey-Dieckmann cyclisation at a late stage have occurred.

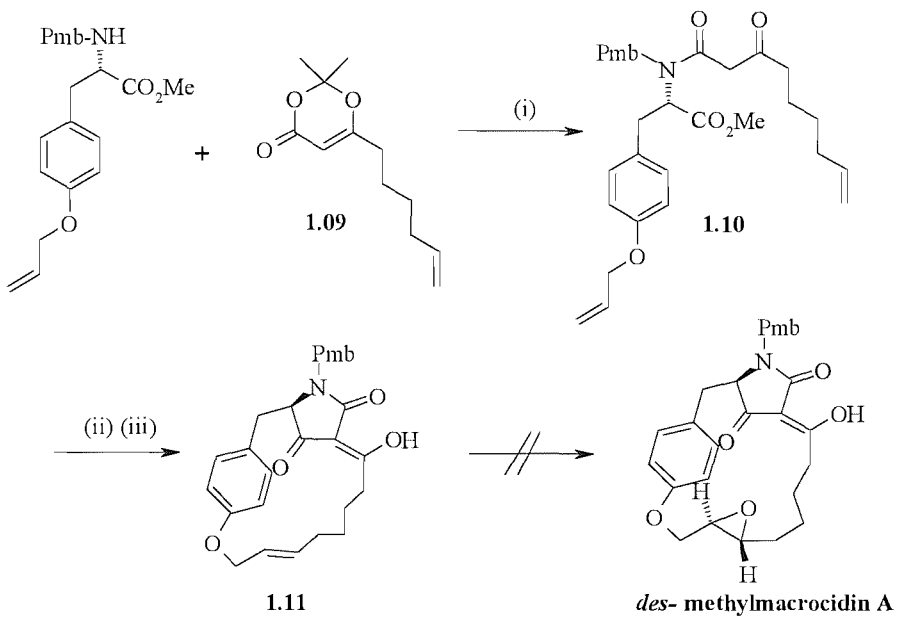
However complex natural products have been successfully completed using this approach. The total synthesis of (+)-cylindramide A (figure 1.3) was completed in 19 steps, in which the final step is the Lacey-Dieckmann condensation (scheme 1.02).[18] After coupling the key fragents (**1.05**) and (**1.06**) *via* a Horner-Wadsworth-Emmons reaction, the *β*-ketoamide (**1.08**) Lacey-Dieckmann precursor, can be formed thermally from (**1.07**) with the loss of acetone. Removal of the *tert*-butyldimethylsilyl ether with HF and treatment with sodium methoxide yielded the protected tetramic acid. Finally

TFA was then used to remove the 2,4-dimethoxybenzyl protecting group to give the natural product cylindramide A.



Scheme 1.2 Reagents and conditions: (i) Dess-Martin periodinane, CH_2Cl_2; (ii) NaHMDS, THF, -78 °C – rt, 90%; (iii) (a) PhMe, 105 °C, 65%; (iii) (b) HF, MeCN, 95%; (iv) (a) NaOMe, MeOH, 90%; (iv) (b) TFA, 67 °C, 65%.

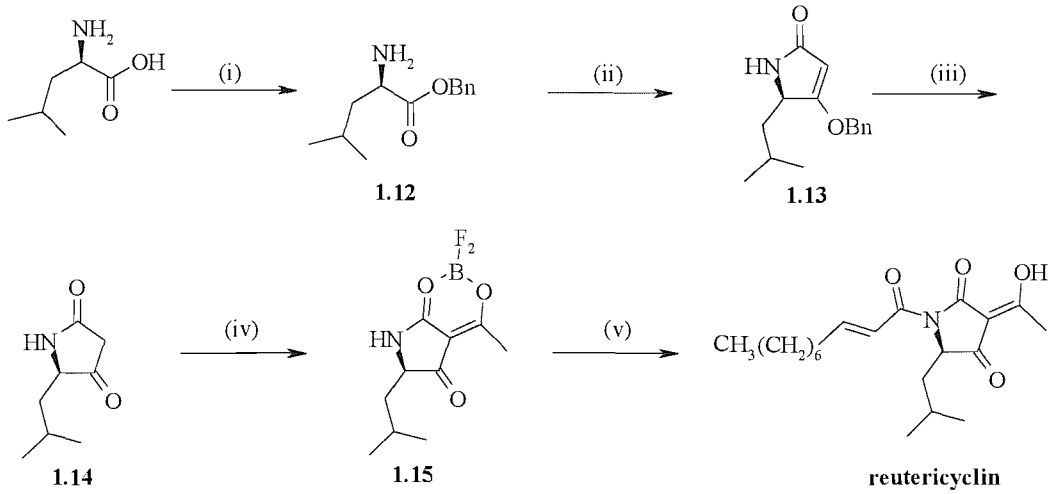
Attempts to synthesise the recently isolated macrocidins (figure 1.3) have also utilized the Lacey-Dieckmann cyclisation.[19] Similar to the synthesis of cylindramide A, the *β*-ketoamide (**1.10**) was formed *via* a [1,3]dioxin-4-one derivative (**1.09**) thermally. The *β*-ketoamide was then successfully converted into the desired protected tetramic acid (**1.11**) *via* a RCM using 1st generation Grubbs catalyst, followed by the addition of potassium *tert*-butoxide in *tert*-butanol in good yield.



Scheme 1.3 Reagents and conditions: (i) PPTS, toluene, reflux, 7 h, 72%; (ii) Grubbs 1st gen (10 mol%), CH_2Cl_2, reflux, 36 h, 63%; (iii) KO^{t}Bu, *t*-BuOH, rt, 0.5 h, 91%.

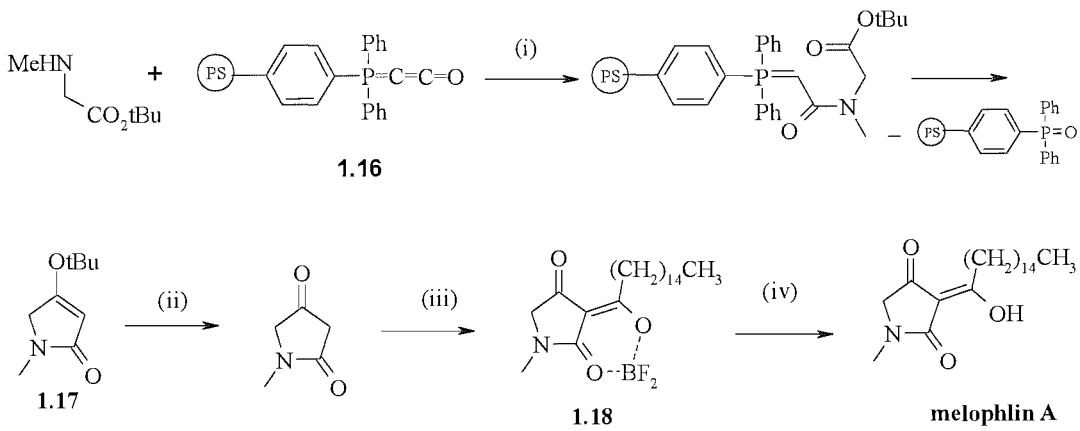
Alternative methods to the Lacey-Dieckmann cyclisation have been reported for the synthesis of tetramic acids.[20-24] Generally, truly pH-neutral methods are rare.

More recent methods have been developed, that are based on phosphorus ylide domino reactions.[20] This method has been applied to the 4 step total synthesis of reutercyclin starting from D-leucine (scheme 1.4). Benzyl D-leucine (**1.12**), formed in near quantitative yield from D-leucine and benzyl alcohol, was cyclised with the phosphorus ylide (triphenylphosphoranylidene)ketene to give the *O*-benzyltetramate (**1.13**). Hydrogenolytic debenzylation yielded the polar tetramic acid (**1.14**). This was then treated wth excess boron trifluoride-diethyl ether complex and acetyl chloride to give the difluoroboryl complex (**1.15**). The complex was stable to base and could be deprotonated with sodium hexamethyldisilazide at low temperatures, immediate quenching with (*E*)-dec-2-enoyl chloride and an aqueous work up yielded reutericyclin (ee >95%).



Scheme 1.4 Reagents and conditions: (i) BnOH, PTSA, benzene, reflux, 16 h; (ii) $Ph_3P{=}C{=}C{=}O$, $PhCO_2H$ (cat), THF, 60 °C, 16 h, 70%; (iii) H_2 (1 bar), Pd/C (5%), MeOH, rt, 1 h, 99% ; (iv) $BF_3{\cdot}OEt_2$ (excess), AcCl (8 equiv), 70 °C, 8 h, 61%; (v) (a) NaHMDS, THF, -78 °C, 5 min, (b) (*E*)-dec-2-enoyl chloride, -65 °C, 1 h, (c) aq 1M $KHSO_4$, 74%

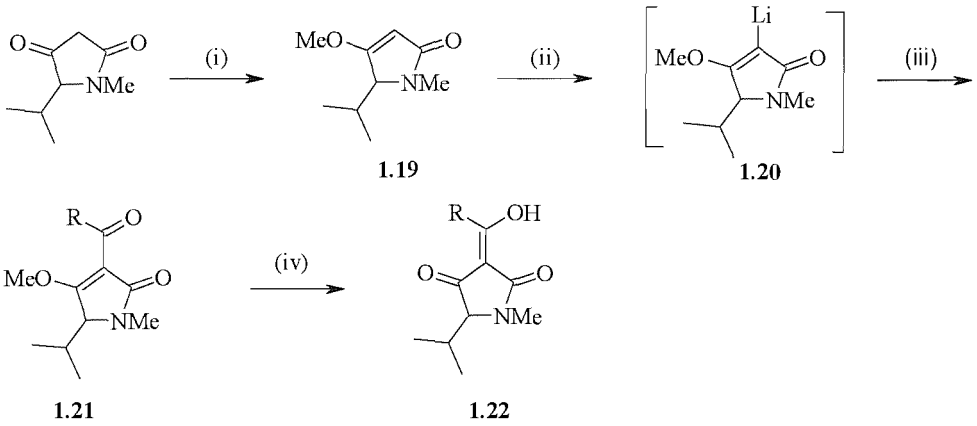
Cyclisation brought about by a domino addition-Wittig olefination reaction can also be applied using immobilized (triphenylphosphoranylidene) ketene (**1.16**) (scheme 1.5). This approach has been used to synthesise the melophlins A (figure 1.3), C and G.



Scheme 1.5 Reagents and conditions: (i) THF, 60 °C, 10 h or microwave, 120 °C, 30 min; (ii) TFA, RT, 3 h, 99%; (iii) $CH_3(CH_2)_{14}COCl$, $BF_3{\cdot}Et_2O$, microwave, 100 °C, 45 min, 47%; (iv) MeOH, reflux, 2 h, 91%.

Melophlin A was prepared in four steps from sarcosine *t*-butyl ester. Treatment with the resin (**1.16**) gave the *N*-methyl-4-t-butoxypyrrolin-2-one (**1.17**) as a product of the domino addition-intramolecular Wittig olefination sequence. After cleavage with TFA, the difluoroboryl complex (**1.18**) was formed using Jones C-3 acylating conditions. The complex was then converted to melophlin A by refluxing in methanol in good yield.[21]

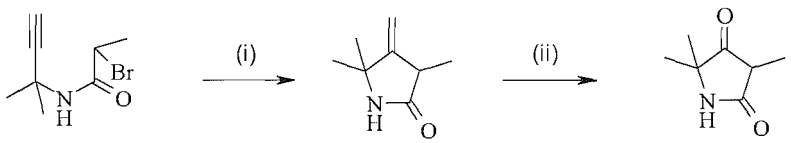
Direct metalation, *via* ortho-lithiation of methyl tetramates (**1.19**) has been reported in the synthesis of 3-acyltetramic acids (scheme 1.6).[22] Metalation with butyllithuim, followed by the addition of various aldehydes affords the aldol adducts. Which in turn are oxidised with manganese dioxide to give the keto-derivatives (**1.21**). The 3-acyl tetramic acids (**1.22**) are isolated upon treatment with sodium hydroxide.



1.19 **1.20** **1.21** **1.22**

Scheme 1.6 Reagents and conditions: (i) *n*-Bu_4NOH, $(MeO)_2SO_2$; (ii) *n*-BuLi, -78 °C; (iii) (a) RCHO; (b) MnO_2; (iv) NaOH.

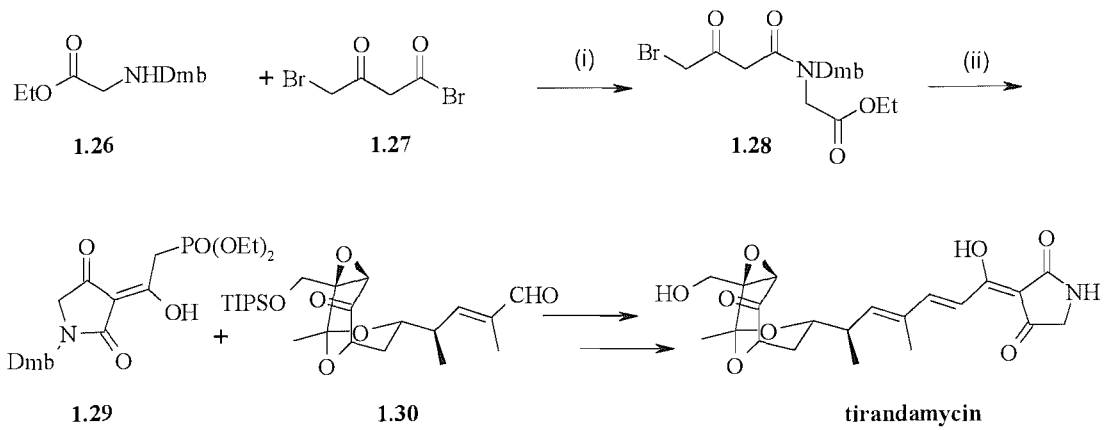
Tetramic acids have also been prepared *via* radical cyclisations of propargyl α-bromoamides (**1.23**) (scheme 1.7).[23] The lactam (**1.24**) was formed by treating the propargyl α-bromoamides (**1.23**) with tri-*n*-butyltin hydride in the presence of AIBN. Ozone was added to a solution of the lactam to give the desired tetramic acid (**1.25**).



1.23 **1.24** **1.25**

Scheme 1.7 Reagents and conditions: (i) Bu_3SnH, AIBN; (ii) ozone, PPh_3, -78 °C.

The total synthesis of the dienoyl tetramic acid antibiotic tirandamycin B was completed *via* the Horner-Wadsworth-Emmons adduct (**1.29**) (scheme 1.8).[24] The adduct was then prepared starting from the 2,4-dimethoxybenzyl protected glycine ethyl ester (**1.26**). Acylation with 1 equivalent of the acid bromide (**1.27**) gave the amide (**1.28**) in 97% yield.



1.26 **1.27** **1.28**

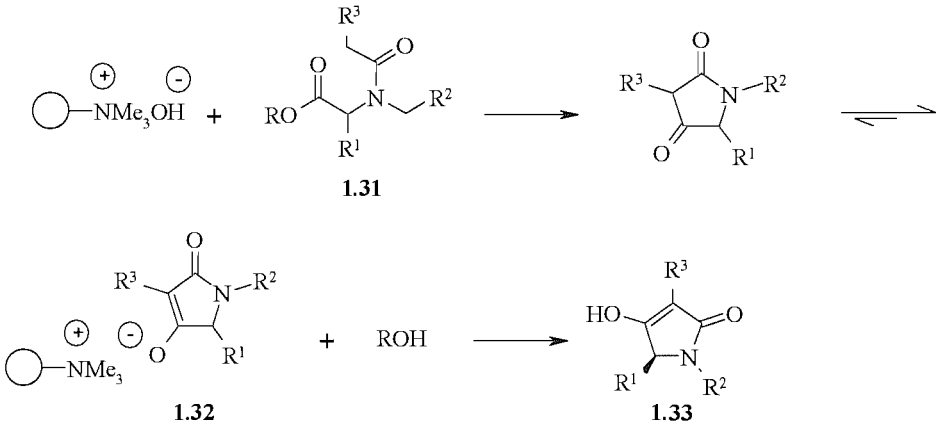
1.29 **1.30** **tirandamycin**

Scheme 1.8 Reagent and conditions: (i) CH_2Cl_2, NEt_3, -40 °C, 97%; (ii) $(EtO)_2POK$, THF, 85%.

Treatment with the potassium salt of diethyl phosphate resulted in the formation of the tetramic acid phosphonate (**1.29**). The Horner-Wadsworth-Emmons reaction with aldehyde (**1.30**) only worked if the ring nitrogen of the tetramic acid phosphonate was alkylated, hence the inclusion of the Dmb protecting group.

The tetramic acid template is an attractive scaffold for combinatorial chemistry as small molecules, particularly heterocycles are prevalent in drug discovey. An approach suitable for solution-phase parallel synthesis was reported by Kulkarni and Ganesan (Scheme 1.9).[25] Amino acid esters are reductively alkylated, and the amine acylated, without purification of these intermediates (**1.31**).

The Lacey-Dieckmann cyclisation is then effected by a quaternary ammonium hydroxide ion-exchange resin (scheme 1.09). The product, being acidic, exchanges with the resin and becomes non-covalently attached (**1.32**). Meanwhile, unreacted starting material and other impurities remain in solution and can be removed by simple filtration. Acidification then releases the tetramic acid (**1.33**) from the beads in high purity. This modular synthesis of tetramic acids uses readily available building blocks; amino acids, aldehydes and carboxylic acids to assemble a library of compounds with three points of diversity. This procedure has also been adapted to a solid phase platform.[26]



Scheme 1.9 A quaternary ammonium resin (OH^- form) catalyseds Lacey-Dieckmann condensation and purifies the products.

I.4 Natural Products as an Antifibrotic Therapy

I.4.1 Postulated Mode of Action

MBP039-06 and MBP049-13 (figure 1.4) are naturally occurring tetramic acids isolated and patented by Mitsubishi Pharmaceuticals as a potential therapeutic for the treatment of liver cirrhosis and fibrosis.[8, 9] The tetramic acid MBP039-06 was isolated from cultures of *Phaeosphaeria* fungus. *Phaeosphaeria* L288 was shake-cultured in a medium containing corn starch, glucose, soybean oil, pharmaceutical media, and soybean powder at 27 °C for 5 days.[8]

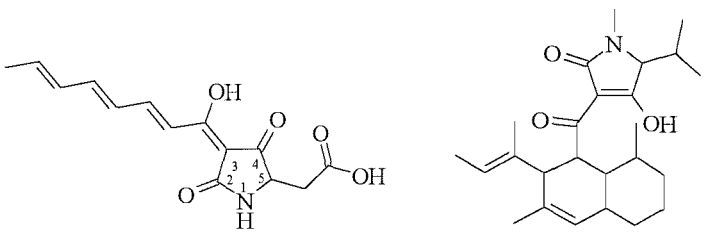


Figure 1.4 Structure of MBP039-06 and MBP049-13.

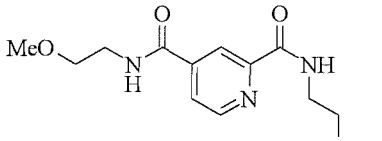
The antifibrobic mechanism of action of MBP039-06 is not known. It is postulated that it is through the inhibition of proline hydroxylase. The natural products MBP039-06

and MBP049-13 are claimed to inhibit proline hydroxylase with an IC_{50} of 16 μM and 19 μM respectfully. [8, 9]

As previously reported liver fibrosis arises from the overproduction of extracellular matrix containing collagen. The synthesis of collagen begins with the translation of collagen messenger RNA (mRNA) followed by hydroxylation of the proline moiety to hydroxyproline catalysed by the enzyme prolyl 4-hydroxylase. After several modifications, procollagen with a triple helix is formed. This triple helix of collagen is stabilised by intramolecular hydrogen bonds involving the hydroxyl groups. Underhydroxylated collagen species are not able to form stable procollagen molecules at physiological temperature and are rapidly degraded. The hydroxylation of the collagens by the enzyme prolyl 4-hydroxylase forming hydroxyproline residues constitutes the major extracellular building blocks of fibrocellular scarring in liver fibrosis. Therefore prolyl 4-hydroxylase is believed to be a key enzyme to target if you wish to regulate collagen synthesis. If hepatic prolyl 4-hydroxylase could be specifically inhibited it might prevent the formation and maintenance of the fibrocellular scar tissue in liver fibrosis patients.[1, 27]

I.4.2 Prolyl 4-Hydroxylase Inhibitors

A number of 'proof of principle' studies of prolyl 4-hydroxylase inhibitors as potential treatments for liver fibrosis have been investigated. It was reported that HOE 077 (**1.34**) (figure 1.5), an inhibitor of prolyl 4-hydroxylase, inhibited CCl_4 induced liver fibrosis in rats, as shown by improved liver histology.[28] A study of pig serum-induced liver fibrosis without hepatcellular necrosis or inflammatory cell infiltration was investigated. This was to show the effectiveness of HOE 077 in a different animal model of liver fibrosis.[29] It was found that it did have a suppressive effect on the model. HOE 077 not only reduced collagen synthesis by inhibiting proline hydroxylation, but also had an antifibrotic effect by suppressing stellate cells. There are several possible mechanisms by which HOE 077 could inhibit stellate cell activation. One is that inhibition of prolyl hydroxylase could result in less deposition of collagens, which may prevent the lack of abnormal cell-matrix interactions and further activation of stellate cells. In summary prolyl hydroxylase inhibitors such as HOE 077 can prevent liver fibrosis induced injury in rats, presumably because the inhibition of stellate cell activation resulted in reduced expression of procollagen mRNA.[1, 28-29]



HOE 077 (1.34)

Figure 1.5 Structure of HOE 077.

HOE 077 is a recently developed prodrug of pyridine 2,4-dicarboxylate. It has no activity itself, but is able to cross the heptocyte plasma membrane and is metabolised to the active compound within the liver cells.[30]

I.4.3 Potential Secondary Indication

The transcription factor HIF (hypoxia inducible factor) has a central role in oxygen homeostasis in animals ranging from nematode worms to man. This factor is regulated by signalling mechanism that involves post-translational hydroxylation. This process is catalysed by a set of non-haem, Fe^{2+}dependent enzymes that belong to the 2-oxoglutarate-dependent-oxygenase superfamily. In mammals, in addition to the hydroxylation of HIF, 2-oxoglutarate-dependent oxgenases catalyse procollagen-extracellular matrix formation. In hypoxia, prolyl hydroxylation is suppressed, which allows the HIFα subunit to escape destruction and therefore to accumulate to high levels. Three 2-oxoglutarate-dependent oxgenases that catalyse HIF prolyl hydroxylation PHD1, PHD2 and PHD3 have been identified. Overall, new insights into the regulation of HIF by protein hydroxylation offer exciting possibilities of understanding the physiological response to hypoxia.[31] Our interest would be that manipulation of these signalling pathways by hydroxylase inhibitors might offer new therapies to ischaemic/ hypoxic disease. It was therefore postulated that tetramic acids like MBP039-06 could be a potential inhibitor of this prolyl hydroxylation process.

I.5 Total Synthesis of MBP039-06

I.5.1 Retrosynthesis

The natural product MBP039-06 is claimed to inhibit proline hydroxylase with an IC_{50} of 16 μM.[8] In comparison the more complex MBP049-13 has an IC_{50} of 19 μM.[9] Therefore the simpler molecule MBP039-06 was chosen as the total synthesis target. Structurally there are some unknowns about the chirality of MBP039-06. The chiral centre at the C-5 position (figure 1.4) is as yet undefined in the naturally occurring antifibrobic compound. As reported a number of solid phase and solution phase synthetic methodologies for similar tetramic acid structures have been investigated.[18-26] This therefore makes MBP039-06 ideally suited for total synthesis and the preparation of analogues. Methodologies would have to be developed in order to investigate and define the absolute stereochemistry at C-5 position of MBP039-06 (**1.33**).

From the retrosynthesis of MBP 039-06 (figure 1.6), we can envisage the creation of the *β*-ketoamide (**1.35**). This can be broken into two further building blocks, the protected amino acid derivative (**1.38**) and the acylating reagent either (**1.36**) or (**1.37**).

It was postulated that the best way to control the stereochemical centre was to start from a naturally occurring chiral pool of amino acids. Starting from this readily available source of protected L and D aspartic acid derivatives looked like a workable approach. The disconnection of the *β*-ketoamide (**1.35**) led to a selection of two possible acylating reagents; these could both be investigated. After amination we envisaged the tetramic acid could then be formed *via* Lacey-Dieckmann condensation reaction of the *β*-ketoamide (**1.35**) under controlled conditions, to minimise the risk of racemisation.[32]

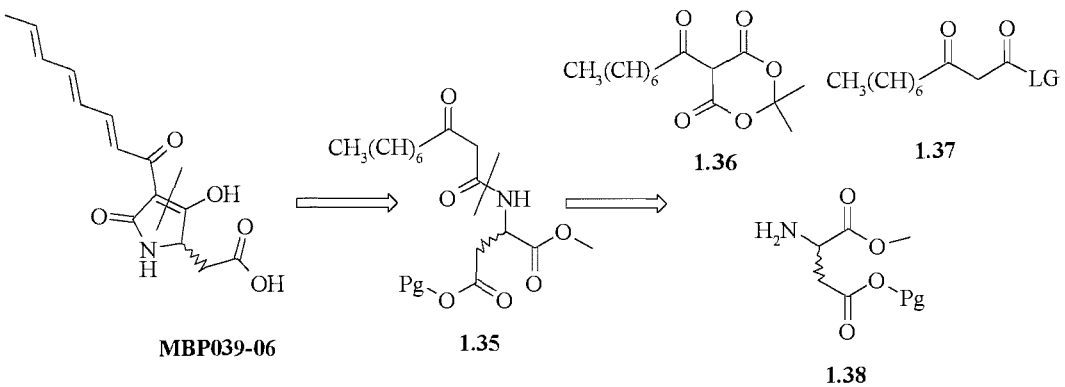
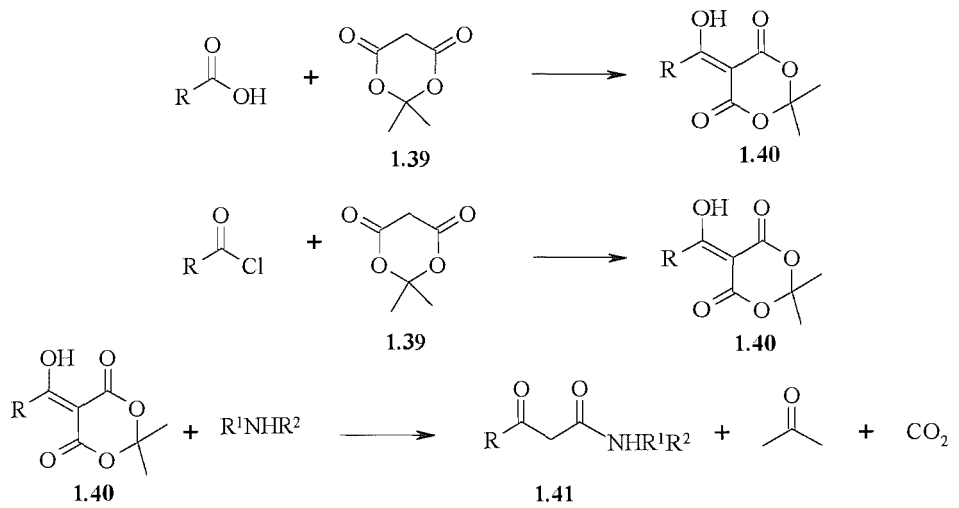


Figure 1.6 Retrosynthesis of MBP 039-06.

Many examples of β-ketoamides have been synthesied *via* 5-acyl Meldrum`s acid derivatives similar to (**1.36**). In recent years, several groups have reported the use of this key building block in the construction of tetramic acid and other heterocyclic combinatorial libraries.[33] Derived $\beta$-ketoamides (**1.41**) can be formed by simply heating the 5-acyl Meldrum`s acid derivative (**1.40**) with a nucleophilic amine analogue in a high boiling solvent like toluene (scheme 1.10). This reaction yields the desired β-ketoamide and liberates acetone and carbon dioxide as by-products.

Two general methods for the preparation of 5-acyl Meldrum`s acid are known and are shown (scheme 1.10). 5-Acyl Meldrum`s acids analogues are readily accessible from the acylation of Meldrum`s acid (**1.39**).[34] They are prepared either by the reaction of a carboxylic acid and Meldrum`s acid with a condensing agent such as DCC or isopropenyl chloroformate together with a base or by the reaction of Meldrum`s acid with an acid chloride and a base. Excess pyridine or DMAP tend to be the bases of choice. It was therefore postulated, that the retrosynthetic acylating agent (**1.36**) (figure 1.6) could be derived from the activated (2*E*,4*E*,6*E*)-octa-2,4,6-trienoic acid and the commercially available Meldrum`s acid. The (2*E*,4*E*,6*E*)-octa-2,4,6-trienoic acid can be synthesised in good yield from commercial 2,4-hexadienal (sorbic aldehyde).[35]

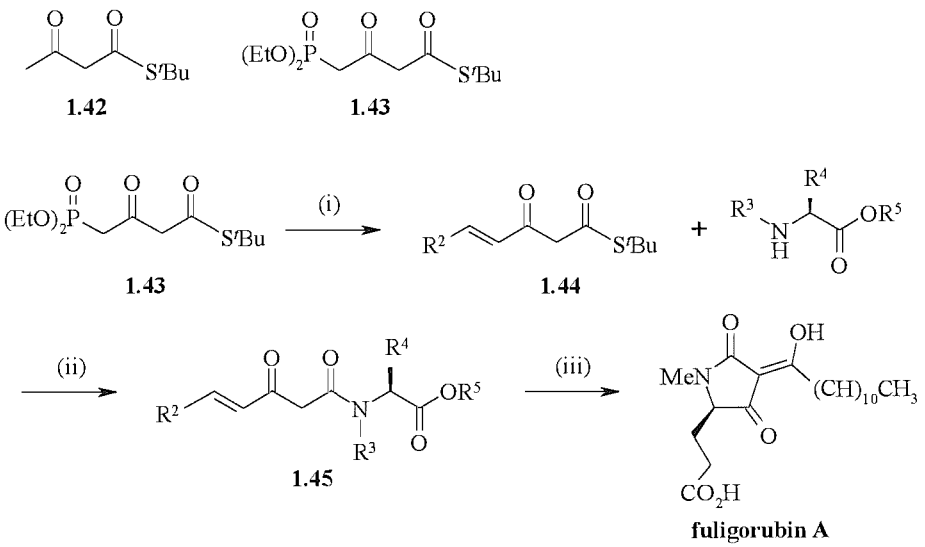


Scheme 1.10 Preparation of 5-acyl Meldrum`s acid analogues and reactions with amine nucleophiles.

An alternative to using an acyl Meldrum`s acid derivative as an acylating agent would be an acyl 1,3-dicarbonyl analogue (**1.37**) where LG is a leaving group. Ley and colleagues developed a strategy centred on *tert*-butyl 3-oxobutanethioate (**1.42**) and

tert-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) (scheme 1.11).[36] These acylating agents proved to be effective in the total synthesis of a variety of natural products, including the tetramic acid fuligorubin A (scheme 1.11).[37] The transformation of the β-ketothioester derivatives to the corresponding β-ketoamides was achieved by using heavy metal salts to promote the acylation process similar to that observed in other thioester to amide reactions.[38, 39]

In the total synthesis of fuligorubin A, the acylating agent *tert*-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) was further functionalised *via* the Horner-Wadsworth-Emmons reaction with the relevant aldehyde ($R^2 = CH_3(CH)_8CHO$) to give the (*E*)-alkene product ($R^2 = (CH)_8CH_3$) **(1.44)**. Conversion to the β-ketoamide ($R^2 = (CH)_8CH_3$, R^3 = Me, $R^4 = (CH_2)_2CO_2{}^t$Bu) **(1.45)** was accomplished by the reaction with a protected *N*-methyl (*R*)-glutamic acid methyl ester (R^3 = Me, $R^4 = (CH_2)_2CO_2{}^t$Bu, R^5 = Me) in the presence of silver (I) trifluoroacetate. Cyclisation was then achieved with TBAF or potassium *tert*-butoxide. A final deprotection of the *tert*-butyl ester with formic acid gave the natural product fuligorubin A with excellent retention of configuration.



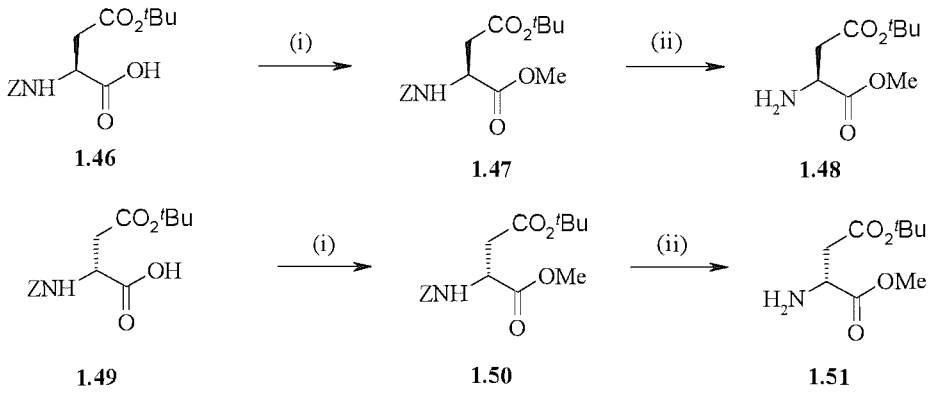
Scheme 1.11 Reagents and conditions: (i) (a) NaH (2.1 equiv), 0 °C, THF, 25 min; R^2CHO, 0°C, 75%; (ii) $AgOCOCF_3$ (1.5 equiv), Na_2HPO_4, THF, rt, 30 min, 67%; (iii) (a) KO^{t}Bu (2 equiv), ^{t}BuOH, rt, 30 min, 75%; (b) formic acid (neat), rt, 1 h, 100%.

Ley and colleagues described that milder bases such as TBAF and shorter reaction times prevented racemisation occurring during the Lacey-Dieckmann cyclisation.[37]

These findings and the versatility of the acylating reagent *tert*-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) make this a possible strategy towards the total synthesis of MBP039-06.

I.5.2 Synthesis of MBP039-06 Building Blocks

It was postulated that the best way to control the stereochemical centre in the total synthesis of MBP039-06 would be to start from a naturally occurring chiral pool of amino acids. The protected (*S*) (**1.48**) & (*R*) (**1.51**) (scheme 1.12) enantiomers of fragments **(1.38)** were obtained in 97% and 77% yields respectively following the removal of the Cbz protecting group by hydrogenolysis using palladium on carbon as a catalyst. The fully protected intermediates (**1.47**) and (**1.50**) were obtained in near quantitative yields starting from the protected commercially available aspartic acids (**1.46**) and (**1.49**). These were readily converted to the desired methyl esters by forming the cesium salt of the acids, which was alkylated upon the addition of iodomethane in DMF to give the (*S*) & (*R*) enantiomers (**1.47**) and (**1.50**) in yields over 85%.[40] The ^{1}H NMR spectra were consistent for the desired products.[41]



1.46 **1.47** **1.48**

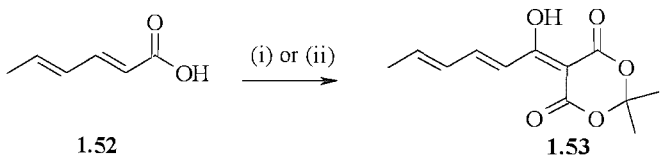
1.49 **1.50** **1.51**

Scheme 1.12 Reagents and conditions: (i) (a) 1.0 M Cs_2CO_3 (0.5 equiv), MeOH, 10 min, rt; (b) MeI (1.1 equiv), DMF, rt, 4 h, (**1.47**) 88%; (**1.50**) 99%; (ii) H_2, Pd/C, MeOH, 40 min, (**1.48**) 97%; (**1.51**) 77%.

The acyl Meldrum's acid fragment **(1.36)** was to be synthesised by conventional conditions, simply reacting an activated acid or acid chloride with Meldrum's acid in the presence of base. After searching the literature, it was found that no such reactions had been attempted with vinylic activated esters, as was present in the desired product. Therefore the chemistry was first attempted on the commercially available acid 2,4-

hexadienoic acid (sorbic acid) **(1.52)** in order not to waste any (2*E*,4*E*,6*E*,)-octa-2,4,6-trienoic acid which had to be prepared.

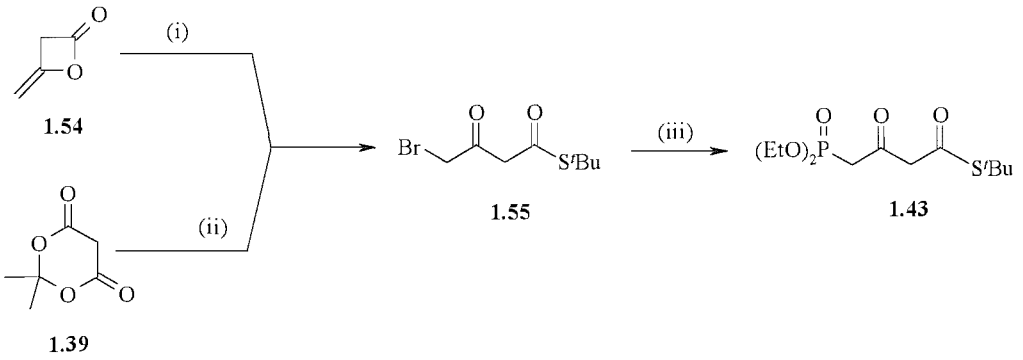
Initially we attempted to form acyl Meldrum`s acid analogue (**1.53**) (scheme 1.13) *via* an activated ester coupling using DCC and DMAP as the base, but this was unsuccessful yielding no desired product.[42] The acid chloride method did not fare much better only yielding less than 2% impure product.[43] As this was such a poor yielding route, it was not deemed to be a viable route to MBP 039-06.



1.52 **1.53**

Scheme 1.13 Reagents and conditions: (i) Meldrum`s acid (1 equiv), DCC (1 equiv), DMAP (1.5 equiv), $CH_2Cl_2$, failed; (ii) $SOCl_2$ (5 equiv), toluene, reflux, 3 h; (b) Meldrum`s acid (1equiv), DMAP (1.5 equiv), CH_2Cl_2, 2%.

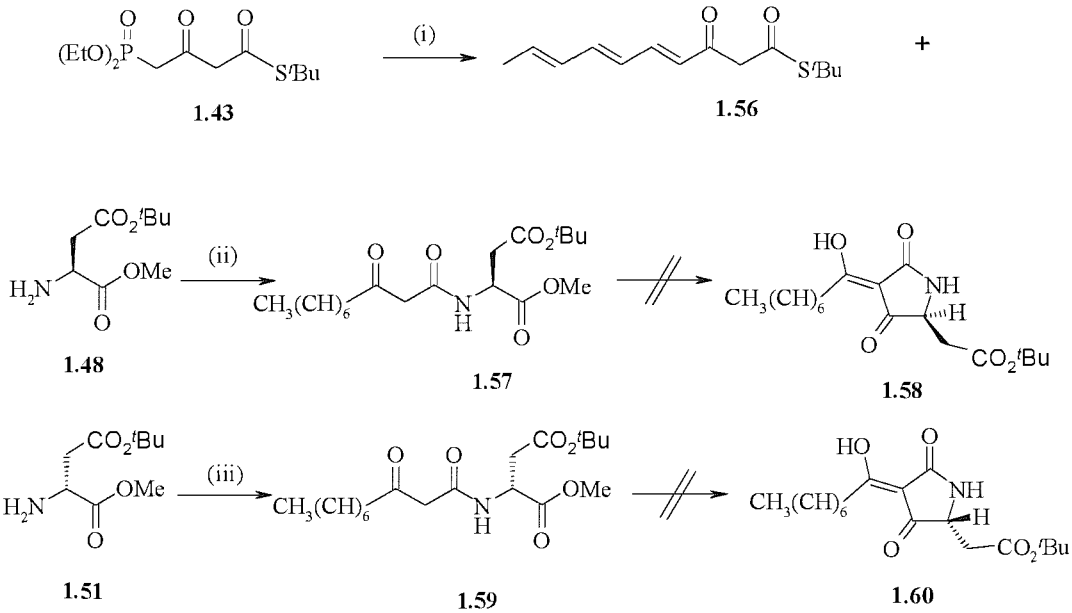
We therefore concentrated on the acyl 1,3-dicarbonyl fragment (**1.37**) using a similar strategy to that of the Ley group's total synthesis of the natural tetramic acid fuligorubin A (scheme 1.11).[37] Ley derived fuligorubin A from the versatile *tert*-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) reagent. The preparation of *tert*-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) was *via* an intermediate bromide, tert-butyl 4-bromo-3-oxobutanethioate (**1.55**). Two routes to this bromide had been published, either staring from diketene (**1.54**) or Meldrum's acid (**1.39**).[36, 44] The literature stated that starting from diketene (**1.54**) gave slightly less yields of the bromide **(1.55)** 73%, compared to staring with Meldrum's acid (**1.39**) 76%. Taking this into consideration along with the fact that diketene is becoming more difficult to source we chose to go the Meldrum's acid route. We prepared the bromide in 42% yield over the two steps. *tert*-Butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) was formed in 41% yield by treatment of the monosodium salt of *tert*-butyl 4-bromo-3-oxobutanethioate (**1.55**) with sodium diethylphophite in THF, which had been prepared from the addition of diethyl phosphate to a suspension of sodium metal in THF at -10 °C (scheme 1.14). The ^1H NMR data were consistent with those published.[36]



Scheme 1.14 Reagents and conditions: (i) (a) Br_2, CCl_4; (b) ^tBuSH, CH_2Cl_2, (Ley's yield, 73%, over 2 steps); (ii) (a) $BrCH_2COBr$, pyridine; (b) ^tBuSH, PhH, (Ley's yield 76%, over 2 steps), 42% over 2 steps; (iii) (a) NaH, THF, -10 °C, 30 min; (b) $(EtO)_2PONa^+$, THF, -10 °C, 1 h, rt, 18 h, (Ley's yield, 85%) 41%.

I.5.3 Lacey-Dieckmann Condensation

The fragment (**1.37**) from the retrosynthesis of MBP039-06, (4*E*,6*E*,8*E*)-3-Oxo-deca-4,6,8-trienethioic acid (S)-*tert*-butylester (**1.56**) was obtained in 89% yield from the commercially available sorbic aldehyde under standard Wadsworth-Emmons olefination conditions with the phosphonate (**1.43**).[45]



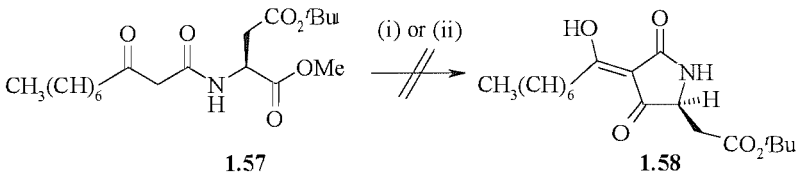
Scheme 1.15 Reagents and conditions: (i) NaH (2.1 equiv), THF 0 °C, 1 h, sobic aldehyde (0.8 equiv), rt, 4 h, 89% (ii) Na_2HPO_4 (3.7 equiv), $AgOCOCF_3$ (1.5 equiv), THF, rt, 40 min, 62%; (iii) Na_2HPO_4 (3.7 equiv), $AgOCOCF_3$ (1.5 equiv), THF, rt, 40 min, 64%.

The (S)-*tert*-butyl ester (**1.56**) was reacted with both D (*R*) (**1.51**) and L (*S*) H-Asp(O^{t}Bu)OMe (**1.48**) in the presence of silver (I) and sodium hydrogen phosphate. The *β*-ketoamides (**1.57**) and (**1.59**) were successfully isolated in 62% and 64% yield

respectively. The phosphate is important as it buffers any trifluoroacetic acid liberated in the reaction, which could cleave the acid sensitive *tert*-butyl ester. Both (**1.57**) & (**1.59**) were treated with KO^{t}Bu in THF at RT for 1 hour. After acidification, extraction with EtOAc, and removal of the solvent the crude products were isolated. These compounds have been difficult to fully characterise. This could be due to the fact that the tetramic acids exist in different congurational and tautomeric forms as a result of enolisation, as described in (figure 1.2).

Recrystallisation (glassy solids) and column chromatography was attempted, but was unsuccessful. After further analysis of the MS data, it appeared that the compounds were not the desired material as first thought. Rather, it was an impure mixture containing mainly the β-ketoamide mono and dicarboxylic acid.

Therefore the expected Lacey-Dieckmann condensation had not occurred under these conditions. This can be explained by the fact that the hydrolysis of the methyl ester occurs in preference to the Lacey-Dieckmann condensation with potassium *tert*-butoxide, by potassium hydroxide being produced by adventitious moisture, at room temperature during the reaction. The ring closure was therefore attempted with a number of bases starting with the (*S*) enantiomer (**1.57**) (scheme 1.16).



1.57 **1.58**

Scheme 1.16 Reagents and conditions: (i) 1M NaHMDS (2 equiv), THF, 0 °C to rt, 1 h, rt, 24 h, reflux, 5 h. (ii) 1M NaOMe (2 equiv), THF, reflux, 3 h.

The strong non-nucleophilic base sodium *bis*(trimethylsilyl)amide had no effect at low temperatures. At room temperature only the acid was observed in the reaction mixture. After 1 h of heating multiple products were observed therefore this approach was abandoned. Literature demonstrates that similar Lacey-Dieckmann condensation occurs in good yield with sodium methoxide in methanol at reflux, but causes epimerisation of the chiral centre.[46] This method was also unsuccessful yielding a multi-component mixture containing none of the desired material.

I.5.4 Alternative Synthetic Strategies Towards MBP039-06

Two approaches were attempted to get round the problematic condensation reaction. One was through an aldol reaction by adding the vinylic side chain to the methyl ketone after the tetramic acid ring (**1.62**) has been formed (figure 1.7).[47] The tetramic acid can again be formed using standard Lacey-Dieckmann conditions from the optically pure β-ketoamide (**1.61**). The methyl ester of MBP039-06 (**1.64**) could then be prepared by an acid catalysed elimination of the diol (**1.63**). Simple saponification of the methyl ester should then yield the desired natural product.

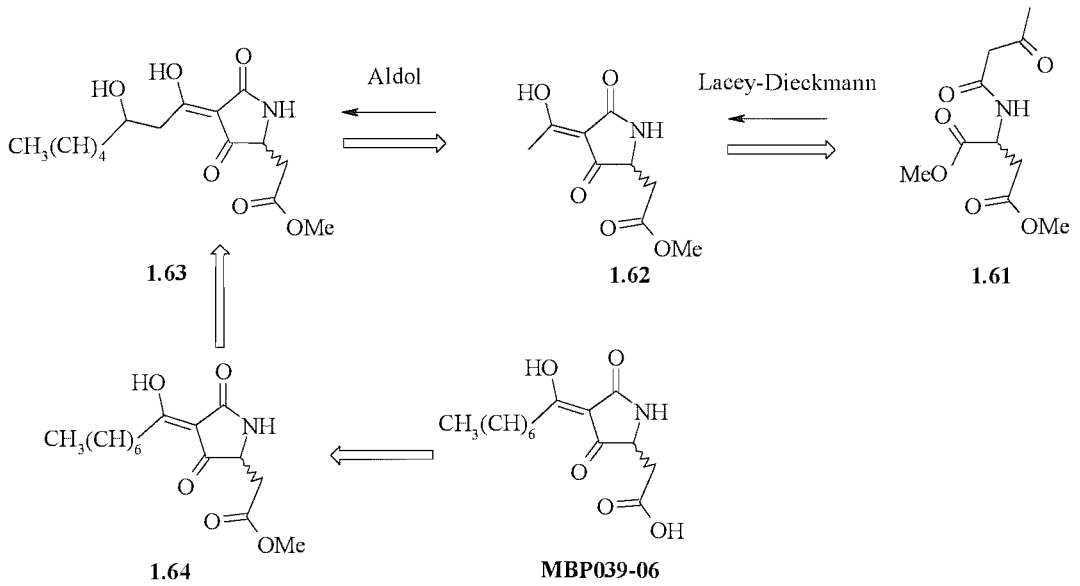


Figure 1.7 Retrosynthesis of MBP039-06: formation *via* an aldol.

The other was to use a protecting group on the amide to aid the Lacey-Dieckmann condensation (figure 1.8). The 2,4-dimethoxybenzyl (Dmb) protecting group has been used in the synthesis of similar tetramic acid compounds and is readily removed under acid conditions.[48] Protection of the amino acid esters (**1.65**) could be achieved *via* reductive alkylation from the commercially available 2,4-dimethoxybenzaldehyde. The β-ketoamide (**1.66**) and tetramic acid (**1.67**) can then be prepared as before. Finally treatment with acid will cleave both the Dmb and *tert*-butyl ester protecting groups in one step.

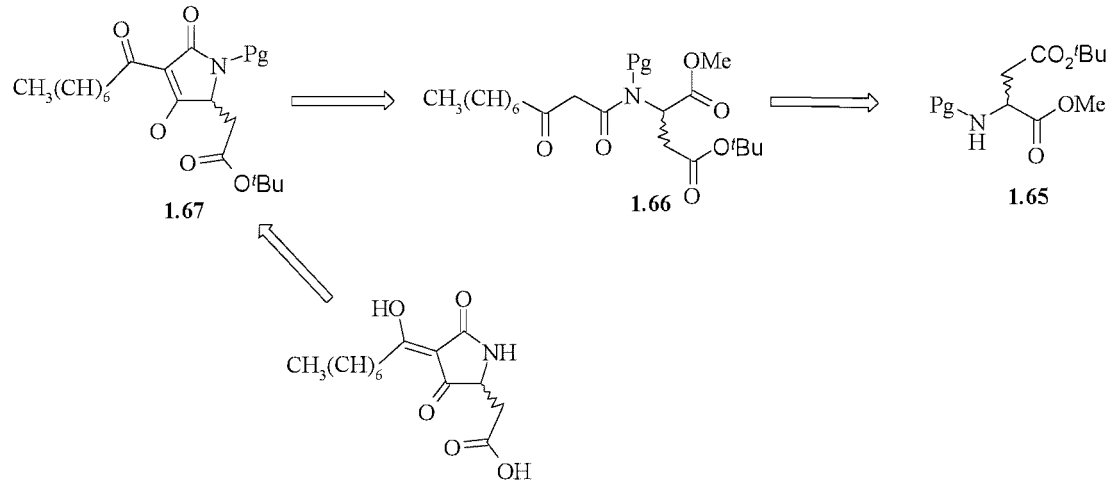


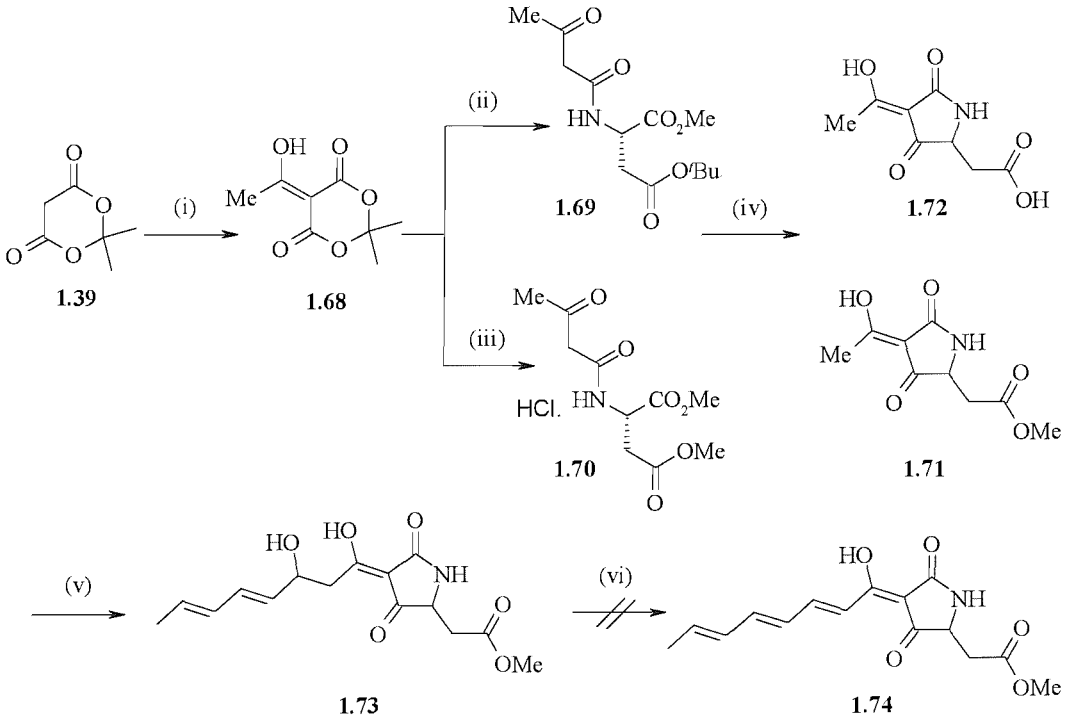
Figure 1.8 Retrosynthesis of MBP039-06: formation *via* protection strategy.

I.5.5 Aldol Approach

Procedures towards the synthesis of the *β*-ketoamide (**1.70**) and the subsequent tetramic acid (**1.62**) had been reported using diketene.[49] As diketene is becoming increasingly difficult to obtain an alternative route was successfully attempted (scheme 1.17). The procedures also started from racemic DL-aspartic acid, because racemisation is likely to occur during the Lacey-Dieckmann cyclisation using the reported conditions, sodium methoxide at reflux. With this in mind we embarked on the synthesis with the natural amino acid derivative (**1.48**) and assumed racemisation during the synthesis. If the aldol route was successful we would investigate alternative Lacey-Dieckmann conditions at a later stage.

Compound (**1.68**) was obtained in 84% yield from Meldrum`s acid. This was then heated at reflux with (**1.48**) in toluene to give the *β*-ketoamide (**1.69**). Subsequently the tetramic acid (**1.71**) was obtained in 50% yield with sodium methoxide as the base. Unfortunately the *tert*-butyl ester could not be obtained by this route. It was found that the *tert*-butyl ester was trans-esterified to the methyl ester with the excess sodium methoxide. The reaction also yielded the carboxylic acid (**1.72**) as the major byproduct due to hydrolysis of the ester. Both these findings could explain why the cyclisation conditions were not successful for the attempted MBP039-06 synthesis.

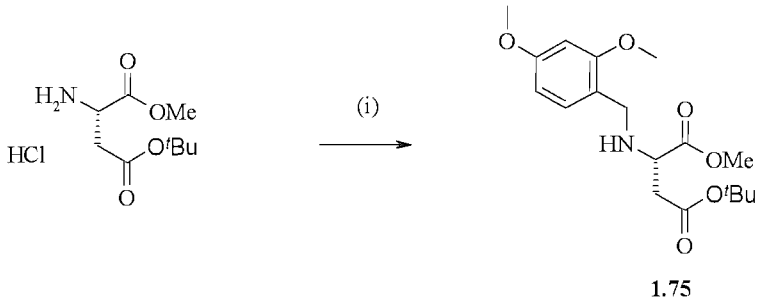
The synthesis was therefore repeated with the commercially available dimethyl aspartate hydrochloride salt, with slightly improved yields. The acyltetramic acid (**1.71**) was lithiated by the addition of 3.2 equivalents of *n*-butyl-lithium in THF, the orange suspension of the presumed trilithioderivative was quenched with hexa-2,4-dienal to afford the crude hydroxyl-adduct (**1.73**) in 93% yield. This crude mixture was then treated with chloroform saturated with hydrogen chloride gas, to effect elimination. The resulting multicomponent material was purified by semi-preparative HPLC purification, but unfortunately did not give the desired product (**1.74**) by MS. Other routes of elimination such as phosphorus oxychloride in chloroform or boron trifluoride etherate in dichloromethane could have been pursued, but the likelihood of developing the required asymmetric synthesis looked poor.



Scheme 1.17 Reagents and conditions: (i) acetyl chloride (1 equiv), pyridine (2 equiv), CH_2Cl_2, 0 °C 15 min, rt, 3 h, 84%; (ii) **1.48** (1 equiv), toluene, 70 °C, 3 h, 53%; (iii) H-Asp(OMe)OMe. HCl (1 equiv), Et_3N (1.1 equiv), toluene, 70 °C 3 h, (61%); (iv) 0.5 M NaOMe (2 equiv), MeOH, reflux 2 h, 56%; (iv) (a) 1.8 M LDA (3.2 equiv), THF, < -78 °C, 1 h, (b) hexa-2,4-dienal (1.4 equiv), rt, 2 h, crude 93%; (vi) sat $HCl_{(g)}$, $CHCl_3$, rt, 24 h.

I.5.6 2,4-Dimethoxybenzyl (Dmb) Protection Strategy

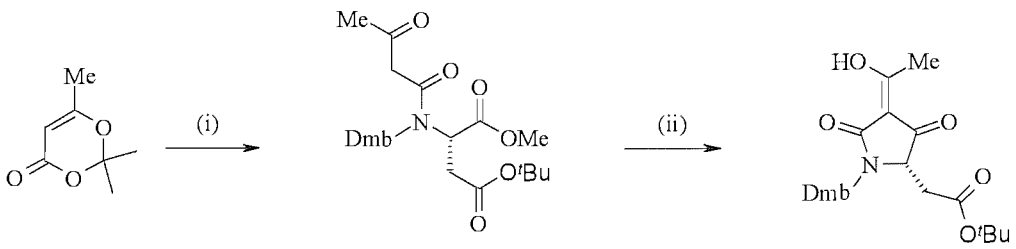
The protected amide DmbAsp(O^{t}Bu)OMe (**1.75**) was obtained *via* a reductive alkylation in an excellent 96% yield (scheme 1.18). The strategy was to investigate whether the Lacey-Dieckmann condensation would proceed under mildly basic conditions in order to retain the stereochemistry of the starting amino acid ester. The Ley group reported potassium *t*-butoxide at room temperature gave acceptable retention of stereochemistry during the cyclisation of similar tetramic acids.[37]



1.75

Scheme 1.18 Reagents and Conditions: (a) (i) 2,4-dimethoxybenzaldehyde (1.05 equiv), EtN(i-Pr)$_2$ (1.1 equiv), CH_2ClCH_2Cl, rt, 40min; (ii) $NaHB(OAc)_3$ (1.4 equiv), rt, 18 h, 96%.

To investigate this, the simpler Dmb protected acyltetramic acid (**1.78**) was successfully obtained using potassium *t*-butoxide at room temperature, albeit in a poor yield (scheme 1.19). The intermediate (**1.77**) was synthesised in one step in 96% yield using another alternative to diketene, 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**1.76**) (diketene acetone adduct).



1.76 **1.77** **1.78**

Scheme 1.19 Reagents and conditions: (i) (**1.75**) (0.7 equiv), toluene, reflux, 2 h, 96%; (ii) KO^{t}Bu (2 equiv), THF, rt, 3 h, 21%.

The (*S*) enantiomer of MBP039-06 (**1.81**) was successfully prepared using this Dmb protecting group approach (scheme 1.20). This could be explained by the protecting

group removing the possible competition between the deprotonation of the amide and the ketone which have similar pK_a values. As this enolate is essential to the Dieckmann condensation removing this possible competition increases the likelihood of the desired cyclisation. The deprotonated amide could, in the presence of any adventitious moisture, lead to the hydrolysis of the ester to the acid explaining the difficulties reported earlier. In addition the Dmb conformationally restricts the β-ketoamide, and may help populate the conformer needed for cyclisation (figure 1.9).

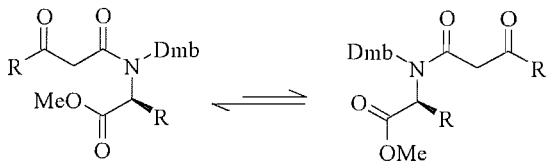
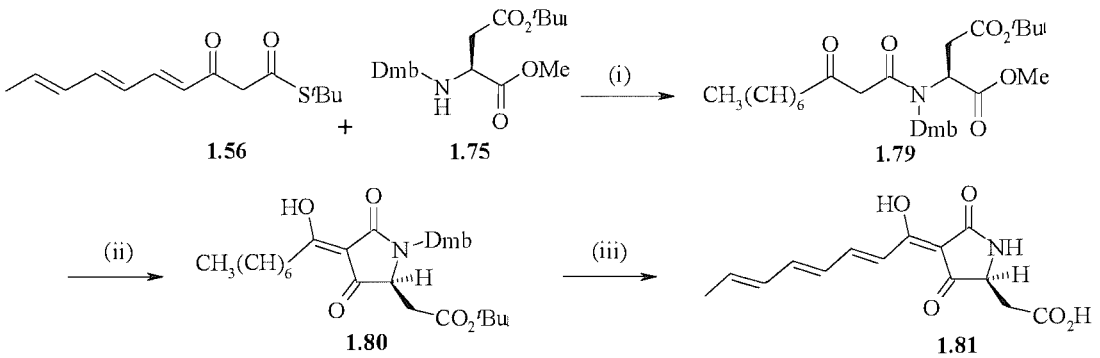


Figure 1.9 Possible conformers of the Dmb protected β-ketoamide.

Using the previously established methods (scheme 1.15) which started from the (S)-*tert*-butyl ester **(1.56),** we synthesised the protected β-ketoamide (**1.79**) in 68% yield in the presence of silver (I) trifluoroacetate. Finally (**1.79**) was converted to the protected triene-tetramic acid (**1.80**) by treatment with potassium *tert*-butoxide in *tert*-butanol in 83% yield. Deprotection with trifluoroacetic acid gave (*S*)-MBP039-06 (**1.81**) as an amorphous yellow solid in 95% yield. This required further purification by semi-prep HPLC. The pure (*S*)-MBP039-06 (**1.81**) was isolated in 55% yield.



Scheme 1.20 Reagents and conditions: (i) **1.75** (1.4 equiv), Na_2HPO_4 (3.7 equiv), $AgOCOCF_3$ (1.5 equiv), THF, THF, 4 Å sieves, rt, 40 min, 68%; (ii) KO^{t}Bu (2 equiv), ^{t}BuOH, rt, 1h, 83% (iii) TFA, 0 °C- rt, 35 min, (95% crude), 55%.

I.5.7 Identifying the Absolute Stereochemistry of MBP039-06

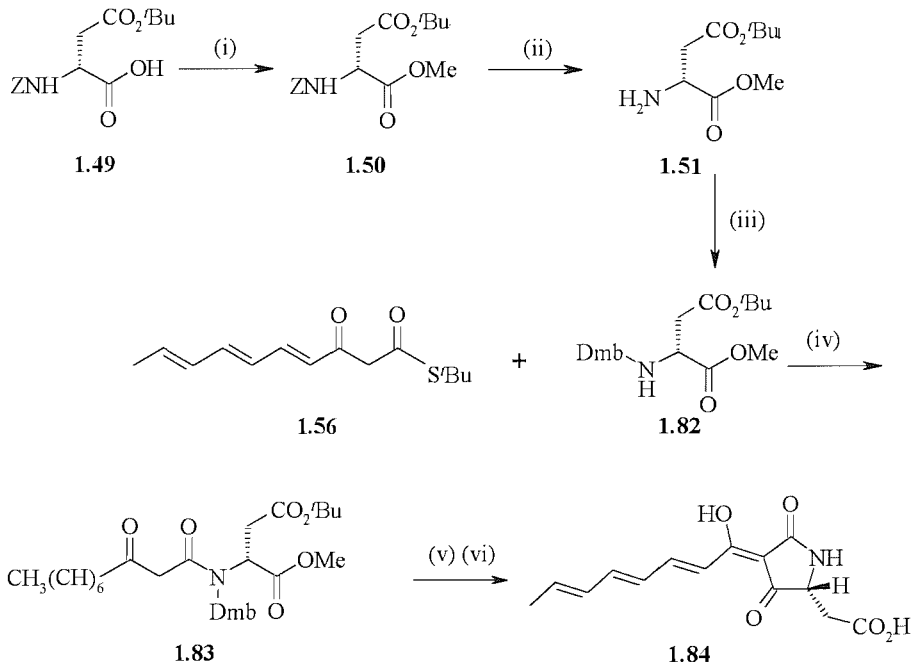
The ^{1}H NMR and UV spectra taken of (**1.81**) are identical to that supplied by the Mitsubishi Corporation of MBP039-06. In their isolation of the natural product, the absolute stereochemistry at C-5 chiral centre was not determined. Neither was optical rotation taken, but they had taken a CD (circular dichroism) spectrum.

CD is a form of spectroscopy based on the differential absorption of left or right handed polarised light. It is used predominantly to determine the structure of macromolecules (including proteins and DNA). In addition to measuring in aqueous systems, CD particularly far UVCD can be measured in organic solvents such as methanol. This has an advantage in that it can induce structure formation of proteins, inducing beta-sheet or alpha helices which they would not show under normal aqueous systems.

A CD spectrum of (**1.81**) in methanol was taken and was found to be consistent with that obtained by Mitsubishi of MBP039-06. Both spectra gave a negative value. The data is conclusive that a total synthesis of MBP039-06 has been completed, and that the absolute stereochemistry at the C-5 chiral centre is (*S*)-stereochemistry.

After successfully completing the synthesis of MBP039-06, it was decided it would be useful to have the opposite (*R*) enantiomer available, in order to compare the biological activities of both the natural (**1.81**) and unnatural analogues.

The route was easily adapted to synthesise the (*R*)-MBP039-06 analogue (**1.84**) (scheme 1.21). The Dmb-DAsp(O^{t}Bu)OMe (**1.81**) was obtained by a reductive alkylation in a similar yield to that of the (*S*) enantiomer from H-DAsp(O^{t}Bu)OMe (**1.51**), which had been prepared from the D (*R*) aspartate derivative (**1.49**) *via* hydrogenolysis as previously reported.



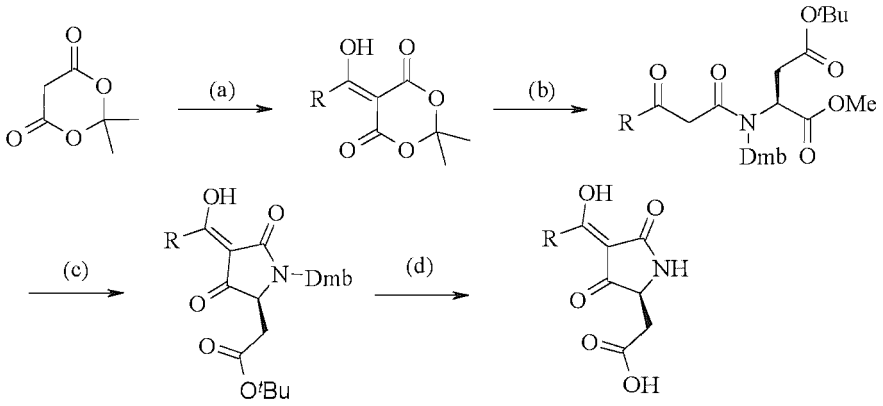
Scheme 1.21 Reagents and conditions: (i) (a) 1.0 M Cs_2CO_3 (0.5 equiv), MeOH, 10 min, rt; (b) MeI (1.1 equiv), DMF, rt, 4 h, 99%; (ii) H_2, Pd/C, MeOH, 40 min, 77%; (iii) (a) 2,4-dimethoxybenzaldehyde (1.05 equiv), $EtN(i\text{-}Pr)_2$ (1.1 equiv), CH_2ClCH_2Cl, rt, 40 min; (b) $NaHB(OAc)_3$ (1.4 equiv), rt, 18 h, 91%; (iv) Na_2HPO_4 (3.7 equiv), $AgOCOCF_3$ (1.5 equiv), THF, 4Å sieves, rt, 40 min, 48%; (v) KO^tBu (2 equiv), ^tBuOH, rt, 1 h, (vi) TFA, 0 °C- rt, 35 min, 15%.

As before, the acylating reagent (**1.56**) was stirred with the amine (**1.82**) in the presence of silver (I) trifluoracetate, 4 Å Mol sieves and sodium hydrogen phosphate as the buffer to give the *β*-ketoamide (**1.83**) in 48% yield. During the synthesis it was observed that the (*R*) intermediate *β*-ketoamide (**1.83**) was far less stable than its opposite enantiomer (**1.79**), hence the lower yields. It was therefore decided that the best way to progress with the synthesis was not to isolate the intermediate Dmb-protected tetramic acid, but to deprotect the crude Lacey-Dieckmann cyclisation product. A small quantity of (*R*) MBP039-06, 2.2 mg (**1.84**) after purification by semi-prep HPLC, was isolated in this way. The analytical data obtained were identical to that of the (*S*) enantiomer (**1.81**).

I.6 Tetramic Acid Library

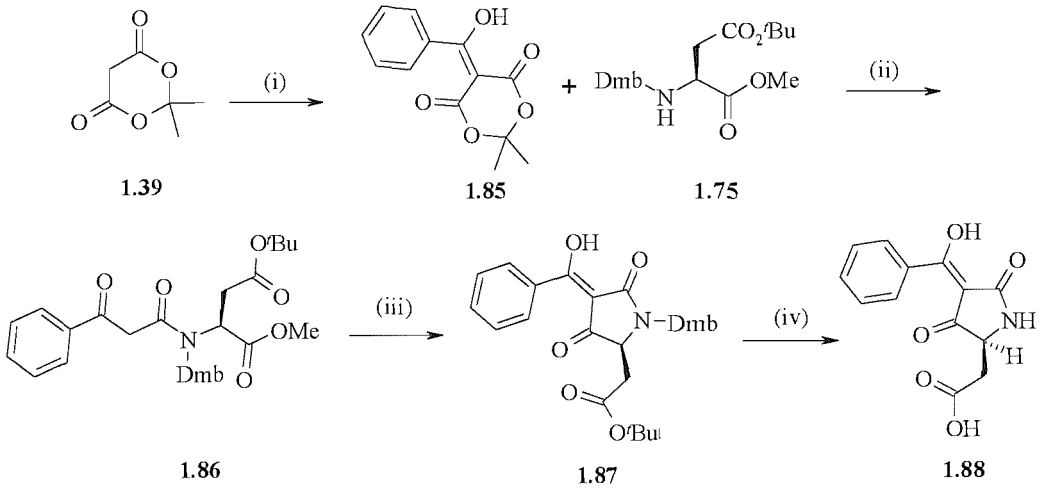
I.6.1 Analogues of MBP039-06

In order to further understand the SAR of this series of compounds, a number of additional analogues of MBP039-06 would be required; a plausible route to these analogues is outlined in (scheme 1.22) derived from Meldrum's acid. This route utilises the protecting group strategy developed for the total synthesis of MBP039-06.



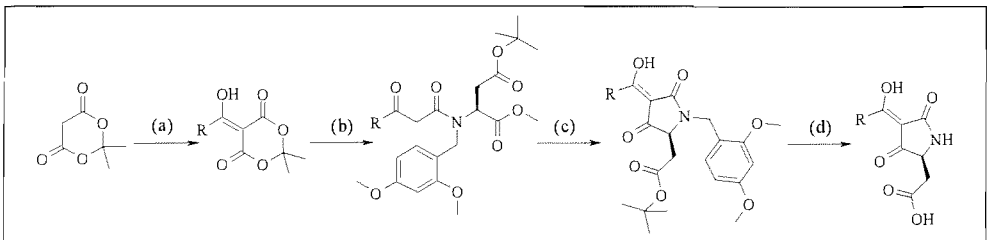
Scheme 1.22 Possible analogue route based on Meldrum's acid: (a) acylation; (b) Nucleophilic addition with protected amino acid; (c) Lacey-Dieckmann condensation; (d) deprotection.

A phenyl derivative was used to test the feasibility of the route (scheme 1.23). The reaction with Meldrum's acid was completed in a good yield using benzoyl chloride and DMAP as the base. This compound (**1.85**) had analytical data consistent with those in the literature.[50]



Scheme 1.23 Reagents and conditions: (i) benzoyl chloride (1.05 equiv), DMAP (1.8 equiv), CH_2Cl_2, 0 °C to r.t , 18 h, 58%; (ii) Dmb-Asp(O^{t}Bu)OMe (1.0 equiv), 1,4-dioxan, 70 °C, 3 h, 90%; (c) KO^{t}Bu (2 equiv), ^{t}BuOH, rt, 1 h, ; (d) TFA 60 min.

The β-ketoamide (**1.86**) was isolated in 90% yield by heating the acyl Meldrum's acid (**1.85**) with (*S*) aspartate derivative (**1.75**) in dioxane at 70° C for 3 hours. The Dmb protected tetramic acid (**1.87**) was subsequently isolated in good yield upon treatment with potassium *tert*-butoxide. The Dmb and *tert* butyl ester protecting groups were then cleaved under acidic conditions, by the addition of trifluoracetic acid. The resulting crude product was purified by semi-preparative HPLC giving (**1.88**) as an off-white amorphous powder. This route was applied to a number of analogues outlined in (table 1.1).



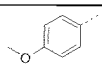
Compound R=	**(a)** **Example** (yield)	**(b)** **Example** (yield)	**(c)** **Example** (yield)	**(d)** **Example** (yield)
	**1.85** (58%)[a, b]	**1.86** (90%)	**1.87** (48%)	**1.88** (69%)
	**1.89** (23%)*	**1.90** (44%)	**1.91** (53%)	**1.92** (56%)
	**1.93** (63%)[a, b]	**1.94** (62%)	**1.95** (86%)	**1.96** (69%)
	**1.97** (78%)[b]	**1.98** (59%)	**1.99** (85%)	**1.100** (38%)
	1.101 (40%)	**1.102** (88%)	**1.103** (90%)	**1.104** (60%)

Table 1.1 Meldrum's acid method applied to tetramic acid analogues: * compound decomposed on isolation after purification; (a) analytical data consistent with literature;[50] (b) analytical data consistent with literature.[51]

In addition to the phenyl analogue (**1.88**), four further analogues were synthesised *via* acyl Meldrums acids in moderate to good yields. We only synthesised the (*S*) enantiomers of each derivative. Difficulties were only encountered for the thiophene Meldrum's acid analogue (**1.89**) which was thermally unstable and the hexyl analogue (**1.101**), which was an oil. This meant it had to be purified by column chromatography,

unlike the other analogues which were all purified by recrystallisation. It was found that the product was not stable to chromatography and was therefore reacted on crude with no loss in yield of the β-ketoamide (**1.102**).

The analogues were selected in order to give a range of physiochemical properties to investigate the importance of the triene side chain of MBP039-06 (table 1.2).

Compound	clogP	clogD (pH 7.4)	Polar surface area (PSA)	MWt (g/mol)	Hydrogen bond acceptor	Hydrogen bond donor	Rotatable bonds
1.81	0.57	-3.42	103.7	277.28	5	3	5
1.84	0.57	-3.42	103.7	277.28	5	3	5
1.88	0.19	-4.63	103.7	261.24	5	3	5
1.92	0.02	-4.89	131.9	267.26	6	3	6
1.96	0.45	-4.44	103.7	275.26	5	3	5
1.100	-0.06	-4.97	112.9	291.26	6	3	6
1.104	0.57	-3.42	103.7	255.27	5	3	5

Table 1.2 Calculations of physiochemical properties using chemaxon software (based on *enol* analogue only).

In addition MBP039-06 and its analogues comply with Lipinski's 'Rule of Five' which states that in general, an orally active drug has:

Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
A molecular weight under 500 g/mol.
A partition coefficient log *P* less than 5.

I.7 Biological Data

I.7.1 Liver Fibrosis Assay Models

In a normal liver, hepatic stellate cells (HSCs) are nonparenchymal quiescent cells with functions to store vitamin A. Following injury of any etiology, HSCs undergo a process of activation and transform from quiescent vitamin A rich cells to proliferative, fibrogenic, contractile myofibroblasts. Activated HSCs lose lipid droplets, feature high level expression of α-smooth muscle actin (α-SMA), and are responsible for the deposition of excess extracellular matrix, leading to scar tissue in the fibrotic liver.[1-2]

The aim was to investigate the effects of the synthesised compound MBP039-06 (**1.81**) on cell proliferation in human hepatic stellate cells and immortalized human hepatic stellate (LX-2) cells, which are ex-vivo models for liver fibrosis. Measuring the expression levels of TIMP-1, MMP-2, collagen 1 and α-SMA gives an indication of the compounds antifibrotic activity.

These experiments were kindly carried out by Annette Hayden at Ferring Research, Chilworth.

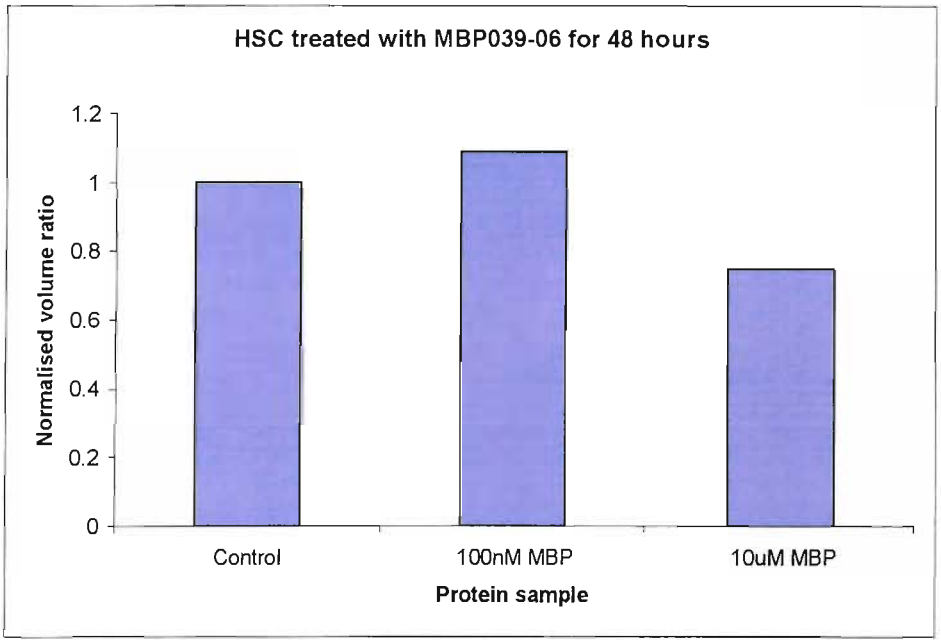
Liver fibrosis assay models, HSC assay protocol:

Day 1; replace media (2% DMEM) and treat HSC cells plated in 100mm dishes (50-60% confluent) with 100 nM and 10 μM doses of MBP039-06 in DMSO. Control plate is treated with 10 μL of 100% DMSO.

Day 3; extract RNA and protein from HSC cells, quantify the RNA and run RT-PCR reactions for TIMP-1, MMP-2, Collagen-1 and GAPDH. Quantify protein and run a western blot for Alpha-SMA and GAPDH. Use phoretix to semi-quantify western blot and PCR images.

I.7.2 HSC *Ex Vivo* Assay Results

The results illustrated in graphs (1.1-1.4) demonstrate no change to the HSC cells when treated with 0.1 μM, and 10 μM MBP039-06 solution in DMSO.

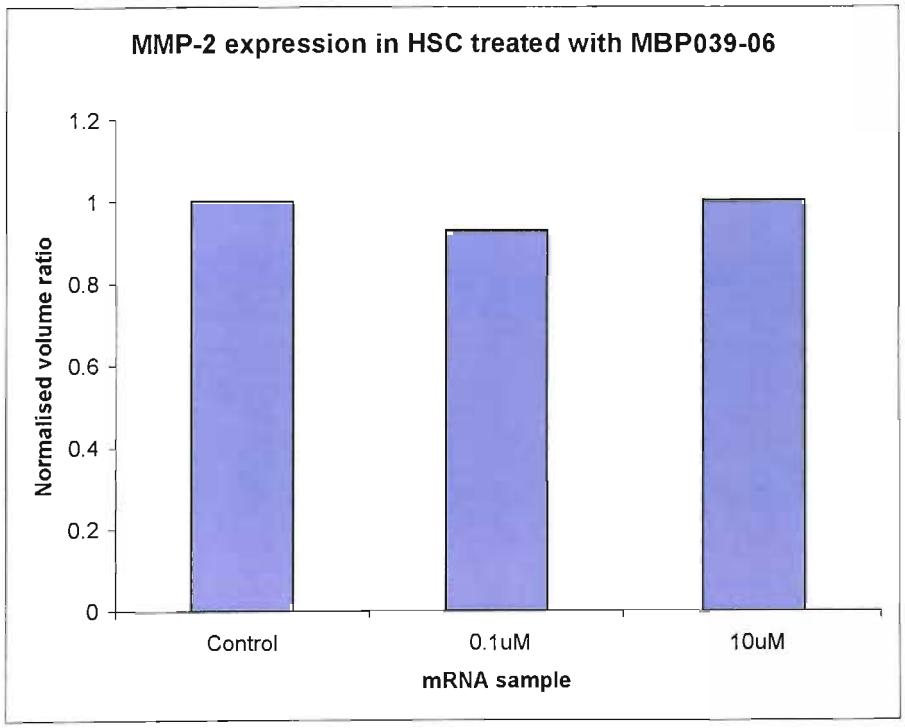


Graph 1.1 HSC treated with 100 nM and 10 μM of MBP039-06 for 48 h.

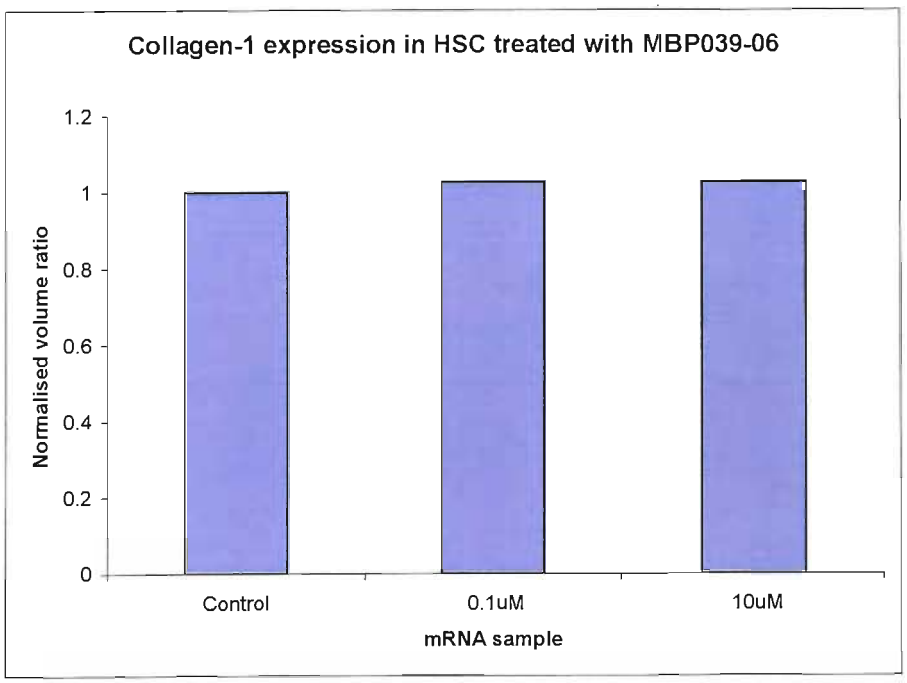
There are a number of possible reasons for this disappointing result: (1) The human stellate cells simply did not respond to the compound MBP039-06. (2) The stellate cells used had changed characteristics after passage and were no longer responding as human stellate cells would *in vivo*. (3) The compound is not stable in the media or in DMSO for 48 h, no stability studies had been carried out on MBP039-06.

In order to answer these possible reasons and maybe clarify the results the assay was repeated with a new batch of primary HSC cells and with an immortalised stellate LX-2 cell line. These cells exhibit typical features of stellate cells and expressed α-SMA under the culture conditions.

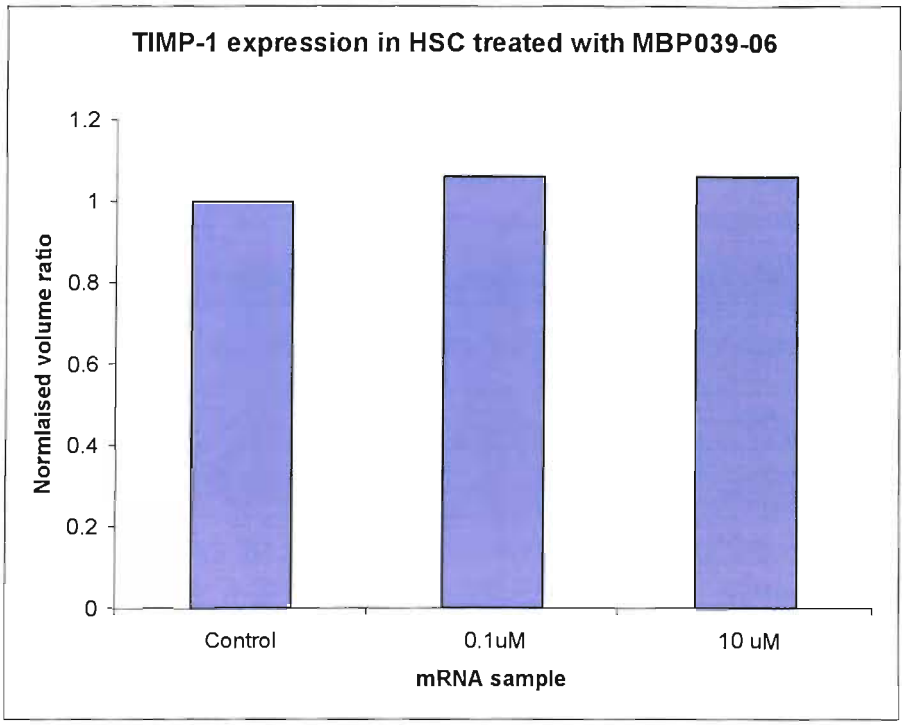
From our findings no change occurred with the initial HSC cell protocol. This was therefore repeated with a new source of primary HSC (graph 1.5). In this experiment we did observe a significant dose dependent reduction in α-SMA consistent with that expected of an antifibrotic compound.[52]



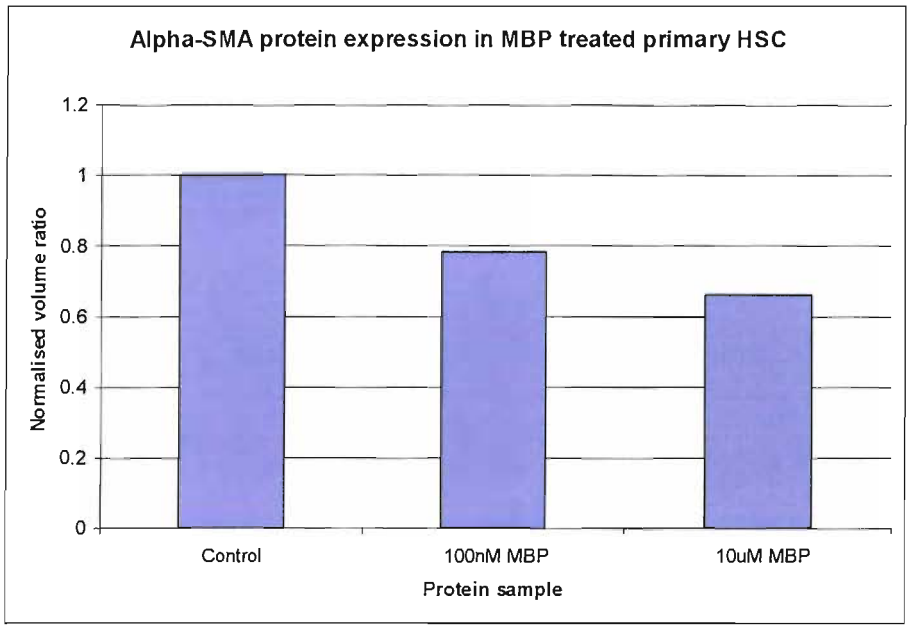
Graph 1.2 MMP-2 expression in HSC treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



Graph 1.3 Collagen-1 expression in HSC treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



Graph 1.4 TIMP-1 expression in HSC treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.

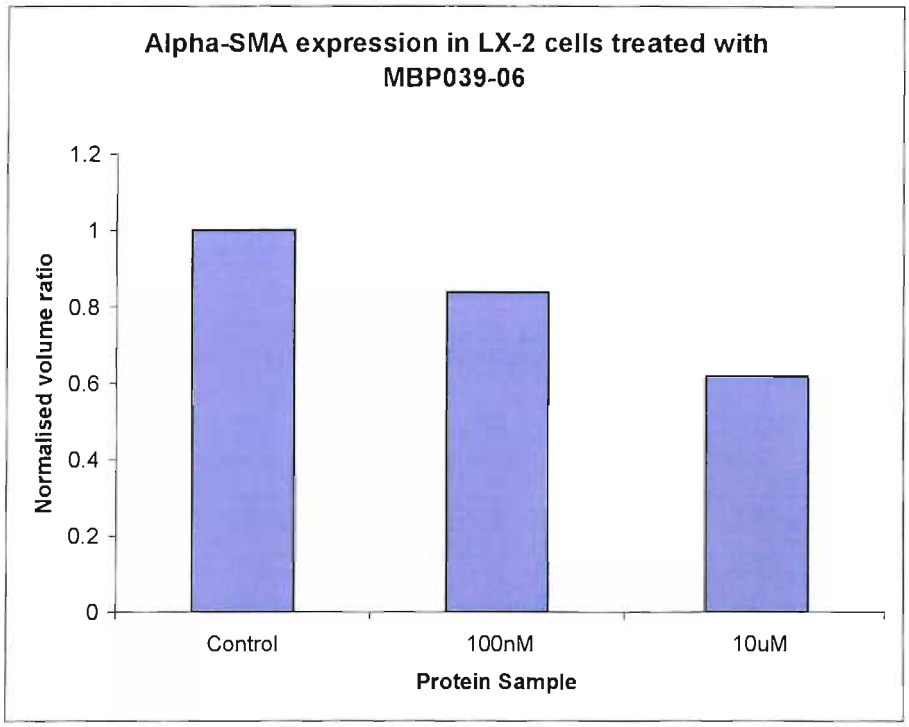


Graph 1.5 α-SMA expression in primary HSC treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.

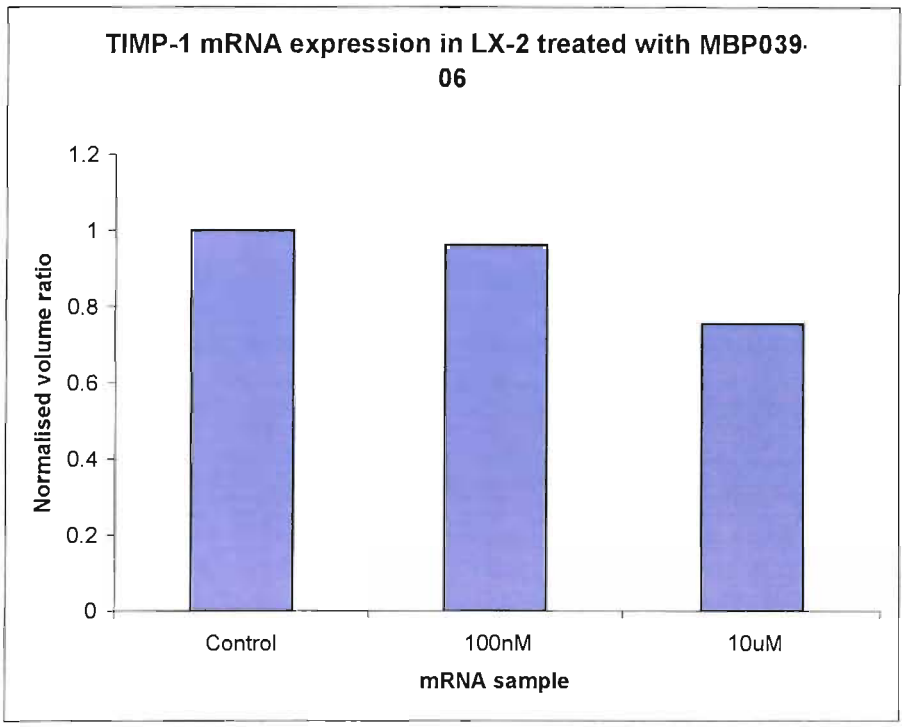
I.7.3 LX-2 Cells *Ex Vivo* Assay Results

The protocol was identical to the HSC cell assay but, immortalised human stellate LX-2 cells were used.

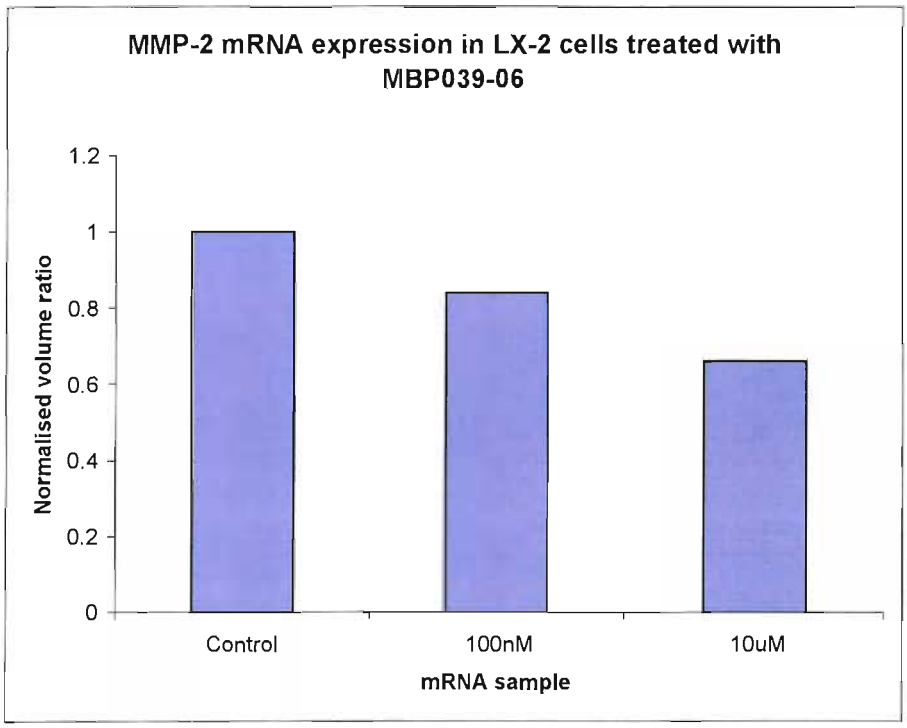
The results with the LX-2 treated cells were consistent with that expected of an antifibrotic compound (graph 1.5-1.10). There appears to be a dose dependent reduction in TIMP-1, MMP-2 and Collagen-1 mRNA expression over the 48h treatment period. The LX-2 cell line experiment was repeated with n = 3 (graph 1.10) and a significant dose dependent reduction in α-SMA was observed.



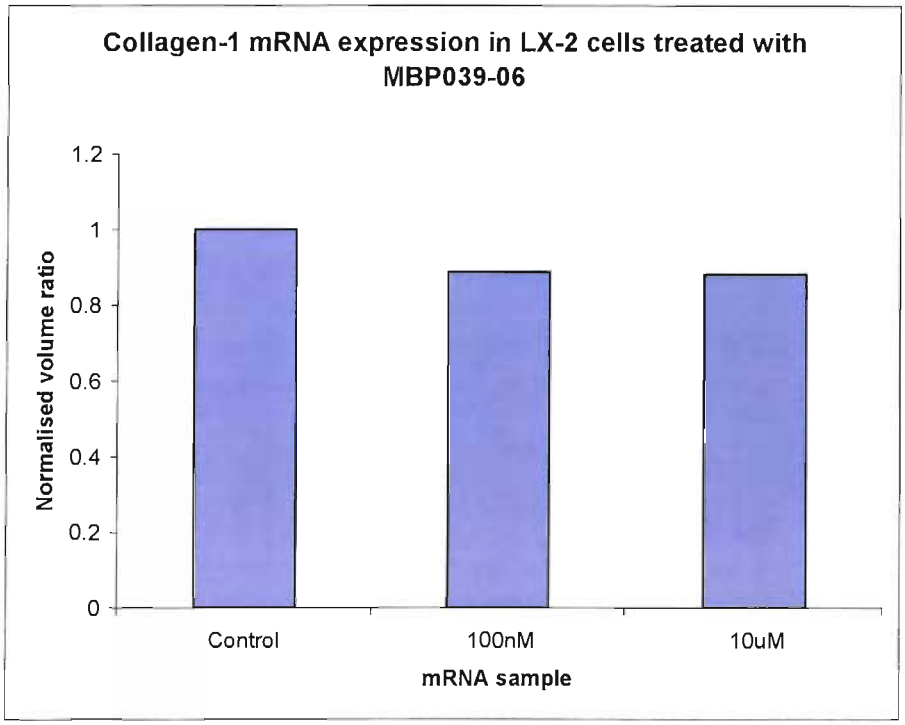
Graph 1.6 α-SMA expression in LX-2 treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



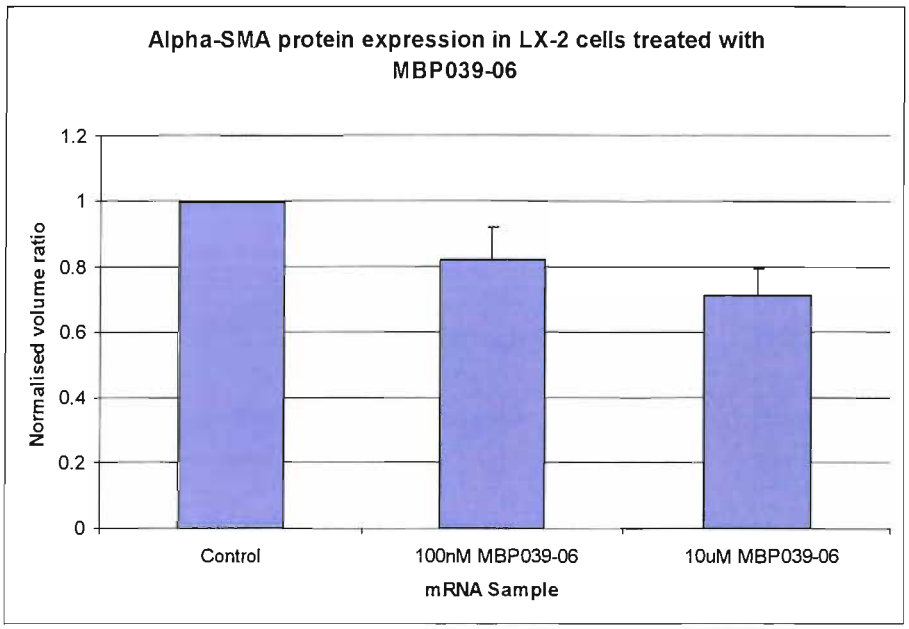
Graph 1.7 TIMP-1 expression in LX-2 treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



Graph 1.8 MMP-2 expression in LX-2 treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



Graph 1.9 collagen-1expression in LX-2 treated with 0.1 μM and 10 μM of MBP039-06 for 48 h.



Graph 1.10 α-SMA expression in LX-2 treated with 0.1 μM and 10 μM of MBP039-06 for 48 h (n = 3).

I.7.4 Conclusions; *Ex vivo* Liver Fibrosis Assay

The activation of the HSC, as a result of liver injury, leads to a downregulation in interstitial collagenase / matrix metalloproteinase-1 (MMP-1) and an increase in matrix type MMP (MT1-MMP) and gelatinase A (MMP-2). Crucially, with respect to fibrosis, the phenotypic change results in an increase in expression of the tissue inhibitors of metalloproteinase-1 and -2, (TIMP-1 and TIMP-2). The results in the LX-2 experiment (graph 1.7 -1.9) show a dose dependent reduction in the expression of TIMP-1 and MMP-2 and collagen-1 in the LX-2 cells. These findings show MBP039-06 reduces the downregulation of these phenotypic markers of activated HSCs and the turnover of collagen in this *ex vitro* model in a dose dependent manner. The α-SMA protein expression is also reduced in a dose dependent manner (graph 1.05, 1.06 and 1.10), which suggests the mechanism of the compound could be to reduce the activation of the HSC and LX-2 cells as well as to reduce the level of collagen and therefore increase the turnover of collagen *in vitro*.

In conclusion, the synthesised batch of MBP039-06 (**1.81**) can significantly reduce phenotypic markers of activated HSCs at doses >10μM in both primary HSC and LX-2 cells indicating that the synthesised batch of MBP039-06 (**1.81**) does have potential antifibrotic properties.

I.7.5 Prolyl Hydroxylase *In Vitro* Assay

In order to access the prolyl hydroxylase activity of MBP039-06 we chose to test MBP039-06 and its analogues in a related prolyl hydroxylation mediated process of HIF (hypoxia inducible factor), of which an *in vitro* assay of HIF was available within Professor Chris Schofield's group at Oxford University.

Ideally we would have liked to have tested the compounds in an assay involving the inhibition of the enzyme prolyl 4-hydroxylase, which catalyses the hydroxylation of proline moiety in the synthesis of collagen. Unfortunately this assay requires specialist equipment to set up and run routinely. In mammals, in addition to the hydroxylation of HIF, 2-oxoglutarate-dependent oxgenases catalyse procollagen-extracellular matrix

formation. The HIF assay would give an indication of the activity of MBPO39-06 against similar prolyl hydroxyses.

The transcription factor HIF (hypoxia inducible factor) has a central role in oxygen homeostasis. This factor is regulated by a signalling mechanism which involves post-translational hydroxylation. This process is catalysed by a set of non-haem, Fe^{2+}dependent enzymes that belong to the 2-oxoglutarate-dependent-oxygenase superfamily. Three 2-oxoglurate-dependent oxgenases that catalyse HIF prolyl hydroxylation have been identified; they are PHD1, PHD2 and PHD3. In hypoxia, prolyl hydroxylation is suppressed, which allows the HIF α subunit to escape destruction and therefore accumulate to high levels. The HIF α subunits produced during hypoxia are regulated by a dual process involving prolyl hydroxylation of HIF as previously stated and the 2-oxoglutarate-dependent oxgenases catalysed asparaginyl hydroxylation of HIF by FIH (factor inhibiting HIF). Both these processes are catalysed by Fe^{2+}dependent enzymes.[31]

Overall, new insights into the regulation of HIF by protein hydroxylation offer exciting possibilities of understanding the physiological response to hypoxia. Our interest would be that manipulation of these signalling pathways by hydroxylase inhibitors might offer new therapies to ischaemic/ hypoxic disease. It was therefore postulated that MBP039-06 could be a potential inhibitor of this proyl hydroxylation process.

I.7.6 HIF *In Vitro* Assay Results (Prolyl Hydroxylation)

The compounds (figure 1.10) were tested in a prolyl hydroxylase assay kindly run by Dr Kirsty Hewitson and Professor Chris Schofield at Oxford University. The assay uses a radioactive substrate targeted for the HIF prolyl hydroxylase. NOG (N-oxalyl glycine) was included as a control for comparison; this compound is an analogue of 2-oxoglutarate (2OG) and is a generic inhibitor of most and possibly all 2OG oxygenases.[31]

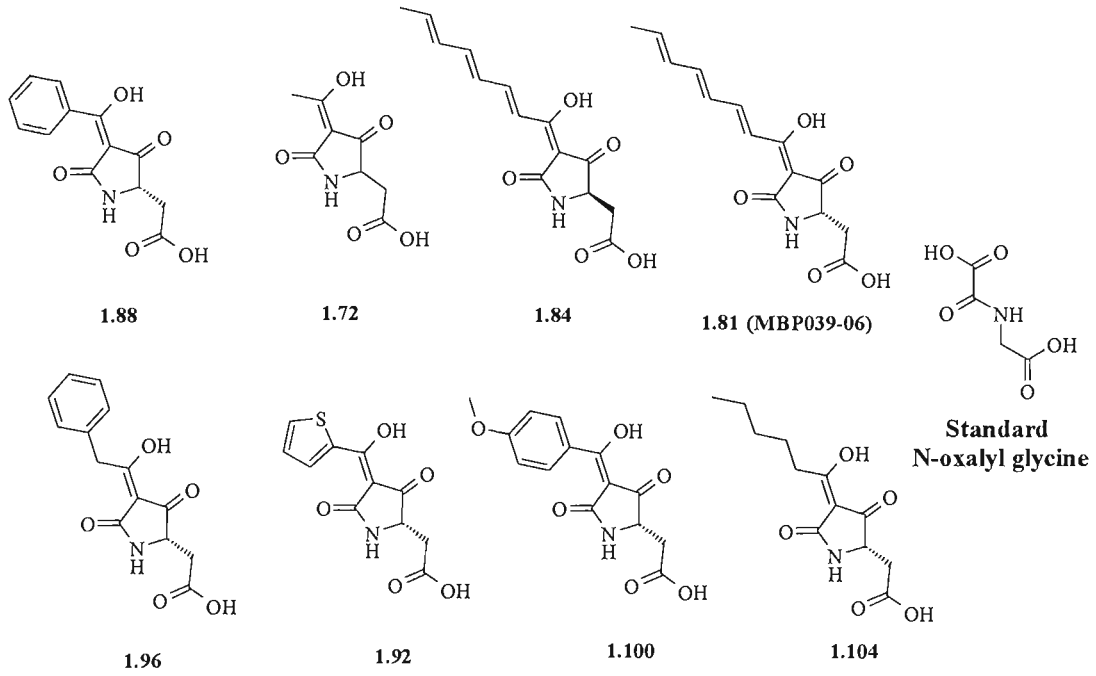
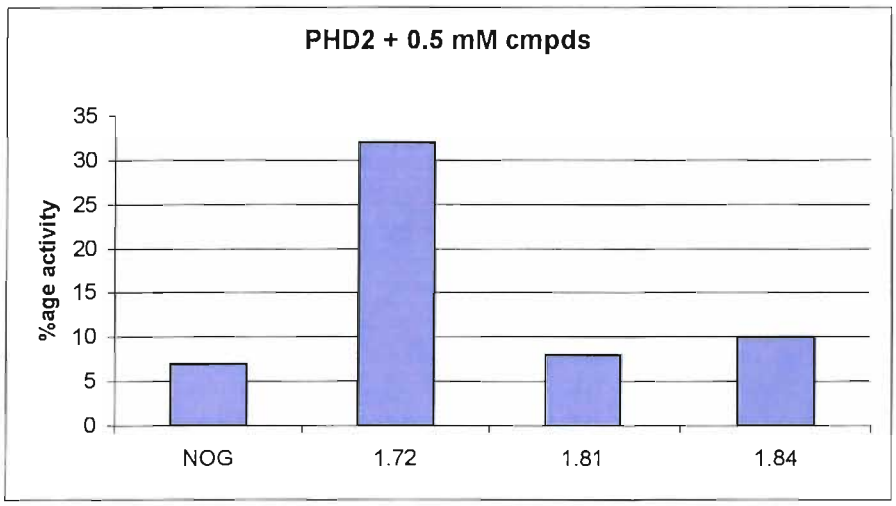


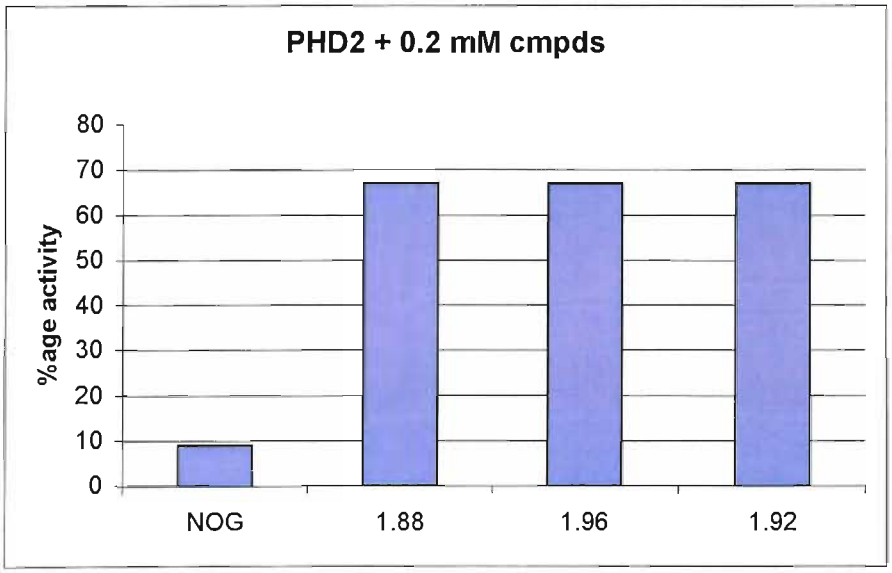
Figure 1.10 Tetramic acid derivatives tested at Oxford, and the control N-oxalyl glycine for the assay.

The first batch of compounds (**1.72, 1.81** & **184**) were assayed against PHD2 181-426 at 0.5 mM final concentration with a fragment of human HIF-1α comprising the C-terminal oxygen dependent degradation domain (CODD) 19mer peptide as substrate (graph 1.11).



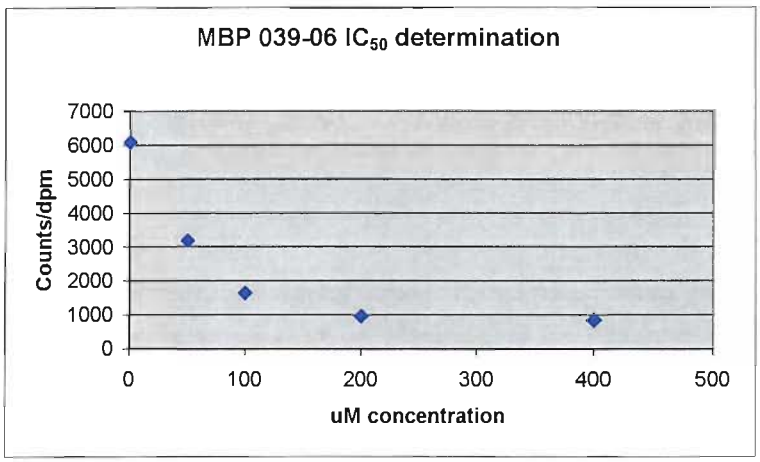
Graph 1.11 PHD2 inhibition of tetramic acids (**1.72, 1.81** & **1.84**).

The second and third batches of compounds (**1.88**, **1.96**, **1.92** & **1.100, 1.104**) were assayed against PHD2 181-426 at 0.2 mM final concentration with HIF-1α CODD 19mer peptide as substrate. NOG was included as control (second batch graph 1.12, no graphical data for batch three was obtained).



Graph 1.12 PHD2 inhibition of tetramic acids (**1.88**, **1.96**, & **1.92**).

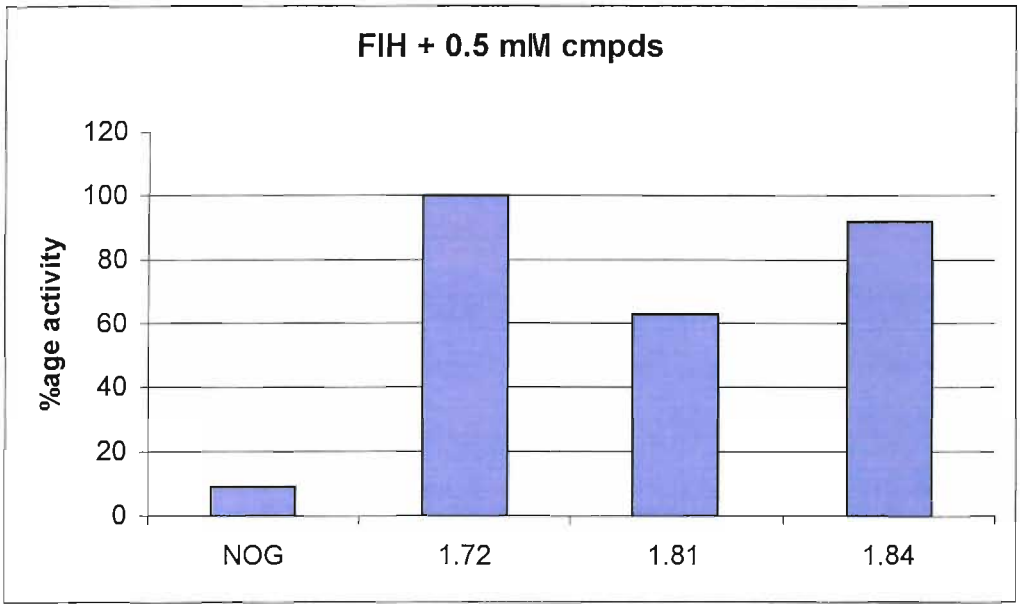
No significant activity was observed with the second batch and third batches of compounds in the PHD2 assays (figure 1.10), therefore IC_{50}s were not pursued. However, MBP039-06 (**1.81**) was analysed further and assayed at various concentrations against PHD2 to determine an IC_{50} (graph 1.13). This gave an approximate IC_{50} of 50 uM, comparable to that of the standard inhibitor NOG.



Graph 1.13 MBP 039-06 (**1.81**) IC_{50} determination.

I.7.7 FIH *In Vitro* Assay Results (Asparaginyl Hydroxylation)

The active PHD2 inhibitors **(1.72),** MBP036-06 **(1.81)** & **(1.84)** were also assayed against factor inhibiting hypoxia inducible factor (a HIF asparaginyl hydroxyase, FIH) at 0.5 mM final concentration with GST HIF-1α 786-826 as substrate. NOG was included as control (graph 1.14). No significant activity was observed with these compounds towards FIH. IC_{50} values therefore were not pursued.



Graph 1.14 FIH inhibition of compounds (**1.72**, **1.81**, & **1.84**).

I.7.8 HIF and FIH *In Vitro* Assay Conclusions

The preliminary conclusions outlined by Professor Chris Schofield at Oxford University group were that some of the compounds were active against PHD2, an *N*-terminally truncated version of the human hypoxia inducible factor (HIF) prolyl-4-hydroxylase. This is consistent with the observations in the Mitsubishi patent.

None of the compounds are active against FIH. This supports the conclusion that the compounds do not work by simple chelation of iron by the tetramic acid. It more importantly demonstrates that the template might be used to develop selective inhibitors. The results for the analogues are disappointing as the hydrophobic

derivatives from the first series were inactive, but we know that the factors that lead to a PHD2 inhibitor are quite subtle.

I.8 Conclusion and Future Work

I.8.1 Conclusions; Total Synthesis of MBP039-06

The total synthesis of MBP039-06 was successfully achieved as two individual enantiomers. The 1H NMR and UV spectra of the enantiomers are identical to that supplied by Dr. Junko Takashima of the Mitsubishi Pharmaceuticals Corporation for naturally isolated MBP039-06. In their isolation of the natural product, the stereochemistry of the chiral centre was not determined. Neither was an optical rotation taken, but they supplied us with the CD spectrum. The CD spectrum of our synthesised (*S*) compound (**1.81**) has been taken and is consistent with that supplied, as both give a negative value. The data showed conclusively that a total synthesis of MBP039-06 had been completed, and that the chiral centre has (*S*)-stereochemistry.

A small library of tetramic acid analogues of MBP039-06 was synthesised utilising the Dmb protection strategy developed during the total synthesis of MBP039-06.

Our compounds were screened against PDH2, the HIF proyl hydroxylase and FIH asparaginyl hydroxylase, by Prof. Chris Schofield at Oxford University. Both enantiomers of MBP039-06 were similar in activity to the standard inhibitor *N*-oxalylglycine, with an IC_{50} = 50 μM. Both enantiomers demonstrated good selectivity against FIH a similar Fe^{2+}dependent enzymes, at which they were inactive demonstrating that the tetramic acids do not work through simple chelation of the iron.

In an *ex-vivo* LX-2 cellular assay undertaken at Ferring Research Ltd; MBP039-06 appears to significantly reduce phenotypic markers of activated HSCs, TIMP-1, MMP-2 and Collagen-1 mRNA expression, over the 48 h treatment period in a dose dependent manner. The Alpha-SMA protein expression is also reduced which suggests the mechanism of the compound could be to reduce the activation of the LX-2 cells as well as reduce the level of collagen and increase the turnover of collagen *in vitro*.

I.8.2 Future Work

Due to time and resources issues only compound (**1.81**) MBP039-06 had been tested in the HSC and LX-2 *ex vivo* assay. The new tetramic acid analogues require further evaluation in a relevant model of fibrosis to access their potential as antifibrotic agents.

Further analogues of MBP039-06 could be synthesised based upon subtle changes to the alkyl triene or modification of the aspartic acid chain to investigate the SAR against HIF. The routes developed during the total synthesis of MBP039-06 would be sufficiently adaptable to accommodate a range of analogues.

Larger quantities of MBP039-06 would also be required in order to fully investigate its antifibrotic mechanism of action.

Chapter II: An Approach to the Total Synthesis of Dithiosilvatin

Chapter II: An Approach to the Total Synthesis of Dithiosilvatin

II.1 Introduction to Epidithiodiketopiperazines (ETPs)

II.1.1 Introduction and Potential Use as Antifibrotic Agents

As reported, liver fibrosis results from chronic injury of the liver by a variety of agents. At present the only effective therapy is transplantation which is limited by donor organ availability and has significant cost implications for the NHS. Recent clinical and experimental work has shown that fibrosis is a reversible process. However, clinically reversion is a rare and slow process that will only be achieved if the underlying cause of the liver disease can be effectively treated. The Southampton Liver Group (SLG) has shown that reversion of fibrosis is associated with the clearance of activated wound healing myofibroblasts from the injured liver by apoptosis. Activation of hepatic stellate cells (HSCs) causes most of the pathology changes in liver fibrosis and cirrhosis. The removal or inactivation of activated HSC from the liver is therefore likely to be a key process before recovery from liver fibrosis can occur.[1, 10]

In proof-of-principle studies the SLG have demonstrated that experimental stimulation of hepatic myofibroblast apoptosis leads to dramatic acceleration of the reversion of rat liver fibrosis. This study utilised the toxic fungal metabolite gliotoxin (figure 2.3) which has been shown to induce apoptosis of HSCs *in vitro*. Administration of gliotoxin to rats *in vivo* resulted in a significant reduction of the number of activated stellate cells in the liver. It has been reported that gliotoxin does decrease liver fibrosis in rats, but did not improve liver function.[10] To further understand the mechanism(s) by which gliotoxin promotes apoptosis and to improve the efficacy of the molecule as a anti-fibrotic therapeutic, gliotoxin and other members of the epidithiodiketopiperazine alkaloid family (ETPs), such as dithiosilvatin (figure 2.3), have become important synthetic targets.

This is emphasized by a recent review that postulated that the toxicity is due to the disulfide bridge in this class of compounds.[53] It is proposed that gliotoxin inactivates proteins *via* reactions with the thiol groups, and leads to the generation of reactive

oxygen species by redox (figure 2.0). However, this cannot account for how gliotoxin causes apoptosis and necrotic death of animal cells. Apoptosis is not inhibited in gliotoxin-treated cells by a range of scavengers of the deleterious reactive oxygen species, which implies that redox cyling is not involved in the induction of apoptosis. Furthermore, the nanomolar concentrations of gliotoxin that induce apoptosis would not be expected to induce oxidative stress.

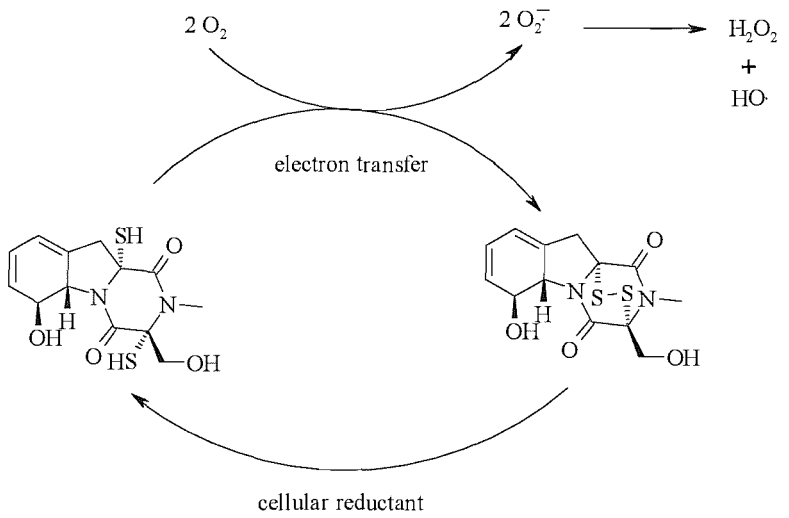


Figure 2.0 redox cycling between the reduced (dithiol) and the oxidised (disulfide) forms of gliotoxin. The oxidation of gliotoxin and presumably other ETPs generates reactive oxygen species.

There is a need to develop an efficient synthetic route to these potential anti-fibrotic therapies. The route will enable us to not only access the natural product, but additional new analogues. Biological testing of such compounds will help elucidate their molecular mechanism of action, which at present is poorly understood.

II.1.2 ETPs as Potential Histone Methyltransferase Inhibitors

Histone methylation plays a key role in establishing and maintaining gene expression patterns during cellular differentiation and embryonic development. During cell life the differential state of a cell is determined by its specific pattern of gene expression, which in turn is established and maintained through the differential packaging of DNA into chromatin. Nucleosomes are the base unit of chromatin. These nucleoprotein particles consist of 147 base pairs of DNA that are wrapped around a proteinaceous core of four histones H2A, H2B, H3 and H4. Post-translational

modifications of the histone *N*-terminal tails, as well as modifications within the globular domain, regulate the level of chromatin condensation and regulate gene expression. Inhibitors of histone acetyltransferases are used as drugs for cancer therapy as they can affect changes in gene expression of specific genes involved in cancer. Methyltransferases have also been shown to be important in establishing stable gene expression. Some known histone methyltransferases (HMTs) are misregulated in tumors.[54] Heterochromatin formation, which can be methyltransferase induced, could also be involved in neurodegenerative diseases such as Alzheimer's.[55]

A specific inhibitor of the histone methyltransferase SU(VAR)3-9 has been recently reported.[56] In this study a library of small molecules was screened for their ability to inhibit the activity of recombinant *Drosophila melanogaster* SU(VAR)3-9 protein, a key player in establishing condensed heterochromatin by specifically methylating Lys9 of the histone H3. During the screen it was found that chaetocin, like gliotoxin a member of the epidithiodiketopiperazine alkaloid family (figure 2.3) affects HMT function. It had an IC_{50} for SU(VAR)3-9 of 0.6 μM. In further tests to investigate its enzyme specificity it was reported that cheaetocin also inhibited the human ortholog of dSU(VAR)3-9 with a similar IC_{50} value of 0.8 μM. It also inhibited other known Lys9-specific HMTs such as mouse G9a with a higher IC_{50} value of 2.5 μM.[56]

II.1.3 Gliotoxin and the Epidithiodiketopiperazine Family

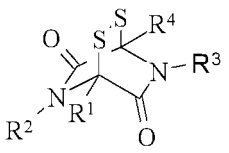


Figure 2.1 Generic structure of an epidithiodiketopiperazine, the most common form of ETP.

Most fungal toxins are secondary metabolites and of low molecular mass. One well characterised class of toxins are the epipolythiodiketopiperazines (ETPs), which is characterised by the presence of an internal disulfide bridge (figure 2.1). In some circumstances the sulfur bridge contains one, three or four sulfur atoms; these compounds are usually co-produced with those containing the disulfide.[53]

Gliotoxin (figure 2.3) was the first isolated ETP reported. It was isolated from cultures of the wood fungus *Gliocladium fimbriatum* in 1936. It has since been isolated from a variety of microorganisms such as the opportunistic animal pathogens *A. fumigatus*, *Penicillium* spp. and *Candida* spp. and mycoparasitic *Trichodrermia* spp.[53]

Gliotoxin is implicated in animal mycoses. As well as its ability to cause apoptosis, it is immunosuppressive and causes necrotic cell death *in vitro*. This toxicity has made the ETPs an important potential therapeutic for diseases like cancer.[57] Even after 50 years of research the biosynthesis or the primary role of the ETPs is not well understood and yet proposed modes of activity, biosynthesis and transport have been made.

There are currently 14 different ETPs known. This diversity stems from the amino acid core. It is predicted that in gliotoxin these amino acids are Phe and Ser and in dithiosilvatin they are Tyr and Gly. The biosynthetic pathway to gliotoxin from these amino acids has been proposed (figure 2.2), but a number of the predicted compounds have not been isolated. All the ETPs isolated to date contain at least one aromatic ring.[53]

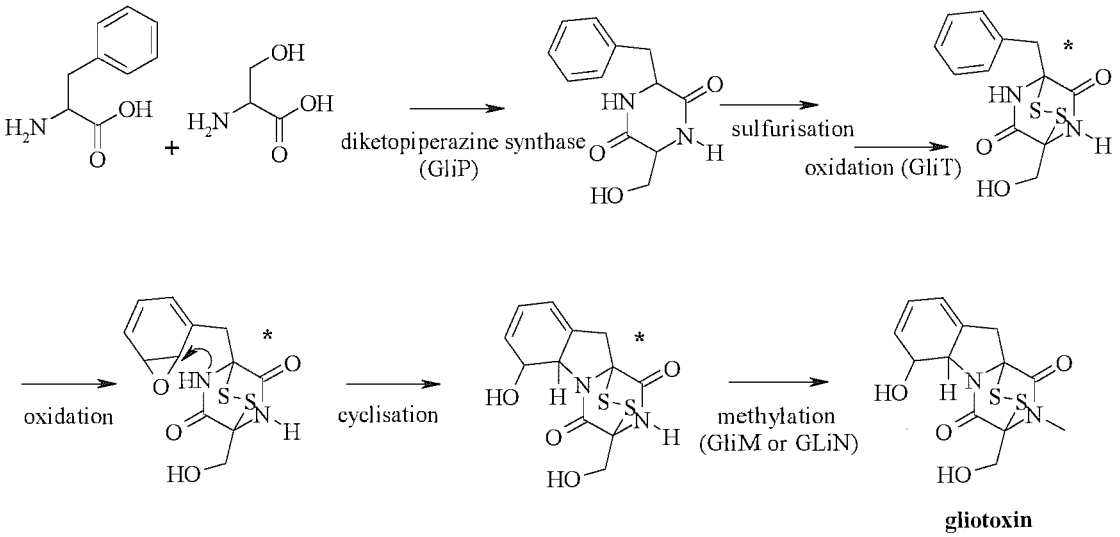


Figure 2.2 Predicted biosynthetic pathway for gliotoxin; * predicted compounds that have not been isolated.

II.2 Synthesis of Epidithiodiketopiperazines (ETPs)

II.2.1 Synthetic Approaches

Very little work in this area has appeared since Kishi's landmark total synthesis of gliotoxin, dehydrogliotoxin and hyalodendrin (figure 2.3).[58] The central problem to the ETPs is the synthesis of the epidithiodiketopiperazine system. When Kishi first embarked on the synthesis of gliotoxin, there were only three ETPs synthesised, this has not changed to any great extent. Still the initial methodologies developed by Trown in 1968, Hino *et al.* in 1971 and Schmidt *et al.* in 1972 (scheme 2.1-2.3) are utilised in ETP synthesis today.[59, 60, 61]

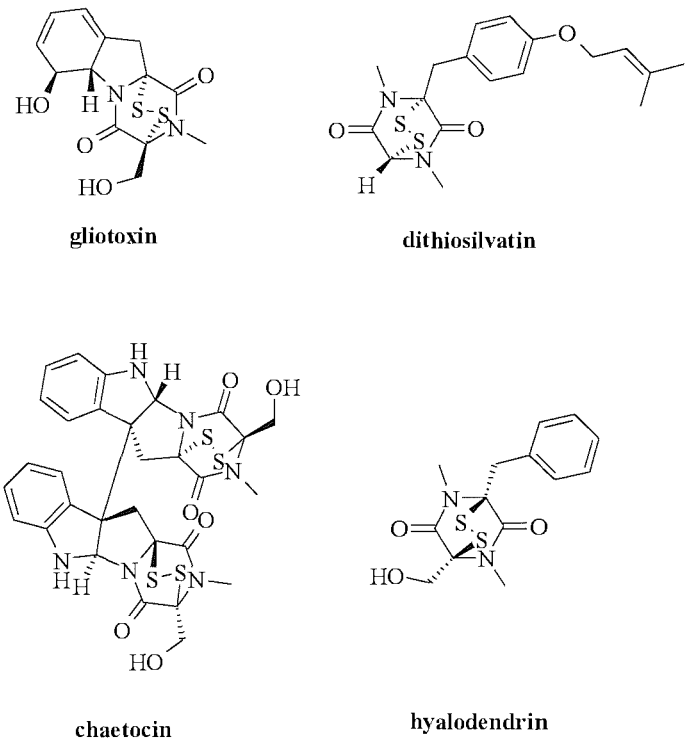
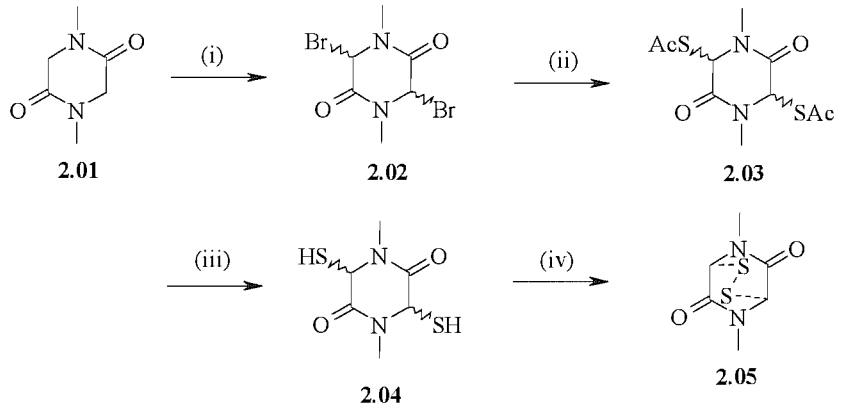


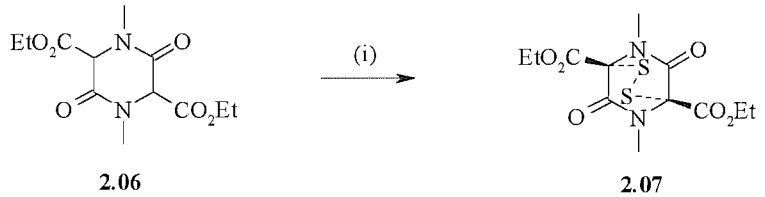
Figure 2.3 Gliotoxin and other members of the epidithiodiketopiperazine alkaloid family.

In the Trown synthesis the sulfur is added *via* nucleophilic substitution of the bromide in the diamide (**2.02**), which is formed by brominating sarcosine anhydride (**2.01**).[59] The acetyl group of DKP (**2.03**) can then be readily cleaved with acid and the resultant dithiol (**2.04**) can be oxidised to the disulfide (**2.05**) with Ellman's reagent.

In both the Hino and Schmidt synthesis the sulfur is introduced using carbanion chemistry. In Hino's approach the carbanion of compound (**2.08**) is quenched with disulfur dichloride resulting in the ETP (**2.07**) in one step.[60]

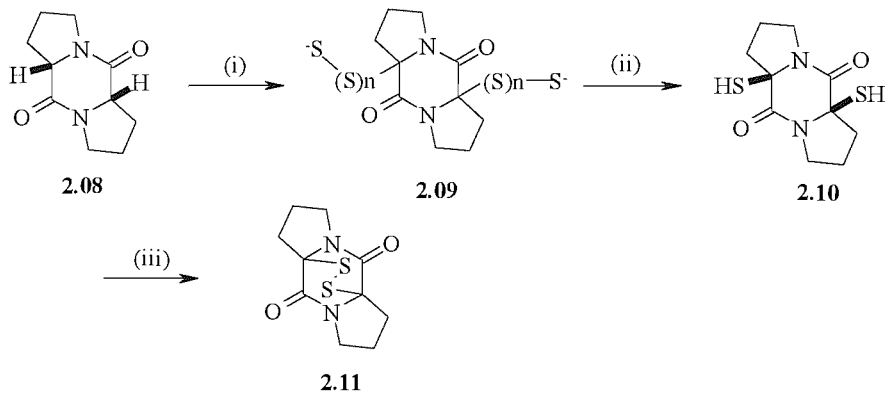


Scheme 2.1 Trown synthesis. Reagents and conditions: (i) Br_2; (ii) AcSK; (iii) HCl/EtOH; (iv) DTNB.



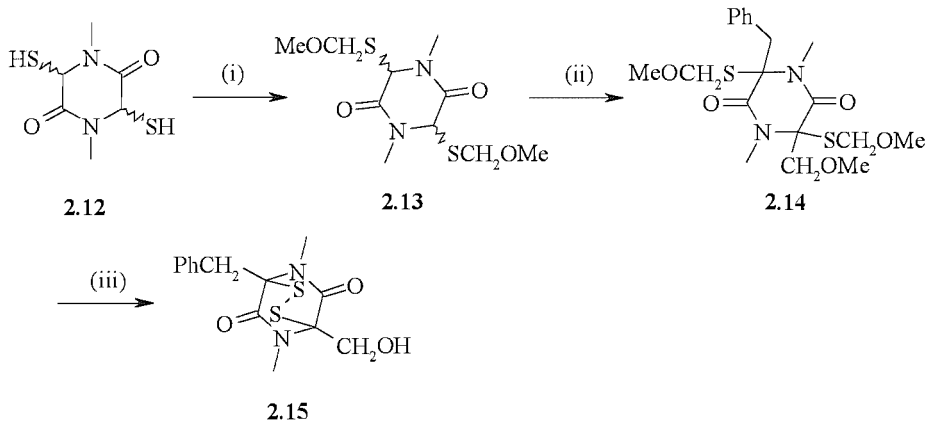
Scheme 2.2 Hino synthesis. Reagents and conditions: (i) S_2Cl_2, NaH.

The Schmidt synthesis is very similar, but the carbanion is trapped with sulfur in this case giving the mixed disulfide (**2.09**).[61] This is subsequently reduced to the desired *cis* dithiol (**2.10**). Finally oxidation with iodine gives the ETP (**2.11**).



Scheme 2.3 Schmidt synthesis. Reagents and conditions: (i) (a) $NaNH_2$; (b) S_8; (c) $NaNH_2$; (d) S_8; (ii) $NaBH_4$; (iii) I_2.

Kishi investigated two routes towards the synthesis of the epidithiodiketopiperazine core during the total synthesis of gliotoxin.[58] Whilst attempting one of these methods he discovered a simple and effective route to the total synthesis of *dl*-hyalodendrin (scheme 2.4).



Scheme 2.4 Reagents and conditions: (i) KO^{t}Bu, CH_3OCH_2OMe, 80%; (ii) LDA (2.3 equiv), $PhCH_2Br$ (5 equiv), $MeOCH_2Br$ (4 equiv), -78°C 63%; (iii) (a)BCl_3, CH_2Cl_2, -78°C; (b) I_2, $CHCl_3$, 28%.

Starting from the Trown intermediate (**2.12**), Kishi, prepared *dl*-hyalodendrin in 28% overall yield. The key step to the route was the alkylation of the protected DKP (**2.13**). They first generated the dicarbanion *via* the addition of 2.3 equivalents of lithium diisopropylaminde. This dicarbanion had a remarkable difference in reactivity to the monocarbanion. When treated with 5 equivalents of benzyl bromide for 20 min at -78 °C and then 4 equivalents of bromoethyl methyl ether for 10 min at -78 °C the dialkyl DKP (**2.14**) was obtained in 63% yield in the desired *cis* configuration. The *cis* configuration was confirmed by the total synthesis of the natural product. After treatment with boron trichloride to remove the protecting groups, only the *cis* dithiol could be oxidised to the ETP (**2.15**).

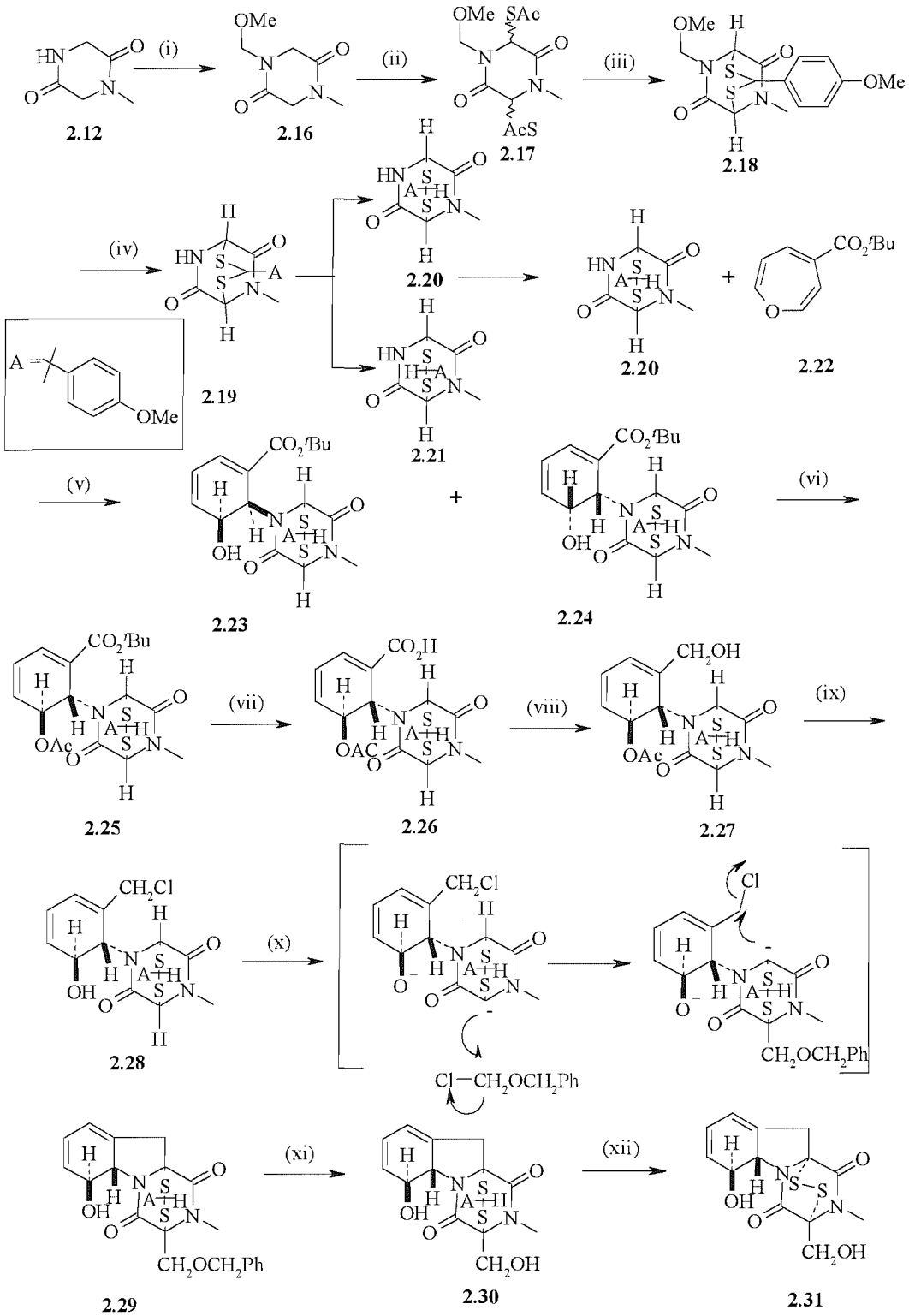
II.2.2 Kishi's Total Synthesis of Gliotoxin

In Kishi`s total synthesis of gliotoxin (scheme 2.5) the amide of glycine-sarcosine anhydride was first protected as a methoxymethyl group.[58] This methoxymethyl derivative (**2.16**) was converted to a *cis* and *trans* mixture of the dithioacetate (**2.17**). This was subjected to methanolysis with methanolic hydrogen chloride at 50 °C to give the dithiol, which was treated with *p*-anisaldehyde and boron trifluoride etherate in dichloromethane to yield methoxymethyl thioacetal (**2.18**). Removal of the

methoxymethyl group was achieved under acid conditions to yield the thioacetal (**2.19**) *via* a crystallisation. The diastereomeric mixture was then resolved by utilising the differences in the reactivity of the two diastereoisomers with benzoyl chloride. A final recrystallisation of the thioacetals gave (**2.20**) pure. This was important in order to get the correct orientation for the Michael reaction with compound (**2.22**), which was prepared by the conditions reported by Berchtold *et al.*[62]

Kishi completed an extensive study of the favourable orientation required for this Michael addition.[63] The Michael addition yielded a 3:1 mixture of the Michael adducts (**2.23**) and (**2.24**). The desired adduct (**2.24**) was treated with acetic anhydride and pyridine to yield the acetate (**2.25**). This was converted to the alcohol (**2.27**) *via* the carboxylic acid (**2.26**) by means of a mixed anhydride and sodium borohydride reduction. The alcohol was converted to the mesylate and treated with lithium chloride. Methanolysis then gave the chloro alcohol (**2.28**).

The critical cyclisation was accomplished by careful addition of 3.2 equivalents of phenyllithium to a solution of benzyl chloromethyl ether in THF at -78 °C, to yield the desired compound (**2.29**) in 52% yield. The free alcohol was essential to prevent aromatisation under these conditions. The benzyl ether was cleaved with boron trichloride to give the diol (**2.30**). Finally the *dl*-gliotoxin thiol acetal (**2.30**) was treated with 1.2 equivalents of *m*-chloroperbenzoic acid in CH_2Cl_2 at 0 °C and then perchloroacetic acid at room temperature to afford *dl*-gliotoxin. Optically active gliotoxin was synthesised by the same route but the diasteroisomer (**2.20**) was resolved with α-(-)-methyl-benzyl isocyanate.

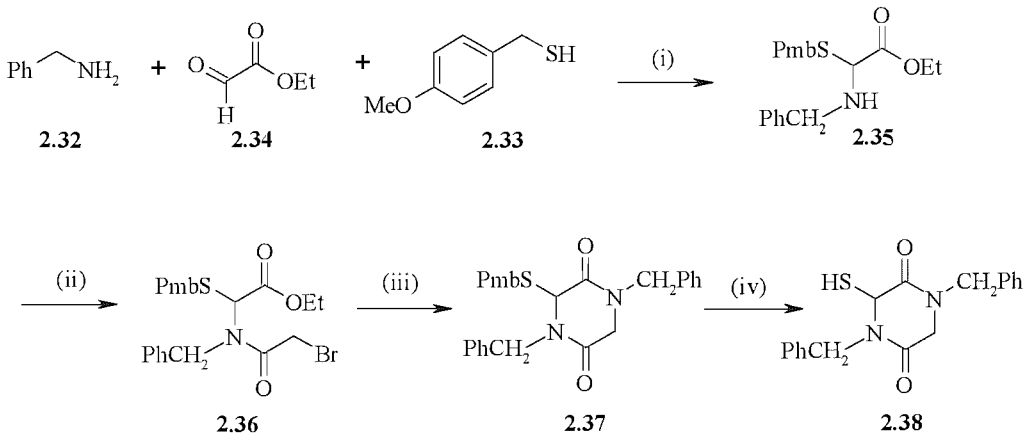


Scheme 2.5 Reagents and conditions: (i) K^{t}OBu, $MeOCH_2Cl$, 0 °C, 75%; (ii) (a) NBS, benzyl peroxide, CCl_4; (b) KSAc, CH_2Cl_2; (iii) (a) HCl, MeOH; (b) *p*-anisaldehyde, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (iv) HCl/EtOH; (v) Triton B, rt, 30 min, 60%; (vi) $(Ac)_2O$, pyridine; (vii) TFA; (viii) (a) Et_3N, EtOCOCl, rt; (b) $NaBH_4$, MeOH; (ix) (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (b) LiCl, DMF, 96% (2 steps); (x) (a) $PhCH_2OCH_2Cl$, PhLi (3.2 equiv), -78 °C, 52%; (xi) BCl_3, 0 °C, 60%; (xii) (a) MCPBA, 0 °C, CH_2Cl_2; (b) $HClO_4$, CH_2Cl_2, 58%.

II.2.3 New Approach to the ETP Core

The Trown methodology has been used by several groups, but such an approach is not tolerant of more sensitive functionality. Motherwell *et al.* published two papers on an amino acid approach whilst we were embarking on the study ourselves. [64, 65]

The first of these papers developed a route to the monomercaptodiketopiperazine nucleus (scheme 2.6) starting with benzylamine (**2.32**), ethyl glyoxalate (**2.34**) and *p*-methoxybenzyl mercaptan (**2.33**) in a three component reaction.[64] The protected mercapto amino acid ethyl ester (**2.35**) was isolated by first mixing benzylamine and ethyl glyoxalate in toluene for 2 minutes. Addition of *p*-methoxybenzyl mercaptan gave the ester in 92% yield. The ester was further elaborated to the DKP (**2.37**) by first treating the ester with bromoacetyl bromide under Schötten-Baumann conditions to give the intermediate amide (**2.36**). This was then reacted with benzylamine in acetonitrile in the presence of triethylamine to give the DKP (**2.37**). Finally the free mono thiol (**2.38**) was isolated upon treatment with boron tribromide at -78 °C in 68% yield.

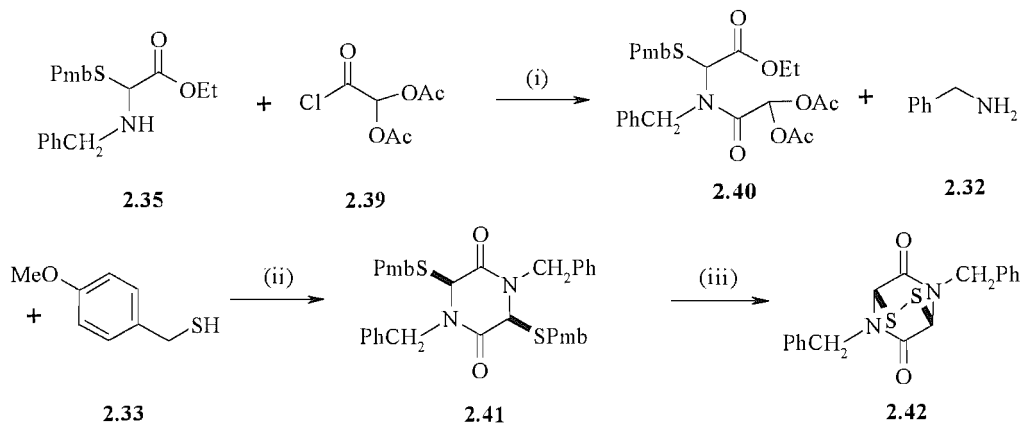


2.32 **2.34** **2.33** **2.35** **2.36** **2.37** **2.38**

Scheme 2.6 Reagents and conditions: (i) Toluene, rt, 92%; (ii) $BrCH_2COBr$, $NaHCO_3$, CH_2Cl_2, H_2O, 99%; (iii) benzylamine, Et_3N, MeCN, 94%; (iv) (a) BBr_3, CH_2Cl_2, -78 °C; (b) aq NH_4Cl, , -78 °C, to rt, 62%.

The follow-up publication by the Motherwell group (scheme 2.7) adapted this three component procedure to the synthesis of the ETP core structure (figure 2.1).[65] The amino acid ester (**2.35**) was synthesised as before (scheme 2.6). It was then reacted with a masked aldehyde reagent, a protected diacetoxyacetyl chloride (**2.39**) under Schötten-Baumann conditions in 94% yield. The *cis* protected dithiodiketopiperazine

was obtained by simply heating diacetate (**2.40**), benzylamine (**2.32**) and *p*-methoxybenzyl mercaptan (**2.33**) in acetonitrile with a catalytic amount of TFA at reflux, exclusively in 64% yield. Initially, this reaction was completed at rt and a 1:1 mixture of *cis*:*trans* pmb protected dithiodiketopiperazine was isolated, but upon prolonged heating only the *cis* was isolated. These findings could be supported by comparing thermodynamic preferences of formation of the *cis* and *trans* Pmb dithiodiketopiperazine *via* the gas phase DFT calculations for a single molecule using the B3LYP/6-13G(d) level of theory. This revealed that the *cis* configuration is 9 kJ mol^{-1} more stable than the alternative *trans* configuration.[65] Efficient deprotection of the *cis* Pmb DKP (**2.41**) was achieved by reaction with boron tribromide in dichloromethane, followed by an oxidation with iodine in the workup yielding the dibenzyl ETP (**2.42**) in 85% yield.



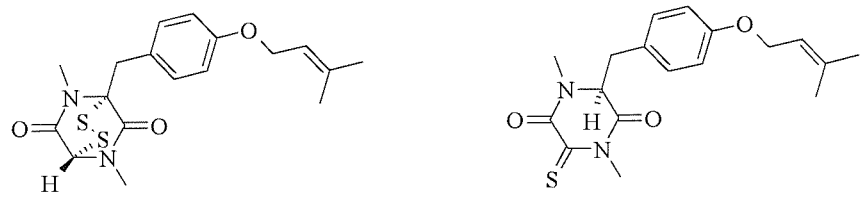
Scheme 2.7 Reagents and conditions: $NaHCO_3$, CH_2Cl_2, H_2O, 94%; (ii) (**2.32**), 3 equiv, (**2.33**), 1.5 equiv, MeCN, TFA, reflux 16 h, 64%; (iii) (a) BBr_3, CH_2Cl_2, -78 °C-rt; (b) aq NH_4Cl; (c) I_2, 85%.

II.3 Dithiosilvatin

II.3.1 Intoduction to Dithiosilvatin

The metabolites dithiosilvatin and silvathione (figure 2.4) were isolated *via* mycelial chloroform extraction and dichloromethane extract of the culture filtrate respectively, of the fungus *Aspergillus silvaticus*. This fungus, which develops an abundance of hülle cell masses, belongs to the *Aspergillus versicolor* group. The full structural elucidation of these compounds has been reported by Kawai *et al.*[66]

Dithiosilvatin is a very rare example of naturally occurring ETPs which appear to be biosynthesized from glycine and tyrosine or phenylalanine but, this is by no means conclusive.

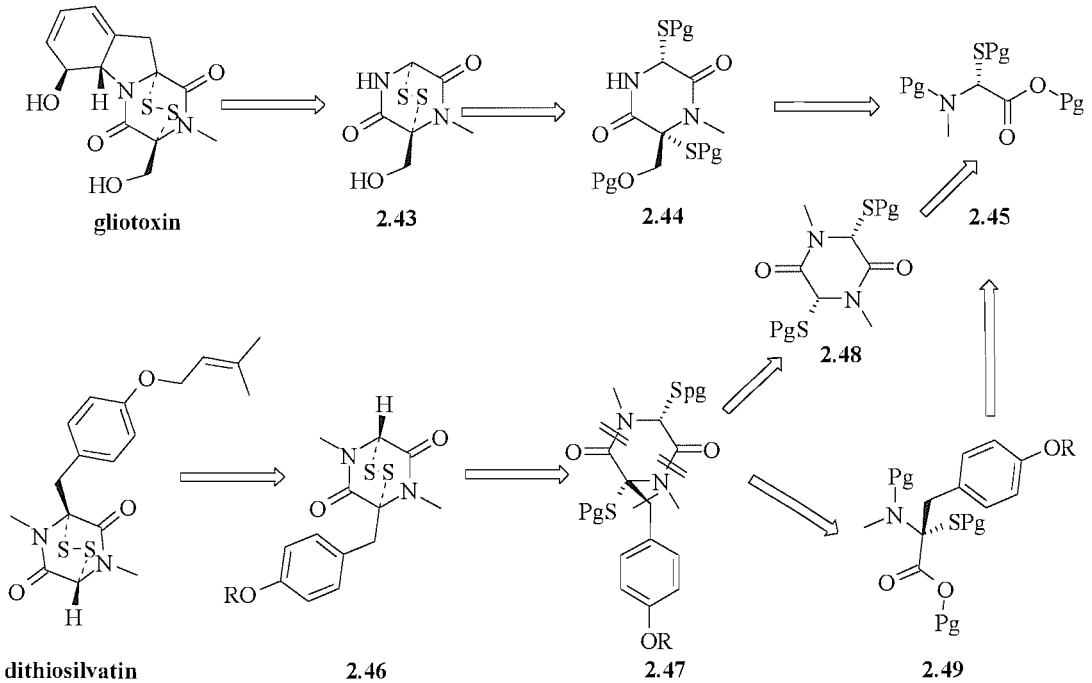


dithiosilvatin **silvathione**

Figure 2.4 Dithiosilvatin, an ETP and silvathione, a diketopiperazinethione isolated from *Aspergillus silvaticus*.

II.3.2 Retrosynthesis of Dithiosilvatin and Gliotoxin

Retrosynthesis of both gliotoxin and dithiosilvatin can lead back to a chiral protected α-mercapto α-amino acid ester (**2.45**). In both examples the key intermediate is the *bis* protected dithiodiketopiperazines (**2.44**) and (**2.47**) in that they must be in the *cis* configuration in order to achieve the desired intramolecular oxidation to form the epidithiodiketopiperazine.



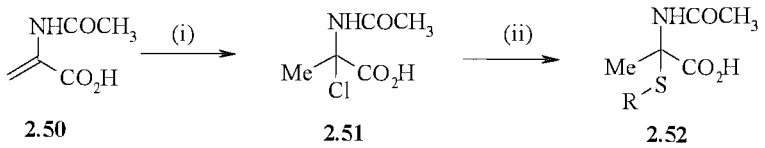
gliotoxin **2.43** **2.44** **2.45**

2.48

dithiosilvatin **2.46** **2.47** **2.49**

Figure 2.5 The disconnection of gliotoxin and dithiosilvatin into chiral thioamino acids building blocks.

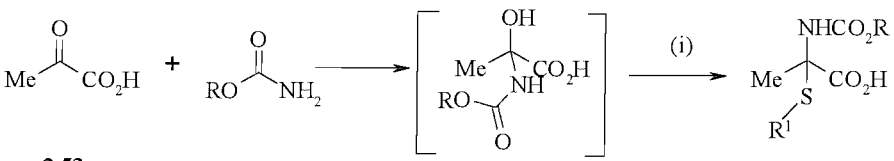
In the retrosynthesis of dithiosilvatin, fragment (**2.47**) could be disconnected in two ways. Directly to the protected α-mercapto tyrosine derived amino acid ester (**2.49**) and subsequently back to the α-mercapto α-amino acid ester (**2.45**) (represented by the double line, figure 2.5) or stepwise through the *bis* protected dithiodiketopiperazine (**2.48**) (represented by the single line, figure 2.5) and subsequently back to the α-mercapto α-amino acid ester (**2.45**). Olsen *et al.* reported the synthesis of similar α-mercapto α-amino acid esters in 1973.[67] He described the synthesis derived from acrylic acids (scheme 2.8). In this route the α-mercaptoalanine derivative (**2.52**) was prepared by treatment of the α-chloro compound (**2.51**) with hydrogen sulfide to give the α-mercaptoalanine (R = H) or by treatment with thioacetic acid to give the α-acetylthioalanine (R = CH_3CO). The α-chloro compound (**2.51**) was formed *in situ* by addition of hydrogen chloride to 2-acetylaminoacrylic acid (**2.50**).



2.50 **2.51** **2.52**

Scheme 2.8 Reagents and conditions: (i) 4 N HCl, TFA, AcOH; (ii) H_2S or AcSH.

These methods were limited to amide protecting groups, as they had to withstand harsh acid reaction conditions in the first step of the synthesis. Further advancements reported by Ottenheijm *et al.* (scheme 2.9) addressed this issue *via* the addition of pyruvic acid (**2.53**) to a carbamate (**2.54**) to form the intermediate 2-hydroxyalanine derivatives (**2.55**) in benzene.[68] The desired amino acid derivative (**2.56**) could then be prepared by the addition of an alkyl thiol, but in low yield.



2.53 **2.54** **2.55** **2.56**

Scheme 2.9 Reagents and conditions: (i) benzene, reflux, R^1-SH, 10-15%.

Although this route could be adapted to more desirable protecting groups, it was extremely low yielding. More recently α-mercapto-amino acids have been prepared by the route previously reported by Motherwell's group (scheme 2.6).[64, 65]

II.4 Synthetic Approach to Dithiosilvatin

II.4.1 Amino Acid Approach

Our initial approach was to adapt the recently published achiral route to similar α-mercapto-amino acid esters by the Motherwell group, so that we could synthesise the desired unnatural amino acid fragments (**2.45**) and (**2.49**) (figure 2.5). Initially like the Motherwell group we chose *p*-methoxybenzyl (Pmb) as our sulfur protecting group. In our desired fragment we required methylamine instead of benzylamine as our amine source in the three component reaction. This analogue was reported by Motherwell, but in very low yields.[64] In light of this publication a disconnection of fragment (**2.57**) can be carried out to give two feasible building blocks (**2.58**) and (**2.59**) (figure 2.6). When combined the target intermediate, Pmb protected dithiodiketopiperazines (**2.57**) could be made. The building block (**2.58**) had been reported by Motherwell so therefore, the route would only require optimisation. It was envisaged that building block (**2.59**) could be synthesised in a similar way, by means of the three component method starting from ketone analogue (**2.62**). This was substituted by ethyl pyruvate (**2.63**) and benzylamine (**2.32**) to investigate the feasibility of this approach. Benzylamine (**2.32**) was used as this gave consistently good yields in the Motherwell publications.[64] If this was successful the conditions would then be repeated with the ketone analogue (**2.62**) and methylamine (**2.61**) to yield the target compound (**2.59**).

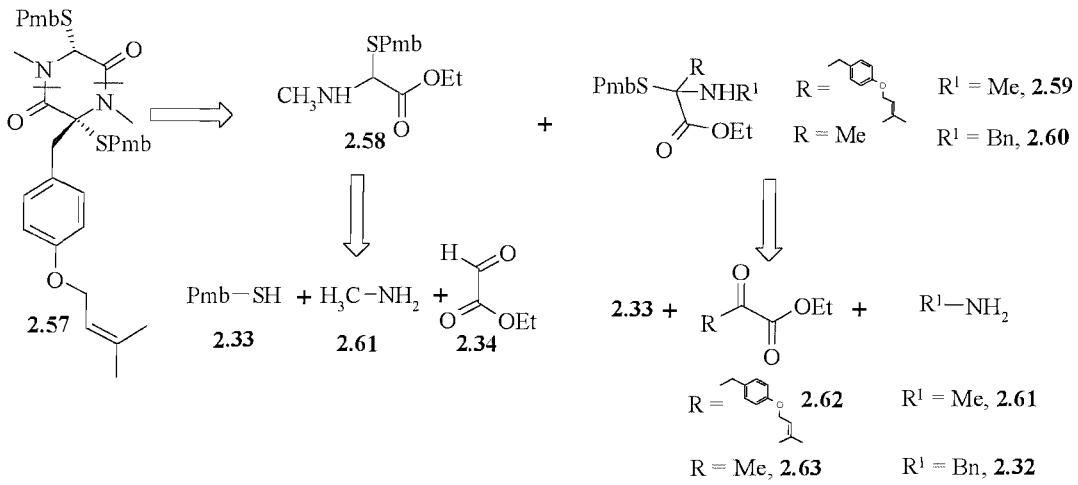
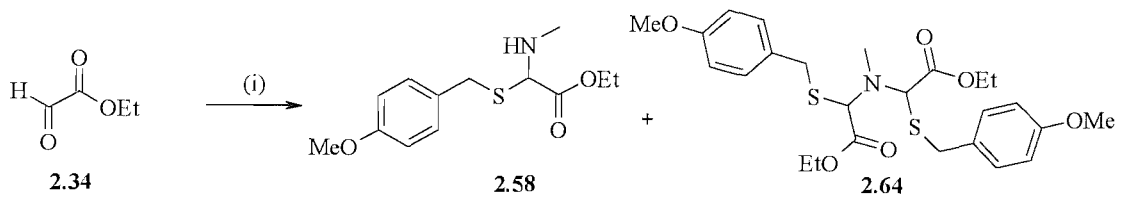


Figure 2.6 Retrosynthesis of dithiosilvatin based on the Motherwell group's findings.

We quickly established that ethyl pyruvate did not react in the desired manner. Benzylamine was treated with ethyl pyruvate and *p*-methoxybenzyl mercaptan in toluene at room temperature and at reflux but neither yielded the desired mercapto amino acid ester (**2.60**). Instead it resulted in a complex reaction mixture. This was also observed in the absence of the mercaptan. This can be explained by the fact that unlike ethyl glyoxalate, the ethyl pyruvate can exists in an enol or keto form. Both of these forms can react with benzylamine leading to a number of reactive species. Due to these findings we focused on optimising the conditions to synthesise the previously reported (4-methoxybenzylsulfanyl)methylaminoacetic acid ethyl ester (**2.58**).

Motherwell *et al.* reported that the desired (4-methoxybenzylsulfanyl)methylamino acetic acid ethyl ester (**2.58**) could be prepared via the three component reaction in 37% yield. The preferred route involved heating the reaction mixture at 110 °C for 5 h. In other examples the elevated reaction temperatures reduced the yields of the desired amino acid esters. Therefore the low yield was rationalised by the possible instability of the compound at higher temperatures.[64]

We therefore thought that microwave heating for shorter times could solve this issue. As the experimental procedure for this particular amino acid ester was not reported, methylamine was liberated *in situ via* the hydrochloride salt using triethylamine as a base. Unfortunately the amino acid ester (**2.58**) was only isolated in 15% yield when heated at 100 °C for 20 min in a CEM microwave system (scheme 2.10). The only other major product isolated was the dimer (**2.64**). There are a number of plausible reasons why this occurred. It could be that the reaction temperature was too high or the concentration of methylamine was too low.



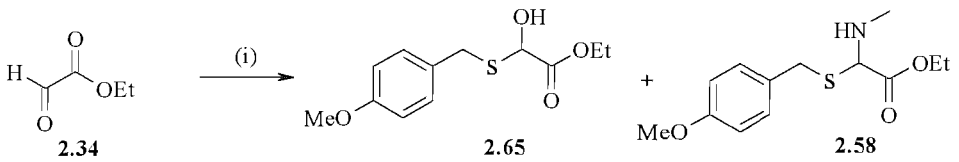
2.34 **2.58** **2.64**

Scheme 2.10 Reagents and conditions: (i) $MeNH_2.HCl$ (1 equiv), Et_3N (1 equiv), *p*-methoxybenzyl mercaptan (1 equiv), toluene, microwave heating to 110 ° C at 100 watts for 20 min, 15% (**2.58**).

In order to address these issues the reaction was repeated at room temperature (scheme

2.11). This fortunately answered both these questions, as the major product isolated was the α-hydroxy derivative (**2.65**). This was clear evidence that the concentration of methylamine was low in the reaction mixture. Therefore the reaction was essentially a two component reaction due to the insolubility of the methylamine hydrochloride in toluene.

It is feasible that the α-hydroxy derivative (**2.65**) could then react with any amino acid ester (**2.58**) formed when the reaction mixture was heated in the microwave. The heating would slightly increase the solubility of the methylamine hydrochloride, increasing the concentration of methylamine in solution but upon heating the amino acid ester (**2.58**) formed could react further with the α-hydroxy derivative (**2.65**), which is formed in excess in the reaction mixture to yield the dimer (**2.64**) as the major product.



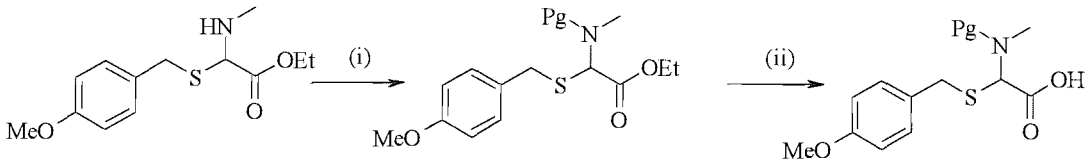
2.34 **2.65** **2.58**

Scheme 2.11 Reagents and conditions: (i) $MeNH_2.HCl$ (1 equiv), Et_3N (1 equiv), *p*-methoxybenzyl mercaptan (1 equiv), toluene, rt, 4 h. 70% (**2.65**)

The good news was the reaction did work at room temperature though, as a small trace of (**2.58**) was isolated. The reaction was therefore repeated, but the methylamine hydrochloride and triethylamine in toluene and ethyl glyoxalate were stirred at rt for 10 minutes before the addition of the *p*-methoxybenzyl mercaptan. This suspension was stirred for 2 h to give predominantly α-hydroxy derivative (**2.65**) as monitored by TLC. Excess methylamine hydrochloride and triethylamine in toluene was then added and the reaction mixture stirred at rt for 18 h to give (**2.58**) in a respectable 68% yield. The method was workable, but not ideal as the yields varied. We simply used a slight excess of 2.0 M methylamine solution in THF, instead of excess methylamine hydrochloride and triethylamine in toluene and found this consistently gave the desired compound (**2.58**) in 66% yield.

We decided that in order to maximise the potential uses of the amine (**2.58**) we would investigate a protection strategy. It was also observed that the amine (**2.58**) was not

stable following prolonged storage even at low temperature. It was postulated that protection of the amine with an amide or preferably a carbamate would solve this problem. Both the Fmoc and Boc protected ethyl esters (**2.66**, **2.67**) were synthesised in good yield (scheme 2.12) under standard conditions. The Boc protected analogue (**2.67**) was readily saponified to the acid (**2.69**) with lithium hydroxide in aqueous THF, at room temperature in almost quantitative yield. This was not the case with the Fmoc derivative (**2.66**). Even at low temperatures the Fmoc group was cleaved in preference to the saponification of the ethyl ester under basic conditions.



2.58

Pg = Fmoc, **2.66**
Pg = Boc, **2.67**

Pg = Fmoc **2.68** (failed)
Pg = Boc = **2.69**

Scheme 2.12 Reagents and conditions: (i) Fmoc-Cl (1 equiv), Na_2CO_3 (1.1 equiv), aq 1,4-dioxan, rt. 4 h, 54% (**2.66**); or (i) Boc_2O (1 equiv), NEt_3 (1.1 equiv), CH_2Cl_2, rt, 18 h, 80% (**2.67**); (ii) $LiOH.H_2O$ (1 equiv), THF, H_2O, rt, (failed) (**2.68**), 0.2M $LiOH_{(aq)}$ (1 equiv), THF,0 °C, 2 h, (failed) (**2.68**); or (ii) $LiOH.H_2O$ (1 equiv), THF, H_2O, rt. 4 h, 98% (**2.69**).

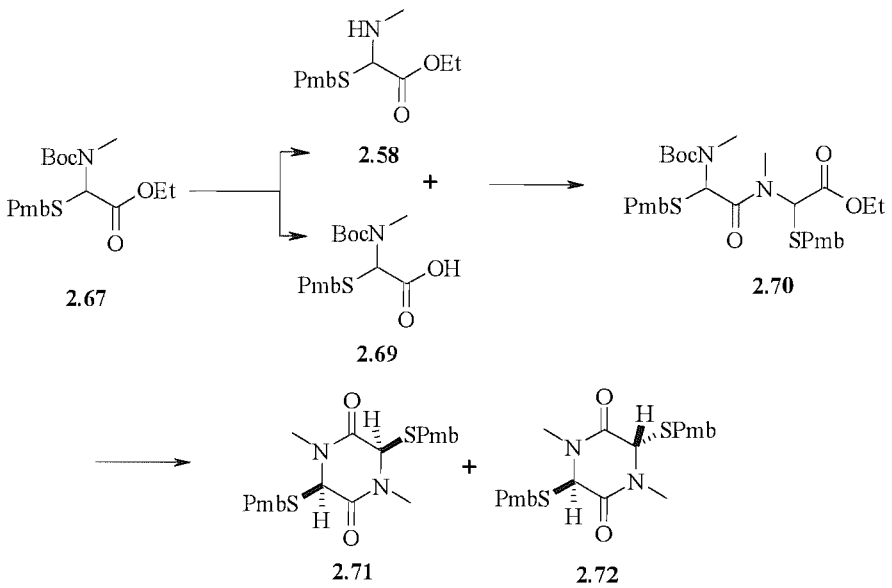
The Fmoc protecting group was the preferred protecting strategy as it was removed rapidly nonhydrolytically, by simple secondary amines, which would not affect the other protecting groups, but it is labile under basic conditions.[69]

The alternative Boc protecting group is potentially more problematic as it is removed under acid conditions. The *para*-methoxybenzyl group on the sulfur is also acid sensitive. With this in mind a number of standard Boc cleavage conditions were attempted. It was found that the Boc group could be cleanly cleaved upon the addition of 4M HCl in dioxane solution. The free amine was cleanly isolated after 30-40 minutes at room temperature, without loss of the *para*-methoxybenzyl protecting group.

The Boc amino acid ester analogue (**2.67**) was found to be stable even when stored at room temperature for prolonged periods of time. It can be selectively saponified to the acid (**2.69**) or converted back to the amine (**2.58**), making it a versatile and durable building block.

II.4.2 Synthesis of Protected Dithiodiketopiperazine

From the retrosynthesis of dithiosilvatin and the retrospective *bis* protected dithiodiketopiperazine (**2.47, 2.57**) (figure 2.5 and figure 2.6) there are two plausible disconnections. Directly to the protected α-mercapto tyrosine derived amino acid ester (**2.49**) and subsequently back to the α-mercapto α-amino acid ester (**2.45**) (represented by the double line, figure 2.5), which relies on the problematic synthesis of the substituted amino acid ester (**2.59**). Or stepwise through the *bis* protected dithiodiketopiperazine (**2.48**) (represented by the single line, figure 2.5) and subsequently back to the α-mercapto α-amino acid ester (**2.45**), which requires a good protecting group strategy. We thought the Boc protected building block (**2.67**) fulfilled these requirements and was the most advantageous approach towards the continued synthesis of the epidithiodiketopiperazine core. In the purposed scheme, (scheme 2.13) both dithio DKPs (**2.71** & **2.72**) can be prepared from the one building block (**2.67**)



Scheme 2.13 Proposed route to *bis* protected dithiodiketopiperazine.

The dipeptide (**2.70**) could be prepared *via* a number of standard peptide coupling methods, (table 2.1) from the acid (**2.69**), and the hydrochloride salt of compound (**2.58**) which in turn was prepared by acid cleavage of the Boc protected analogue (**2.68**). The best yields were achieved by first forming a mixed anhydride with *iso*butyl chloroformate in THF at -10 °C in the presence of NMM as base. After 40 minutes the reaction mixture was filtered and the filtrate added to a solution of the hydrochloride

salt (**2.58**) and NMM in dichloromethane at 0 °C. This was then stirred at room temperature for 4 hours giving the dipeptide (**2.70**) as a mixture of diasteroisomers in 58% yield. We changed from THF to dichloromethane as the solvent of choice due to the insolublity of the hydrochloride salt of (**2.58**) in THF. This resulted in an improved yield from 38% to 58%.

Reaction conditions and reagents	**Yield**
Pybrop ® (1.1 equiv), DIEA, CH_2Cl_2, rt, 18h	10%
HBTU (1.0 equiv), Et_3N, CH_2Cl_2, rt, 18 h,	12%
*iso*BuOCOCl (1.1 equiv), NMM, THF -10 °C – rt 4 h,	38%
*iso*BuOCOCl (1.1 equiv), NMM, THF & CH_2Cl_2 -10 °C – rt 4 h	58%

Table 2.1 Amino acid coupling conditions attempted.

Small scale trial reactions showed the amine of the dipeptide (**2.70**) could be unmasked in a similar manner to the Boc amino acid ester (**2.67**) previously reported. We opted not to isolate the hydrochloride salt of the amine dipeptide to limit the risk of decomposition. Compound (**2.70**) was treated with 4.0 M HCl in dioxane for 50 minutes. The solvent was removed and the residue azeotroped with toluene to remove any residual hydrochloric acid. The resultant hydrochloride salt was then heated at 50 °C in a 5% triethylamine, isopropanol solution for 18 hours, to exclusively give the *cis* dithiodiketopiperazine (**2.71**) in 45% yield as a crystalline solid. The *cis* configuration was identified by X-ray crystallography of the resultant crystals. Unfortunately the yield could not be improved, staying consistently at 45%. This made us believe that the yield (**2.71**) was determined by the subsequent stability of the deprotected dipeptide free base. This was confirmed by the presence of *p*-methoxybenzyl mercaptan by TLC of the crude reaction mixture. The *cis* isomer was exclusively isolated during the reaction. This can be explained by the *cis* isomer (**2.71**) being thermodynamically more stable than the *trans* isomer (**2.72**), consistent with Motherwell's findings. The thermodynamic preference for formation of the *cis* isomer was supported by gas phase DFT calculations using Gaussian 03 software, for a single molecule using the B3LYP/6-31G(d) level of theory which revealed that the *cis* isomer configuration is approximately 9 kJ mol^{-1} more stable than the alternative *trans* arrangement.[65]

II.4.3 Alkylation Strategy

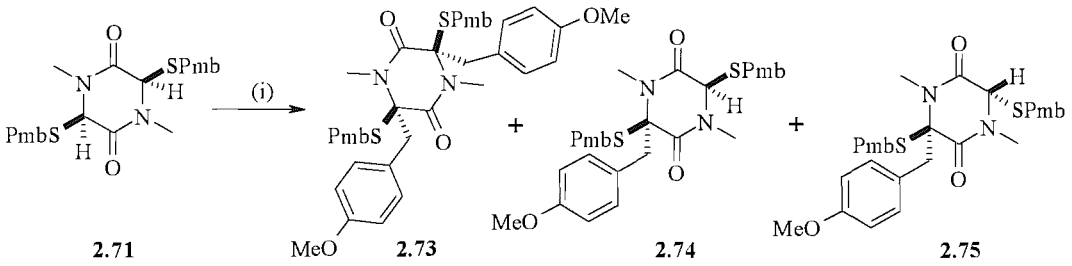
Kishi *et al.* reported the synthesis of a number of symmetrical and unsymmetrical disubstituted and mono substituted ETPs *via* clean *cis* alkylation of 3,6-*bis* methylthio-1,4-dimethylpiperazine-2,5-dione.[70] We repeated these methodologies on the 3,6-*bis*-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (**2.71**) but our observation differed slightly from that reported by Kishi. We completed the alkylation experiments with *p*-methoxybenzyl bromide which, with further elaboration could be converted to the desired dithiosilvatin.

The diketopiperazine (**2.71**) was treated with 2.2 equiv of LDA at -78 °C in THF and stirred for one hour. One equivalent of *p*-methoxybenzyl bromide was added in THF. Kishi aways added five or more equivalents of alkylating agent in his analoguous experiments. After one hour one equivalent of acetic acid in THF was added, upon workup three new products, one major and two minor were observed. Unfortunately a lot of starting DKP was also recovered (**2.71**). Kishi only isolated the *cis* mono alkylated product under similar reaction conditions.[70]

The major product isolated was a crystalline solid. X-ray crystallography, ^{1}H and ^{13}C NMR analyses confirmed the structure to be the *cis* dialkylated analogue (**2.73**). ^{1}H, ^{13}C NMR and MS analysis identified the two remaining products to be the desired mono-alkylated analogues. The more polar of the two remaining products was again crystalline. X-ray crystallography determined it to be the *trans* isomer (**2.75**). Therefore the remaining less polar product was assigned to be the desired *cis* isomer (**2.74**).

By gradually increasing the amounts of LDA and adding the *p*-methoxybenzyl bromide slowly as a dilute solution in THF under similar reaction conditions, the amount of the two mono alkylated DKPs could be enhanced. Using LDA (3 equiv), *p*-methoxybenzyl bromide (1.5 equiv) and acetic acid (3 equiv) the two mono alkylated DKPs were isolated in 64% yield in a ratio of 3:1 (**2.74**: **2.75**). Only a trace amount of compound (**2.73**) was isolated under these conditions (scheme 2.14). The reaction was also attempted with LDA (1.2 equiv) and an excess of the *p*-methoxybenzyl bromide, but only recovered starting material was isolated. This was consistent with Kishi`s

observations on the differences in the reactivity of the mono and dicarbanion, reported in the total synthesis of hyalodendrin.[58]

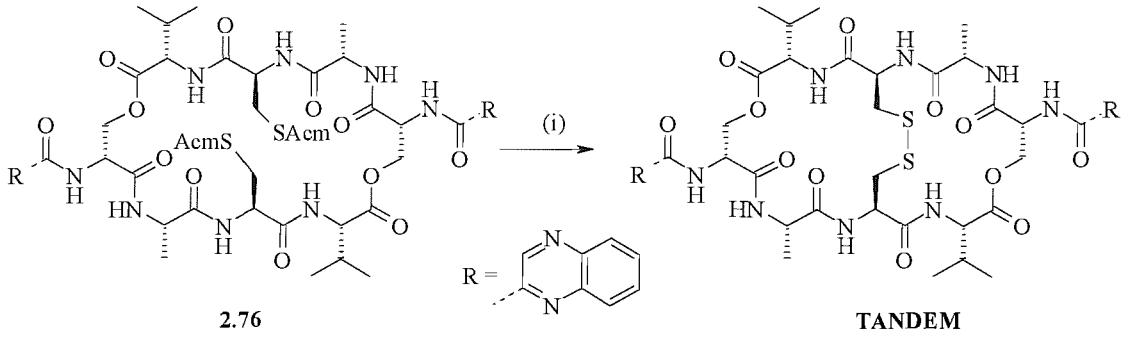


Scheme 2.14 Reagents and conditions: (i) LDA (3 equiv), THF, -78 ° C, *p*-methoxybenzyl bromide (1.5 equiv) in THF (2 ml), AcOH (3 equiv) 64 % (**2.74** & **2.75**).

II.4.4 Epidithiodiketopiperazine (ETP) Synthesis

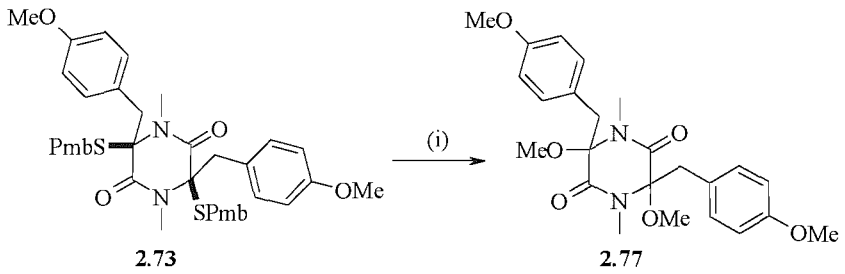
The disulfide bridge can be found in a large number of natural products. Many of these natural products and their analogues have been produced synthetically. One such example is the macrocycle TANDEM, a synthetic analogue of triostin A (Scheme 2.15).[71, 72] In TANDEM like many macrocyclic disulfide analogues, the disulfide bridge was formed by addition of iodine to the *bis*-Acm protected compound (**2.76**) in DMF. Iodine oxidation conditions of this kind have also been reported for trityl (Tr) protected analogues. These reaction conditions are a convenient method to form the disulfide without having to first cleave the protecting group. Traditionally this one step procedure, simultaneously removing the sulfur protecting groups and forming the disulfide bridge *via* treatment with iodine in DMF or MeOH, is carried out at high dilution.

No such procedures had been reported for this kind of disulfide formation *via* the Pmb protecting group, but we saw this as an easy and convenient way to form the ETP in a one step procedure.



Scheme 2.15 Reagents and conditions: (i) I_2, DMF, rt, 1 h.

This approach was therefore initially attempted on the *cis* dialkylated DKP (**2.73**) in order not to waste the *cis*-3,6-*bis*-(4-methoxybenzylsulfanyl)-3-(4-methoxybenzyl-1,4-dimethylpiperazine-2,5-dione analogue (**2.74**) (scheme 2.16). The DKP (**2.73**) was treated with iodine in a methanol, DMF solution for one hour. A rapid colour change was observed and TLC analysis showed a spot to spot conversion. Upon workup with aqueous sodium thiosuphate solution to remove any excess iodine, we isolated a single compound. Spectral analysis identified the product was not the desired ETP, but 3,6-dimethoxy-3,6-*bis*-(4-methoxybenzyl)-1,4-dimethylpiperazine-2,5-dione (**2.77**), which was isolated in near quantitative yield.



Scheme 2.16 Reagents and conditions: (i) I_2 (1.5 equiv), MeOH (10 ml), DMF (1 ml) rt 1 hr, 80%.

The mechanism outlined (figure 2.7) demonstrates the importance of the protecting group to aid the one step deprotection and oxidation to the disulfide. The Tr group stabilises the carbocation, which leads to the activated thiol and subsequent disulfide. This is a plausible reason why the *bis* Pmb compound (**2.73**) did not proceed in the desired manner. The activation with iodine resulted in the SPmb acting as a leaving group for nucleophilic attack by methanol yielding compound (**2.86**).

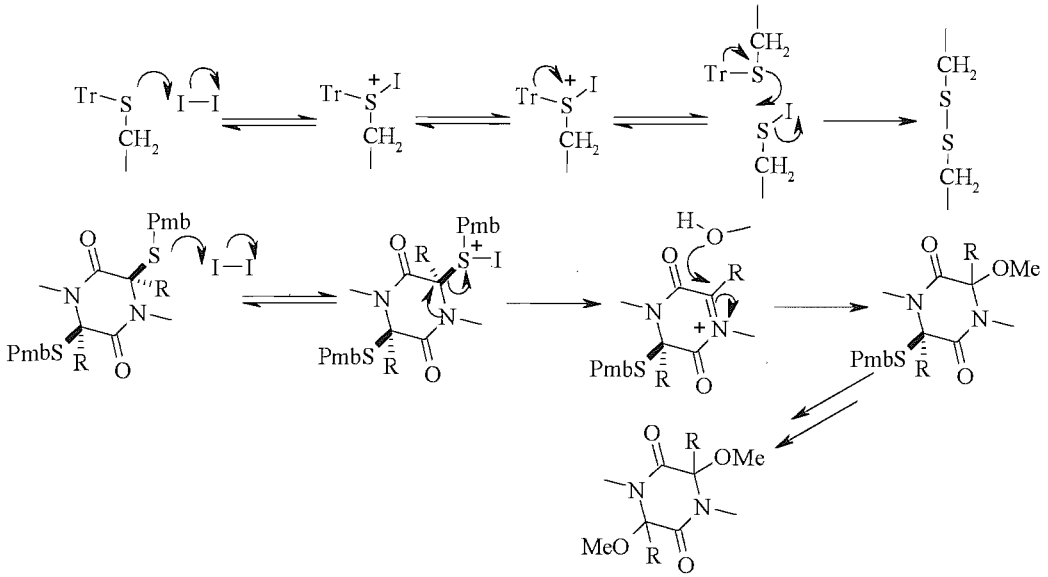
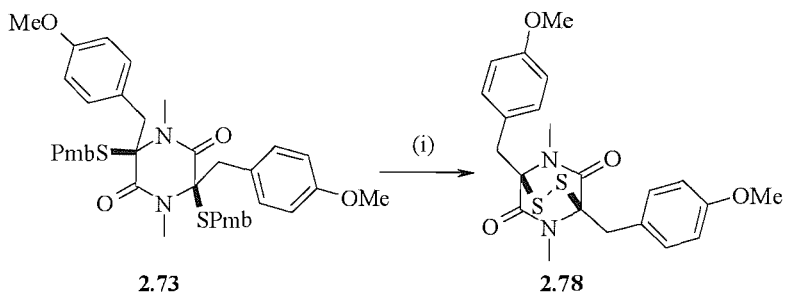


Figure 2.7 Iodine oxidation method to form disulfide bridge and plausible nucleophilic attack of activated SPmb group.

Taking this observation into consideration, an alternative approach was attempted which was to deprotect the sulfides then form the disulfide bridge *via* oxidation. As before the dialkylated DKP (**2.73**) was used to investigate the feasibility of the new reaction conditions

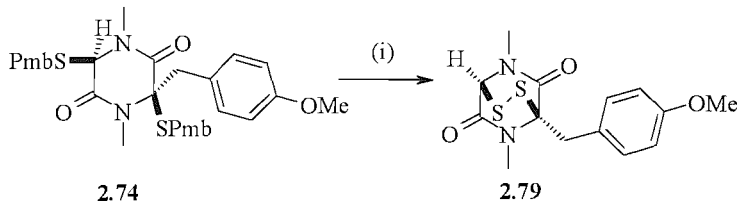
The ETP (**2.78**) was isolated in 38% yield (scheme 2.17) using the method first developed by Kishi in his synthesis of hyalodendrin.[58] The DKP (**2.73**) was first treated with 1 M boron trichloride solution in dichloromethane at 0 °C for 10 minutes to remove the sulfur protecting groups. Oxidation to the disulfide was accomplished *via* the addition of iodine in a 10% methanol dichloromethane solution, after the excess boron trichloride, dichloromethane solution had been removed by evaporation *in vacuo.*



2.73 **2.78**

Scheme 2.17 Reagents and conditions: (i) (a) 1.0 M BCl_3 (2.4 equiv) 0 °C 10 min. evaporate; (b) I_2 (2.0 equiv), 10% MeOH, CH_2Cl_2, rt, 20 min, 38%.

The method was successfully applied to the desired mono alkylated analogue (**2.79**), which was prepared in 46% yield from the *cis* DKP (**2.74**) (scheme 2.18).

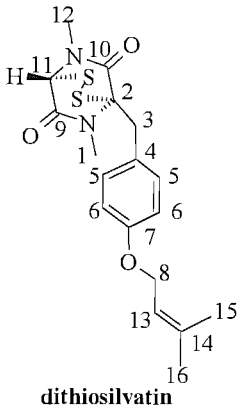


2.74 **2.79**

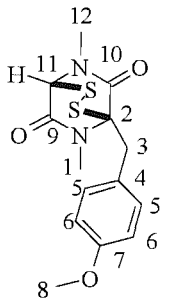
Scheme 2.18 Reagents and conditions: (i) (a) 1.0 M BCl_3 (2.4 equiv) 0 °C 10 min, evaporate; (b) I_2 (2.0 equiv), 10 % MeOH, CH_2Cl_2, rt, 20 min, 38%.

The ^1H and ^{13}C NMR spectroscopic data were comparable to that published for the isolated dithiosilvatin.[66] The ^1H signal for H11 (figure 2.8) at δ_H 5.33 ppm was characteristic for the disulfide bridge structure (table 2.3). The appropriate ^{13}C signals for the carbons found in both dithiosilvatin and compound (**2.79**) were identical (table 2.2).

The MS data were also consistent to that reported for dithiosilvatin, in that the ion minus the two sulfurs was observed in both examples: *m/z* $[M\text{-}S_2]^+$ 314 (dithiosilvatin), *m/z* $[M+H\text{-}2S]^+$ 261.1 (**2.79**).



dithiosilvatin
M.W. 378.52
$C_{18}H_{22}N_2O_3S_2$



2.79
M.W. 324.42
$C_{14}H_{16}N_2O_3S_2$

Figure 2.8 Dithiosilvatin and compound (**2.79**).

Carbon	Dithiosilvatin (δ_C)	(2.79) (δ_C)
1	28.13	28.5
2	77.14	76.9
3	36.97	36.3
4	125.83	126.1
5	130.53	130.8
6	114.78	114.4
7	158.24	159.1
8	64.80	55.4 (methyl)
9	164.89	165.1
10	165.51	165.7
11	67.14	67.3
12	32.26	32.7
13	119.76	n/a
14	137.98	n/a
15	18.17	n/a
16	25.76	n/a

Table 2.2 ^{13}C NMR chemical shifts for dithiosilvatin and compound (**2.79**).

The final steps towards dithiosilvatin require the demethylation of compound (**2.79**) to give the phenolic derivative (**2.80**). It was foreseen that this could then be further elaborated by simple alkylation of the phenol (figure 2.9).

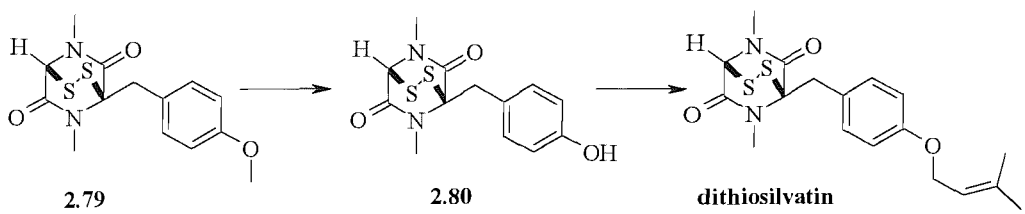


Figure 2.9 Synthetic steps required towards dithiosilvatin.

Proton type	Dithiosilvatin (δ_H)	(2.79) (δ_H)
Olefinic Me	3H, s,1.74	n/a
Olefinic Me	3H, s, 1.79	n/a
NMe	3H, s, 2.97	3H, s, 2.97
NMe	3H, s, 3.17	3H, s, 3.16
CH2	1H, d, *J* 15Hz, 3.45	1H, d, *J* 15Hz, 3.58
CH2	1H, d, *J* 15Hz, 3.94	1H, d, *J* 15Hz, 3.92
OC*H2*CH=	2H, d, *J* 7Hz, 4.48	n/a
COCHN	1H, s, 5.33	1H, s, 5.33
OCH2C*H*=	1H, br t, *J* 7Hz, 5.45	n/a
ArH	2H, d, *J* 9Hz, 6.84	2H, d, *J* 9Hz, 6.83
ArH	2H, d, *J* 9Hz, 7.24	2H, d, *J* 9Hz, 7.25

Table 2.3 ^{1}H NMR chemical shifts for dithiosilvatin and compound (**2.79**).

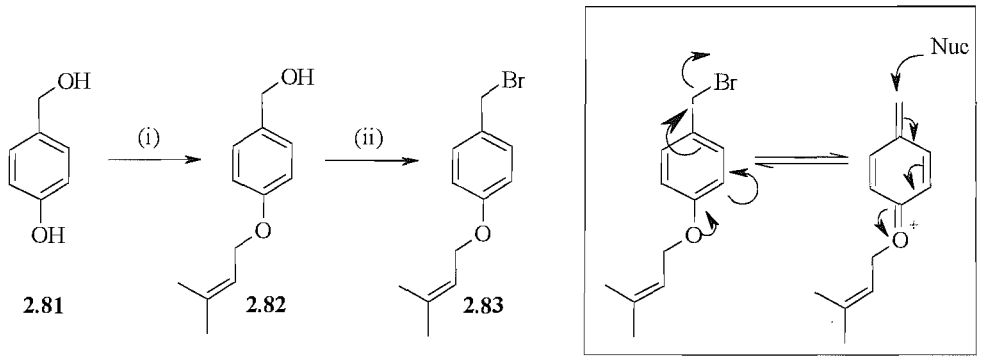
A number of demethylation conditions were attempted (table 2.4) without success. This was possibly due to the relative instability of compound (**2.79**) under standard demethylation conditions.[73]

Reagents and conditions	Result
1.0 M BCl_3 in CH_2Cl_2 at 0 °C, 1 h	60% recovered S.M (**2.79**)
1.0 M BCl_3 in CH_2Cl_2 at 0 °C 1 h, rt, 2 h	30% recovered S.M (**2.79**)
1.0 M BBr_3 in CH_2Cl_2 at 0 °C 1 h, rt, 2 h	No recovered S.M or products isolated

Table 2.4 Demethylation reaction conditions attempted.

II.4.5 Isoprenyl Approach

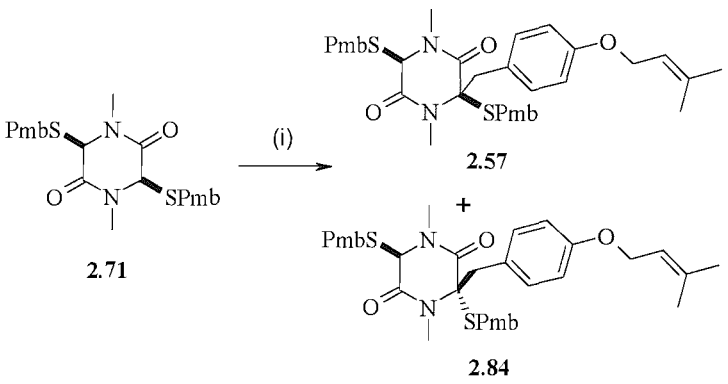
From the synthetic route developed thus far a workable route to the disulfide bridge had been successfully achieved. Unfortunately the proposed demethylation method was not viable as decomposition of the ETP (**2.79**) was observed. We thought the best approach was to have the isoprenyl group in place and form the disulfide at the final step. We first required synthesis of the relevant bromide. This proved to be difficult. The bromide (**2.83**) was extremely unstable although it could be prepared from 4-hydroxybenzoyl alcohol (**2.81**) in two steps (scheme 2.19). The more acidic phenolic group was selectively deprotonated by the addition of one equivalent of sodium hydride in DMF at 0 °C. This was then quenched with 3,3-dimethylallyl bromide to give the alcohol (**2.82**) in 61% yield. Conversion to the bromide was achieved under standard Appel reaction conditions.[74]



Scheme 2.19 Reagents and conditions: (i) NaH (1.0 equiv), DMF, 0 °C 30 min, 61% (ii) 3,3-dimethyl allyl bromide (1.0 equiv), rt, 2 h; (ii) $P(Ph)_3$ (1.27 equiv), CBr_4 (1.2 equiv), CH_2Cl_2, 0 °C – rt 6 h, crude 60%; Box = plausible decomposition of bromide.

Attempted purification of the crude product was by trituration with cold petroleum ether to remove any triphenylphosphine oxide followed by flash column chromatography through a short silica plug. However, we observed the material was still always impure, approximately 90% bromide, but quickly degraded further on standing. This could be explained by the inherent instability of the bromide. If the bromine is eliminated the resultant stabilised charged species formed can be attacked by any nucleophiles. The bromide had to be used the day it was prepared.

Alkylation of the DKP (**2.71**) with crude bromide (**2.83**) was completed as before, but not as cleanly. The *trans* isomer (**2.84**) was isolated pure, but unfortunately the desired *cis* isomer (**2.57**) could not be isolated in greater than 60% purity after several columns (scheme 2.20).



Scheme 2.20 Reagents and conditions: (i) (a) LDA (3.0 equiv), THF, -78 °C, 30 min; (i) (b) **2.83** (1.5-2.0 equiv), THF, -78 °C, 2 h; (i) (c) AcOH (2.0 equiv), THF, -78 °C rt, 1 min, 21%, (**2.57**:**2.84**, 2:1).

This was due to a co-eluting impurity from the bromide, so further purification by column chromatography was not possible. The intramolecular disulfide formation was attempted on the impure *cis* analogue without success. Once again this resulted in multiple products.

Due to the instability of the bromide, other leaving groups were investigated such as the mesylate. Unfortunately any attempts to form the mesylate were also unsuccessful. This can be explained by the mesylate being a better leaving group than the bromide thereby increasing the formation of the charged reactive species which can react with any nucleophile present such as the alcohol or water (scheme 2.19).

II.4.6 Phenol Protection Strategy

Taking into consideration the stability issues with the isoprenyl bromide (**2.83**), it was thought the best approach would be to proceed with a more stable bromide. This could be achieved by protecting the oxygen in such a manor that it could no longer stabilise a positive charge. This could be achieved *via* the carbonate, acetate or sulfonate functionality. The strategy was to revert back to the original approach of cleaving the

protecting group on the phenol after the disulfide bridge has been formed. This would leave the free phenol to be alkylated in the final step.

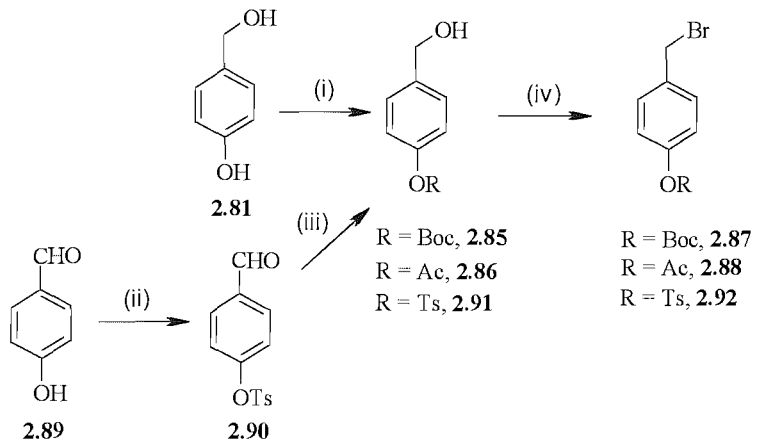
Three phenolic protecting groups were investigated; the *tert*-butyl carbonate (Boc), acetyl (Ac) and the tosyl (Ts). The desired chlorides or bromides of all three protecting groups had been reported *via* various methods.[75, 76, 77] We chose to use a standardized procedure to form the bromides.

The mono Boc protected alcohol (**2.85**) and mono Ac protected alcohol (**2.86**) were prepared *via* 4-hydroxybenzyl alcohol (**2.81**). The Ts analogue (**2.91**) was prepared from 4–hydroxybenzaldehyde (**2.89**). The alcohols were then converted to the relevant bromide in a two step procedure *via* the mesylate, which upon treatment with lithium bromide in THF yielded the desired bromide (scheme 2.21).

The Boc alcohol (**2.85**) was synthesised by the addition of one equivalent of di *tert*-butyl dicarbonate in the presence of DMAP as base in acetonitrile in 81% yield.[78] The alcohol was readily converted to the bromide (**2.87**) in 57% yield after chromatography. As predicted the bromide (**2.87**) was extremely stable and was purified by standard chromatography conditions. A stored sample remained unchanged after 1 month storage in a freezer under nitrogen.

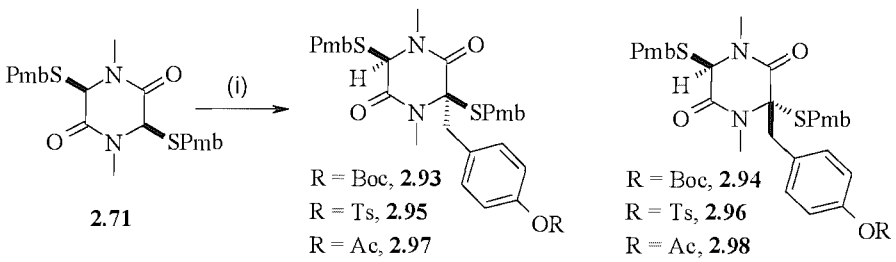
The acetyl analogue (**2.86**) was prepared by the careful addition of one equivalent of acetyl chloride to a solution of 4-hydroxybenzyl alcohol (**2.81**) and triethylamine in ethyl acetate at 0 °C. As before the alcohol was converted to the bromide (**2.88**) *via* a mesylate in 65% yield.[79] The Ac protected bromide (**2.88**) was also stable to chromatography, but does decompose after approximately two weeks storage under nitrogen in a freezer.

4-Hydroxybenzaldehyde (**2.89**) in acetonitrile and potassium carbonate was treated with tosyl chloride for 18 h at room temperature to give the aldehyde (**2.90**). Subsequent reduction with sodium borohydride in methanol yielded the alcohol (**2.91**) in 96% yield.[77] The stable bromide (**2.92**) was once again formed under the reported conditions in 62% yield.



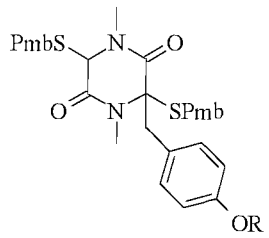
Scheme 2.21 Reagents and conditions: (i) Boc_2O (1.05 equiv), DMAP (0.05 equiv), CH_3CN, rt 1 h, 81%, (**2.85**);or (i) AcCl (1.05 equiv), TEA (1.05 equiv), EtOAc, 0 °C – rt, 5 h, 54%, (**2.86**); (ii) K_2CO_3 (1.4 equiv), tosyl chloride (1.2 equiv), CH_3CN, reflux 18 h, 96% (**2.90**); (iii) $NaBH_4$ (1.0 equiv), MeOH, 0 °C, 3 h, 96% (**2.91**); (iv) MsCl (1.1 equiv), Et_3N (2.0 equiv), THF, -40 °C, 45 min; (iv) (b) LiBr (4.0 equiv), THF, 0 °C, 1.5 h, 54 % (**2.87**); 65% (**2.88**); 62% (**2.92**).

As speculated, both the Boc and Ts protected bromide (**2.87** & **2.92**) cleanly reacted with the double deprotonated DKP (**2.71**) to give the mono alkylated products (**2.93**, **2.94** & **2.95**, **2.96**) with fewer by-products than had been previously observed (scheme 2.22). As the stable bromides could be purified it was also observed that the yields became more consistent under these reaction conditions, averaging around 50%. Unfortunately, due to the basic nature of the reaction conditions the acetyl protected bromide (**2.88**) gave a messier reaction mixture. The less polar desired *cis* DKP (**2.97**) could be isolated pure, but the more polar *trans* (**2.98**) co-eluted with the starting DKP (**2.71**) and the deprotected unassigned free phenol derivative. If the reaction time was increased the yield of the Ac analogue (**2.97**) decreased, indicating hydrolysis of the acetyl to the phenol.



Scheme 2.22 Reagents and conditions: (i) 2.0 M LDA (3.0 equiv), THF, -78 °C 30 min. (i) (a) bromide (1.2 equiv), THF, -78 °C, 2 h; (b) AcOH (1.5 equiv), THF, -78 °C rt, 44%, (**2.93**: **2.94**, 3:1); 63%, (**2.95**: **2.96**, 2:1), 46%, (**2.97** ; impure **2.98**, 2:1).

Unfortunately, no X-ray data were collected on the new analogues and therefore the configurations of these analogues, *cis* or *trans* was assigned by a combination of factors. Simply by comparing the polarity by TLC of the new analogues with that previously assigned by X-ray, the *cis* analogue is much less polar (more non polar) than the *trans*. Similar observations have been reported by Kawai *et al.*[66] This polarity difference can also be observed by LCMS, by comparison of the LC spectra (reverse phase column) of the *cis* and *trans* analogues in all the examples synthesised. The longer the retention time the more nonpolar (lipophilic) the product. (table 2.5).

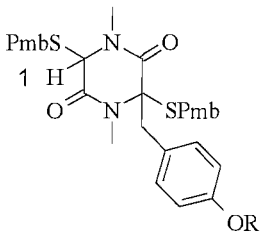


R group	*Cis* retention time	*Trans* retention time
Me	8.02 min(**2.74**)	7.58 min (**2.75**)
Isoprenyl	9.13 min (**2.57**)	8.78 min(**2.84**)
Boc	8.82 min (**2.93**)	8.25 min (**2.94**)
Ac	8.97 min (**2.97**)	(impure) (**2.98**)
Ts	8.57 min (**2.95**)	8.03 min (**2.96**)
H	7.14 min (**2.99**)	6.49 min (**2.100**)

Table 2.5 LCMS retention times recorded of cis and trans analogues synthesized.

Secondly the ^{1}H NMR chemical shift δ_H of the DKP proton (H1) is a characteristic singlet. In all examples the observed chemical shift δ_H for H1 in the *cis* isomer is higher than 4.00 ppm and the *trans* isomer is less than 4.00 ppm (table 2.6).

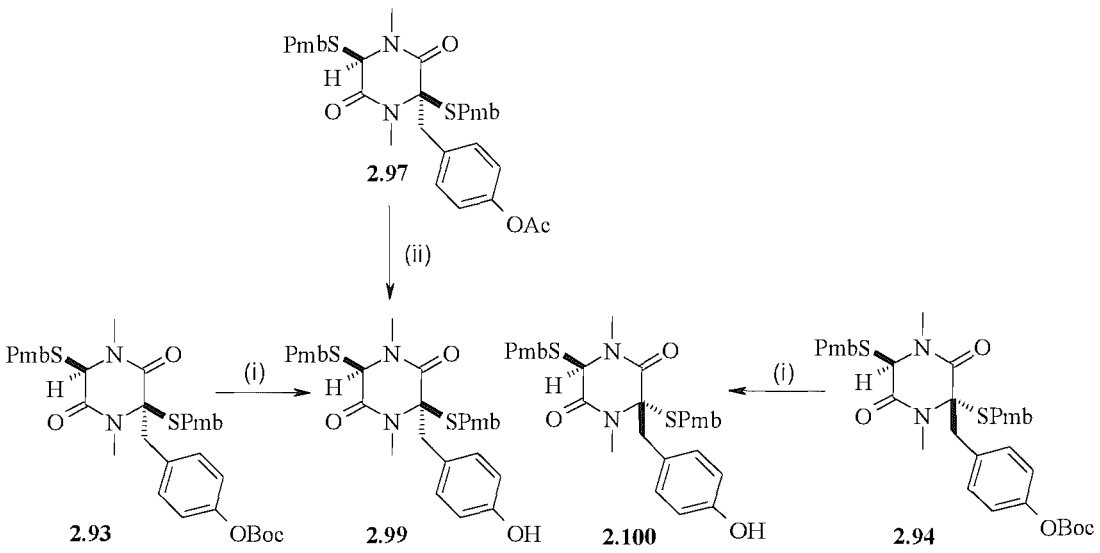
In addition they could be assigned by chemical means; the *cis* isomers, acetyl (**2.97**) and Boc (**2.93**) were deprotected. Compound (**2.93**) *via* acidic conditions gave the phenol (**2.99**) and compound (**2.97**) *via* basic conditions yielding the same product (**2.99**) as the major product plus a minor amount of a less polar product, later identified as the *trans* isomer (**2.100**). The *trans* Boc analogue (**2.94**) could also be deprotected under acid conditions and gave the less polar product (**2.100**) consistent for the expected *trans* assignment (scheme 2.23).



R group	δ_H (H1) *Cis* (ppm)	δ_H (H1) *Trans* (ppm)
Me	4.11 (**2.74**)	3.99 (**2.75**)
Isoprenyl	4.05 (**2.57**)	3.99 (**2.84**)
Boc	4.17 (**2.93**)	3.88 (**2.94**)
Ac	4.15 (**2.97**)	3.91 (impure) (**2.98**)
Ts	4.10 (**2.95**)	3.87 (**2.96**)
H	4.12 (**2.99**)	3.94 (**2.100**)

Table 2.6 Chemical shift δ_H for H1, of analogues synthesized.

The newly formed *cis* and *trans* phenols were individually treated with sodium hydride in DMF at 0 °C. Addition of the 3,3-dimethylallyl bromide yielded the previously reported isoprenyl compounds (**2.57** & **2.84**).

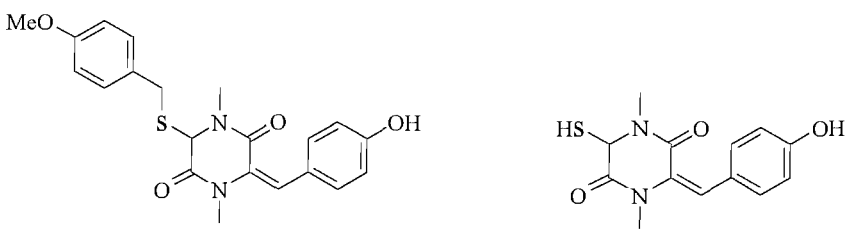


Scheme 2.23 Reagents and conditions: (i) 4 M HCl/ 1,4-dioxan, 0 °C, 18 h, 89%; (ii) 1.0 M KOH (2 equiv), MeOH, 0 °C – rt, 65% (**2.99**:**2.100**, 5:1).

We attempted to cleave the Pmb groups with a Lewis acid as before, and oxidise the resulting dithiols to the desired ETPs. We found that the *cis* acetyl (**2.97**) and Boc

(**2.93**) compounds did not give the desired disulfide products, but a complex mixture as before. Both the Boc and acetyl groups appeared to be cleaved with boron trichloride or tribromide. In fact the Boc group was cleaved at -78 °C within minutes. This discovery provided a new rapid, clean method of preparing the phenol (**2.99**).

In addition we tried the deprotection on the free phenol (**2.99**), but this was also unsuccessful. To further understand why the reaction mixtures were so complex, we first monitored the deprotection step with the Lewis acid. This was carried out on both the Ac compound (**2.97**) and the phenol (**2.99**). Both examples were treated with boron tribromide at -78 °C in dichloromethane for 10 minutes then allowed to warm to 0 °C for 20 minutes and room temperature for 10 minutes. The reactions were monitored by LCMS at each time point. For the phenol analogue (**2.99**) after 20 minutes at 0 °C only one peak was observed at 3.5 min. *m/z* $[M+H]^+$ = 279.1 and $[M+H+CH_3CN]^+$ = 320.0. This mass ion identified could be the thiol (**2.102**) Mwt = 278.33 (figure 2.10). This mass ion was also observed for the compound (**2.97**) but in addition after 10 minutes we also observed a earlier peak at 5.42 min. *m/z* $[M+H]^+$ = 399.1 & $[M+H+CH_3CN]^+$ = 440.1. This peak had completely gone after the 20 minute time point and only the one peak at 3.5 minutes was recorded. This earlier product at 5.42 minutes had been previously observed and isolated as a minor by-product during the acid deprotection of the *tert*-butyl carbonate (**2.93**) to the phenol (**2.99**). ^1H NMR and MS data of this material identified the structure to be eliminated SPmb phenolic product (**2.101**). These findings explain why the reaction is not giving the desired ETP, first the protecting group is cleaved from the phenol (in the acetyl analogue), subsquently the *p*-methoxybenzyl mercaptan group is eliminated to give the mono SPmb protected thiol (**2.101**). Finally this is deprotected to yield the mono thiol (**2.102**).



2.101
M.W. 398.48
$C_{21}H_{22}N_2O_4S$

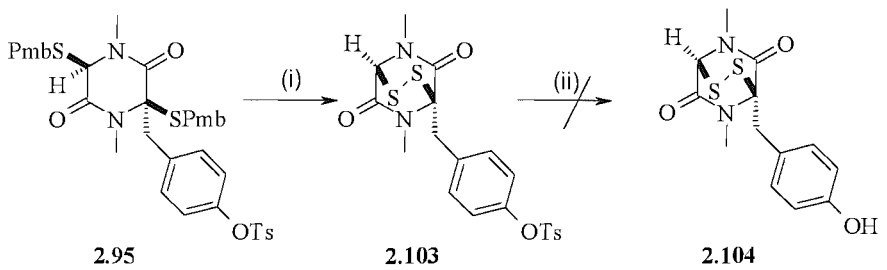
2.102
M.W. 278.33
$C_{13}H_{14}N_2O_3S$

Figure 2.10 Plausible structures for the mass ions observed during SPmb deprotection reaction.

The acid stable Ts compound (**2.95**) could be readily converted to the ETP (**2.103**) in 71% yield by this two step procedure, indicating the *cis* and *trans* isomers had been correctly assigned (scheme 2.24).

The evidence indicates thus far that in order to effect the desired deprotection of the Pmb group with boron trichloride the phenol must be protected by an acid stable group. The problem with this is that the acid stable protecting groups generally require harsh conditions to remove them as demonstrated by the tosyl ETP (**2.103**).

The standard methods for cleaving the tosyl are *via* strong base hydrolysis, refluxing the substrate in potassium hydroxide for several hours.[80] An alternative method was by reductive conditions such as magnesium in methanol.[81] Both these conditions were attempted without success. This was not unforeseen as Kishi had reported that ETPs were not stable to base or strong acid conditions. The base instability of gliotoxin was reported by Woodward *et al.*[82]



2.95 **2.103** **2.104**

Scheme 2.24 Reagents and conditions: (i) (a) 1.0 M BCl_3 (2.4 equiv) 0 °C 10 min, evaporate; (i) (b) I_2 (2.0 equiv), 10% MeOH/ CH_2Cl_2, rt, 20 min, 71%; (ii) 2.0M KOH (aq), reflux, failed; or Mg, MeOH, failed.

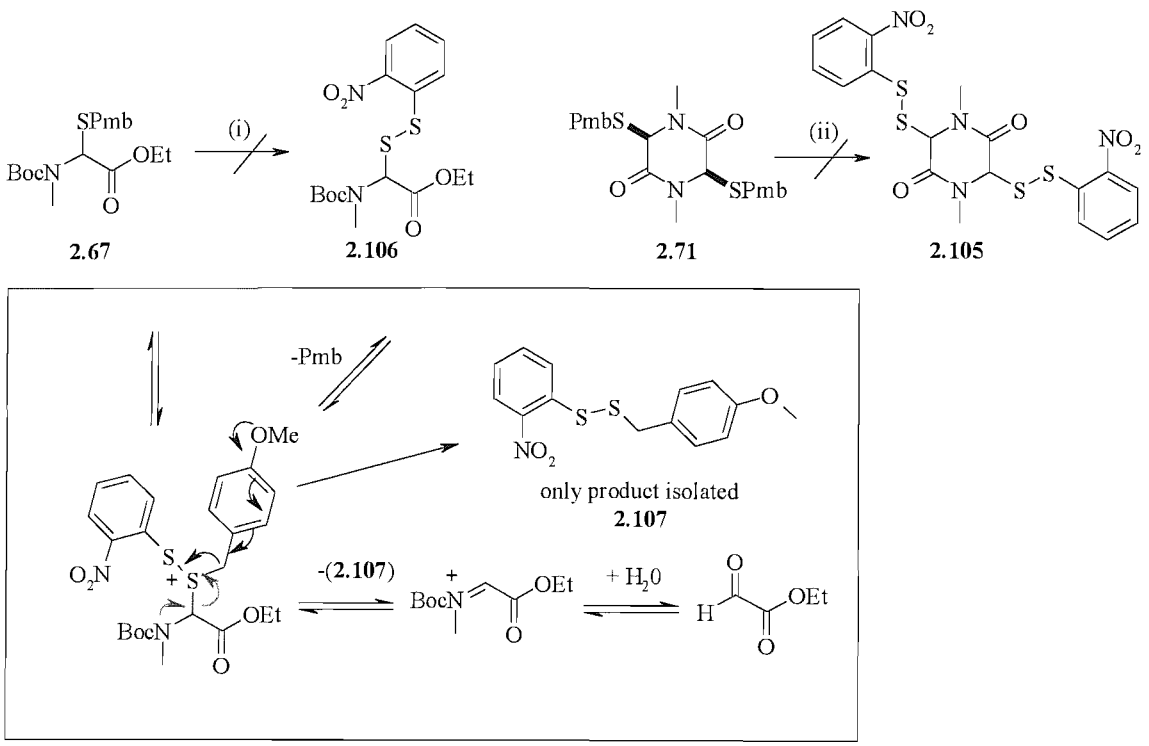
II.4.7 Synthesis of Alternative *Bis* Protected Dithiodiketopiperazines

To avoid the instability issues of the epidithiodiketopiperazine under both base and acid conditions, we concentrated on investigating new strategies in which the disulfide bridge is once again formed in the last step of the synthesis.

We investigated approaches that avoided the problematic Pmb group, which requires harsh acidic conditions to remove it. One possible route was *via* 2-nitrobenzenesulfenyl chloride as outlined by Reese *et al.*[83] They reported similar

problems with the Pmb group and simply replaced it with a disulfide analogue in a one step procedure under mild conditions.

Two trial reactions on the protected amino acid ester (**2.67**) and the DKP (**2.71**) were attempted without success. In both cases it was found that the undesired disulfide of SPmb (**2.107**) was formed when the Pmb protected substrate was reacted with 2-nitrobenezenesulfenyl chloride. No protecting group transfer had occurred to give the desired compounds (**2.105**) and (**2.106**). Only cleavage of the sulfur unit was observed. This can be explained by the Pmb protecting group on the sulfur not being eliminated as expected, as it was not sufficiently electron rich. The desired electron movement as indicated by the black arrows does not occur. Instead electron movement indicated by the blue arrows leads to the elimination of the disulfide (**2.107**) (scheme 2.25).

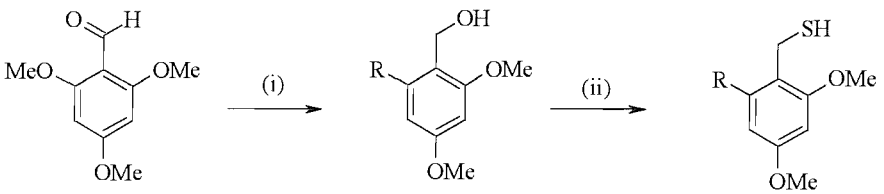


Scheme 2.25 Reagents and conditions: (i) 2-nitrobenzenesulfenyl chloride (1.1 equiv), 10% AcOH/CH_2Cl_2, 0 °C, 1 h; (ii) 2-nitrobenzenesulfenyl chloride (2.1 equiv), CH_2Cl_2, 0 °C, 1 h. Box: plausible reaction mechanisms

4.8 Synthesis of Electron Rich Protecting Groups

In light of these findings we chose to investigate more electron rich protecting groups. If the protecting group was more electron rich it would be possible to use milder deprotection conditions than currently used to remove the Pmb. This could be achieved by adding further methoxy groups to the phenyl ring. Therefore synthetic routes towards the 2,4-dimethoxybenzyl (Dmb) and 2,4,6-trimethoxybenzyl (Tmob) DKP`s were undertaken.

Both 2,4-dimethoxybenzyl thiol (**2.111**) and 2,4,6-trimethoxybenzyl thiol (**2.112**) are not commercially available, but were readily synthesised. The 2,4-dimethoxybenzyl thiol (**2.111**) was synthesised in a one step procedure *via* commercial alcohol in 84% yield. Likewise, the 2,4,6-trimethoxybenzyl thiol (**2.112**) was synthesised in two steps from the aldehyde (**2.108**) also in good yield (scheme 2.26).[84]



2.108

R = H, **2.109**
R = OMe, **2.110**

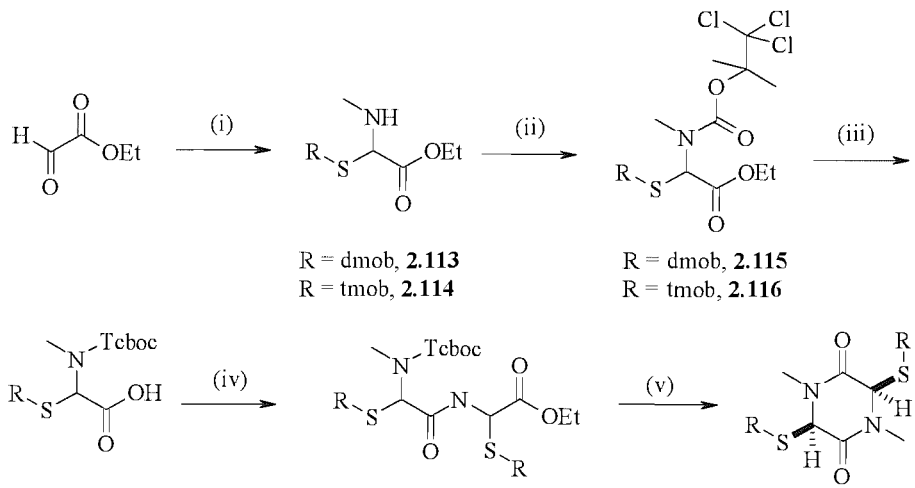
R = H, **2.111**
R = OMe, **2.112**

Scheme 2.26 Reagents and conditions: (i) $NaBH_4$ (1 equiv), MeOH, 0 °C, 3 h, 94% (**2.110**); (ii) (a) thiourea (2 equiv), 5N HCl (15 ml), acetone / water 1:1 (100 ml); (b) NaOH (3 equiv), 84% (**2.111**), 77% (**2.112**).

Both the thiols progressed smoothly to the corresponding amino acid esters (**2.113** & **2.114**) *via* the three component procedure previously reported in 62% and 38% respectively.

Both the dmob and tmob protecting groups are increasingly more acid sensitive than the Pmb. This meant the acid cleaved carbonate, Boc protection strategy could lead to undesired cleavage of the thiol protection. Therefore an alternative approach was developed (scheme 2.27). We achieved this *via* 2,2,2-trichloro-1,1-dimethylethyl carbamate (Tcboc) protection of the amine, which is readily removed under mild conditions by the addition of activated zinc powder.[85] The amino acid esters (**2.113** &

2.114) were smoothly converted to the carbonates (**2.115** & **2.116**) by the addition of 2,2,2-trichloro-1,1-dimethylethyl chloroformate in the presence of triethylamine in dichloromethane in almost quantitative yield. Further elaboration to the dipeptides (**2.119** & **2.120**) was completed under similar reaction conditions as previously reported. The acids (**1.117** & **1.118**) were synthesised by saponification of the esters (**2.113** & **2.114**) in 73% and 83% yield, then converted to the dipeptide (**2.119** & **2.120**) *via* a mixed anhydride coupling. We observed that the diketopiperazines (**2.121** & **2.122**) could be synthesized in a one step process from the starting Tcboc analogues (**2.119** & **2.120**). In both examples the DKP (**2.121** & **2.122**) were isolated when the dipeptides (**2.119** & **2.120**) were treated with activated zinc powder in 10% acetic acid, IPA solution at room temperature for 18 h. This resulted in yields of 50% and 34% respectively. Once again only one isomeric form was isolated, it was arbitrarily assigned *cis*. This could not be confirmed as crystals of good enough quality for X-ray experiments could not be obtained.

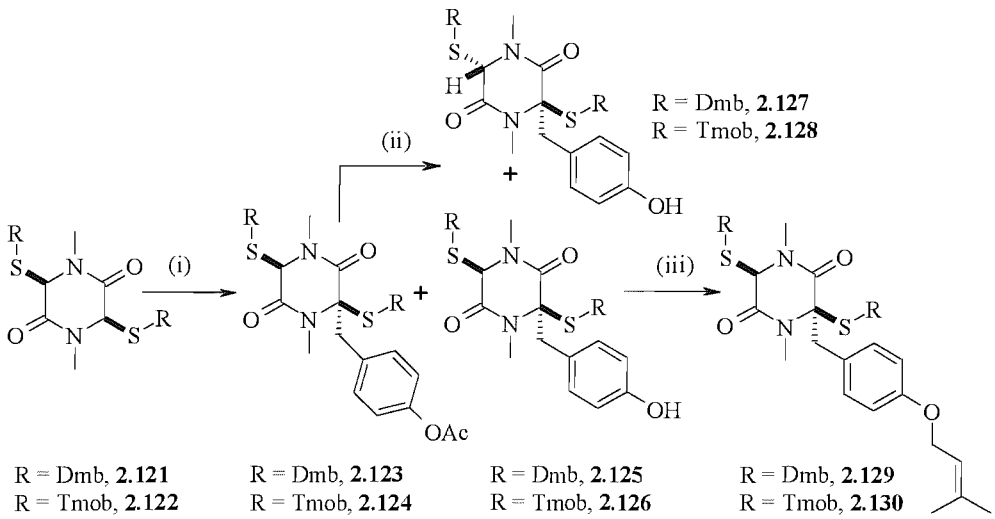


Scheme 2.27 Reagents and conditions: (i) 2.0 M methylamine in THF (1 equiv), (**2.111**) or (**2.112**) (1 equiv), Na_2SO_4 anhydrous (excess), rt, 18 h, 62% (**2.113**), 38% (**2.114**); (ii) 2,2,2 trichloro-1,1-dimethylethyl chloroformate (1.1 equiv), triethylamine (1.2 equiv), CH_2Cl_2, rt, 18 h, 94%, (**2.115**), 97% (**2.116**); (iii) $LiOH.H_2O$ (1.5 equiv), aq 1,4-dioxane, rt, 18 h, 89%, (**2.117**), 83% (**2.118**); (iv) (a) *iso*BuOCOCl (1.1 equiv), NMM (1.2 equiv), THF, -10 °C, 1 h; (iv) (b) (**2.113**) or (**2.114**) (1.1 equiv), NMM (1.2 equiv), THF, rt, 18 h, 41% (**2.119**), 45% (**1.120**); (v) zinc powder (20 equiv), 10% AcOH/IPA, rt, 18 h, 50% (**2.121**), 34% (**2.122**).

Both the dmob and tmob protected, isoprenyl analogues (**2.129** & **2.130**) were synthesised. The DKP`s were treated with 3 equivalents of LDA as before and

alkylated with acetic acid 4-bromomethylphenyl ester (**2.88**), upon quenching the reaction mixture with acetic acid, to give the *cis* acetyl compounds (**2.123** & **2.124**) and the *cis* phenol analogues (**2.125** & **2.126**). In addition the *cis* acetyl analogues (**2.123** & **2.124**) were deprotected under basic conditions to give a 9:1 mixture of the *cis*:*trans* phenols (**2.125**: **2.127**) & (**2.126**:**2.128**).

As previously reported (table 2.5 & 2.6) the configuration of the isomers could be arbitrarily assigned by comparing the relative polarities of the two isomers by LCMS and TLC and the proton shifts δ_H by ^1H NMR. The *cis*-phenolic compounds (**2.125**) and (**2.126**) were treated with sodium hydride at -10 °C in DMF and alkylated with 3,3-dimethylallyl bromide to give isoprenyl compounds (**2.129** & **2.130**) in 41% and 45% yield (scheme 2.28).



Scheme 2.28 Reagents and conditions: (i) (a) 2.0 M LDA (3.0 equiv), THF, -78 °C, 30 min; (i) (b) Acetic acid 4-bromomethylphenyl ester (1.5 equiv), THF, -78 °C, 2 h; (i) (c) AcOH (1.5 equiv), THF, -78 °C, rt, 30% (**2.123**) & 31% (**2.125**): 21% (**2.124**) & 31% (**2.126**); (ii) 1.0 M KOH (2 equiv), MeOH, 0 °C – rt, 71% (**2.125: 2.127,** 9:1), 96% (**2.126**: **2.128**, 9:1); (iii) (a) NaH (1.05 equiv), DMF, -10 °C, (iii) (b) 3,3-dimethylallyl bromide (1.2 equiv), rt, 4 h, 41% (**2.129**), 45% (**2.130**).

In both cases a small trace of the *trans* isomer was observed by TLC and LCMS, but material of sufficient purity for full characterisation was not isolated. A number of conditions were attempted for the final disulfide step to form dithiosilvatin. In the literature both the Tmob and Dmb can be cleaved under acid conditions.[86, 87] Therefore the acid stability of the isoprenyl group was investigated (table 2.7).

Conditions	Products observed by LCMS
50% formic acid / DCM	Loss of isoprenyl (10 min). Multiple products after 1 h
50% TFA / DCM	Loss of isoprenyl (10 min). Multiple products after 1 h
2% TFA / DCM	No change (10 min). Loss of isoprenyl after 1 h
1% TFA / DCM	No change (1 h). Loss of isoprenyl after 24 h

Table 2.7 Acid stability of Tmob protected analogues (**2.129 & 2.130**).

Unfortunately it was observed that the isoprenyl group was extremely acid labile. During all the test experiments no fragments relating to the desired cleavage of the thiol protecting group was seen, in either the Dmb (**2.129**) or Tmob (**2.130**) compounds.

Similar findings were observed when the Tmob protected analogue (**2.130**) was treated with boron tribromide (2 equiv) at -78 °C in dichloromethane. The only product observed by LCMS was the *m/z* $[M+H]^+$ 673.1, $[M+Na]^+$ 695.1 peak at 8.56 min, which is identical to that observed for the hydroxyl analogue (**2.126**), indicating the undesired loss of the isoprenyl group, even at low temperatures.

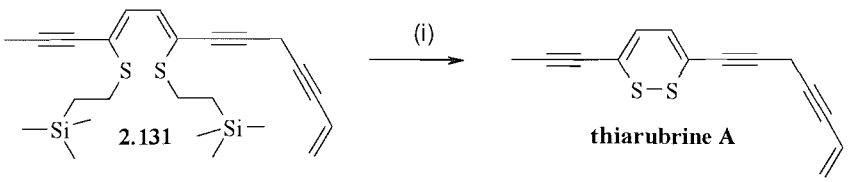
In parallel a number of oxidation methods were attempted to form the disulfide (table 2.8).[88-91] Once again at no stage was any dithiosilvatin ever observed by LCMS. Under all the conditions attempted many products were formed and no starting material remained.

Conditions	Products observed
I_2 (4 eq) $EtOH/CH_2Cl_2$, rt[88]	Multiple products after 1 h, no desired mass ion
I_2 (2-4 eq) DMF, rt	Products after 1 h loss of thiol, no desired mass ion
PhSOPh, CH_3SiCl_3, CH_2Cl_2[89]	Loss of thiol observed major product
DMSO/10% TFA[90]	Loss of isoprenyl after 10 min, multiple products
$Tl(CF_3COO)_3$, DMF, anisole[91]	Multiple products after 1 h, no desired mass ion

Table 2.8 Investigation of disulfide bond forming reactions by oxidation (reagent **2.130**).

II.4.9 Synthesis of *Bis* Silyl Analogue

It was becoming clear that no acidic reaction conditions were tolerated for the final steps of the synthesis of dithiosilvatin, as the isoprenyl group was extremely acid sensitive. This vastly limited our choice of thiol protecting groups. We chose to investigate possible silyl protection strategies. As the silicon-sulfur bond is weaker than a silicon-oxygen bond it is more susceptible to hydrolysis, therefore direct silyl protection of the sulfur was not a viable option, but indirect β-silyl protection of sulfur has been reported.[92] The novel use of the 2-(trimethylsilyl)ethyl as the thiol protecting group was reported in the construction of thiarubrine A. The *bis* silyl protected compound (**2.131**) was successfully cleaved with TBAF as a source of F^- and oxidation with iodine yielded the desired natural product (scheme 2.29).[92]

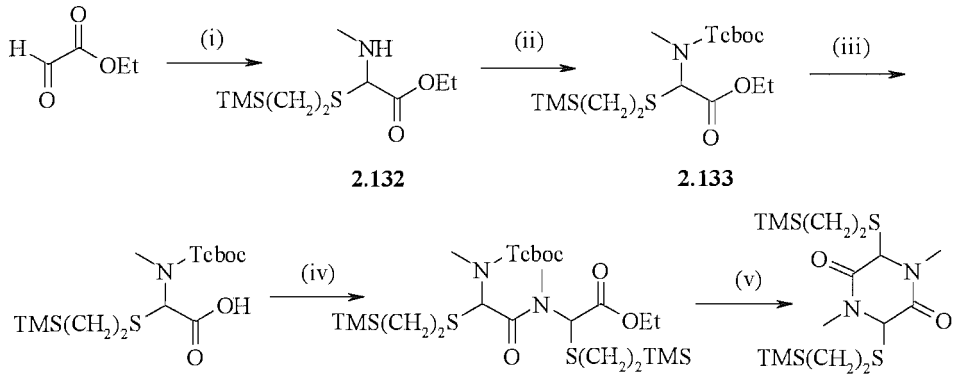


Scheme 2.29 Reagents and conditions: (i) (a) TBAF (8.0 equiv), 3 Å mol. sieves/ THF, rt, 1h; (b) I_2 (10 equiv), rt, 30 min, 53%.

Initially we attempted to adopt the same methodology we had developed in the synthesis of a number of *bis* protected dithiodiketopiperazines. Once again we utilised the three component reaction to form the key building blocks. The three component reaction progressed to the desired amino acid analogue (**2.132**) albeit in a lower yield than previous derivatives. We opted for the Tcboc route, as we presumed the silyl protecting group may be acid sensitive. The Tcboc protected ester (**2.133**) was synthesised in quantitative yield and was readily saponified to the acid (**2.134**) in 70% yield. The coupling reaction to the dipeptide (**2.135**) proved to be problematic and poor yielding. A similar result was observed for the DKP (**2.136**). The best yield obtained was an extremely poor 14% (scheme 2.30).

Due to the two successive poor yielding steps the route was not feasible to synthesise sufficient material to investigate the new protection strategy further. We therefore attempted alternative routes. Two routes were investigated, one using the Trown

methodology previously reported and the other *via* the alkylation of a DKP by a disulfide or electrophilic source of sulfur, hence adding the 2-(trimethylsilyl)ethanethiol protecting group to the DKP at the latter steps of the synthesis.[59, 93]

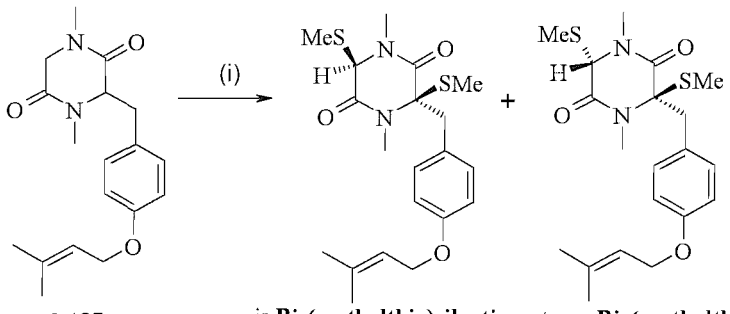


2.132 **2.133** **2.134** **2.135** **2.136**

Scheme 2.30 Reagents and conditions: (i) 2.0 M methylamine in THF (1 equiv), 2-(trimethylsilyl) ethanethiol (1 equiv), tolune, rt, 18 h, 49% (**2.132**); (ii) 2,2,2 trichloro-1,1-dimethylethyl chloroformate (1.1 equiv), triethylamine (1.2 equiv), CH_2Cl_2, rt, 18 h, 91%, (**2.133**); (iii) $LiOH.H_2O$ (1.5 equiv), aq 1,4-dioxan, rt, 18 h, 70%, (**2.134**); (iv) (a) *iso*BuOCOCl (1.1 equiv), NMM (1.2 equiv), THF, -10 °C, 1 h; (iv) (b) **2.132** (1.1 equiv), NMM (1.2 equiv), THF, rt, 18 h, 24% (**2.135**); (v) zinc powder (20 equiv), 10% AcOH/IPA, rt, 18 h, 14% (**2.136**).

II.4.10 Thiosulfonate Reagent

This alkylation approach had been achieved in the total synthesis of *cis* and *trans bis*(methylthio)silvatin, a ring opened version of dithiosilvatin. The synthesised DKP (**2.137**) was successfully alkylated with dimethyl disulfide (scheme 2.31) to give the *cis* and *trans* analogues in good yield (scheme 2.31).[94] In our case, we would use a thiosulfonate or disulfide derivative as an electrophile.[95]

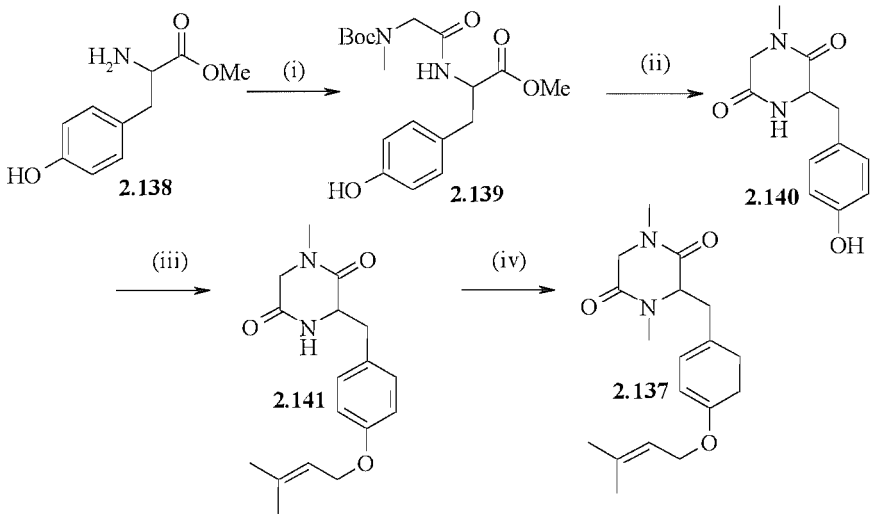


2.137 ***cis* Bis(methylthio)silvatin** ***trans* Bis(methylthio)silvatin**

Scheme 2.31 Reagents and conditions: LDA (3 equiv), Me_2S_2 (6 equiv), THF/HMPA, -78 °C, 2 h, 83% (*cis:trans* 1:1).

The DKP (**2.137**) was synthesised using a slightly modified route to that outlined in the total synthesis of *bis*(methylthio)silvatin (scheme 4.2). Starting from BocSarOSu and H-D,L-TyrOMe (**2.138**) to form BocSar-D,L-TyrOMe (**2.139**) in 92% yield. This was readily cyclised to the phenolic DKP (**2.140**) using the Nitecki method.[96] The Boc dipeptide ester (**2.139**) was first treated with formic acid at room temperature for 1 h. The resultant formate salt obtained after removal of any excess formic acid was converted to the DKP (**2.140**) by prolonged heating at reflux in a 20% toluene/ *sec*-butanol solution in 82% yield. Finally two alkylations yielded the desired building block (**2.137**), firstly with 3,3-dimethylallyl bromide yielding the intermediate (**2.141**) and then iodomethane giving the DKP (**2.137**) in 86% yield (scheme 2.32).

For the electrophile there were two possible options; we could simply form the disulfide of 2-(trimethylsilyl)ethanethiol or using a leaving group approach such as a thiosulfonate. We chose the latter due to the increasing difficultly to source the starting 2-(trimethylsilyl)ethanethiol, which would be required to prepare the disulfide. The thiosulfonate (**2.144**) was readily prepared by reacting potassium thiotosylate (**2.142**) with 2-(trimethylsilyl)bromoethane (**2.143**) in acetone at room temperature to give the toluene-4-thiosulfonic acid S-(2-trimethylsilonylethyl) ester (**2.144**) as a volatile oil in 40% yield (scheme 2.33).



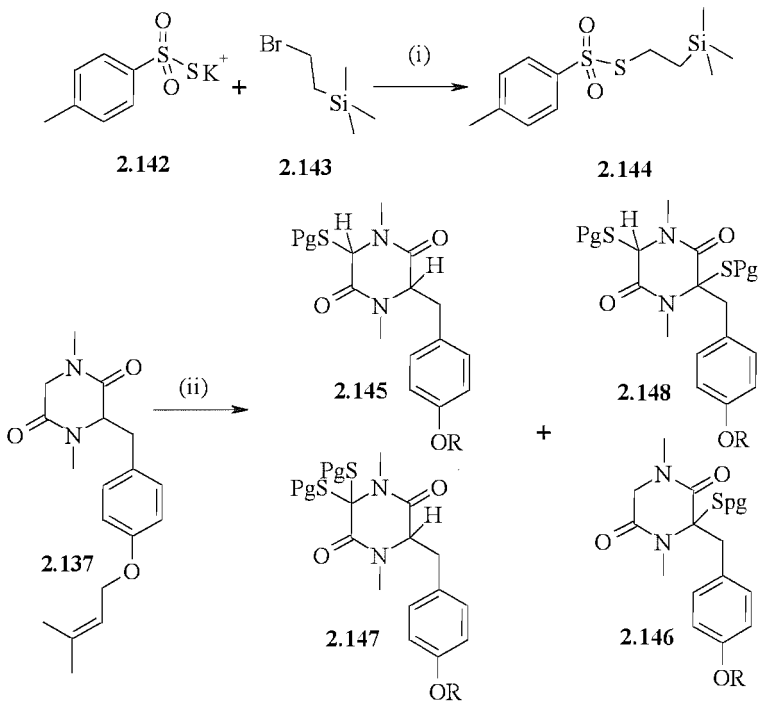
Scheme 2.32 Reagents and conditions: (i) BocSarOSu (1 equiv), CH_2Cl_2, NMM, rt, 18 h, 92%; (ii) (a) formic acid (neat), rt, 1 h; (b) 20% toluene/*sec*-BuOH, reflux, 3 days, 82%; (iii) NaH (1.05 equiv), 3,3-dimethylallyl bromide (1 equiv), DMF, -10 °C, - rt, 18 h, 90%; (iv)) NaH (1.2 equiv), MeI (1.2 equiv), DMF, -10 °C, - rt, 18 h, 86%.

The alkylation of the DKP (**2.137**) was carried out under identical reaction conditions to that applied to the synthesis of *bis*(methylthio)silvatin (scheme 2.33). The DKP (**2.137**) was added to a freshly prepared solution of LDA (3 equiv) in a mixture of HMPA/ THF at -78 °C for 1 h. To this solution was added the electrophile (**2.144**) (6 equiv) neat drop by drop, to give a mixture of products. We observed the major products were the two mono sulfanylated derivates (**2.145** and **2.146**).

After comparing the experimental data of the mono protected compound (**2.145**) with the spectral data published for methylthiosilvatin we arbitrarily assigned the configuration as *cis*.

The next major product was the undesired *bis* protected analogue (**2.147**), this was not consistent with the findings of Shin *et al.* though, they had not observed dialkylation of this kind with dimethyl disulfide.[94] This could possibly be explained by the bulky nature of the electrophile we were using in comparison to that of dimethyl disulfide, hence reacting at the least hindered site on the DKP yielding the *bis* analogue (**2.147**) in preference to the desired compound (**2.148**). A small amount of the desired *bis* compound (**2.148**) was isolated. Initially we could not determine the configuration, but it was a single isomer either *cis* or *trans*.

In parallel we had completed the synthesis of this compound (**2.148**) and its isomer (**2.150**) *via* an alternative route. After comparing the spectral data we found this analogue (**2.148**) to be in fact, the *trans* isomer and not the desired *cis* isomer. It was possible that the *cis* isomer was also produced during the reaction conditions used. Unfortunately it was not isolated, as it co-eluted with the excess alkylating agent (**2.144**) used in the reaction.



Scheme 2.33 Reagents and conditions: (i) acetone, rt, 18 h, 40%; (ii), LDA (3 equiv), HMPA, THF -78 °C, 1 h, (**2.144**) (6 equiv), -78 °C- rt, 3 h, 1 h, 8% (**2.148**), 14% (**2.17**), 14% (**2.146**), 20% (**2.145**).

II.4.11 Trown Approach

The Trown synthesis as previously reported builds the dithiodiketopiperazine *via* radical bromination and nucleophilic substitution with a thiol. Starting from sarcosine anhydride (**2.01**) we formed the intermediate dibromide upon treatment with NBS and AIBN in carbon tetrachloride (scheme 2.34). The dibromide was then reacted without purification with initially *p*-methoxybenzyl mercaptan in the presence of triethylamine as base, to give the *cis* isomer (**2.71**) as the major product and the *trans* isomer (**2.72**) as the minor product in 31% yield over the two steps. The isomers were assigned by comparison of the two new analogues with the *cis* analogue identified by X-ray crystallography, previously synthesised *via* the amino acid approach.

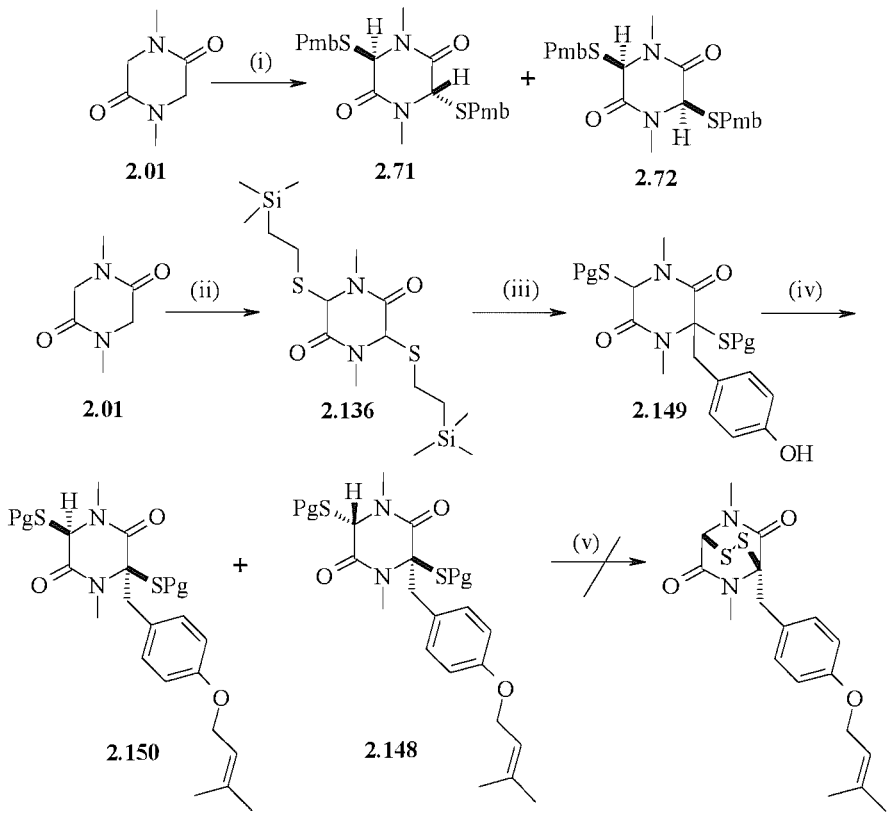
These conditions were then successfully repeated with 2-(trimethylsilyl)ethanethiol. The major product isolated from the resultant reaction mixture was identical to that isolated *via* the previously attempted amino acid approach (**2.136**). As we had no X-ray data (oil) or the alternative isomer available for spectral comparison we could not assign its configuration but, we were confident it was a single isomer. The DKP (**2.136**) was alkylated with the acetyl protected bromide (**2.88**) under the standard conditions with a

modified workup (scheme 2.34). By stirring the final reaction mixture with 1 M KOH solution for 15 minutes at rt the major product isolated was the deprotected phenol (**2.149**), which could be directly alkylated. Once again this isomer was not assigned. We observed during the alkylation with 3,3-dimethylallyl bromide two products. The major product isolated was extremely non polar. The minor more polar product was identical to that previously isolated by the protecting group alkylation strategy.

We arbitrarily assigned the major product as the *cis* isomer (**2.150**) and the remaining product as the *trans* isomer (**2.148**) by comparing the LCMS retention times and TLC rf values of the two products. However the attempted transformation to the natural product dithiosilvatin, was undertaken on both isomers anyway.

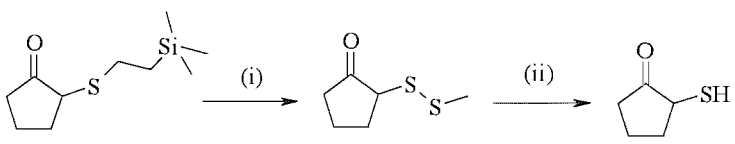
Initially we attempted a method in which the free thiol would be formed by the addition of TBAF. This could then be oxidized to the disulfide with iodine. We observed upon addition of TBAF in THF and 4Å mol sieves that the *bis* protecting group still remained. After 1 h, further TBAF was added and the reaction stirred at room temperature for 18 h. The resultant reaction mixture, which contained no starting material by TLC, was oxidised with iodine. After workup the resulting residue contained multiple products, but LCMS analysis identified no desired mass ion was observed. Trial deprotection reactions were therefore completed on the mono protected thiol (**2.145**) with TBAF and it was observed that at no point was the free thiol detected by LCMS. Prolonged treatment with TBAF in THF in the presence of 4Å mol sieves, resulted in decomposition of the starting material to a multi-component mixture.

The difficult fluoride mediated cleavage observed was also reported by Fuchs *et al.*[94] Fuchs solved the problem by cleaving the protecting group and forming an unsymmetrical disulfide *in situ* (scheme 2.35). Their objective was to synthesise a range of α-sulfenylated ketones. This was achieved by the simple addition of (methylthio)dimethylsulfonium tetrafluoroborate to the protectected ketone (**2.151**) in the presence of excess dimethyl disulfide at 0 °C yielding the unsymmetrical disulfide (**2.152**), which they then reduced to the desired thiol (**2.153**).



Scheme 2.34 Reagents and conditions: (i) (a) NBS (2.1 equiv), AIBN (0.2 equiv), CCl_4; (b) *p*-methoxybenzyl mercaptan (2.1 equiv), Et_3N (2.1 equiv), 31% (**2.71**:**2.72,** 1:5); (ii) (a) NBS (2.1 equiv), AIBN (0.2 equiv), CCl_4; (b) 2-(trimethylsilyl)ethanethiol (2.1 equiv), Et_3N (2.1 equiv), 31%; (iii) 2.0 M LDA (3.0 equiv), THF, -78 °C, 30 min; (b) Acetic acid 4-bromomethylphenyl ester (1.5 equiv), THF, -78 °C, 2 h; (i) (c) AcOH (1.5 equiv), THF, -78 °C, rt; (d) KOH, 15 min, 40%; (iv) (a) NaH (1.05 equiv), DMF, -10 °C, 30 min; (b) 3,3-dimethylallyl bromide (1.2 equiv), rt, 4 h, 74% (**2.150**:**2.148**, 7:1); (v) TBAF (8 equiv), 4Å mol sieves, THF, 18 h; (b) I_2 THF, failed.

During their investigation they also observed the symmetrical disulfide and this decreased with the addition of further equivalents of dimethyl disulfide.



Scheme 2.35 Reagents and conditions: (i) $MeS^+SMe_2BF_4^-$ (1 equiv), CH_2Cl_2, 0 °C, 60%; (ii) *n*-Bu_3P, 10% aqueous MeOH, rt, 2 h, 80%.

We therefore predicted we could modify the reaction conditions in order to form the desired internal disulfide ring. We chose not to add any dimethyl disulfide to the

reaction mixture. Unfortunately once again the reaction did not progress as predicted. Addition of 2.2 equivalents of (methylthio)dimethylsulfonium tetrafluoroborate to (**2.150**) in dichloromethane at 0 °C led to multiple products, the major product was extremely nonpolar. This nonpolar product was isolated as a mixture of three co-eluting products. ^{1}H NMR spectra of this mixture identified it to be a mixture of symmetrical and unsymmetrical disulfides of 2-(trimethylsilyl)ethanethiol indicating the protecting group had once again been eliminated.

II.5 Biological Data

II.5.1 Histone Methyltransferase (HMTs) Assay

As previously reported a member of the epipolydithiodiketopiperazine family chaetocin, has been shown to be a specific inhibitor of the histone methyltransferase SU(VAR)3-9. It was also shown to inhibit other known Lys9-specific HMTs such as mouse G9a.[56]

HMTs have been shown to be important in establishing stable gene expression, hetereochromatin formation can be HMT induced could be involved in Alzheimer`s. In addition some known histone methyltransferases (HMTs) are misregulated in tumors, this makes HMT inhibitors an attractive biological target.

A Lys9-specific HMTs G9a ELISA assay was developed by Yoshida`s group at the RIKEN Institute in Japan. Their aim was to see if gliotoxin, a member of the ETP alkaloid family was also an inhibitor of Lys9-specific HMTs. In collaboration we selected two of our synthesised ETP`s (**2.78**) and (**2.79**) and a *bis* Pmb protected DKP (**2.74**) to test alongside the natural product in the Lys9-specific HMTs G9a ELISA assay (figure 3.3).

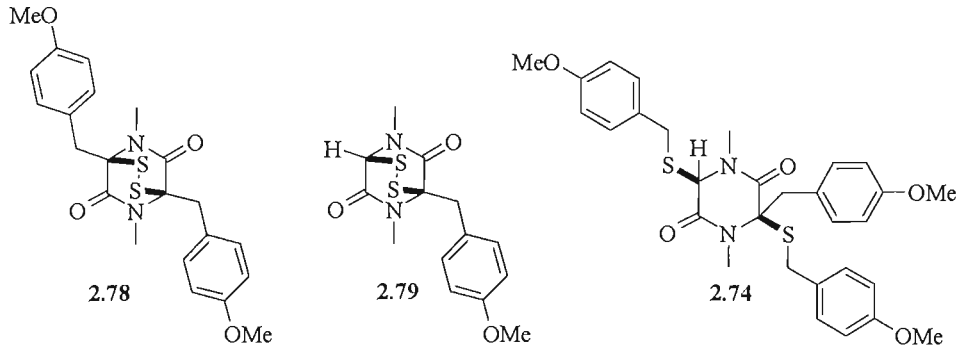
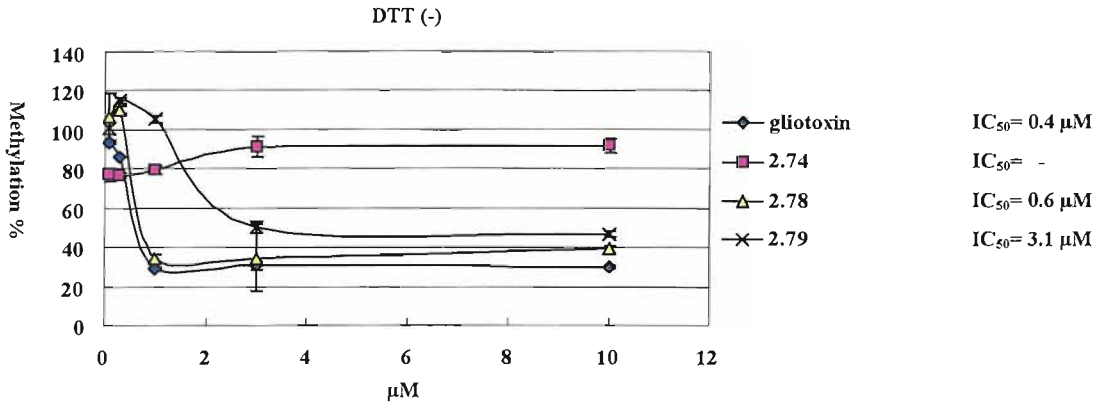
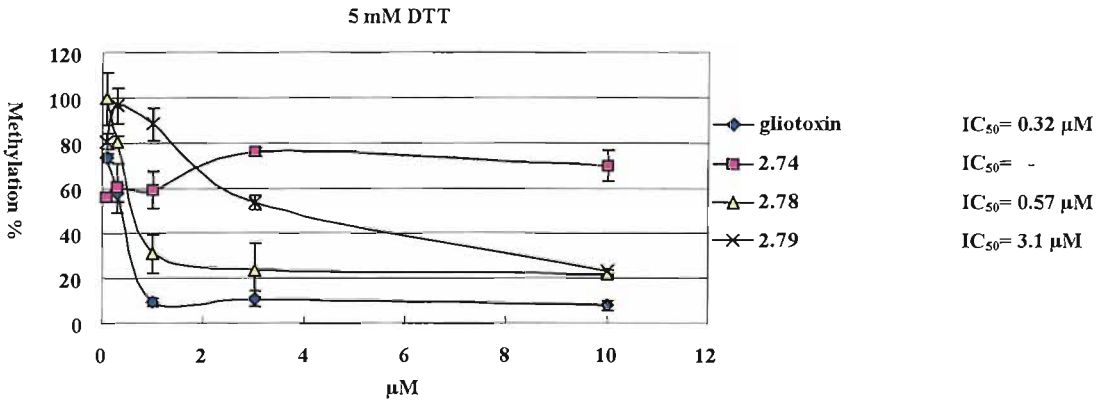


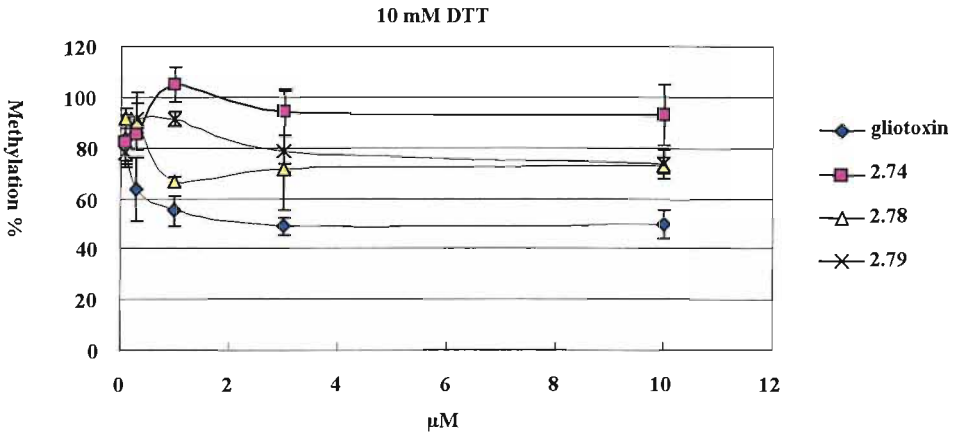
Figure 2.11 Compounds selected for Lys9-specific HMTs G9a ELISA assay.



Graph 2.1 Gliotoxin, and compounds (**2.74**), (**2.78**) & (**2.79**) in ELISA assay without DTT.



Graph 2.2 Gliotoxin, and compounds (**2.74**), (**2.78**) & (**2.79**), in ELISA assay 5mM DTT.



Graph 2.3 Gliotoxin, and compounds (**2.74**), (**2.78**) & (**2.79**), in ELISA assay 10 mM DTT.

The assay was initially run with the compounds alone and IC_{50} value calculated (graph 2.1). It was observed that gliotoxin and compounds (**2.78**) and (**2.79**) were inhibitors of the Lys-specific histone methyltransferase G9a. Gliotoxin inhibited the Lys-specific histone methyltransferase G9a with an IC_{50} value of 0.4 μM, likewise compound (**2.78**) was an inhibitor with an IC_{50} value of 0.6 μM. Compound (**2.79**) was also found to be an inhibitor, but with a slightly higher value of 3.1 μM, which is similar to that reported for chaetocin (IC_{50} value of 2.5 μM) for Lys9-specific HMTs G9a.

The assay was then repeated with increasing doses of the reducing agent DTT (graphs 2.2-2.3). This was to investigate the importance of the disulfide bridge in gliotoxin and the ETPs, compounds (**2.78**) and (**2.79**). Compound (**2.74**) does not have the disulfide bridge functionality and was therefore used as a control in all three assays. It was shown that the IC_{50} value was reduced in all three of the ETP compounds (gliotoxin, **2.78**, **2.79**) when 10 mM of DTT was added, little effects was observed when only 5 mM of DTT was added to the assay.

Finally a SDS-PAGE analysis of *in vitro* methylation of H3 was completed to investigate the dose dependent effect of the inhibitor on methylation of the H3 molecules at Lys9 (figure 2.12). When gliotoxin was assayed it was shown that dimethylation (αH3K9me2) was reduced at concentrations above 500 nM to methylation (αH3K9me). At higher concentrations of gliotoxin (2 μM) a slight drop in methylation (αH3K9me) was reported. A similar trend in the reduction of

dimethylation (αH3K9me2) was reported for ETPs (**2.78**) and (**2.79**) but at higher concentrations (500 nM).

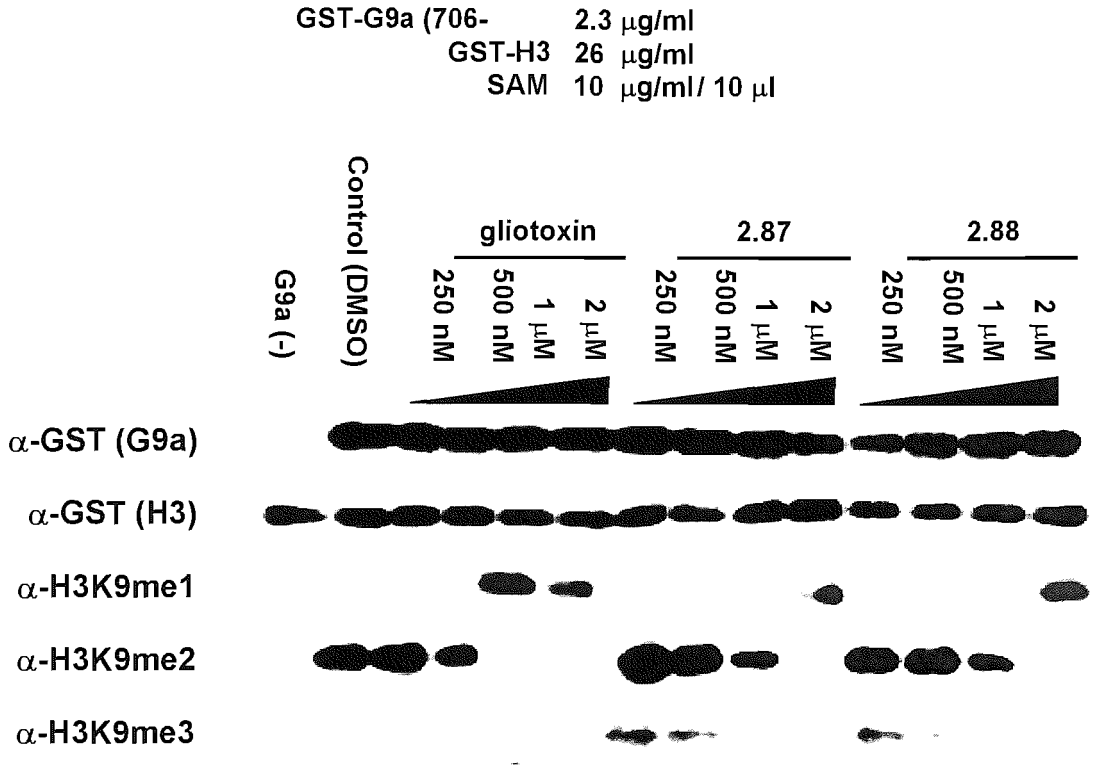


Figure 2.12 Dose-dependent inhibition assay with gliotoxin and compounds (**2.78**) & (**2.79**).

II.5.2 Conclusions; HMT ELISA Assay

The assay data results clearly demonstrated that gliotoxin, another member of the ETP alkaloid family is a potent inhibitor of Lys9-specific histone methyltransferase G9a. In addition the two novel synthesized ETPs (**2.78**) and (**2.79**) were also potent inhibitors. The addition of the reducing agent DTT, led to a reduction in potency in all three examples of the ETPs. We can therefore conclude that the activity is due to the presence of the disulfide bridge functionality. This can also be supported by the fact that the *bis* protected analogue (**2.74**), which does not have this functionality, was inactive in the assay.

Chaetocin and gliotoxin (figure 2.11) are both *bis* substituted at the epidithiodiketopiperazine core. It was therefore interesting that the most potent analogue we synthesised was again *bis* substituted (**2.78**) with an IC_{50} value of 0.6 μM, comparable to the natural product gliotoxin (IC_{50} 0.4 μM). The second analogue (**2.79**) is not *bis* substituted but, has comparable potency (IC_{50} value of 3.1 μM) to that reported for chaetocin (IC_{50} value of 2.5 μM).[56]

We have therefore discovered two novel inhibitors of Lys9-specific histone methyltransferase G9a. These examples tested could lead to a series of *bis* and mono substituted ETPs based on the ETP core.

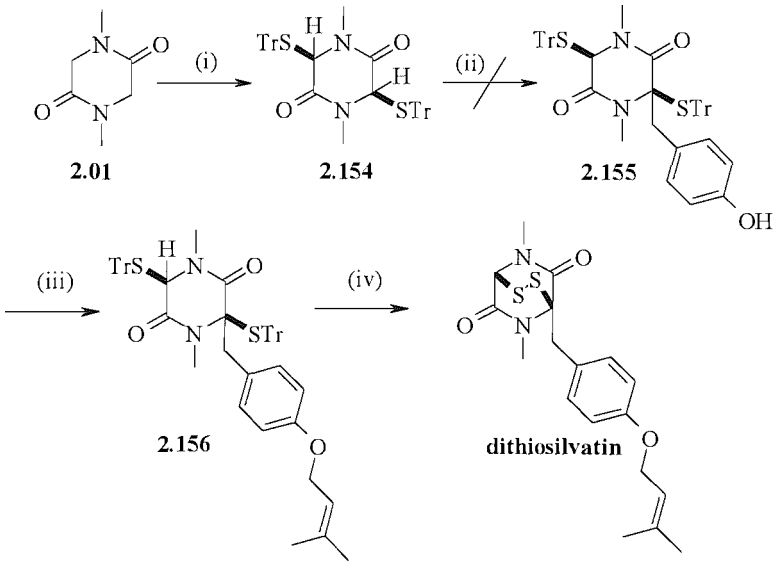
II.6 Conclusions and Future Work

II.6.1 Conclusions

Unfortunately we were not successful in our attempted total synthesis of dithiosilvatin. We approached the total synthesis from a number of directions, but mainly concentrated on the amino acid approach to the dithiodiketopiperazine core. This was highly successful yielding a new diverse set of protected α-mercaptoamino acids and esters, which can be selectively deprotected for further elucidation. In addition we developed routes to a range of *bis* protected dithiodiketopiperazines. Our investigations have now given us a better understanding of the sequence of synthetic steps in the work towards dithiosilvatin. It is clear that the disulfide bridge has to be assembled at the final stage of the synthesis. We observed the ETP core is not stable in basic media or prolonged exposure to acid conditions as reported by Woodward *et al.* We can also conclude that the isoprenyl group is extremely labile under acid reaction conditions, therefore acid must be avoided at the final step. Another important finding is that the *bis* protected thiol is prone to elimination when fully substituted. During the synthesis a number of ETPs were successfully synthesized, two of these (**2.78**) and (**2.79**) have been found to be potent inhibitors of Lys9-specific HMTs G9a.

II.6.2 Future Work; Total Synthesis of Dithiosilvatin

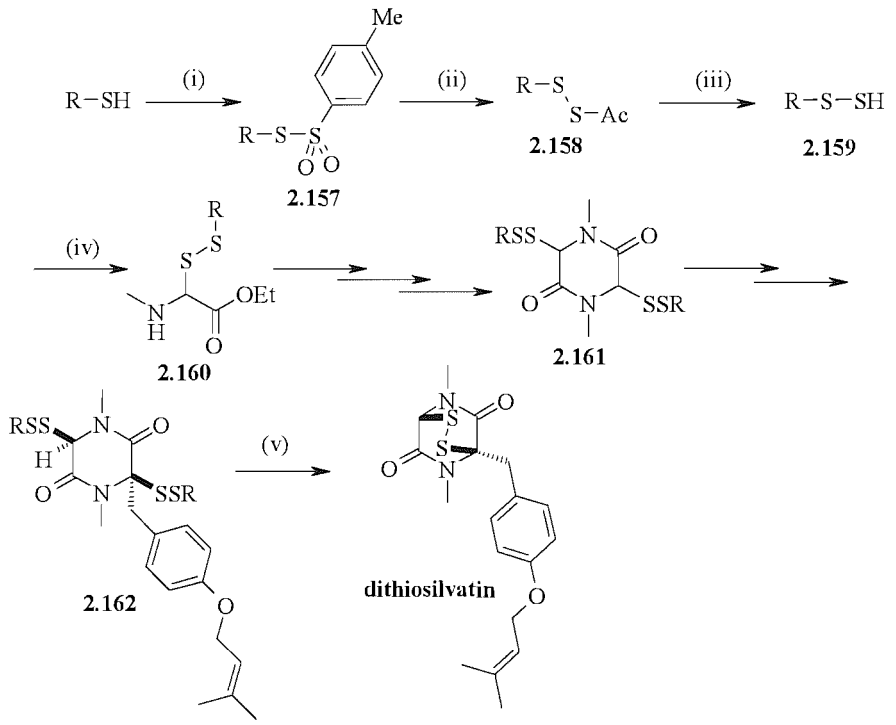
Nearing the end of our investigation we synthesised some bis protected DKP *via* the Trown methodology (scheme 2.36). One example that was not reported was the *bis* trityl (Tr) protected DKP (**2.154**). We intended to carry this through using the standard routes developed to the bis protected isoprenyl analogue (**2.156**), which we envisaged could be converted to the natural product by the addition of iodine (scheme 2.36). Unfortunately the initial step was poor yielding, but by no means optimised. The major problem was the DKP (**2.154**) which was extremely insoluble. We found the alkylation step therefore was not viable in the traditional solvents (THF or diethyl ether). This approach was therefore abandoned.



Scheme 2.36 Reagents and conditions: (i) (a) NBS (2.1 equiv), AIBN (0.2 equiv), CCl_4; (b) triphenyl - methyl mercaptan (2.1 equiv), Et_3N (2.1 equiv), 17%; (ii) 2.0 M LDA (3.0 equiv), THF, -78 °C, 30 min; (b) acetic acid 4-bromomethylphenyl ester (1.5 equiv), THF, -78 °C, 2 h; (c) AcOH (1.5 equiv), THF, -78 °C, rt; (d) KOH, 15 min, (failed); (iii) (a) NaH (1.05 equiv), DMF, -10 °C, 30 min; (b) 3,3-dimethylallyl bromide (1.2 equiv), rt; (iv) I_2 , DMF.

One approach that we would have liked to have attempted would be *via* a disulfide protection strategy (scheme 2.37). It would be envisaged that this could then be simply reduced to the dithiol, which upon re-oxidation would give the desired disulfide bridge. The problem is how to introduce the disulfide protection. One possible solution would be to introduce the disulfide as before *via* the three component reaction with glyoxalate and methylamine in toluene, to form the protected amino acid ester (**2.160**). The thiol (**2.159**) would require synthesis and could be prepared by utilising a thiosulfonate

analogue (**2.157**) similar to that reported previously (**2.144**). This could be formed by reacting potassium thiotosylate (**2.142**) with the desired alkylthiol or aromatic thiol (R-SH) in acetone to give the thiosulfonate (**2.157**). The thiol (**2.159**) could then be prepared in two steps. First reacting the thiosulfonate (**2.157**) with the sodium salt of thioacetic acid to give the acetyl protected disulfide, and then cleaving the acetyl group with base, to give the thiol (**2.159**). It is envisaged that the disulfide protected amino acid ester (**2.160**) could then be converted to the isoprenyl DKP (**2.162**) using the standard procedure previously reported. Finally reduction with a mild reducing agent such as tributylphosphine, in order not to affect the isoprenyl group and oxidation should yield the dithiosilvatin. The major foreseen problem would be the stability of the disulfide under the proposed reaction scheme.

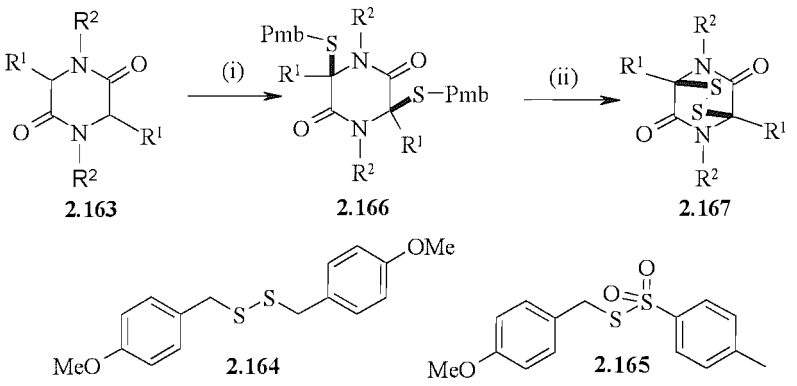


Scheme 2.37 Purposed reagents and conditions: (i) potassium thiotosylate, acetone; (ii) thioacetic acid sodium salt, THF; (iii) 0.2 M NaOH[97]; (iv) ethyl glyoxylate, 1.0 M methylamine THF; (v) Bu_3P, trifluoroethanol, water;[98] (b) I_2, MeOH, CH_2Cl_2.

II.6.3 Future Work: HMT Inhibitors

It is feasible to synthesise a range of ETPs similar in structure to (**2.79**) using the routes developed towards the synthesis of dithiosilvatin, but this would not be the case for the *bis* substituted analogue (**2.78**). This was synthesized from the minor by-product, due to undesired dialkylation of the *bis* protected Pmb DKP (**2.71**). Therefore

a new route would be required (scheme 2.38). The simplest approach would be to treat the DKP (**2.163**) with more than 2 equivalents of LDA or n-BuLi and react the resultant dicarbanion with a sulfur electrophile such as Pmb-disulfide (**2.164**) or a Pmb-thiosulfonate (**2.165**) to give the desired *bis* Pmb protectected DKP (**2.166**). The Pmb could then be cleaved with BCl_3 in dichloromethane, and oxidised to the new ETPs (**2.167**) as previously reported. Alternative protecting groups such as acetyl, can be used for acid sensitive analogues. A number of DKP's of this kind are commercially available. Any additional analogues can be readily synthesised.



Scheme 2.38 Purposed reagents and conditions: (i) LDA (> 2 equiv), THF -78 °C, 1 h; (b) (**2.164**) or (**2.165**) (4-8 equiv); (ii) (a) 1.0 M BCl_3 (2.4 equiv) 0 °C 10 min. evaporate; (b) I_2 (2.0 equiv), 10% MeOH, CH_2Cl_2, rt, 20 min.

Chapter III: Experimental

III.1 Experimental

III.1.1 General

All reactions were carried out under an inert atmosphere, in oven-dried glassware and were magnetically stirred unless otherwise stated. Benzene, 1,4-dioxane, ether and THF were distilled from sodium, with benzophenone as an internal indicator, immediately before use. Toluene was distilled from sodium. Chloroform and dichloromethane were distilled from calcium hydride immediately prior to use. Where appropriate, all other solvents and reagents were purified according to standard methods.

Reactions were monitored by TLC using aluminium-backed sheets coated with silica gel 60 containing a fluorescence indicator active at 254 nm or Merck silica gel 60 F_{254} precoated plates; the chromatograms were visualised under UV light (254 nm) and by staining with, most commonly, 20% phosphomolybdic acid in ethanol or 10% aqueous $KMnO_4$. Where flash chromatography was undertaken, Apollo silica gel (0.040-0.063 mm, 230-400 mesh) or Merck silica gel 60 (0.040-0.063 mm) was used, slurry packed and run at low pressure.

Infrared (IR) spectroscopy was performed using a BioRad FT-IR Goldengate spectrometer or Thermo Mattson Satellite FT-IR spectrometer. Positions of absorption maxima are quoted in cm^{-1}. Letters after give an indication of the relative strength of the peak (w = weak, m = moderate, s = strong, br. = broad).

^{1}H and ^{13}C spectroscopy was performed on a Bruker AC/AM300, Jeol EX 270 (270 MHz) or DPX400 spectrometer at operating frequencies indicated in the text. Chemical shifts (δ_H, δ_C) are reported in parts per million relative to residual $CHCl_3$ (δ_H = 7.27 ppm, δ_C = 77.2 ppm). Multiplicities are reported using the following notation: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet, app. = apparent, br. = broad, obs. = obscured. In order to aid the assignment of chemical shifts in both ^{1}H and ^{13}C NMR spectra, the structures are arbitrarily assigned numbers. These numbers do not conform to any naming conventions.

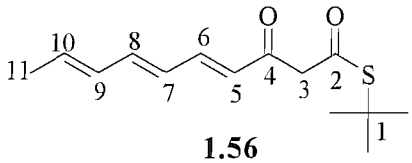
Chemical ionisation (CI) and electron ionisation (EI) mass spectroscopy was performed on a Thermoquest Trace GCMS spectrometer. Electrospray (ES) mass spectroscopy was performed on a Micromass Platform (MP) spectrometer.

LCMS was carried out using a Chromolith Speedrod RP-18e column, 50 x 4.6 mm, with a linear gradient 10% to 90% 0.1% HCO_2H/MeCN into 0.1% HCO_2H/H_2O over 11 min, flow rate 1.5 mL/min. Data was collected using a Thermofinnigan Surveyor MSQ mass spectrometer with electrospray ionization in conjunction with a Thermofinnigan Surveyor LC stystem.

Mass spectroscopic data are reported as values in atomic mass units with peak intensities reported relative to the base peak (100%). Elemental analysis was performed by Medac Ltd. Melting points were carried out using a Griffin melting point apparatus and are uncorrected.

III.1.2 Chapter I Experimental

(4*E*,6*E*,8*E*)-3-Oxodeca-4,6,8-trienethioic acid S-*tert*-butylester (1.56)

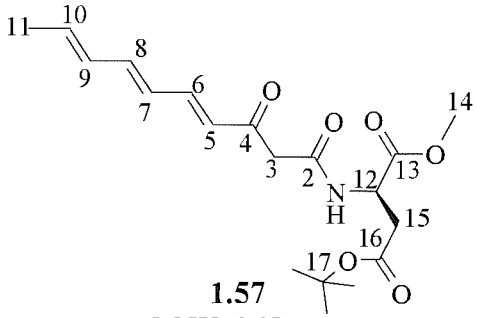


1.56
M.W. 252.38
$C_{14}H_{20}O_2S$

tert-butyl 4-diethylphosphono-3-oxobutanthioate (**1.43**) (560 mg, 1.80 mmol) was added to a stirred suspension of sodium hydride (127 mg, 2.68 mmol of 60% oil disp.) in THF (20 mL) at 0 °C. The suspension was stirred for 30 min. whereupon the 2,4-hexadienal (138 mg, 1.44 mmol) in THF (5 mL) was added. The reaction was allowed to warm to room temperature and stirred overnight. The dark brown mixture was then poured into saturated NH_4Cl (aq) solution (10 mL), the organic phase was separated, and the aqueous extracted with ether (3 x 10 mL). The combined organics were washed with water (10 mL) and brine (10 mL), dried over Na_2SO_4, evaporated *in vacuo* and the residue purified by column chromatography (SiO_2, 2% EtOAc/Hexane) to give (**1.56**) as a yellow solid (323 mg, 89%).

Mp 71.6-73.1 °C (EtOAc/Hexane); **^{1}H NMR** (300 MHz, $CDCl_3$, >60% *enol* form) δ_H 12.49 (d, 0.6H, *J* 1.8 Hz, OH, *enol*), 6.99-7.21 (m, 1H, H5), 5.63-6.59 (m, 5H, H6-H10), 5.28 (s, 0.6H, H3, *enol*), 3.63 (s, 0.7H, H3, *keto*), 1.78-1.74 (m, 3H, H11), 1.44 & 1.39 (s, 9H, H1); **^{13}C NMR** (75MHz) δ_C 220.0 (C2, Cq), 196.1 (C4, Cq), 145.3, 143.3, 139.2, 138.7, 136.4, 134.1, 131.5, 131.3, 128.7, 127.9, 127.4, 124.0 (C5-C10, 6 x CH), 101.3 (C3, *enol* CH), 56.8 (C3, CH_2), 30.2, 29.6 (C1, 3 x CH_3), 18.6, 18.5 (C11, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 275.2 (100), $[2M+Na]^+$ 527.3 (50), $[M+H]^+$ 253.2 (25); **FTIR** ν_{max} (neat, cm^{-1}) 1604 (m), 1554 (m), 1158 (m) cm^{-1}.

(*R*)-2-((4*E*,6*E*,8*E*)-3-Oxodeca-4,6,8-trienoylamino)succinic acid 4-*tert*-butyl ester 1-methyl ester (1.57)



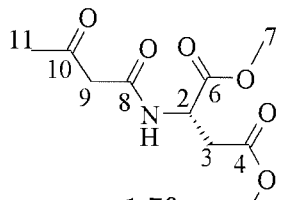
1.57
M.W. 365.43
$C_{19}H_{27}NO_6$

A mixture of H-DAsp(O^{t}Bu)OMe (120.7 mg, 0.59 mmol), Na_2HPO_4 (215 mg, 1.51 mmol) and powdered 4A sieves (215 mg) in THF (5 mL) was stirred at room temperature for 10 min. A solution of **(1.56)** (100 mg, 0.39 mmol) in THF (10 mL) was added, followed by the addition of silver (I) trifluoroacetate (131.2 mg, 0.59 mmol). The resultant suspension was stirred in the dark for 35 min. The solid was filtered though celite, and washed with diethyl ether (20 mL). Evaporation *in vacuo* and purification by column chromatography (SiO_2, 50% EtOAc/Hexane) gave **(1.57)** as a yellow solid (90 mg 62%).

Mp 88.1-89.7 °C (EtOAc/Hexane); **^{1}H NMR** (300 MHz, $CDCl_3$, >95 % *keto* form) δ_H 7.90 (d, 1H, *J* 8.8 Hz, NH), 7.31 (dd, 1H, *J* 15.3, 10.6 Hz, H5-H10), 6.64 (dd, 1H, *J* 14.6, 10.6 Hz, H5-H10), 6.27-5.91 (m, 4H, H5-H10), 4.88-4.86 (m, 1H, H12), 3.76 (s, 3H, H14), 3.58 (s, 2H, H3), 2.76 & 2.95 (dd, 1H, *J* 16.8, 4.7 Hz; dd, 1H, *J* 16.8, 4.7 Hz, H15), 1.86 (d, 3H, *J* 6.6 Hz, H11), 1.45 (s, 9H, H17); **^{13}C NMR** (75 MHz) δ_C 194.4 (C4, Cq), 170.9, 170.0 (C13 & C16, 2 x Cq) 167.0 (C2, Cq), 145.6, 143.8, 136.8, 131.3, 127.7, 127.6 (C5-C10, 6 x CH), 81.9 (C17, Cq), 52.6 (C14, CH_3), 48.8 (C12, CH), 46.7 (C3, CH_2), 37.6 (C15, CH_2), 28.0 (C17, 3 x CH_3), 18.6 (C11, CH_3); **LRMS** ES+ *m/z* $[2M+Na]^+$ 753.5 (100), $[M+Na]^+$ 388.2 (80); **FTIR** ν_{max} (neat, cm^{-1}) 1722 (m), 1532 (m), 1367 (m), 1214 (m), 1151 (s) cm^{-1}; $[\alpha]_D$ = +7.5 (c = 0.01, MeOH, 25 °C).

(*S*) Enantiomer of **(1.59),** prepared in the same way from H-DAsp(O^{t}Bu)OMe with identical spectroscopic data. $[\alpha]_D$ = -4.1 (c = 0.006, MeOH, 25 °C).

(*S*)-2-(3-Oxobutyrylamino)succinic acid dimethyl ester (1.70)

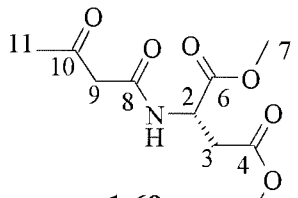


1.70
M.W. 245.23
$C_{10}H_{15}NO_6$

A solution of 5-acetyl-2,2-dimethyl[1,3]dioxinone-4,6-dione (900 mg, 4.80 mmol), H-Asp(OMe)OMe.HCl (950 mg, 4.80 mmol), and triethylamine (533 mg, 5.28 mmol) in toluene (50 ml) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated and partitioned between ethyl acetate and $KHSO_4$ (0.3 M). The organics were separated, washed with sat $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 75% EtOAc/Hexane) to give (**1.70**) as a colourless oil (490 mg, 53%).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.63 (d, 1H, *J* 6.2 Hz, NH), 4.88-4.86 (m, 1H, H2), 3.77 (s, 3H, H7), 3.71 (s, 3H, H5), 3.45 (s, 2H, H9), 3.03 & 2.86 (dd, 1H, *J* 17.2, 4.8 Hz; dd, 1H, *J* 17.2, 4.8 Hz, H3), 2.27 (s, 3H, H11); **^{13}C NMR** (75 MHz) δ_C 203.3 (C10, Cq), 171.2 (C4, Cq), 170.8 (C6, Cq), 165.5 (C8, Cq), 52.8, 52.1 (C5 & C7, 2 x CH_3), 49.7 (C9, CH_2), 48.5 (C2, CH), 36.0 (C3, CH_2), 30.8 (C11, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 268 (100); **FTIR** ν_{max} (neat, cm^{-1}) 2970 (w), 1718 (s), 1635 (m), 1195 (s), 1151 (s), 1025 (m) cm^{-1}.

(*S*)-2-(3-Oxobutyrylamino)succinic acid 4-*tert*-butyl ester 1-methyl ester (1.69)



1.69
M.W. 287.32
$C_{13}H_{21}NO_6$

A solution of 5-acetyl-2,2-dimethyl[1,3]dioxinone-4,6-dione (600 mg, 3.22 mmol), H-Asp(O^{t}Bu)OMe. HCl (772 mg, 3.22 mmol) and triethylamine (358 mg, 3.54 mmol) in toluene (30 mL) was stirred at reflux for 3 h. After cooling, the reaction mixture was concentrated and partitioned between ethyl acetate and 0.3 M $KHSO_4$ solution .The organics were separated, washed with sat $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 50% EtOAc/Hexane) to give (**1.69**) as a colourless oil (490 mg, 53%).

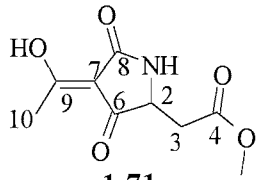
^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.64 (d, 1H, *J* 7.7 Hz, NH), 4.88-4.81 (m, 1H, H2), 3.76 (s, 3H, H7), 3.45 (s, 2H, H9), 2.98 & 2.73 (dd, 1H, *J* 16.8, 4.4 Hz; dd, 1H, *J* 16.8, 4.4 Hz, H3), 2.27 (s, 3H, H11), 1.45 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 203.3 (C10, Cq), 171.0 (C4, Cq), 169.6 (C6, Cq), 165.4 (C8, Cq), 81.9 (C5, Cq), 52.8 (C7, CH_3), 49.7 (C9, CH_2), 48.7 (C2, CH), 37.4 (C3, CH_2), 30.8 (C11, CH_3), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[2M\text{-}2O^tBu]^+$ 428.9 (100); **FTIR** ν_{max} (neat, cm^{-1}) 2978 (w), 2942 (w), 1716 (s), 1630 (m), 1209 (s), 1152 (s), 1025 (m) cm^{-1}.

(*R,S*)-(4-Acetyl-3,5-dioxopyrrolidin-2-yl)acetic acid methyl ester (1.71) and (*R,S*)-(4-acetyl-3,5-dioxopyrrolidin-2-yl)acetic acid (1.72)

Sodium methoxide in methanol (0.5M, 11.4 ml, 5.7 mmol) was added to a solution of (*S*)-2-(3-oxobutyrylamino)succinic acid dimethyl ester (700 mg, 2.85 mmol) in methanol (10 mL) and the reaction mixture stirred at reflux for 2 h. After cooling, the reaction mixture was concentrated and partitioned between dichloromethane (15 mL) and 2 N HCl (aq) solution (5 mL) pH < 7 .The organics were separated and the aqueous extracted with dichloromethane (2 x 20 mL). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give (**1.71**) as a pink solid (340 mg, 56%). The aqueous layer was acidified further to pH < 2 by the addition of 5 N HCl

(aq) solution (10 mL) and extracted with ethyl acetate (2 x 10 mL) to give (**1.72**) as an off white solid (100 mg).

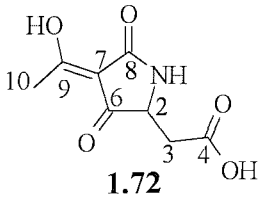
(*R,S*)-(4-Acetyl-3,5-dioxopyrrolidin-2-yl)acetic acid methyl ester (1.71)



1.71
M.W. 213.19
$C_9H_{11}NO_5$

Mp 106.7-107.4 °C; **^{1}H NMR** (300 MHz, $CDCl_3$) δ_H 12.31 (br s, 1H, OH), 7.08 (br s, 1H, NH), 4.16-4.11 (m, 1H, H2), 3.72 (s, 3H, H5), 2.99 & 2.50 (dd, 1H, *J* 17.0, 2.9 Hz; dd, 1H, *J* 17.0, 2.9 Hz, H3), 2.45 (s, 3H, H10); **^{13}C NMR** (75 MHz) δ_C 193.7 (C6, Cq), 186.1 (C9, Cq), 175.3 (C8, Cq), 171.6 (C4, Cq), 101.3 (C7, Cq), 58.7 (C2, CH), 52.5 (C5, CH_3), 36.4 (C3, CH_2), 20.1 (C10, CH_3); **LRMS ESI+** *m/z* $[M+Na]^+$ 235.8 (100), $[M+H]^+$ 213.8 (50), $[M+Na+CH_3CN]^+$ 276.8 (20); **LCMS ESI+** *m/z* $[M+H]^+$ 214.2 peak at 2.26 min; **FTIR** ν_{max} (neat, cm^{-1}) 3211 (w), 1732 (m), 1667 (s), 1618 (s), 1413 (m), 1319 (m), 1213 (s), 1172 (s) cm^{-1}; ***Anal.*** Calcd for $C_9H_{11}NO_5$: C, 50.71; H, 5.20; N, 6.57. Found: C, 50.42; H, 5.18; N, 6.49.

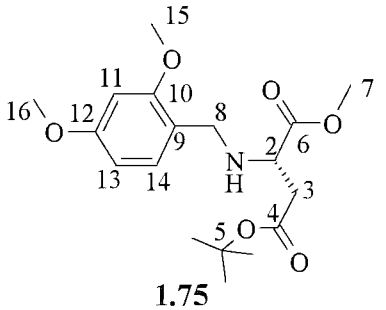
(*R,S*)-(4-Acetyl-3,5-dioxopyrrolidin-2-yl)acetic acid (1.72)



1.72
M.W. 199.16
$C_8H_9NO_5$

Mp 161.8-162.5 °C (DCM); **^{1}H NMR** (300 MHz, CD_3OD, acid not observed) δ_H 8.20 (br s, 1H, NH) 4.04 (dd, 1H, *J* 7.7, 3.7 Hz, H2) 2.74 & 2.53 (dd, 1H, *J* 17.2, 3.7 Hz; dd, 1H, *J* 17.2, 7.7 Hz, H3), 2.32 (s, 3H, H10); **^{13}C NMR** (75 MHz) δ_C 197.5 (C6, Cq), 187.1 (C9, Cq), 175.3 (C8, Cq), 173.6 (C4, Cq), 103.0 (C7, Cq), 59.5 (C2, CH), 36.8 (C3, CH_2), 20.0 (C10, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 200.3 peak at 1.68 min; **FTIR** ν_{max} (neat, cm^{-1}) 3215 (w), 1716 (m), 1680 (s), 1610 (s), 1423 (m), 1319 (m), 1210 (s) cm^{-1} ***Anal.*** Calcd for $C_8H_9N_2O_5$: C, 48.25; H, 4.55; N, 7.03. Found: C, 48.35; H, 4.56; N, 6.76. Data consistent with literature.[99]

(*S*)-2-(2,4-Dimethoxybenzylamino)succinic acid 4-*tert*-butyl ester 1-methyl ester (1.75)

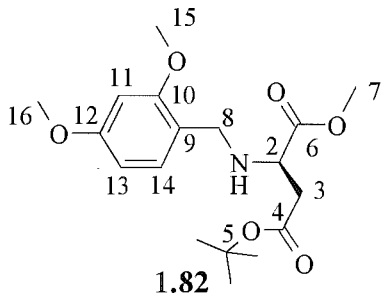


1.75
M.W. 353.42
$C_{18}H_{27}NO_6$

To a solution of H-Asp(O^{t}Bu)OMe.HCl (1.00 g, 4.1 mmol), *N,N*-diisopropylethylamine (0.58 g, 4.50 mmol) in 1,2-dichloroethane (10 mL) was added 2,4-dimethoxybenzaldehyde (0.715 g, 4.30 mmol) and the reaction mixture stirred at room temperature for 40 min. Sodium triacetoxyborohydride (1.21 g, 5.0 mmol) was added portionwise and the reaction mixture stirred at room temperature for 18 h. The reaction mixture was concentrated and partitioned between ethyl acetate (30 mL) and saturated $NaHCO_3$ (aq) solution (10 mL). The organics were separated and washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 60% EtOAc/Hexane) to give (**1.75**) as a colourless gum (1.41 g, 96%).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.17-7.14 (m, 1H, H14), 6.48-6.42 (m, 2H, H11 & H13), 3.81 (s, 3H, H15), 3.79 (s, 3H, H16), 3.76-3.74 (m, 2H, H8), 3.68 (s, 3H, H7), 3.66-3.61 (m, 1H, H2), 2.67 & 2.62 (dd, 1H, *J* 15.7, 6.6 Hz; dd, 1H, *J* 15.7, 6.6 Hz, H3), 1.42 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 173.9 (C4, Cq), 170.0 (C6, Cq), 160.3 (C12, Cq), 158.6 (C10, Cq), 130.5 (C14, CH), 119.9 (C9, Cq), 103.9 (C13, CH), 98.5 (C11, CH), 81.0 (C5, Cq), 57.2 (C2, CH), 55.4, 55.3 (C15 & C16, 2 x CH_3), 51.9 (C7, CH_3), 46.8 (C8, CH_2), 39.1 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 375.8 (100), $[M+H]^+$ 353.8 (30); $[\alpha]_D$ = -6.5 (c = 0.02, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2976 (w), 1727 (s), 1588 (m), 1506 (m), 1287 (m), 1151 (s), 1034 (s) cm^{-1}.

(*R*)-2-(2,4-Dimethoxybenzylamino)-succinic acid 4-*tert*-butyl ester 1-methyl ester (1.82)

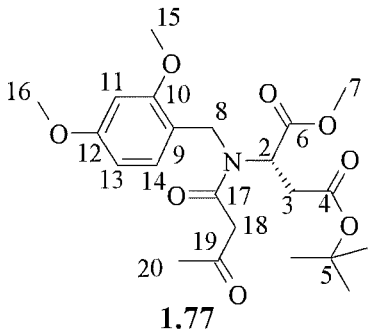


1.82
M.W. 353.42
$C_{18}H_{27}NO_6$

To a solution of H-DAsp(O^{t}Bu)OMe.HCl (219.2 mg, 0.65 mmol), in 1,2-dichloroethane (10 mL) was added 2,4-dimethoxybenzaldehyde (113.4 mg, 0.65 mmol) and the reaction mixture stirred at room temperature for 40 min. Sodium triacetoxyborohydride (193 mg, 0.91 mmol) was added and the reaction mixture stirred at room temperature for 18 h. The reaction mixture was concentrated and partitioned between ethyl acetate (20 mL) and saturated $NaHCO_3$ (aq) solution (10 mL). The organics were separated and washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 60% EtOAc/Hexane) to give (**1.82**) as a colourless gum (210 mg, 91%).

Spectroscopic data are identical to (**1.75**).
$[\alpha]_D$ = +2.1 (c = 0.015, MeOH, 25 ° C).

(*S*)-2-[-(2,4-Dimethoxybenzyl)-(3-oxo-butyryl)-amino]-succinic acid 4-*tert*-butyl ester 1-methyl ester (1.77)

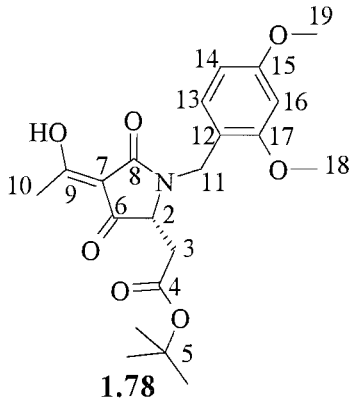


1.77
M.W. 437.49
$C_{22}H_{31}NO_8$

A solution of (**1.75**) (700 mg, 1.98 mmol) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (366 mg, 2.57 mmol) in toluene (15 mL) was stirred at reflux for 2 h. After cooling, the reaction mixture was concentrated. The resultant residue was purified by column chromatography (SiO_2, 55% EtOAc/Hexane) to give (**1.77**) as a colourless gum (830 mg, 96%).

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.23-7.17 (m, 1H, H14), 6.48-6.39 (m, 2H, H13, H11), 4.43 (s, 2H, H18), 3.81 (s, 3H, H15), 3.80 (s, 3H, H16), 3.76-3.74 (m, 2H, H8), 3.68 (s, 3H, H7), 3.66-3.61 (m, 1H, H2), 3.18 & 2.40 (dd, *J* 16.8, 7.7 Hz; dd, 1H, *J* 16.8, 5.5 Hz, H3), 2.26 (s, 3H, H20), 1.42 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 202.3 (C19, Cq), 170.4, 170.2 (C6 & C4, 2 x Cq), 167.3 (C17, Cq), 161.1 (C12, Cq), 158.7 (C10, Cq), 130.5 (C14, CH), 116.0 (C9, Cq), 103.9 (C13, CH), 98.7 (C11, CH), 81.0 (C5, Cq), 56.2 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH_3), 52.2 (C7, CH_3), 50.0 (C18, CH_2), 49.1 (C8, CH_2), 39.1 (C3, CH_2), 30.0 (C20, CH_3), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 459.8 (100); **FTIR** ν_{max} (neat, cm^{-1}) 2978 (w), 2942 (w), 1716 (s), 1630 (m), 1610 (m), 1454 (m), 1295 (m), 1152 (s), 1024 (s) cm^{-1}.

[(*S*)-4-Acetyl-1-(2,4-dimethoxybenzyl)-3,5-dioxo-pyrrolin-2-yl]acetic acid *tert*-butyl ester (1.78)

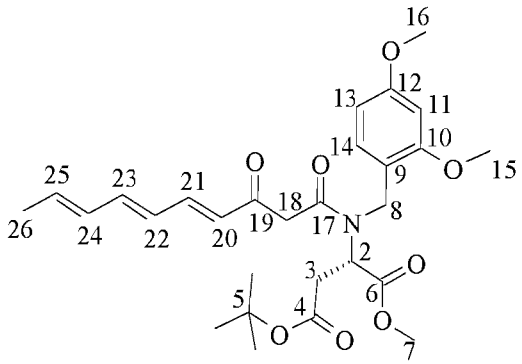


1.78
M.W. 405.45
$C_{21}H_{27}NO_7$

Potassium *tert*-butoxide (399 mg, 3.56 mmol), was added to (**1.77**) (780 mg, 1.78 mmol), in tetrahydrofuran (20 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred at room temperature for 3 h. The reaction mixture was concentrated and partitioned between ethyl acetate and 0.3 M $KHSO_4$ (aq) solution. The aqueous was separated and extracted with further ethyl acetate (2 x 20 ml). The organics were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 5% MeOH/DCM) to give compound (**1.78**) as a red glass (150 mg, 21%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 7.20-7.12 (m, 1H, Ar-H), 6.40-6.36 (m, 2H, Ar-H), 4.91-4.83 (m, 1H, H11), 4.15-4.11 (m, 1H, H11), 3.81-3.74 (m, 7H, H8, H2 & H19), 2.94-2.20 (m, 5H, H3 & H10), 1.32 (s, 9H, H5); **^{13}C NMR** (100 MHz, CD_3OD) δ_C 196.1 (C6, Cq), 177.0 (C9, Cq), 172.8 (C8, Cq), 171.2 (C4, Cq), 161.8 (C15, Cq), 159.7 (C17, Cq), 132.5 (C13, CH), 118.7 (C12, Cq), 105.7 (C14, CH), 101.0 (C7, Cq), 99.3 (C13, CH), 82.1 (C5, Cq), 61.7 (C19, CH_3), 57.3 (C2, CH), 55.9, 55.9 (C18 & C19, 2 x CH_3), 48.5 (C11, CH_2), 36.7 (C3, CH_2), 30.7 (C10, CH_3), 28.3 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 428 (100); **$[\alpha]_D$** = -10.5 (c = 0.01, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2970 (w), 2938 (w), 1724 (m), 1589 (s), 1467 (s), 1197 (m) cm^{-1}.

[(*S*)-2-[2,4-Dimethoxybenzyl-((4*E*,6*E*,8*E*)-oxodeca-4,6,8-trienoyl)amino]-succinic acid 4-*tert*-butyl 1-methyl ester (1.79)

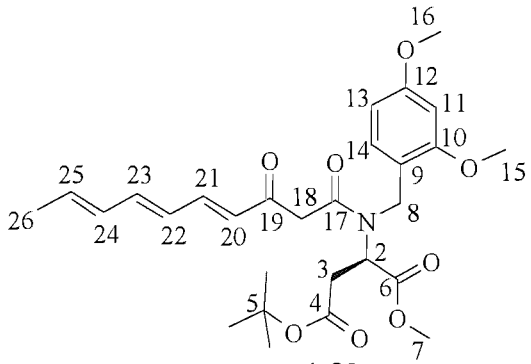


1.79
M.W. 515.61
$C_{28}H_{37}NO_8$

A mixture of (**1.75**) (252 mg, 0.71 mmol), Na_2HPO_4 (257 mg, 1.81 mmol) and powdered 4Å sieves (257 mg) was stirred in tetrahydrofuran (5 mL) at room temperature for 10 min. A solution of (**1.56**) (150 mg, 0.59 mmol) in tetrahydrofuran (5 mL) was added, followed by silver (I) trifluoroacetate (158 mg, 1.81 mmol) and the resultant suspension was stirred in the dark for 35 min. The solid was filtered through celite® and washed with ethyl acetate (2 x 10 mL). The organics were concentrated and residue was purified by column chromatography (SiO_2, 40% EtOAc/Hexane) to give (**1.79**) as a yellow glass (210 mg, 68%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed) δ_H 7.32-7.00 (m, 2H, Ar-H & H20-H25), 6.64-6.41 (m, 3H, Ar-H & H20-H25), 6.30-6.14 (m, 2H, H20-H25), 6.09-5.79 (m, 2H, H20-H25), 4.81-4.41 (m, 3H, H8 & H2), 3.81-3.74 (m, 8H, H15, H16 & H18), 3.65 & 3.59 (s, 3H, H7), 3.22 & 3.16 (dd, 1H, *J* 7.7, 4.0 Hz, H3), 2.48 & 2.38 (dd, 1H, *J* 16.8, 5.5 Hz, H3), 1.85-1.80 & 1.68-1.61 (m, 3H, H26), 1.42 & 1.38 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 202.3 (C19, Cq), 170.4, 170.2 (C4 & C6, 2 x Cq), 167.3 (C17, Cq), 162.5 (C12, Cq), 158.0 (C10, Cq), 138.2, 136.4, 133.2, 131.6 (C20-C25, 4 x CH), 130.5 (C14, CH), 128.8, 125.5 (C20-C25, 2 x CH), 116.0 (C9, Cq), 103.9 (C13, CH_3), 98.7 (C11, CH), 81.0 (C5, Cq), 56.2 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH_3), 52.2 (C7, CH_3), 50.0 (C18, CH_2), 47.1 (C8, CH_2), 36.0 (C3, CH_2), 28.0 (C5, 3 x CH_3), 18.6 (C26, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 538 (100), $[M+H]^+$ 516 (30); **LCMS ESI+** *m/z* $[M+H]^+$ 516.4, $[M+Na]^+$ 538.4 peak at 8.08 min; $[\alpha]_D$ = -18.3 (c = 0.01, EtOAc, 25 °C). **FTIR** ν_{max} (neat, cm^{-1}) 2942 (w), 1718 (s), 1635 (m), 1207 (s), 1145 (s), 1025 (m) cm^{-1}.

[(*R*)-2-[2,4-Dimethoxybenzyl-((4*E*,6*E*,8*E*)-oxodeca-4,6,8-trienoyl)amino]succinic acid 4-*tert*-butyl 1-methyl ester (1.83)



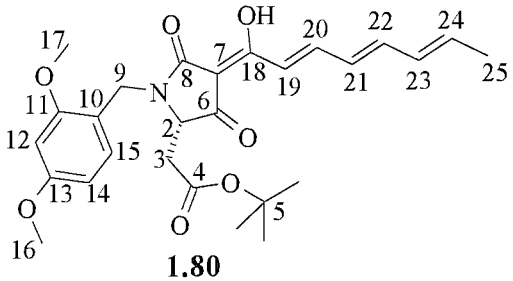
1.83
M.W. 515.61
$C_{28}H_{37}NO_8$

A mixture of (**1.82**) (200 mg, 0.56 mmol), Na_2HPO_4 (200 mg, 1.41 mmol) and powdered 4Å sieves (200 mg) was stirred in tetrahydrofuran (5 mL) at room temperature for 10 min. A solution of (**1.56**) (100 mg, 0.39 mmol) in tetrahydrofuran (10 mL) was added, followed by silver (I) trifluoroacetate (125 mg, 0.56 mmol) and the resultant suspension was stirred in the dark for 35 min. The solid was filtered through celite® and washed with ethyl acetate (2 x 10 mL). The organics were concentrated and the residue was purified by column chromatography (SiO_2, 40% EtOAc/Hexane) to give (**1.83**) as a yellow glass (100 mg, 48%).

Spectroscopic data are identical to (**1.79**).

$[\alpha]_D$ = +27.7 (c = 0.03, EtOAc, 25 °C).

[(*S*)-1-(2,4-Dimethoxybenzyl)-4-((2*E*,4*E*,6*E*)-octa-2,4,6-trienoyl)-3,5-dioxo-pyrrolidin-2-yl]acetic acid *tert*-butyl ester (1.80)

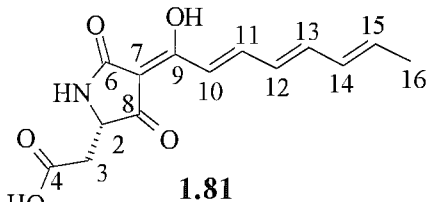


1.80
M.W. 483.57
$C_{27}H_{33}NO_7$

Potassium *tert*-butoxide (16.8 mg, 0.15 mmol), was added to a solution of (**1.79**) (39.0 mg, 0.07 mmol) in *tert*-butanol (5 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 ml) and saturated citric acid solution (1 mL) was added. The organics were separated, and the aqueous was extracted with ethyl acetate (2 x 10 mL).The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated, to give (**1.80**) as a red glass (30 mg, 83%).

^{1}H NMR (400 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 7.48-7.14 (m, 3H, Ar-H & H19-H24), 6.66-6.35 (m, 4H, Ar-H & H19-H24), 6.37-5.99 (m, 2H, H19-H24), 4.99-4.94 (m, 1H, H9), 4.23-4.14 (m, 1H, H9), 3.82-3.79 (m, 7H, H16, H17 & H2), 3.00-2.89 & 2.78-2.74 (m, 2H, H3), 1.88-1.85 (m, 3H, H25), 1.35 (s, 9H, H5); **LRMS ES+** *m/z* $[M+Na]^+$ 506 (100) $[2M+Na]^+$ 989 (30); **LCMS ESI+** *m/z* $[M+H]^+$ 484.3, $[M+Na]^+$ 506.2 peak at 9.56 min; $[\alpha]_D$ = -99 (c = 0.03, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2930 (w), 1726 (m), 1506 (m), 1592 (s) cm^{-1}.

[(*S*)-4-((2*E*,4*E*,6*E*)-Octa-2,4,6-trienoyl)-3,5-dioxopyrrolidin-2-yl]acetic acid, MBP039-06 (1.81)



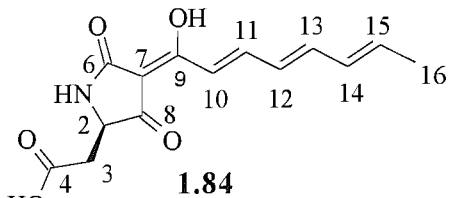
1.81
M.W. 277.28
$C_{14}H_{15}NO_5$

Trifluoroacetic acid (4 ml) was added (**1.80**) (22 mg, 0.45 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 35 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give MBP039-06 (**1.81**) as a dark yellow amorphous solid (11.8 mg, 95%). The compound was purified by preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give a yellow amorphous solid (6.9 mg, 55%).

Mp 212 °C (dec); **^{1}H NMR** (400 MHz, DMSO-d6, tautomers observed, acid not observed) δ_H 12.39 (br s, 1H, OH), 8.81 (br s, 1H, NH), 7.48 (dd, 1H, *J* 14.6, 11.6 Hz, H11), 7.06 (d, 1H, *J* 11.6 Hz, H10), 6.85 (dd, 1H, *J* 14.6, 11.6 Hz, H12), 6.49 (dd, 1H, *J* 14.6, 11.6 Hz, H13), 6.35-6.26 (m, 1H, H14), 6.08 (dq, 1H, *J* 14.6, 6.5 Hz, H15), 4.06 (apparent br s, 1H, H2), 2.62-2.59 (m, 2H, H3), 1.83 (d, 3H, *J* 6.5 Hz, H16); **LRMS ES+** *m/z* $[M+Na]^+$ 300 (100), $[M+H]^+$ 278 (80); **LCMS ESI+** *m/z* $[M+H]^+$ 278.1, $[M+Na]^+$ 301.1 at 5.81 min; **$[\alpha]_D$** = -24.0 (c = 0.001, MeOH, 25 °C); **UV** λ_{max} 395 nM; **FTIR** ν_{max} (neat, cm^{-1}) 3269 (w), 2970 (w), 1589 (s), 1548 (m), 1434 (m), 1205 (m), 1001 (m) cm^{-1}. **CD** see appendix.

Data identical to ^{1}H NMR, UV and CD spectra supplied by Dr Junko Takashima of Mitsubishi Pharma Corporation.

[(*R*)-4-((2*E*,4*E*,6*E*)-Octa-2,4,6-trienoyl)-3,5-dioxopyrrolidin-2-yl]acetic acid, (*R*)-MBP 039-06 (1.84)



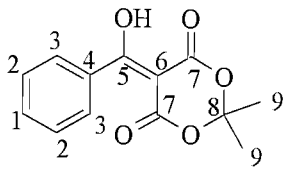
1.84
M.W. 277.28
$C_{14}H_{15}NO_5$

Potassium *tert*-butoxide (19 mg, 0.15 mmol) was added to a solution of (**1.82**) (40 mg, 0.08 mmol) in *tert*-butanol (5 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) was added, the organics were separated and the aqueous layer extracted with (2 x 10 mL) ethyl acetate. The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered and concentrated. Trifluoroacetic acid (4 mL) was added to the residue at 0 °C. The resulting red solution was stirred at room temperature for 35 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give (**1.84**) as a dark yellow amorphous powder (2.2 mg, 15%) after semi-preparative HPLC purification (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min).

Spectroscopic data identical to (**1.81**).

$[\alpha]_D$ = +34 (c = 0.0014, EtOAc, 25 °C).

5-Benzoyl-2,2-dimethyl[1,3]dioxane-4,6-dione (1.85)

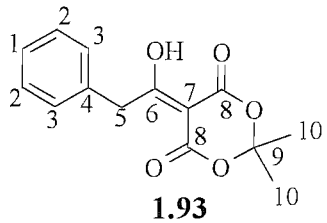


1.85
M.W. 248.24
$C_{13}H_{12}O_5$

A solution of Meldrum's acid (1.00 g, 6.90 mmol) and 4-*N,N*-dimethylaminopyridine (1.5 g, 1.20 mmol) in dichloromethane (10 mL) was cooled to 0 °C. To this solution was added dropwise benzoyl chloride (1.02 g, 7.30 mmol) in dichloromethane (5 mL). The resulting solution was stirred at 0 °C to room temperature for 18 h. The reaction mixture was partitioned between dichloromethane (50 ml) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous layer extracted with further dichloromethane (2 x 20 mL). The organics were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated. The resulting brown residue was triturated with diethyl ether yielding (**1.85**) as a cream solid (1.0 g, 58%).

Mp 98-99 °C (Ether), (lit. 114 °C);[100] **^{1}H NMR** (300 MHz, $CDCl_3$, OH not observed) δ_H 7.68-7.44 (m, 5H, Ar-H), 1.84 (s, 6H, H9); **^{13}C NMR** (100 MHz) δ_C 189.5 (C7, 2 x Cq), 171.2 (C5, Cq), 133.6 (CH), 133.0 (C4, Cq), 129.7, 128.3 (4 x CH), 105.2 (C6, Cq), 90.2 (C8, Cq), 26.9 (C9, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-}OC(CH_3)_2+H]^+$ 191.11, $[M\text{-}O]^+$ 232.08 at 4.20 min; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1736 (s), 1658 (m), 1430 (m), 1200 (m) cm^{-1}. Data consistent with literature.[50]

2,2-Dimethyl-5-phenylacetyl[1,3]dioxane-4,6-dione (1.93)

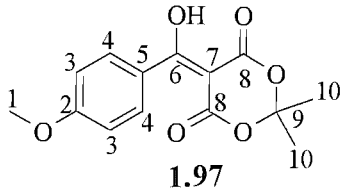


1.93
M.W. 262.62
$C_{14}H_{14}O_5$

A solution of Meldrum's acid (1.00 g, 6.9 mmol) and 4-*N,N*-dimethylaminopyridine (1.5 g, 1.20 mmol) in dichloromethane (10 mL) was cooled to 0 °C. To this solution was added dropwise phenylacetyl chloride (1.13 g, 7.3 mmol) in dichloromethane (5 mL). The resulting solution was stirred at 0 °C to room temperature for 18 h. The reaction mixture was partitioned between dichloromethane (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous layer extracted with further dichloromethane (2 x 20 mL). The organics were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated. The resulting brown residue was triturated with diethyl ether yielding (**1.93**) as a cream solid (1.2 g, 63 %).

Mp 87-88.6 °C (Ether), (lit. 90-92 °C);[101] **^{1}H NMR** (300 MHz, $CDCl_3$, OH not observed) δ_H 7.39-7.24 (m, 5H, Ar-H), 4.42 (s, 2H, CH_2), 1.71 (s, 6H); **^{13}C NMR** (100 MHz) δ_C 194.9 (C8, 2 x Cq), 170.2 (C6, Cq), 134.6 (C4, Cq), 129.8, 128.9, 127.3 (5 x CH), 105.2 (C6, Cq), 91.6 (C8, Cq), 41.0 (C5, CH_2), 27.0 (C10, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-}OC(CH_3)_2+H]^+$ 205.12, $[M\text{-}O]^+$ 246.08 at 7.69 min; **FTIR** ν_{max} (neat, cm^{-1}) 3000 (w), 1742 (s), 1650 (m), 1430 (m), 1203 (m), 1104 (m) cm^{-1}. Data consistent with literature.[50]

5-(4-Methoxy-benzoyl)-2,2-dimethyl[1,3]dioxane-4,6-dione (1.97)

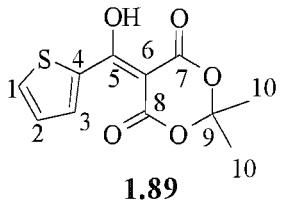


1.97
M.W. 278.26
$C_{14}H_{14}O_6$

A solution of Meldrum's acid (1.00 g, 6.9 mmol) and 4-*N,N*-dimethylaminopyridine (1.5 g, 1.20 mmol) in dichloromethane (10 mL) was cooled to 0 °C. To this solution was added dropwise *p*-methoxybenzoyl chloride (1.24 g, 7.3 mmol), in dichloromethane (5 mL). The resulting solution was stirred at 0 °C to room temperature for 18 h. The reaction mixture was partitioned between dichloromethane (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous layer extracted with further dichloromethane (2 x 20 mL). The organics were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated. The resulting brown residue was triturated with diethyl ether yielding (**1.97**) as a cream solid (1.6 g, 78%).

Mp 101.2-101.8 °C (EtOAc), (lit. 111-112 °C (dec);[51] **^{1}H NMR** (400 MHz, $CDCl_3$, OH not observed) δ_H 7.75 (d, 1H, *J* 8.9 Hz, Ar-H), 6.96 (d, 1H, *J* 8.9 Hz, Ar-H), 3.90 (s, 3H, OCH_3), 1.86 (s, 6H); **^{13}C NMR** (100 MHz) δ_C 188.4 (C8, 2 x Cq), 170.8 (C6, Cq), 164.4 (C2, Cq), 132.6 (C4, 2 x CH), 124.7 (C5, Cq), 113.6 (C3, 2 x CH), 104.9 (C7, Cq), 89.8 (C9, Cq), 55.8 (C1, CH_3), 26.9 (C10, 2 x CH_3); **LCMS ESI+** *m/z* [M-$OC(CH_3)_2$+H]$^+$ 121.10, [M-O]$^+$ 262.09 at 5.94 min; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1740 (s), 1650 (m), 1230 (m), 1100 (m) cm^{-1}. Data consistent with literature.[51]

2,2-Dimethyl-5-(thiophene-2-carbonyl)-[1,3]dioxane-4,6-dione (1.89)



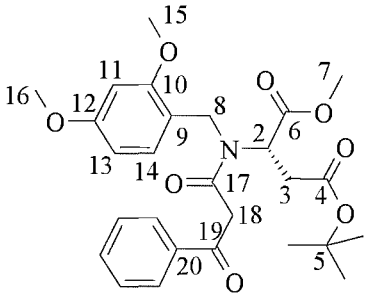
1.89
M.W. 254.26
$C_{11}H_{10}O_5S$

A solution of Meldrum's acid (1.00 g, 6.90 mmol) and 4-*N*,*N*-dimethylaminopyridine (1.5 g, 1.2 mmol), in dichloromethane (10 mL), was cooled to 0 °C. To this solution was added dropwise 2-thiophenecarbonyl chloride (1.1g, 7.30 mmol), in dichloromethane (5 mL). The resulting solution was stirred at 0 °C to room temperature for 18 h. The reaction mixture was partitioned between dichloromethane (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous layer extracted with further dichloromethane (2 x 20 mL). The organics were combined, washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (SiO_2, 20:80:1, EtOAc/Hexane/AcOH) to give (**1.89**) as an orange gum (400 mg, 23%).

^{1}H NMR (300 MHz, $CDCl_3$, OH not observed) δ_H 8.47 (dd, 1H, *J* 4.0, 1.1 Hz, H3), 7.84 (dd, 1H, *J* 5.1, 1.1 Hz, H1), 7.20 (dd, 1H, *J* 5.1, 4.4 Hz, H2), 1.80 (s, 6H, H10); **^{13}C NMR** (75 MHz) δ_C 196.1 (C7 & C8, 2 x Cq), 179.4 (C5, Cq), 104.0 (C6, Cq), 138.6, 137.2 (C2 & C3, 2 x CH), 134.7 (C4, Cq) , 128.2 (C1, CH), 104.7 (C6, Cq), 88.7 (C9, Cq), 27.6 (C10, 2 x CH_3); **FTIR** ν_{max} (neat, cm^{-1}) 3010 (w), 1725 (s), 1643 (m), 1588 (m), 1254 (m) cm^{-1}.

* The compound decomposes when heated > 40 ° C.

(*S*)-2-[(2,4-Dimethoxybenzyl)-3-oxo-3-phenylpropionyl)-amino]succinic acid 4-*tert*-butyl ester 1-methyl ester (1.86)

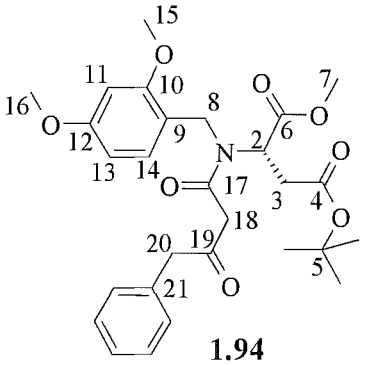


1.86
M.W. 499.57
$C_{27}H_{33}NO_8$

A solution of (**1.85**) (220 mg, 0.886 mmol), and (**1.75**) (313 mg, 0.89 mmol) in 1,4-dioxan (20 mL) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated. The residue was purified by column chromatography (SiO_2, 35% EtOAc/Hexane) to give (**1.86**) as a yellow gum (>80% pure + <20% (**1.75**) 400 mg, 90%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed) δ_H 8.00-7.97 (m, 1H, Ar-H), 7.71-7.69 (m, 1H, Ar-H), 7.57-7.30 (m, 4H, Ar-H), 6.49-6.43 (m, 2H, Ar-H), 4.80-4.42 (m, 3H, H2 & H8), 3.82 (s, 3H, H15), 3.80 (s, 3H, H16), 3.79-3.77 (m, 2H, H18), 3.67 & 3.55 (s, 3H, H7), 3.20 & 2.47 (dd, 1H, *J* 16.8, 7.7 Hz; dd, 1H, *J* 16.8, 5.5 Hz, H3), 1.44 & 1.42 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 202.0 (C19, Cq), 170.4, 170.3 (C6 & C4, 2 x Cq), 167.4 (C17, Cq), 161.1 (C12, Cq), 158.7 (C10, Cq), 133.0 (C20, Cq), 130.5, 128.7, 128.4, 126.0 (6 x CH), 116.0 (C9, Cq), 103.9 (C13, CH), 98.7 (C11, CH), 85.7 (C5, Cq), 56.2 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH3), 52.2 (C7, CH_3), 49.0, 47.3 (C8 & C18, 2 x CH_2), 36.0 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 522 (100), $[M+H]^+$ 500 (10); **LCMS ESI+** *m/z* $[M+Na]^+$ 522.4, $[M+H]^+$ 500.5 peak at 7.55 min; $[\alpha]_D$ = -29.3 (c = 0.09, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2977 (w), 1725 (s), 1613 (m), 1588 (m), 1454 (m), 1208 (m), 1152 (s) cm^{-1}.

(*S*)-2-[(2,4-Dimethoxybenzyl)-3-oxo-4-phenylbutyryl)-amino]succinic acid 4-*tert*-butyl ester 1-methyl ester (1.94)

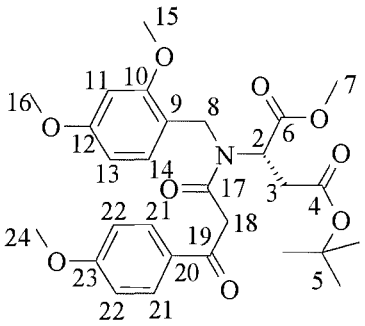


1.94
M.W. 513.59
$C_{28}H_{35}NO_8$

A solution of (**1.93**) (222.3 mg, 0.85 mmol) and (**1.75**) (300 mg, 0.85 mmol) in 1,4-dioxan (20 mL) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated. The residue was purified by column chromatography (SiO_2, 35% EtOAc/Hexane) to give (**1.94**) as a pale yellow gum (>80 % pure + <20% (**1.75**) (271 mg, 62%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed) δ_H 7.34-7.12 (m, 6H, Ar-H), 6.45-6.39 (m, 2H, Ar-H), 4.46-4.38 (m, 3H, H2 & H8), 3.91-3.53 (m, 13H, H15, H16, H7, H20, & H18), 3.20 & 2.40 (dd, 1H, *J* 16.8, 7.7 Hz; dd, 1H, *J* 16.8, 5.5Hz, H3), 1.43 & 1.42 (s, 9H, H5); **^{13}C NMR** (75MHz) δ_C 202.0 (C19, Cq), 170.5, 170.2 (C6 & C4, 2 x Cq), 167.0 (C17, Cq), 161.1 (C12, Cq), 158.8 (C10, Cq), 133.8 (C21, Cq), 130.5, 129.7, 129.2, 128.7, 128.4, 127.1 (6 x CH), 116.7 (C9, Cq), 103.9 (C13, CH), 98.7 (C11, CH), 80.7 (C5, Cq), 56.4 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH_3), 52.1 (C7, CH_3), 49.8, 46.7 (C8 & C18, 2 x CH_2), 39.3 (C20, CH_2), 35.6 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 514.4, $[M+Na]^+$ 536.4 peak at 7.66 min; **LRMS ES+** *m/z* $[M+Na]^+$ 536 (100), $[M+H]^+$ 514 (5); $[\alpha]_D$ = -15.5 (c = 0.05, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2977 (w), 1725 (m), 1613 (m), 1588 (m), 1508 (m), 1208 (m) cm^{-1}.

(*S*)-2-{(2,4-Dimethoxybenzyl)-[3-(4-methoxyphenyl)-3-oxopropionyl]-amino}succinic acid 4-*tert*-butyl ester 1-methyl ester (1.98)

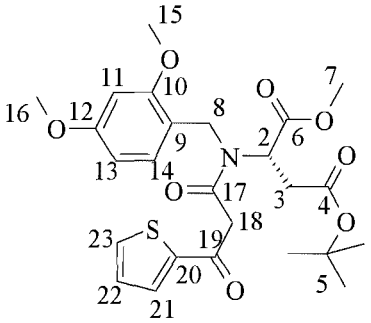


1.98
M.W. 529.59
$C_{28}H_{35}NO_9$

A solution of (**1.97**) (267.7 mg, 0.96 mmol) and (**1.75**) (340 mg, 0.96 mmol) in 1,4-dioxan (20 mL) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated. The residue was purified by column chromatography (SiO_2, 35% EtOAc/Hexane) to give (**1.98**) as a yellow gum (>80% pure + <20% (**1.75**) 300 mg, 59%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed) δ_H 7.98-7.95 (m, 2H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 6.94-6.88 (m, 2H, Ar-H), 6.48-6.46 (m, 2H, Ar-H), 4.62-4.38 (m, 3H, H2 & H8), 4.17-4.16 (m, 1H, H18), 4.10-3.66 (m, 10H, H15, H16, H24 & H18), 3.67 & 3.53 (s, 3H, H7), 3.21 & 2.47 (dd, 1H, *J* 16.8, 7.7 Hz; dd, 1H, *J* 16.8, 5.1 Hz, H3), 1.42 & 1.41 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 192.3 (C19, Cq), 170.6, 170.4 (C6 & C4, 2 x Cq), 167.8 (C17, Cq), 163.8, 161.0 (C23 & C12, 2 x Cq), 158.8 (C10, Cq), 131.1, 130.6, 127.6 (C14 & C21, 3 x CH), 124.2, (C20, Cq), 116.4 (C9, Cq), 113.8 (C22, 2 x CH), 103.9 (C13, CH), 98.7 (C11, CH), 80.7 (C5, Cq), 56.2 (C2, CH), 55.4, 55.3, 55.2 (C24, C16 & C15, 3 x CH_3), 52.1 (C7, CH_3), 49.0, 45.6 (C8 & C18, 2 x CH_2), 36.0 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 530.2 $[M+Na]^+$ 552.2 peak at 8.06 min; **LRMS ES+** *m/z* $[M+Na]^+$ 552 (100); $[\alpha]_D$ = -23.4 (c = 0.03, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2978 (w), 2933 (w), 1732 (m), 1597 (s), 1503 (m), 1258 (s), 1205 (s), 1152 (s), 1025 (m) cm^{-1}.

(*S*)-2-[(2,4-Dimethoxybenzyl)-3-oxo-3-thiophen-2-yl-propionyl)-amino]succinic acid 4-*tert*-butyl ester 1-methyl ester (1.90)

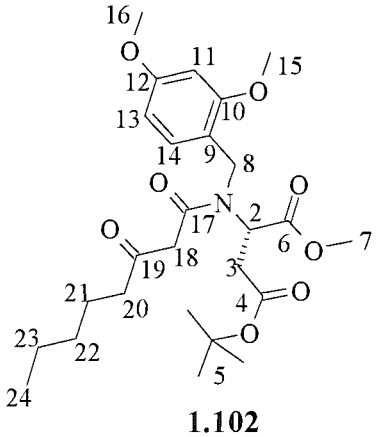


1.90
M.W. 505.59
$C_{25}H_{31}NO_8S$

A solution of (**1.89**) (215mg, 0.848 mmol) and (**1.75**) (300 mg, 0.85 mmol) in 1,4-dioxan (20 mL) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated. The residue was purified by column chromatography (SiO_2, 35% EtOAc/Hexane) to give (**1.90**) as a yellow glass (188 mg, 44%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed) δ_H 7.82 (dd, 1H, *J* 4.0, 1.1 Hz, H21), 7.66 (dd, 1H, *J* 5.1, 1.1 Hz, H23), 7.28-7.25 (m, 1H, Ar-H), 7.12 (dd, 1H, *J* 5.0, 4.0 Hz, H22), 6.48-6.44 (m, 2H, Ar-H), 4.42-4.37 (m, 1H, H2), 4.17-4.08 (m, 2H, H8), 3.82 - 3.76 (m, 8H, H15, H16, H18), 3.56 & 3.55 (s, 3H, H7), 3.19 (dd, 1H, *J* 16.8, 7.7 Hz, H3), 2.40 (dd, 1H, *J* 16.8, 5.5 Hz, H3), 1.44 & 1.42 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 186.4 (C19, Cq), 170.5, 170.2 (C6 & C4, 2 x Cq), 167.0 (C17, Cq), 161.1 (C12, Cq), 158.8 (C10, Cq), 134.5, 133.7 (C21, C22, 2 x CH), 130.7 (C14, CH), 128.2 (C23, CH), 127.1 (C20, Cq), 116.0 (C9, Cq), 103.9 (C13, CH_3), 98.7 (C11, CH_3), 80.7 (C5, Cq), 56.4 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH_3), 52.1 (C7, CH_3), 49.1, 46.5 (C8 & C18, 2 x CH_2), 35.6 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 528 (100), $[M+H]^+$ 506 (10); **LCMS ESI+** *m/z* $[M+Na]^+$ 528.3, $[M+H]^+$ 506.3 peak at 7.35 min; $[\alpha]_D$ = -35 (c = 0.007, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2980 (w), 1732 (s), 1643 (m), 1613 (m), 1589 (m), 1209 (s), 1154 (s), 1042 (s) cm^1.

(*S*)-2-[(2,4-Dimethoxybenzyl)-(3-oxo-octanoyl)-amino]-succinic acid 4-*tert*-butyl ester 1-methyl ester (1.102)

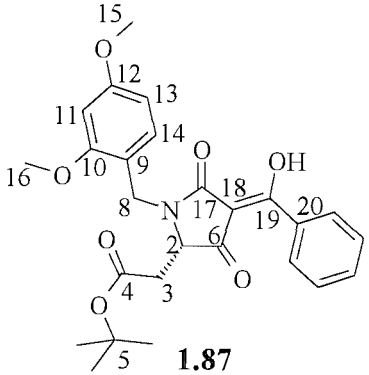


1.102
M.W. 493.60
$C_{26}H_{39}NO_8$

A solution of (**1.101**) (205 mg, 0.85 mmol) and (**1.75**) (300 mg, 0.85 mmol) in 1,4-dioxan (20 mL) was stirred at 70 °C for 3 h. After cooling, the reaction mixture was concentrated. The residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**1.102**) as a yellow gum (368 mg, 88%).

^{1}H NMR (400 MHz, $CDCl_3$, tautomers observed) δ_H 7.21-7.12 (m, 1H, Ar-H), 6.46-6.39 (m, 2H, Ar-H), 4.89-4.82 (m, 1H, H2), 4.49-4.41 (m, 2H, H8), 3.79-3.68 (m, 8H, H15 & H16 & H18), 3.64 & 3.60 (s, 3H, H7), 3.21-3.19 (m, 1H, H20-H23), 2.68-2.65 (m, 1H, H20-H23), 2.59-2.48 (m, 1H, H20-H23), 2.43-2.25 (m, 1H, H20-H23), 1.61-1.56 (m, 2H, H20-H23), 1.43 (s, 9H, H5), 1.31-1.25 (m, 4H, H20-H23), 0.89-0.87 (m, 3H, H24); **^{13}C NMR** (100 MHz) δ_C 204.8 (C19, Cq), 170.6, 170.3 (C6 & C4, 2 x Cq), 167.9 (C17, Cq), 161.0 (C12, Cq), 159.0 (C10, Cq), 130.7 (C14, CH), 116.0 (C9, Cq), 104.3 (C13, CH), 98.8 (C11, CH), 81.2 (C5, Cq), 56.4 (C2, CH), 55.6, 55.5 (C15 & C16), 52.2 (C7, CH_3), 49.8 (C18, CH_2), 47.3 (C8, CH_2), 37.8 (CH_2), 36.1 (C3, CH_2), 31.6 (CH_2), 28.3 (C5, 3 x CH_3), 23.4, 22.6 (2 x CH_2), 14.5 (C24, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 516.4 (100); **LCMS ESI+** *m/z* $[M+H]^+$ 494.5, $[M+Na]^+$ 516.5 peak at 8.20 min; $[\alpha]_D$ = -24.7 (c = 0.01, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2937 (w), 1724 (m), 1612 (m), 1507 (m), 1207 (m), 1150 (s), 1032 (m) cm^{-1}.

[(*S*)-4-Benzoyl-1-(2,4-dimethoxybenzyl)-3,5-dioxopyrrolidin-2-yl]acetic acid *tert*-butyl ester (1.87)

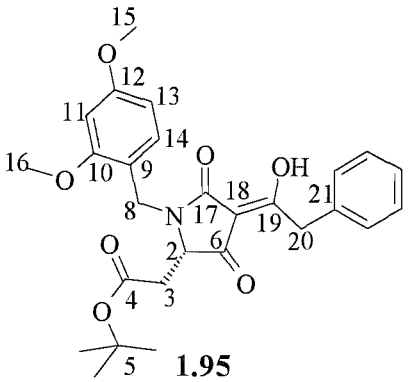


1.87
M.W. 467.52
$C_{26}H_{29}NO_7$

Potassium *tert*-butoxide (71.7 mg, 0.80 mmol) was added to a solution of (**1.86**) (200 mg, 0.40 mmol) in *tert*-butanol (10 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) were added, the organics were separated and the aqueous was extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 60% EtOAc/Hexane) to give (**1.87**) as a pink solid (90 mg, 48%).

M p 148 °C (Dec) (EtOAc/Hexane); **^{1}H NMR** (300 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 7.50-7.15 (m, 6H, Ar-H), 6.32-6.14 (m, 2H, Ar-H), 4.91-4.65 (m, 1H, H8), 4.13-3.95 (m, 1H, H8), 3.79-3.56 (m, 7H, H15, H16 & H2), 2.95-2.70 (m, 1H, H3), 2.16-2.10 (m, 1H, H3), 1.27 (br s, 9H, H5); **LRMS ES+** *m/z* $[M+Na]^+$ 490 (100), $[M+H]^+$ 468 (5), **LCMS ESI+** *m/z* $[M+H]^+$ = 468.2 peak at 8.96 min; $[\alpha]_D$ = -8.8 (c = 0.012, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2930 (w), 1726 (m), 1506 (m), 1592 (s), 1455 (s), 1433 (s), 1207 (m), 1143 (m), cm^{-1}.

[(*S*)-1-(2,4-Dimethoxybenzyl)-3,5-dioxo-4-phenylacetylpyrrolidin-2-yl]acetic acid *tert*-butyl ester (1.95)

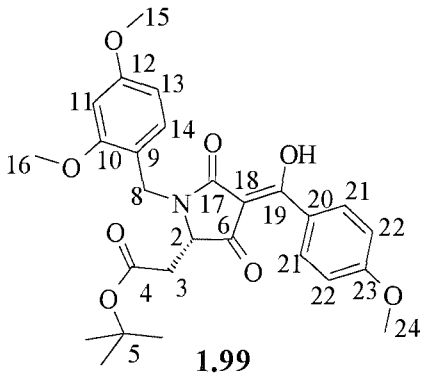


1.95
M.W. 481.55
$C_{27}H_{31}NO_7$

Potassium *tert*-butoxide (65.42mg, 0.58 mmol), was added to a solution of (**1.94**) (150 mg, 0.29 mmol) in *tert*-butanol (10 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) were added, the organics were separated, and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 80% EtOAc/Hexane) to give (**1.95**) as a pale yellow glass (120 mg, 86%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 7.26-7.06 (m, 6H, Ar-H), 6.64-6.41 (m, 2H, Ar-H), 4.95-4.85 (m, 1H, H8), 4.21-3.95 (m, 3H, H2, H20), 3.80-3.61 (m, 7H, H15, H16 & H8), 2.91-2.80 (m, 1H, H3), 2.70-2.62 (m, 1H, H3), 1.27 & 1.24 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 202.1 (C6, Cq), 193.8 (C19, Cq), 171.2 (C17, Cq), 168.6 (C4, Cq), 160.3 (C12, Cq), 158.2 (C10, Cq), 133.7 (C21, Cq), 131.8, 131.0, 129.7, 128.4, 127.9, 126.1 (6 x CH), 116.9 (C9, Cq), 104.5 (C13, CH), 101.1 (C18, *enol* Cq), 98.7 (C11, CH), 81.1 (C5, Cq), 63.4 (C2, CH), 55.4, 55.2 (C15 & C16, 2 x CH_3), 44.1 (C8, CH_2), 38.0 (C20, CH_2), 35.2 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 504 (100); **LCMS ESI+** *m/z* $[M+Na]^+$ 504.3, $[M+H]^+$ 482.3 peak at 8.64 min; $[\alpha]_D$ = -8.3 (c = 0.03, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2970 (w), 2925 (m), 1720 (m), 1589 (s), 1454 (s), 1360 (m), 1144 (s), 1025 (m), cm^{-1}.

[(*S*)-1-(2,4-Dimethoxybenzyl)-4-(4-methoxybenzoyl)-3,5-dioxopyrrolidin-2-yl]acetic acid *tert*-butyl ester (1.99)

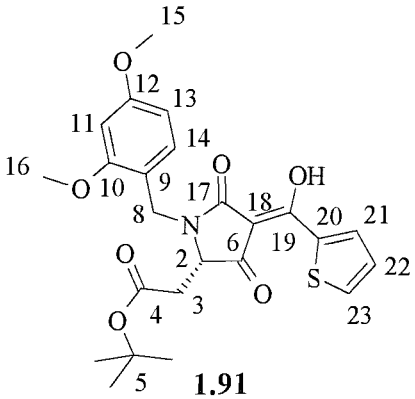


1.99
M.W. 497.55
$C_{27}H_{31}NO_8$

Potassium *tert*-butoxide (93.3 mg, 0.83 mmol), was added to a solution of (**1.98**) (220 mg, 0.415 mmol) in *tert*-butanol (10 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) were added, the organics were separated, and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 75% EtOAc/Hexane) to give (**1.99**) as a pink glass (175 mg 85%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 8.41-8.35 (m, 2H, Ar-H), 7.68-7.60 (m, 1H, Ar-H), 7.01-6.70 (m, 2H, Ar-H), 6.44 (br s 2H, Ar-H), 5.01-4.96 (m, 1H, H8), 4.24 (d, 1H, *J* 15.0 Hz, H8), 3.86-3.74 (m, 10H, H16, H15, H24 & H2), 2.98-2.93 (m, 1H, H3), 2.83-2.71 (m, 1H, H3), 1.33 (s, 9H, H5); **^{13}C NMR** (100 MHz) δ_C 192.2 (C6, Cq), 180.6 (C19, Cq), 171.9 (C17, Cq), 168.4 (C4, Cq), 163.1, 161.0 (C23 & C12, 2 x Cq), 158.7 (C10, Cq), 131.2, 130.5, 129.2 (3 x CH), 125.1 (C20, Cq), 116.3 (C9, Cq), 113.6 (C22, 2 x CH), 104.7 (C13, CH), 98.7 (C11, CH), 81.1 (C5, Cq), 61.2 (C16, CH_3), 56.4 (C2, CH), 55.8, 55.5 (C15 & C24, 2 x CH_3), 48.4 (C8, CH_2), 35.2 (C3, CH_2), 28.0 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 520 (100); **LCMS ESI+** *m/z* $[M+H]^+$ 498.3, $[M+H+CH_3CN]^+$ 561.3 peak at 8.71 min; $[\alpha]_D$ = -23.2 (c = 0.005, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2970 (w), 2938 (w), 1728 (m), 1585 (s), 1511 (s), 1450 (s), 1254 (s), 1140 (m), 1033 (m), cm^{-1}.

[(*S*)-1-(2,4-Dimethoxybenzyl)-3,5-dioxo-4-(thiophene-2-carboyl)-pyrrolidin-2-yl]-acetic acid *tert*-butyl ester (1.91)

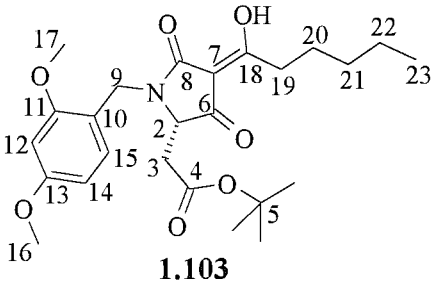


1.91
M.W. 473.55
$C_{24}H_{27}NO_7S$

Potassium *tert*-butoxide (66 mg, 0.59 mmol), was added to a solution of (**1.90**) (140 mg, 0.28 mmol) in *tert*-butanol (10 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) were added, the organics were separated, and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 70% EtOAc/Hexane) to give (**1.91**) as a colourless glass (70 mg, 53%).

^{1}H NMR (300 MHz, $CDCl_3$, tautomers observed, OH not observed) δ_H 9.20-8.88 (m, 1H, Ar-H), 7.74-6.91 (m, 3H, Ar-H), 6.44-6.03 (m, 2H, Ar-H), 4.91-4.65 (m, 1H, H8), 4.13-3.95 (m, 1H, H8), 3.79-3.56 (m, 7H, H15, H16 & H2), 2.95-2.70 (m, 1H, H3), 2.16-2.10 (m, 1H, H3), 1.27 (s, 9H, H5); **^{13}C NMR** (75 MHz) δ_C 205.3, 191.8 (C6 & C19, 2 x Cq), 160.0 (C12, Cq), 157.8 (C10, Cq), 137.6, 137.2 (2 x CH), 134.7 (C20, Cq), 129.3, 128.0 (2 x CH), 104.4 (C13, CH), 98.7 (C11, Cq), 55.2 (C15 & C16, 2 x CH_3), 48.1 (C8, CH_2), 36.1 (C3, CH_2), 27.8 (C5, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 496 (100), $[2M+Na]^+$ 970 (80), $[M+H+CH_3CN]^+$ 537 (40), $[M+H]^+$ 474 (5); **LCMS ESI+** *m/z* $[M+H]^+$ 474.2, $[M+H+CH_3CN]^+$ 537.2 peak at 8.69 min; $[\alpha]_D$ = -25.8 (c = 0.022, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2974 (w), 2929 (w), 1716 (m), 1585 (s), 1442 (s), 1205 (m), 1152 (m), 1029 (m), cm^{-1}.

(*S*)-1-(2,4-Dimethoxybenzyl)-4-hexanoyl-3,5-dioxopyrrolidin-2-yl]acetic acid *tert*-butyl ester (1.103)

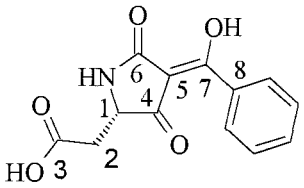


1.103
M.W. 461.56
$C_{25}H_{35}NO_7$

Potassium *tert*-butoxide (68 mg, 0.60 mmol), was added to a solution of (**1.102**) (150 mg, 0.30 mmol) in *tert*-butanol (10 mL) at room temperature. The resultant red suspension was stirred at room temperature for 1 h. Ethyl acetate (20 mL) and saturated citric acid (aq) solution (1 mL) were added, the organics were separated, and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organics were washed with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (SiO_2, 75% EtOAc/Hexane) to give (**1.103**) as a yellow gum (126 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$, tautomers observed) δ_H 7.22-7.21 (m, 1H, H15), 6.47-6.44 (m, 2H, H12), 4.91 (d, 1H, *J* 15.1 Hz, H9), 4.19 (d, 1H, *J* 15.1 Hz, H9), 3.80-3.79 (m, 7H, H16, H17 & H2), 2.86-2.74 (m, 5H, H3, H19-H22), 2.34 (t, 1H, *J* 7.5 Hz, H19), 1.69-1.62 (m, 3H, H20-H22), 1.37-1.35 (m, 10H, H5, H20-H22), 0.90-0.87 (m, 3H, H23); **^{13}C NMR** (100 MHz) δ_C 193.3, 187.0 (C6 & C18, 2 x Cq), 173.5 (C18, Cq), 168.3 (C4, Cq), 160.9 (C13, Cq), 158.3 (C11, Cq), 131.2 (C15, CH), 116.0 (C10, Cq), 104.3 (C14, CH), 101.1 (C7, Cq), 98.8 (C12, CH), 81.2 (C5, Cq), 61.8 (C2, CH), 55.3, 55.2 (C17 & C18, 2 x CH_3), 47.3 (C9, CH_2), 35.4, 33.0, 31.8 (3 x CH_2), 28.3 (C5, 3 x CH_3), 25.9, 22.7 (2 x CH_2), 14.2 (C23, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 484.4 (100), $[2M+Na]^+$ 945.7 (40); **LCMS ESI+** *m/z* $[M+H]^+$ 462.5, $[M+Na+CH_3CN]^+$ 525.5 peak at 9.24 min; $[\alpha]_D$ = -25.0 (c = 0.01, EtOAc, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 2970 (w), 1720 (m), 1565 (s), 1451 (s), 1205 (m), 1110 (m) cm^{-1}.

((*S*)-4-Benzoyl-3,5-dioxo-pyrrolidin-2-yl)acetic acid (1.88)

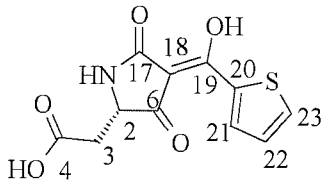


1.88
M.W. 261.24
$C_{13}H_{11}NO_5$

Trifluoroacetic acid (5 mL) was added to (**1.87**) (68 mg, 0.14 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 60 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give as a pink amorphous solid (37 mg, 98%). The compound was purified by Preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give (**1.88**) a pale pink amorphous solid (26 mg, 69%).

Mp 201.9-203.1 °C; **^{1}H NMR** (400 MHz, DMSO-d_6, acid not observed) δ_H 12.61 (br s, 1H, enol OH), 9.26 (br s, 1H, NH), 8.20 (d, 2H, *J* 7.5 Hz, Ar-H), 7.77-7.74 (m, 1H, Ar-H), 7.65-7.62 (m, 2H, Ar-H), 4.26-4.23 (m, 1H, H1), 2.81 & 2.75 (dd, 1H, *J* 17.1, 4.5 Hz; dd, 1H, 17.1, 6.5 Hz, H2); **^{13}C NMR** (100 MHz) δ_C 192.3 (C4, Cq), 180.4 (C7, Cq), 176.4 (C6, Cq), 171.2 (C4, Cq), 133.2 (CH), 132.5 (C8, Cq), 129.0 (2 x CH), 128.1 (2 x CH), 99.8 (C5, Cq), 57.6 (C1, CH), 35.9 (C2, CH_2); **LRMS ES+** *m/z* $[M+Na]^+$ 284 (100), $[M+H]^+$ 262 (5); **LCMS ESI+** *m/z* $[M+H]^+$ 262.1 peak at 3.68 min; $[\alpha]_D$ = -34 (c = 0.001, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 3200 (w), 1655 (m), 1594 (m), 1561 (m), 1449 (m), 1147 (m), cm^{-1}; ***Anal.*** Calcd for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.24; N, 5.36. Found: C, 59.42; H, 4.22; N, 5.16.

[(*S*)-3,5-Dioxo-4-(thiophene-2-carbonyl)-pyrrolidin-2-yl]acetic acid (1.92)

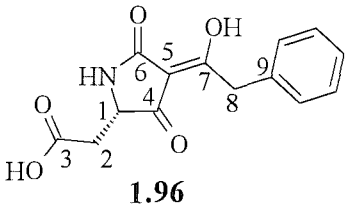


1.92
M.W. 267.26
$C_{11}H_9NO_5S$

Trifluoroacetic acid (5 mL) was added to (**1.91**) (66 mg, 0.14 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 60 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give a pink amorphous solid (35 mg, 94%). The compound was purified by semi-preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give (**1.92**) a pale pink amorphous solid (21 mg, 56%).

Mp 205.6-207.8 °C; **^{1}H NMR** (400 MHz, DMSO-d_6, acid not observed) δ_H 12.61 (br s, 1H, *enol* OH), 9.33 (br s, 1H, NH), 9.23 (br d, 1H, *J* 2.5 Hz, H21), 8.22 (br d, 1H, *J* 5.0 Hz, H23), 7.45-7.43 (m, 1H, H22), 4.28-4.26 (m, 1H, H2), 2.84 & 2.74 (dd, 1H, *J* 17.0, 4.5 Hz; dd, 1H, *J* 17.0, 7.0 Hz, H3); **^{13}C NMR** (100 MHz) δ_C 192.8 (C6, Cq), 179.2 (C19, Cq), 173.4 (C17, Cq), 171.8 (C4, Cq), 136.6, 135.9 (C21 & C22, 2 x CH), 134.7 (C20, Cq), 129.4 (C23, CH), 99.7 (C18, Cq), 58.1 (C2, CH), 36.4 (C3, CH_2); **LRMS ES+** *m/z* $[M+Na]^+$ 290 (100); **LCMS ESI+** *m/z* $[M+H]^+$ 268.0 peak at 4.01 min; $[\alpha]_D$ = -44.8 (c = 0.006, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 3200 (w), 1707 (m), 1688 (m), 1656 (m), 1565 (s), 1439 (m), 1408 (s), cm-1; ***Anal.*** Calcd for $C_{11}H_9NO_5S$: C, 49.44; H, 3.39; N, 5.24; S, 12.00. Found: C, 49.66; H, 3.31; N, 4.91; S, 11.70.

((*S*)-3,5-Dioxo-4-phenylacetyl-pyrrolidin-2-yl]-acetic acid (1.96)

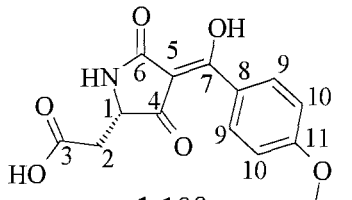


1.96
M.W. 275.26
$C_{14}H_{13}NO_5$

Trifluoroacetic acid (5 mL) was added to (**1.95**) (98.0 mg, 0.20 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 60 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give a pink amorphous solid (56mg, 100%). The compound was purified by semi-preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give (**1.96**) as an off-white amorphous solid (38.4 mg, 69%).

Mp 166.2-168.4 °C; **^{1}H NMR** (400 MHz, DMSO-d_6, acid not observed) δ_H 12.59 (br s, 1H, enol OH), 8.98 (br s, 1H, NH), 7.43-7.34 (m, 5H, Hz, Ar-H), 4.25-4.22 (m, 1H, H1), 4.19 (br d, 2 x 1H, *J* 7.5 Hz, H8), 2.80 & 2.74 (dd, 1H, *J* 17.1, 5.0 Hz ; dd, 1H, *J* 17.1, 6.0 Hz, H2); **^{13}C NMR** (100 MHz) δ_C 194.6 (C4, Cq), 184.7 (C7, Cq), 174.1 (C6, Cq), 171.2 (C3, Cq), 135.2 (C9, Cq), 129.3, 129.2, 128.5, 128.2, 126.9 (5 x CH), 101.1 (C5, Cq), 58.1 (C1, CH), 39.0 (C8, CH_2), 35.6 (C2, CH_2); **LRMS ES+** *m/z* $[M+Na]^+$ 298 (100); **LCMS ESI+** *m/z* $[M+H]^+$ 276.1 peak at 4.17 min; $[\alpha]_D$ = -12.8 (c = 0.001, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 3205 (w), 2921 (m), 1708 (m), 1659 (s), 1611 (s), 1595 (s), 1593 (s), 1565 (s), 1238 (m), 1118 (m), cm^{-1}; ***Anal.*** Calcd for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09. Found: C, 60.63; H, 4.72; N, 4.99.

[(*S*)-4-(4-Methoxybenzoyl)-3,5-dioxo-pyrrolidin-2-yl]-acetic acid (1.100)



1.100

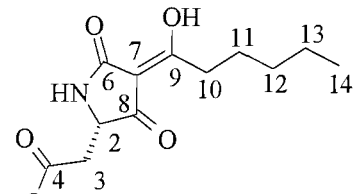
M.W. 291.26

$C_{14}H_{13}NO_6$

Trifluoroacetic acid (5 mL) was added to (**1.99**) (130 mg, 0.26 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 60 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give a pink amorphous solid. The compound was purified by semi-preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give (**1.100**) as an off-white amorphous solid (29 mg, 38%).

Mp 215.0-216.3 °C; **^{1}H NMR** (400 MHz, DMSO-d_6, acid not observed) δ_H 12.61 (br s, 1H, enol OH), 9.21 (br s, 1H, NH), 8.38 (d, 2H, *J* 7.6 Hz, H9), 7.19 (d, 2H, *J* 8.5 Hz, H10), 4.23-4.20 (m, 1H, H1), 3.98 (s, 3H, H12), 2.80 & 2.71 (dd, 1H, *J* 17.1, 4.5 Hz; dd, 1H, *J* 17.1, 6.5 Hz, H2); **^{13}C NMR** (100 MHz) δ_C 192.3 (C4, Cq), 179.3 (C7, Cq), 177.6 (C6, Cq), 171.2 (C3, Cq), 163.1 (C11, Cq), 131.6 (C9, 2 x CH), 124.5 (C8, Cq), 113.6 (C10, 2 x CH), 98.5 (C5, Cq), 57.6 (C1, CH), 55.6 (C12, CH_3), 35.9 (C2, CH_2); **LRMS ES+** *m/z* $[M+Na]^+$ 314; **LCMS ESI+** *m/z* $[M+H]^+$ 292.2 at 4.20 min; $[\alpha]_D$ = -36.4 (c = 0.001, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 3215 (w), 1708 (m), 1581 (s), 1556 (s), 1511 (m), 1266 (s), 1180 (s), 1119 (m), cm^{-1}; ***Anal.*** Calcd for $C_{14}H_{13}NO_6$: C, 57.73; H, 4.50; N, 4.81. Found: C, 57.36; H, 4.35; N, 4.54.

((*S*)-4-Hexanoyl-3,5-dioxopyrrolidin-2-yl)acetic acid (1.104)



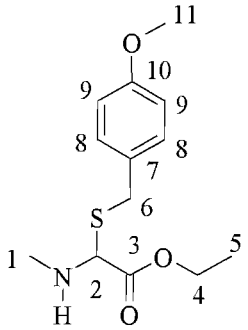
1.104
M.W. 255.27
$C_{12}H_{17}NO_5$

Trifluoroacetic acid (5 mL) was added to (**1.101**) (100 mg, 0.22 mmol) at 0 °C. The resulting red solution was stirred at room temperature for 60 min. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give a white amorphous solid. The compound was purified by semi-preparative HPLC (Linear gradient 10% to 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 45 min, flow rate 3.0 mL/min) to give (**1.104**) as a white amorphous solid (33 mg, 60%).

Mp 143.1-144.3 °C; **^{1}H NMR** (400 MHz, $CDCl_3$, acid not observed) δ_H 12.37 (br s, 1H, enol OH), 8.74 (br s, 1H, NH), 4.00-3.98 (m, 1H, H2), 2.67 (t, 2H, *J* 7.5 Hz, H10), 2.56 & 2.50 (dd, 1H, *J* 17.1, 4.5 Hz; dd, 1H, 17.1, 6.5 Hz, H3), 2.44-2.43 (m, 1H, H11-H13), 1.51-1.48 (m, 2H, H11-H13), 0.81-0.77 (m, 3H, H14); **^{13}C NMR** (100 MHz) δ_C 194.5 (C8, Cq), 187.7 (C9, Cq), 175.1, (C6, Cq), 171.1 (C4, Cq), 101.0 (C7, Cq), 58.0 (C2, CH), 35.7 (C3, CH_2), 32.2, 30.9, 24.9, 21.7 (4 x CH_2), 13.7 (C14, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 256.2, $[2M+H]^+$ 511.4 at 4.87 min; $[\alpha]_D$ = -25.6 (c = 0.001, MeOH, 25 °C); **FTIR** ν_{max} (neat, cm^{-1}) 3217 (br w), 2931 (w), 1708 (s), 1661 (s), 1607 (s), 1235 (m), 876 (m), cm^{-1}; ***Anal.*** Calcd for $C_{12}H_{17}NO_5$: C, 56.46; H, 6.71; N, 5.48. Found: C, 56.65; H, 6.73; N, 5.28.

III.3 Chapter II Experimental

(4-Methoxybenzylsulfanyl)methylaminoacetic acid ethyl ester (2.58)



2.58
M.W. 269.37
$C_{13}H_{19}NO_3S$

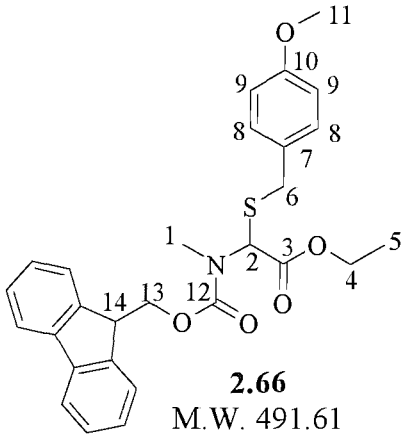
Methylamine hydrochloride (396 mg, 4.89 mmol) and triethylamine (700 μL, 4.89 mmol) was added to a solution of ethyl glyoxalate (1 mL of a 50% solution in toluene, 4.89 mmol) in toluene (5 mL) and stirred at room temperature for 10 min. *p*-Methoxybenzyl mercaptan (750 mg, 680 μL, 4.89 mmol) was added and the resulting suspension was stirred at room temperature for 2 h. Further methylamine hydrochloride (792 mg, 9.78 mmol) and triethylamine (1400 μL 9.78 mmol) was added and the reaction mixture stirred at room temperature for 18 h. The resulting suspension was concentrated, pre-adsorbed onto silica and purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.60**) as a colourless gum (903 mg, 68%).

Alternative method: Methylamine (4.8 ml of a 2.0 M solution in THF, 9.78 mmol) was added to a solution of ethyl glyoxalate (2 ml of a 50 % solution in toluene, 9.78 mmol) in toluene (5 mL) and stirred at room temperature for 10 min. *para*-Methoxybenzyl mercaptan (1.5 g, 1.36 mL, 4.89 mmol) was added and the resulting cloudy solution was stirred at room temperature for 24 h. The crude reaction mixture was concentrated, pre-adsorbed onto silica and purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.58**) as a colourless gum (1.73 g, 66%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.16 (d, 2H, *J* 8.6 Hz, H8), 6.73 (d, 2H, *J* 8.6 Hz, H9), 4.13 (s, 1H, H2), 4.13-4.09 (m, 2H, H4), 3.67 (s, 3H, H11), 3.62 (s, 2H, H6), 2.24 (s, 3H, H1), 1.74 (br s, 1H, NH), 1.19 (t, 3H, *J* 7.1 Hz, H5); **^{13}C NMR** (100 MHz) δ_C 170.0 (C3, Cq), 158.8 (C10, Cq), 130.1 (C8, 2 x CH), 129.8 (C7 , Cq), 114.0 (C9, 2 x CH),

66.2 (C2, CH), 61.3 (C4, CH_2), 53.5 (C11, CH_3), 33.1 (C6, CH_2), 32.1 (C1, CH_3), 14.1 (C5, CH_3); **LRMS ES+** *m/z* $[M+H]^+$ 270 (100), $[M+Na]^+$ 292 (5); **FTIR** ν_{max} (neat, cm^{-1}) 3353 (w), 1715 (s), 1630 (m), 1515 (s), 1289 (m), 1216 (s), 1153 (s), cm^{-1}

[9H-Fluoren-9-ylmethoxycarbonyl)-methylamino]-(4-Methoxybenzylsulfanyl)-acetic acid ethyl ester (2.66)

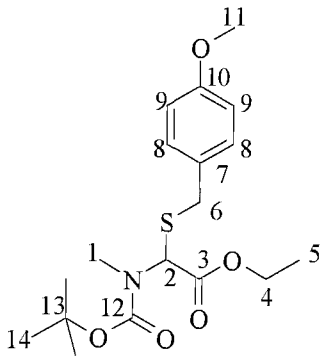


2.66
M.W. 491.61
$C_{28}H_{29}NO_5S$

Fmoc-Cl (180 mg, 0.694 mmol) and sodium carbonate (81 mg, 0.76 mmol) were added to a solution of (**2.58**) (187 mg, 0.69 mmol), in 1,4-dioxan (8 mL) and water (0.5 mL). The resulting suspension was stirred at room temperature for 4 h. Water (50 mL) and EtOAc (30 mL) was added, the organics were separated, dried ($MgSO_4$), filtered, and concentrated to give (**2.66**) as a colourless gum (184 mg, 54%).

^{1}H NMR (300 MHz, $CDCl_3$, rotamers observed) δ_H 7.76 (d, 2H, *J* 7.3 Hz, Ar-H), 7.61-7.50 (m, 2H, Ar-H), 7.41-7.21 (m, 6H, Ar-H), 6.81 (d, 2H, *J* 8.4 Hz, Ar-H), 6.14 & 5.71 (s, 1H, H2), 4.46-4.44 (m, 2H, H13), 4.27-4.11 (m, 3H, H4 & H14), 3.72 (s, 3H, H11), 3.68-3.50 (m, 2H, H6), 2.96 & 2.93 (s, 3H, H1), 1.27 (t, 3H, *J* 7.3 Hz, H5); **^{13}C NMR** (75 MHz) δ_C 168.3 (C3, Cq), 158.8 (C10, Cq), 156.5 (C12, Cq), 143.7, 141.3 (4 x Cq), 130.3 (C7, Cq), 130.0, 127.8, 127.1, 125.0, 120.0, 114.0 (12 x CH), 67.9 (C13, CH_2), 62.6 (C2, CH), 62.1 (C4, CH_2), 55.2 (C11, CH_3), 47.2 (C14, CH), 35.3 (C6, CH_2), 30.2 (C1, CH_3), 14.1 (C5, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 514, $[M+H]^+$ 492; **FTIR** ν_{max} (neat, cm^{-1}) 2953 (w), 1737 (m), 1689 (s), 1510 (m), 1450 (m), 1300 (m), 1244 (m), 1023 (m), cm^{-1}. ***Anal.*** Calcd for $C_{28}H_{29}NO_5S$: C, 68.41; H, 5.95; N, 2.85; S, 6.52. Found: C 68.73; H, 6.04; N, 2.42; S 6.05.

(*tert*-Butoxycarbonylmethylamino)-(4-methoxybenzylsulfanyl)acetic acid ethyl ester (2.67)

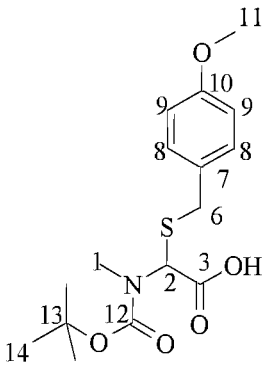


2.67
M.W. 369.48
$C_{18}H_{27}NO_5S$

Di-*tert*-butyl dicarbonate (1.43 g, 6.5 mmol) was added portionwise to a solution of (**2.58**) (1.73 g, 6.4 mmol), and triethylamine (1.01 mL, 7.1 mmol) in dichloromethane (20 mL) and stirred at room temperature for 18 h. The resulting solution was concentrated and the residue purified by column chromatography (SiO_2, 20% EtOAc/Hexane) to give (**2.67**) as a colourless gum (1.9 g, 80%).

^{1}H NMR (300 MHz, $CDCl_3$, rotamers observed) δ_H 7.24 (d, 2H, *J* 8.4 Hz, H8), 6.83 (d, 2H, *J* 8.4 Hz, H9), 6.21 & 5.68 (s, 1H, H2), 4.22-4.08 (m, 2H, H4), 3.79 (s, 3H, H11), 3.75-3.62 (m, 2H, H6), 2.88 (br s, 3H, H1), 1.50-1.42 (m, 9H, H14), 1.29-1.23 (m, 3H, H5); **^{13}C NMR** (75 MHz) δ_C 168.3 (C3, Cq), 158.8 (C10, Cq), 156.2 (C12, Cq), 130.0 (C8, 2 x CH), 129.4 (C7, Cq), 114.0 (C9, 2 x CH), 80.7 (C13, Cq), 62.6 (C2, CH), 62.0 (C4, CH_2), 55.3 (C11, CH_3), 35.4 (C6, CH_2), 30.2 (C1, CH_3), 28.3 (C14, 3 x CH_3), 14.1 (C5, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 392 (100), $[2M+Na]^+$ 761 (90), $[M+H]^+$ 370 (5); **LCMS ESI+** *m/z* $[M+Na+CH_3CN]^+$ 433.2, $[M+Na]^+$ 392.2 peak at 8.04 min; **FTIR** ν_{max} (neat, cm^{-1}) 2973 (w), 1742 (s), 1689 (s), 1509 (s), 1369 (s), 1135 (s), 1246 (s) cm^{-1}; ***Anal.*** Calcd for $C_{18}H_{27}NO_5S$: C, 58.51; H, 7.37; N, 3.79; S, 8.68. Found: C, 58.53; H, 7.45; N, 3.86; S, 9.00.

(*tert*-Butoxycarbonylmethylamino)-(4-methoxybenzylsulfanyl)acetic acid (2.69)

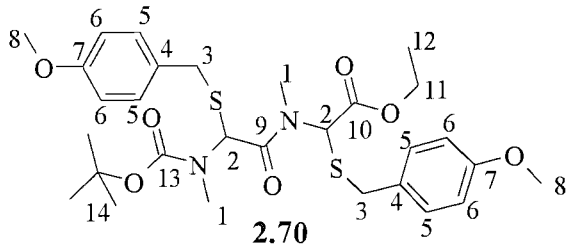


2.69
M.W. 341.43
$C_{16}H_{23}NO_5S$

Lithium hydroxide (41.5 mg, 1.01 mmol) was added to a solution of (**2.67**) (369 mg, 1.0 mmol) in THF (10 mL), water (1 mL) and the reaction mixture was stirred at room temperature for 4 h. EtOAc (15 mL) and saturated citric acid solution (1 mL) was added. The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed well with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated to give (**2.69**) as a colourless gum (334 mg, 98%).

^{1}H NMR (300 MHz, CD_3OD, rotamers observed) δ_H 7.51 (br s, 2H, H8), 6.77-6.75 (m, 2H, H9), 5.83 & 5.48 (s, 1H, H2), 3.68 (s, 3H, H11), 3.60-3.57 (m, 2H, H6), 2.8 (br s, 3H, H1), 1.38-1.28 (m, 9H, H14); **^{13}C NMR** (75 MHz) δ_C 173.5 (C3, Cq), 160.4 (C10, Cq), 156.0 (C12, Cq), 131.3 (C9, 2 x CH), 129.8 (C7, Cq), 115.0 (C8, 2 x CH), 81.1 (C13, Cq), 63.2 (C2, CH), 55.7 (C11, CH_3), 35.8 (C6, CH_2), 30.8 (C1, CH_3), 28.3 (C14, 3 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 364 (100), $[M\text{-Boc}+H]^+$ 242 (5); **LCMS ESI+** *M/Z* $[M+Na+CH_3CN]^+$ 405.1, $[M+Na]^+$ 364.2 peak at 6.31 min; **FTIR** ν_{max} (neat, cm^{-1}) 2973 (w), 1736 (m), 1690 (m), 1509 (s), 1241 (s), 1174 (s), 1136 (s), cm^{-1}; ***Anal.*** Calcd for $C_{16}H_{23}NO_5S$: C, 56.29; H, 6.79; N, 4.10; S, 9.39. Found: C 56.36; H, 6.87; N, 4.18; S 9.45.

{[2-(tert-Butoxycarbonylmethylamino)-2-(4-methoxybenzylsulfanyl)-acetyl]-methylamino}-(4-methoxybenzylsulfanyl)acetic acid ethyl ester (2.70)



2.70

M.W. 592.78

$C_{29}H_{40}N_2O_7S_2$

(**2.67**) (1.5 g, 4.02 mmol) was dissolved in 4 M HCl dioxane solution and stirred at room temperature for 40 minutes. The reaction mixture was concentrated and azeotroped with toluene (3 x 20 mL) to give a white solid hydrochloride salt. To a stirring solution of (**2.69**) (1.25 g, 3.66 mmol) and NMM (406 mg, 4.02 mmol) in THF (10 mL) at -10 °C was added isobutyl chloroformate (549 mg, 4.02 mmol) in THF (5 mL) dropwise over 5 minutes. The resulting suspension was then stirred at -10 °C for 40 minutes. The reaction mixture was filtered and the filtrate added to a solution of the hydrochloride salt and NMM (406 mg, 4.02 mmol) in dichloromethane at 0 °C. The resulting reaction mixture was stirred at room temperature for 4 h, concentrated and partitioned between EtOAc (50 ml) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 25% EtOAc/ Hexane) to give (**2.70**) as a colourless gum (1.25 g, 58%).

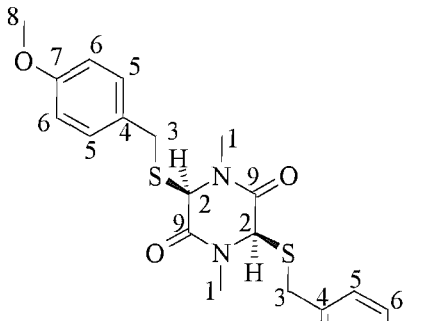
^{1}H NMR (400 MHz, $CDCl_3$, rotamers observed) δ_H 7.30-7.17 (m, 4H, H5), 6.85-6.80 (m, 4H, H6), 6.36-6.29 & 6.13-5.78 (m, 2H, H2), 4.16-4.09 (m, 2H, H11), 3.80 & 3.78 (s, 6H, H8), 3.65-3.61 (m, 4H, H3), 3.14-2.75 (m, 6H, H1), 1.48-1.43 (m, 9H, H13), 1.30-1.20 (m, 3H, H12); **^{13}C NMR** (100 MHz) δ_C 169.4 (C10, Cq), 167.9 (C9, Cq), 159.3 (C7, 2 x Cq), 156.0 (C13, Cq), 130.5, 130.2 (C5, 4 x CH), 129.3 (C4, 2 x Cq), 114.0 (C6, 2 x CH), 81.1 (C14, Cq), 62.8 (C11, CH_2), 61.1, 60.8 (C2, 2 x CH), 55.7 (C8, 2 x CH_3), 35.4, 35.3, (C3, 2 x CH_2), 31.3, 30.9 (C1, 2 x CH_3), 28.7 (C14, 3 x CH_3), 14.6 (C12, CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 615.3 (100); **LCMS ESI+** *m/z* $[M+H]^+$ 593.5, $[M+Na]^+$ 615.4 split peak at 9.05 min; **FTIR** ν_{max} (neat, cm^{-1}) 2960 (w), 1750 (s), 1650 (s), 1510 (s), 1399 (s), 1145 (s), 1146 (s) cm^{-1}; *Anal.* Calcd for $C_{29}H_{40}N_2O_7S_2$: C, 58.76; H, 6.80; N, 4.72; S, 10.82. Found: C, 58.46; H, 6.93; N, 4.63; S, 10.82.

***Cis*-3,6-*Bis*-(4-Methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.71) and *Trans*-3,6-*Bis*-(4-Methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.72)**

(**2.70**) (2.0 g, 3.37 mmol) was dissolved in 4 M HCl dioxane solution and stirred at room temperature for 50 minutes. The reaction mixture was concentrated and azeotroped with toluene (2 x 50 mL). The resulting residue was dissolved in 5% triethylamine isopropanol solution and stirred at 50 °C for 18 h, then concentrated and partitioned between EtOAc (50 mL) and 1 M $KHSO_4$ solution (50 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 40% to 60% EtOAc/Hexane) to give (**2.72**) as a white solid (618 mg, 46%).

Alternative method: To a stirred suspension of sarcosine anhydride (1 g, 7.0 mmol), in carbon tetrachloride (68 mL), was added AIBN (230 mg, 1.41 mmol) and NBS (2.63 g, 14.8 mmol). The suspension was brought to reflux for 3 h, cooled to room temperature, filtered, and concentrated. The resultant crude dibromide was dissolved in THF (100 mL), a solution of *p*-methoxybenzyl mercaptan (2.1 ml, 14.7 mmol) and triethylamine (2.1 ml, 14.7 mmol) in THF (20 mL) was carefully added over 10 min. The resultant red solution was stirred at room temperature for 18 h, then concentrated and partitioned between EtOAc (50 mL) and 0.3 M $KHSO_4$ (aq) solution (20 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 30 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, eluant 30% EtOAc/Hexane) to give (**2.71**) and (**2.72**) as white solids (180 mg and 800 mg, 31%, 5:1 (**2.71**:**2.72**)

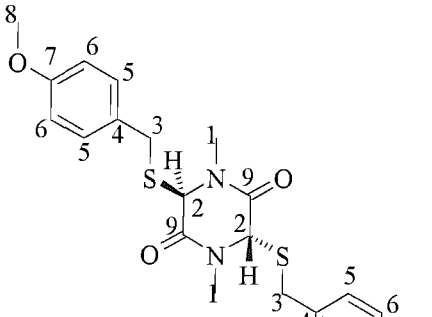
***Cis*-3,6-*Bis*-(4-Methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.71)**



2.71
M.W. 446.59
$C_{22}H_{26}N_2O_4S_2$

Mp 114.1-115.9 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.36 (d, 4H, *J* 8.5 Hz, H5), 6.87 (d, 4H, *J* 8.5 Hz, H6), 4.34 (s, 2H, H2), 3.97 (d, 2H, *J* 13.6 Hz, H3), 3.85 (d, 2H, *J* 13.6 Hz, H3), 3.80 (s, 6H, H8), 2.68 (s, 6H, H1); **^{13}C NMR** (100 MHz) δ_C 165.5 (C9, 2 x Cq), 159.4 (C7, 2 x Cq), 131.0 (C5, 4 x CH), 129.0 (C4, 2 x Cq), 114.3 (C6, 4 x CH), 61.8 (C2, 2 x CH), 55.6 (C8, 2 x CH_3), 37.0 (C3, 2 x CH_2), 31.9 (C1, 2 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 469.2 (100), $[2M+Na]^+$ 915.5 (80); **LCMS ESI+** *m/z* $[M+H]^+$ 447.0, $[M+Na+CH_3CN]^+$ 510.0 peak at 7.81 min; **FTIR** ν_{max} (neat, cm^{-1}) 2933 (w), 1656 (s), 1607 (m), 1508 (s), 1398 (m), 1236 (s), 1170 (m) cm^{-1}; ***Anal.*** Calcd for $C_{22}H_{26}N_2O_5S_2$: C, 59.17; H, 5.87; N, 6.27; S, 14.36. Found: C, 59.29; H, 5.97; N, 6.22; S, 14.05; **X-ray** see appendix.

***Trans*-3,6-*Bis*-(4-Methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.72)**



2.72
M.W. 446.59
$C_{22}H_{26}N_2O_4S_2$

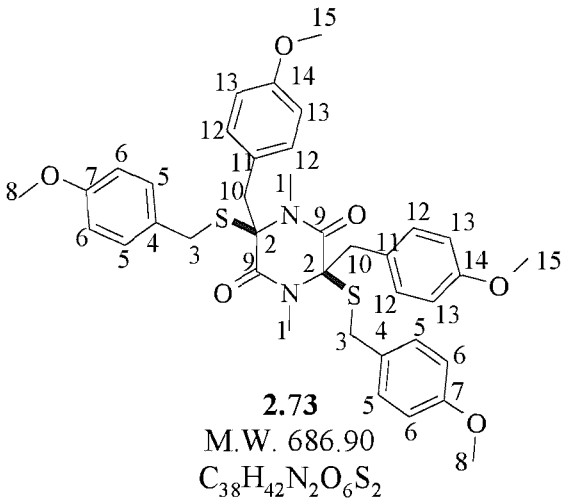
Mp 197.7-198.4 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.18 (d, 4H, *J* 8.9 Hz, H5), 6.76 (d, 4H, *J* 8.9 Hz, H6), 4.36 (s, 2H, H2), 3.75 (d, 2H, *J* 18.6 Hz, H3), 3.71 (s, 6H, H8), 3.63 (d, 2H, *J* 18.6 Hz, H3), 2.67 (s, 6H, H1); **^{13}C NMR** (100 MHz) δ_C 165.5 (C9, 2 x Cq), 159.4 (C7, 2 x Cq), 131.0 (C5, 4 x CH), 129.0 (C4, 2 x Cq), 114.3 (C6, 4 x CH), 61.8 (C2, 2 x CH), 55.6 (C8, 2 x CH_3), 37.0 (C3, 2 x CH_2), 31.9 (C1, 2 x CH_3); **LRMS ES+** *m/z* $[M+Na]^+$ 469.2 (100), $[2M+Na]^+$ 915.5 (80); **LCMS ESI+** *m/z* $[M+H]^+$ 447.0, $[M+Na+CH_3CN]^+$ 510.0 peak at 6.85 min; **FTIR** ν_{max} (neat, cm^{-1}) 2930 (w), 1643 (s), 1611 (m), 1508 (s), 1396 (m), 1239 (s), 1168 (m) cm^{-1}; ***Anal.*** Calcd for $C_{22}H_{26}N_2O_5S_2$: C, 59.17; H, 5.87; N, 6.27; S, 14.36. Found: C, 59.07; H, 5.87; N, 6.53; S, 14.21;

3,6-*Bis*-(4-Methoxybenzyl)-3,6-*bis*-(4-methoxybenzylsulfanyl)-1,4-dimethylpip eazine-2,5-dione (2.73), *cis*-3,6-*bis*-(4-Methoxybenxylsulfanyl)-3-(4-methoxybenzyl -1,4-dimethylpiperazine-2,5-dione (2.74) and *trans*-3,6-*bis*-(4-Methoxy benxylsulfanyl)-3-(4-methoxybenzyl-1,4-dimethylpiperazine-2,5-dione (2.75)

To a solution of (**2.71**) (100 mg, 0.23 mmol) in THF (10 ml) was added a solution of 2.0 M LDA (344 µL, 0.69 mmol) at -78 °C and the reaction mixture stirred at -78 °C for 15 minutes. To the resulting solution was added dropwise a solution of 4-methoxybenzyl bromide (69 mg, 0.34 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature < -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for a further 40 minutes. A solution of acetic acid (22 mg, 0.604 mmol) in THF (1 mL) was then added and the resulting reaction mixture was allowed to warm to room temperature. The reaction solution was quenched at room

temperature with the addition of sat. NaCl (20 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% to 60% EtOAc/Hexane) to give (**2.73**) as a white solid (10 mg, 7%), (**2.74**) as a colourless gum and (**2.75**) as a white solid (52 mg and 15 mg, 64%, 3.5:1 (**2.74**:**2.75**).

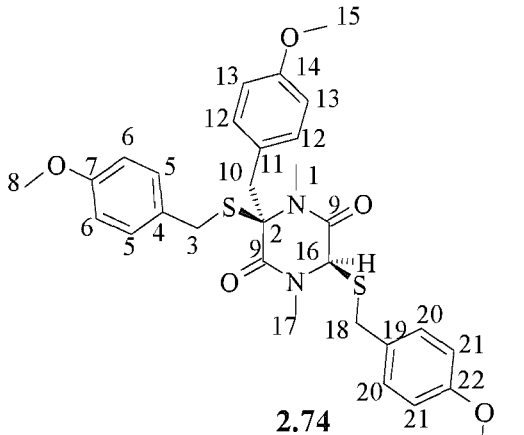
3,6-*Bis*-(4-Methoxybenzyl)-3,6-*bis*-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.73)



2.73
M.W. 686.90
$C_{38}H_{42}N_2O_6S_2$

Mp 127.4-128.6 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.20 (d, 4H, *J* 8.5 Hz, Ar-H), 6.83-6.78 (m, 8H, H13, Ar-H), 6.62 (d, 4H, *J* 9.0 Hz, Ar-H), 3.96 (d, 2H, *J* 12.0 Hz, H3), 3.92 (d, 2H, *J* 12.0 Hz, H3), 3.76 & 3.74 (s, 12H, H8 & H15) 3.47 (d, 2H, *J* 14.5 Hz, H10), 3.17 (s, 6H, H1), 2.96 (d, 2H, *J* 14.5 Hz, H10); **^{13}C NMR** (100 MHz) δ_C 165.7 (C9, 2 x Cq), 159.1, 158.9 (C7 & C14, 4 x Cq), 131.3, 130.7 (C5 & C12, 8 x CH), 128.4, 126.6 (C4 & C11, 4 x Cq), 114.4, 114.2 (C6 & C13, 8 x CH), 73.8 (C2, 2 x Cq), 55.7, 55.4 (C8 & C15, 4 x CH_3), 43.0 (C10, 2 x CH_2) 35.8 (C3, 2 x CH_2), 31.9 (C1, 2 x CH_3); **LRMS ES+** *m/z* $[M\text{-SPmb}]^+$ 533.2 (100), $[M+Na]^+$ 709.2 (25); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 533.1, $[M+Na]^+$ 709.4, $[M+Na+CH_3CN]^+$ 750.2 peak at 8.94 min; **FTIR** ν_{max} (neat, cm^{-1}) 2952 (w), 2931 (w), 1650 (s), 1607 (m), 1508 (s), 1307 (s), 1240 (s), 1173 (m) cm^{-1}; **X-ray** see appendix.

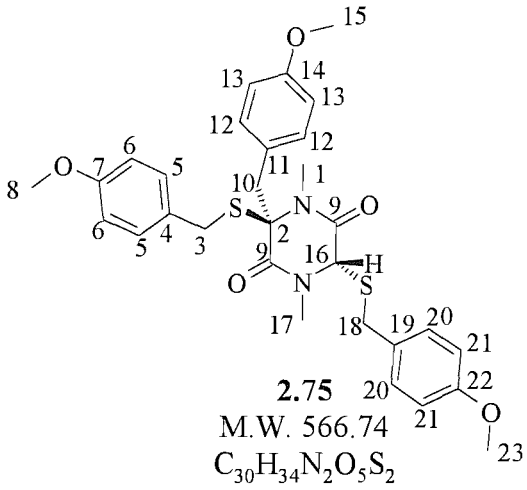
Cis-3-(4-Methoxybenzyl)-3,6-*bis*-(4-methoxybenxylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.74)



2.74
M.W. 566.74
$C_{30}H_{34}N_2O_5S_2$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.30 (d, 2H, *J* 8.5 Hz, Ar-H), 7.24 (d, 2H, *J* 9.0 Hz, Ar-H), 6.95 (2H, d, *J* 8.5 Hz, Ar-H), 6.86-6.82 (m, 4H, Ar-H), 6.74 (d, 2H, *J* 9.0 Hz, Ar-H), 4.11 (s, 1H, H16), 4.07 (d, 1H, *J* 13.5 Hz, H10 or H3 or H18) 3.86-3.82 (m, 2H, H10 or H3 or H18), 3.79 (s, 3H, H8 or H15 or H23), 3.78 (s, 3H, H8 or H15 or H23) 3.75 (s, 3H, H8 or H15 or H23), 3.61 (d, 1H, *J* 14.0 Hz, H10 or H3 or H18), 3.29 (d, 1H, *J* 16.5 Hz, H10 or H3 or H18), 3.23 (s, 3H, H1 or H17), 3.05 (d, 1H, *J* 14.5 Hz, H10 or H3 or H18), 2.63 (s, 3H, H1 or H17); **^{13}C NMR** (100 MHz) δ_C 166.1, 164.8 (C9, 2 x Cq), 159.4, 159.3, 159.2, (C7, C14 & C22, 3 x Cq), 131.3, 130.9, 130.8 (C5, C20 & C12, 6 x CH), 128.9, 128.3, 126.6 (C4, C11 & C19, 3 x Cq), 114.5, 114.4, 114.3 (C6, C13 & C21, 6 x CH), 75.2 (C2, Cq), 61.6 (C16, CH), 55.7, 55.5, 55.1 (C8, C15 & C23, 3 x CH_3), 42.3 (C10, CH_2), 37.6, 35.8 (C18 & C3, 2 x CH_2), 33.4, 30.6 (C1 & C17, 2 x CH_3); **LRMS ES+** *m/z* $[M\text{-SPmb}]^+$ 413.2 (100), $[M+Na]^+$ 589.2 (95), $[M+H]^+$ 567.2 (20); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 413.0, $[M+Na]^+$ 589.1, $[M+H]^+$ 567.1 peak at 8.02 min; **FTIR** ν_{max} (neat, cm^{-1}) 2932 (w), 1656 (s), 1508 (s), 1437 (m), 1237 (s), 1173 (m) cm^{-1}; ***Anal.*** Calcd for $C_{30}H_{34}N_2O_5S_2$: C, 63.58; H, 6.05; N, 4.94; S, 11.32. Found: C, 63.27; H, 6.05; N, 4.80; S, 10.59.

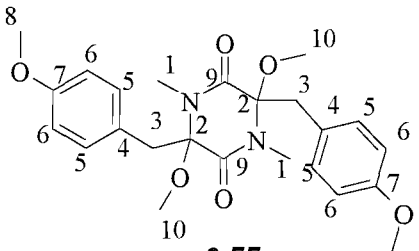
***Trans*-3-(4-Methoxybenzyl)-3,6-*bis*-(4-methoxybenxylsulfanyl)-1,4-dimethylpiperazine -2,5-dione (2.75)**



2.75
M.W. 566.74
$C_{30}H_{34}N_2O_5S_2$

Mp 136.6-138.2 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.17-7.11 (m, 4H, Ar-H), 6.98 (d, 2H, *J* 9.0 Hz, Ar-H), 6.83 (2H, d, *J* 8.5 Hz, Ar-H), 6.76-6.71 (m, 4H, Ar-H), 3.99 (s, 1H, H16), 3.79 (s, 3H, H8 or H15 or H23), 3.72 (s, 3H, H8 or H15 or H23) 3.63 (s, 3H, H8 or H15 or H23), 3.61 (d, 1H, *J* 3.5 Hz, H10 or H3 or H18), 3.57 (d, 1H, *J* 3.5 Hz, H10 or H3 or H18), 3.53 (d, 1H, *J* 4.0 Hz, H10 or H3 or H18), 3.50 (d, 1H, *J* 4.0 Hz, H10 or H3 or H18), 3.31 (s, 3H, H1 or H17), 3.30 (d, 1H, *J* 14.0 Hz, H10 or H3 or H18), 3.04 (d, 1H, *J* 14.0 Hz, H10 or H3 or H18), 2.30 (s, 3H, H1 or H17); **^{13}C NMR** (100 MHz) δ_C 166.1, 164.8 (C9, 2 x Cq), 159.6, 159.3, 159.2, (C7, C14 & C22, 3 x Cq), 131.7, 130.9, 130.2 (C5, C12 & C20, 6 x CH), 128.9, 128.0, 126.3 (C4, C11 & C19, 3 x Cq), 114.7, 114.3, 114.2 (C21, C6 & C13, 6 x CH), 76.5 (C2, Cq), 62.1 (C16, CH), 55.7, 55.5, 54.6 (C8, C15 & C23, 3 x CH_3), 41.6 (C10, CH_2) 36.3, 35.1 (C18 & C3, 2 x CH_2), 33.1, 30.9 (C1 & C17, 2 x CH_3); **LRMS ES+** *m/z* [M- SPmb]+ 413.2 (100), $[M+Na]^+$ 589.2 (80), $[M+H]^+$ 567.2 (50); **LCMS ESI+** *m/z* $[M+H]^+$ 567.1, $[M+Na]^+$ 589.1 peak at 7.58 min; **FTIR** ν_{max} (neat, cm^{-1}) 2914 (w), 1647 (s), 1608 (m), 1509 (s), 1245 (s), 1184 (m) cm^{-1}; ***Anal.*** Calcd for $C_{30}H_{34}N_2O_5S_2$: C, 63.58; H, 6.05; N, 4.94; S, 11.31. Found: C 63.64; H, 6.06; N, 4.63; S 11.39; **X-ray** see appendix.

3,6-Dimethoxy-3,6-*bis*-(4-methoxybenzyl)-1,4-dimethyl-piperazine-2,5-dione (2.77)

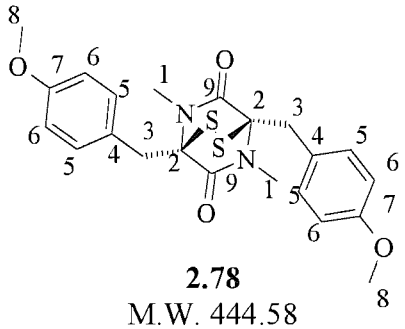


2.77
M.W. 442.52
$C_{24}H_{30}N_2O_6$

A solution of iodine (3.23 mg, 0.03 mmol) in MeOH (10 mL) was slowly added dropwise to (**2.73**) (12 mg, 0.02 mmol) in DMF (1 mL) over a period of 1 hr. The resulting solution was concentrated and partitioned between EtOAc (50 ml) and 1 M $Na_2S_2O_3$ (aq) solution (10 mL). The organics were separated and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 80% EtOAc/Hexane) to give (**2.77**) as a colourless gum (6 mg, 80%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.98 (d, 4H, *J* 8.5 Hz, H5), 6.81 (d, 4H, *J* 8.5 Hz, H6), 3.73 (s, 6H, H8), 3.08 (s, 6H, H10), 2.82 (s, 6H, H1), 2.70 (d, 2H, *J* 14.0 Hz, H3), 2.47 (d, 2H, *J* 14.0 Hz, H3); **^{13}C NMR** (100 MHz) δ_C 165.2 (C9, 2 x Cq), 159.4 (C7, 2 x Cq), 132.3 (C5, 4 x CH), 126.5 (C4, 2 x Cq), 114.1 (C6, 4 x CH), 91.4 (C2, 2 x Cq), 55.6 (C8, 2 x CH_3), 51.6 (C10, 2 x CH_3), 43.5 (C3, 2 x CH_2), 28.7 (C1, 2 x CH_3); **LRMS ES+** *m/z* $[2M+Na]^+$ 907.5 (100), $[M+Na]^+$ 465.2 (70), $[M+Na+CH_3CN]^+$ 506.3 (20), $[M-OCH_3]^+$ 411.2 (10); **LCMS ESI+** *m/z* $[M-OCH_3]^+$ 411.2, $[M+Na+CH_3CN]^+$ 506.3, $[M+H]^+$ 443.3 peak at 5.52 min; **FTIR** ν_{max} (neat, cm^{-1}) 2952 (w), 1662 (s), 1599 (m), 1500 (s), 1307 (s), 1230 (s), 1173 (m) cm^{-1}.

1,4-*Bis*-(4-Methoxybenzyl)-5,7-dimethyl-2,3-dithia-5,7-diazabicyclo[2.2.2]octane-6,8-dione (2.78)

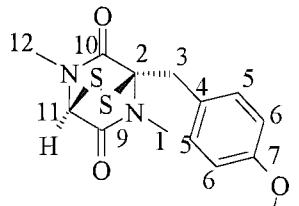


2.78
M.W. 444.58
$C_{22}H_{24}N_2O_4S_2$

A solution of (**2.73**) (50 mg, 0.07 mmol) in dichloromethane (7.5 mL) was cooled to 0 °C. To the stirred solution was added a solution of 1.0 M boron trichloride (0.17 mL, 0.17 mmol) in dichloromethane after stirring at 0 °C for 10 minutes, the resulting solution was concentrated to give a red residue. The residue was dissolved in a solution of iodine (18.5 mg, 0.15 mmol) in 10% MeOH/DCM (5 mL) and stirred at rt. After 10 minutes the solution was poured into a mixture of sat $NaHCO_3$ (2 mL) and 1.0 M $Na_2S_2O_3$ (aq) solution (2 mL), the organics were separated and the aqueous extracted with further dichloromethane (4 x 10 mL). The organics were combined, washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified twice by column chromatography (SiO_2, 25% EtOAc/Hexane) and (2% MeOH/DCM) to give (**2.78**) as a colourless gum (12.1 mg, 38%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.27 (d, 4H, *J* 9.0 Hz, H5), 6.85 (d, 4H, *J* 9.0 Hz, H6), 4.01 (d, 2H, *J* 15.5 Hz, H3), 3.79 (s, 6H, H8), 3.60 (d, 2H, *J* 15.5 Hz, H3) 3.01 (s, 6H, H1); **^{13}C NMR** (100 MHz) δ_C 165.5 (C9, 2 x Cq), 159.2 (C7, 2 x Cq), 130.9 (C5, 4 x CH), 126.7 (C4, 2 x Cq), 114.4 (C6, 4 x CH), 77.1 (C2, 2 x Cq), 55.6 (C8, 2 x CH_3), 36.8 (C3, 2 x CH_2), 29.5 (C1, 2 x CH_3); **LRMS ES+** *m/z* [2M+Na]+ 911.1 (100), [M+Na+ CH_3CN]$^+$ 508.1 (50), [M+Na]$^+$ 467.1 (50); **LCMS ESI+** *m/z* [M+H]$^+$ 445.1, [M+Na]$^+$ 508.1 peak at 8.41 min; **FTIR** ν_{max} (neat, cm^{-1}) 2923 (w), 1674 (s), 1607 (m), 1509 (s), 1240 (s), 1177 (m) cm^{-1}.

1-(4-Methoxybenzyl)-5,7-dimethyl-2,3-dithia-5,7-diazabicyclo[2.2.2]octane-6,8-dione (2.79)

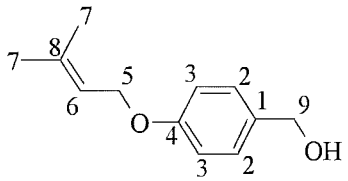


2.79
M.W. 324.42
$C_{14}H_{16}N_2O_3S_2$

A solution of (**2.74**) (160 mg, 0.28 mmol) in dichloromethane (20 mL) was cooled to 0 °C. To the stirred solution was added a solution of 1.0 M boron trichloride (0.68 mL, 0.68 mmol) in dichloromethane after stirring at 0 °C for 10 min, the resulting solution was concentrated to give a red residue. The residue was dissolved in a solution of iodine (71 mg, 0.56 mmol) in 10% MeOH/DCM (10 mL) and stirred at room temperature. After 10 minutes the solution was concentrated. The resulting residue was dissolved in EtOAc (20 mL) and partitioned between EtOAc and a mixture of sat.$NaHCO_3$ (aq) solution (10 mL) and 1M $Na_2S_2O_3$ (aq) solution (5 mL), the organics were separated and the aqueous extracted with further EtOAc (4 x 10 mL). The organics were combined washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 2% MeOH/DCM) to give (**2.79**) as a colourless gum (42.1 mg, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.25 (d, 2H, *J* 9.0 Hz, H5), 6.83 (d, 2H, *J* 9.0 Hz, H6), 5.33 (s, 1H, H11), 3.92 (d, 1H, *J* 15.5 Hz, H3), 3.78 (s, 3H, H8), 3.58 (d, 1H, *J* 15.5 Hz, H3) 3.16 (s, 3H, H1 or H12), 2.97 (s, 3H, H1 or H12); **^{13}C NMR** (100 MHz) δ_C 165.7 , 165.1 (C9 & C10, 2 x Cq), 159.1 (C7, Cq), 130.8 (C5, 2 x CH), 126.1 (C4, Cq), 114.4 (C6, 2 x CH), 76.9 (C2, Cq), 67.3 (C11, CH), 55.4 (C8, CH_3), 36.3 (C3, CH_2), 32.7, 28.5 (C1 & C12, 2 x CH_3); **LRMS ES+** *m/z* $[2M+Na-2S]^+$ 607.2, (100), $[M+Na-S+CH_3CN]^+$ 356.1, (50), $[M+Na-S]^+$ 315.1 (25), $[M+H-S]^+$ 293.2; **LCMS ESI+** *m/z* $[M+H-2S]^+$ 261.1 $[M+H]^+$ 325.0 peak at 6.22 min; **FTIR** ν_{max} (neat, cm^{-1}) 1675 (s), 1511 (m), 1374 (w), 1240 (m) cm^{-1}

[4-(3-Methylbut-2-enyloxy)-phenyl]methanol (2.82)

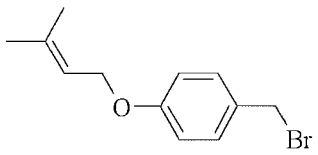


2.82
M.W. 192.26
$C_{12}H_{16}O_2$

Sodium hydride 60% (0.4 g, 0.01 mol) was added portion wise to a stirring solution of 4-hydroxybenzyl alcohol (1.5 g, 0.01 mol) in DMF (15 mL) at 0 °C over 5 min. After 30 minutes 3,3-dimethylallyl bromide was added and the resultant brown suspension stirred at rt for 2 h, EtOAc (40 mL) and water (20 mL) were added. The organics were separated and the aqueous further extracted with EtOAc (40 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 35% EtOAc/ Hexane) to give (**2.82**) as a white solid (1.4 g, 61%).

Mp 40.9-41.5 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.26 (d, 2H, *J* 8.5 Hz, H2), 6.89 (d, 2H, *J* 8.5 Hz, H3), 5.50-5.54 (m, 1H, H6), 4.60 (d, 2H, *J* 5.6 Hz, H9), 4.50 (d, 2H, *J* 6.5 Hz, H5), 1.79 (s, 3H, H7), 1.74 (s, 3H, H7), 1.60 (t, 1H, *J* 5.6 Hz, OH); **^{13}C NMR** (100 MHz) δ_C 157.6 (C4, Cq), 137.2 (C8, Cq), 132.2 (C1, Cq), 127.7 (C2, 2 x CH), 118.8 (C6, CH), 113.8 (C3, 2 x CH), 64.2 , 64.0 (C9 & C5, 2 x CH_2), 24.9, 17.3 (C7, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-}OH]^+$ 175.5 peak at 6.01 min; **FTIR** ν_{max} (neat, cm^{-1}) 3230 (br w), 1260 (s), 1219 (m) cm^{-1}; ***Anal.*** Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.56; H, 8.38.

1-Bromomethyl-4-(3-methylbut-2-enyloxy)benzene (2.83)



2.83
M.W. 255.16
$C_{12}H_{15}BrO$

(**2.82**) (200 mg, 1.04 mmol), Triphenylphosphine (346.5 mg, 1.32 mmol), carbon tetrabromide (414.56 mg, 1.25 mmol) and dichloromethane (10 mL) were combined and stirred at rt in the dark for 5 h. The resulting solution was concentrated and the residue was treated with 3% EtOAc/Hexane. The resulting solid was filtered and the filtrate passed through a short silica plug. Fractions 2-4 were concentrated to give (**2.83**) as an unstable colourless oil (260 mg, 98%).

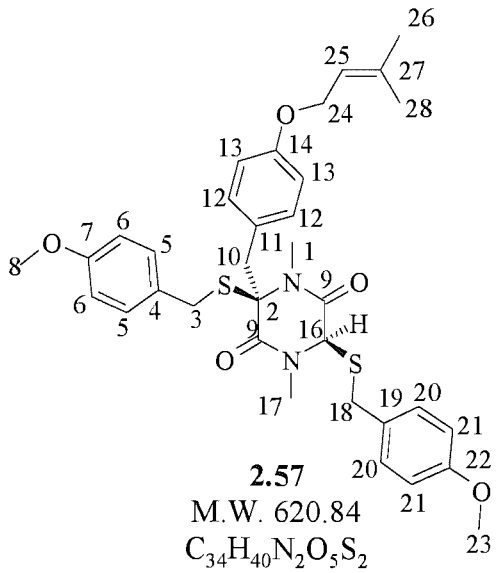
^{1}H NMR (270 MHz, $CDCl_3$) δ_H 7.30 (d, 2H, *J* 8.6 Hz, Ar-H), 6.86 (d, 2H, *J* 8.6 Hz, Ar-H), 5.50-5.54 (m, 1H, CH), 4.49 (s, 4H, 2 x CH_2), 1.79 (s, 3H, CH_3), 1.74 (s, 3H, CH_3), **LCMS ESI+** *m/z* $[M+H]^+$ 256.1 peak at 6.37 min.

***Cis*-3,6-Bis-(4-Methoxybenzylsulfanyl)-1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]piperazine-2,5-dione (2.57) and *trans*-3,6-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]piperazine-2,5-dione (2.84)**

To a solution of **(2.71)** (150 mg, 0.33 mmol) in THF (10 ml) was added a solution of 2.0 M LDA (500 μL, 1.0 mmol) at -78 °C. After stirring at -78 °C for 25 minutes, to the resulting solution was added dropwise a solution of **(2.83)** (168.4 mg, 0.66 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature below -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C to for 5 h. A solution of acetic acid (34 mg, 1.0 mmol) in THF (1 mL) was then added and the resulting reaction mixture was warmed to room temperature. The reaction solution was quenched with the addition of sat. NaCl (20 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% to 60% EtOAc/Hexane) to give **(2.57)** approx. 70% pure as a colourless gum and **(2.84)** as a white solid (46mg and 30 mg, 22% (2:1 **(2.57**:**2.84)**.

Alternative method: Sodium hydride (60% oil disp, 8.4 mg, 0.25 mmol) was added to a stirring solution of **(2.99)** (5:1 *cis*:*trans*) (130 mg, 0.24 mmol) in DMF (3 mL) at -10 °C. The resultant suspension was stirred at -10 °C for 30 minutes. 3,3-Dimethylallyl bromide (30.5 μL, 0.26 mmol) was added at -10 °C. The resultant suspension was allowed to warm and was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (10 mL) and 1 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 10 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/ Hexane) to give to give **(2.57)** as a colourless gum and **(2.84)** as a white solid (100 mg and 30 mg, 89%).

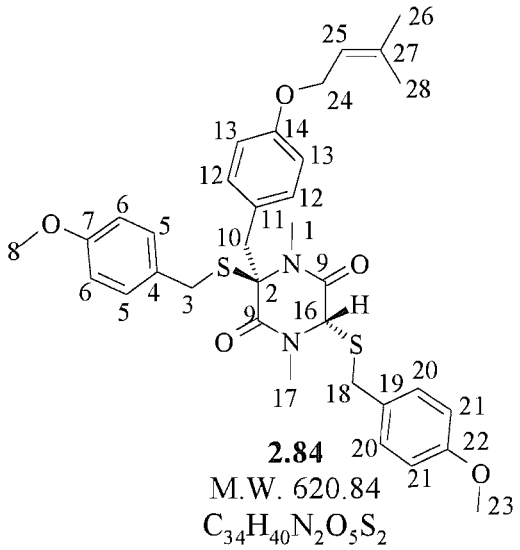
***Cis*-3,6-Bis-(4-Methoxybenzylsulfanyl)-1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]piperazine-2,5-dione (2.57)**



2.57
M.W. 620.84
$C_{34}H_{40}N_2O_5S_2$

^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.24 -7.16 (m, 4H, Ar-H), 6.88-6.85 (m, 2H, Ar-H), 6.77-6.73 (m, 4H, Ar-H), 6.75 (d, 2H, *J* 8.6 Hz, Ar-H), 5.40-5.36 (m, 1H, H25), 4.37 (d, 2H, *J* 6.5 Hz, H24), 4.05 (s, 1H, H16), 4.00 (d, 1H, *J* 13.6 Hz, H10 or H3 or H18), 3.79-3.71 (m, 9H, H8 & H23, H10 or H3 or H18), 3.53 (d, 1H, *J* 14.2 Hz, H10 or H3 or H18), 3.17 (s, 3H, H1 or H17), 2.98 (d, 1H, *J* 14.2 Hz, H10 or H3 or H18), 2.56 (s, 3H, H1 or H17), 1.71 & 1.65 (s, 6H, H26 or H28); **^{13}C NMR** (100 MHz) δ_C 166.8, 164.4 (C9, 2 x Cq), 159.0, 158.9, 158.1 (C7, C14 & C22, 3 x Cq), 138.4 (C27, Cq), 131.6, 130.5, 130.4 (C5, C12 & C20, 6 x CH), 128.5, 127.8, 126.0 (C4, C11 & C19, 3 x Cq), 119.5 (C25, CH), 114.6, 114.5, 114.0 (C6, C13 & C21, 6 x CH), 75.9 (C2, Cq), 64.7 (C24, CH_2), 61.1 (C16, CH), 55.3 (C8 & C23, 2 x CH_3), 41.9 (C10, CH_2) 37.2, 34.7 (C18 & C3, 2 x CH_2), 33.1, 30.3 (C1 & C17, 2 x CH_3), 25.9, 18.2 (C26 & C28, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 467.3, $[M+H]^+$ 621.4, $[M+Na+CH_3CN]^+$ 684.4 peak at 9.13 min; **FTIR** ν_{max} (neat, cm^{-1}) 2920 (w), 1655 (s), 1619 (m), 1508 (s), 1237 (s), 1176 (m) cm^{-1}; ***Anal.*** Calcd for $C_{34}H_{40}N_2O_5S_2$: C, 65.78; H, 6.49; N, 4.51; S, 10.33. Found: C 65.41; H, 6.83; N, 4.82; S 9.91.

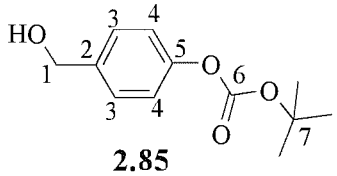
***Trans*-3,6-Bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3-[4-(3-methylbut-2-enyl oxy)-benzyl]piperazine-2,5-dione (2.84)**



2.84
M.W. 620.84
$C_{34}H_{40}N_2O_5S_2$

Mp 115.9-117.5 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.16 (d, 2H, *J* 8.5 Hz, Ar-H), 7.13 (d, 2H, *J* 8.5 Hz, Ar-H), 6.97 (d, 2H, *J* 8.5 Hz, Ar-H) 6.82 (d, 2H, *J* 8.5 Hz, Ar-H), 6.76 (d, 2H, *J* 8.5 Hz, Ar-H), 6.73 (d, 2H, *J* 8.5 Hz, Ar-H), 5.39-5.36 (m, 1H, H25), 4.31 (d, 2H, *J* 6.5 Hz, H24), 3.99 (s, 1H, H16), 3.79 (s, 3H, H8 or H23), 3.75 (d, 1H, *J* 7.0 Hz, H10 or H3 or H18), 3.72 (s, 3H, H8 or H23), 3.63-3.49 (m, 4H, H10 or H3 or H18), 3.30 (s, 3H, H1 or H17), 3.06 (d, 1H, *J* 14.0 Hz, H10 or H3 or H18), 2.29 (s, 3H, H1 or H17), 1.76 (s, 3H, H26 or H28), 1.67 (s, 3H, H26 or H28); **^{13}C NMR** (100 MHz) δ_C 166.1, 164.9 (C9, 2 x Cq), 159.4, 159.3 158.9 (C7, C14 & C22, 3 x Cq), 138.1 (C27, Cq), 131.6, 130.8, 130.5 (C5, C12 & C20, 6 x CH), 128.9, 127.9, 126.2 (C4, C11 & C19, 3 x Cq), 120.2 (C25, CH), 114.4, 114.3, 114.2 (C6, C13 & C21, 6 x CH), 65.1 (C24, CH_2), 62.2 (C16, CH), 55.7, 55.6 (C8 & C23, 2 x CH_3), 41.7 (C10, CH_2), 36.2, 35.5 (C18 & C3, 2 x CH_2), 33.1, 30.8 (C1 & C17, 2 x CH_3), 26.1, 18.5 (C26 & C28, 2 x CH_3); **LCMS ESI** *m/z* $[M\text{-SPmb}]^+$ 467.3, $[M+H]^+$ 621.5, $[M+Na+CH_3CN]^+$ 684.6, $[M+Na]^+$ 643.5 peak at 8.78 min; **FTIR** ν_{max} (neat, cm^{-1}) 2910 (w), 1650 (s), 1609 (m), 1508 (s), 1237 (s) cm^{-1}; ***Anal.*** Calcd for $C_{34}H_{40}N_2O_5S_2$: C, 65.78; H, 6.49; N, 4.51; S, 10.33. Found: C, 64.91; H, 6.48; N, 4.74; S, 9.85.

Carbonic acid *tert*-butyl ester 4-hydroxymethylphenyl ester (2.85)

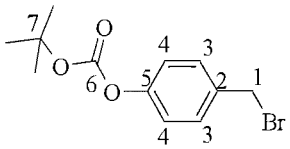


2.85
M.W. 224.26
$C_{12}H_{16}O_4$

4-Hydroxybenzyl alcohol (2.00 g, 0.02 mol), DMAP (97.6 mg, 0.001 mol), di-*tert*-butyl dicarbonate (3.68 g, 0.02 mol) and acetonitrile (20 mL) were combined and stirred at rt for 1 hr. The resulting solution was concentrated and partitioned between EtOAc (50 mL) and 0.3 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 40% EtOAc/Hexane) to give **(2.85)** as a white solid (2.9 g, 81%).

Mp 40.0-41.1 °C; **^{1}H NMR** (270 MHz, $CDCl_3$) δ_H 7.33 (d, 2H, *J* 8.6 Hz, Ar-H), 7.12 (d, 2H, *J* 8.6 Hz, Ar-H), 4.64 (d, 2H, *J* 5.9 Hz, H1), 1.90 (t, 1H, *J* 5.9 Hz, OH), 1.57 (s, 9H, 3 x CH_3); **^{13}C NMR** (100 MHz) δ_C 152.1 (C6, Cq), 150.4 (C5, Cq), 138.7 (C2, Cq), 128.0 (C3, 2 x CH), 121.3 (C4, 2 x CH), 83.7 (C7, Cq), 64.3 (C1, CH_2), 28.0 (C7, 3 x CH_3); **LCMS ESI** *m/z* $[M\text{-Boc}]^+$ 107.0, $[M\text{-Boc+ }CH_3CN]^+$ 148.0, $[2M+Na]^+$ 471.0 peak at 6.46 min; **FTIR** ν_{max} (neat, cm^{-1}) 3246 (br w), 2984 (w), 1747 (s), 1277 (s), 1224 (m), 1144 (s) cm^{-1} ; ***Anal.*** Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.69; H, 7.20.

Carbonic acid 4-bromomethylphenyl ester *tert*-butyl ester (2.87)

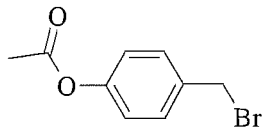


2.87
M.W. 287.16
$C_{12}H_{15}BrO_3$

To a stirred solution of **(2.85)** (150 mg, 0.66 mmol), and methanesulfonyl chloride (66 µL, 0.86 mmol) in anhydrous THF (3.5 mL) was added triethylamine (183 µL, 1.32 mmol) at -40 °C. After 45 minutes a solution of lithium bromide (229 mg, 2.64 mmol) in THF (3.5 mL) was added and the resultant reaction mixture stirred at 0 °C for 1.5 h, EtOAc (40 mL) and H_2O (20 mL) was added. The organics were separated and the aqueous further extracted with EtOAc (40 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by a short silica column (SiO_2, 5% EtOAc/Hexane) to give (**2.87**) as a white solid (92 mg, 49%).

Mp 47.5-47.9 °C; **^{1}H NMR** (270 MHz, $CDCl_3$ δ_H 7.33 (d, 2H, *J* 8.6 Hz, Ar-H), 7.12 (d, 2H, *J* 8.6 Hz, Ar-H), 4.46 (s, 2H, CH_2), 1.57 (s, 9H, 3 x CH_3); **^{13}C NMR** (100 MHz) δ_C 151.9 (C6, Cq), 151.1 (C5, Cq), 135.2 (C2, Cq), 130.3 (C3, 2 x CH), 121.8 (C4, 2 x CH), 84.0 (C7, Cq), 32.9 (C1, CH_2), 27.9 (C7, 3 x CH_3); **FTIR** ν_{max} (neat, cm^{-1}) 2964 (w), 1749 (s), 1270 (s), 1220 (m) cm^{-1}; ***Anal.*** Calcd for $C_{12}H_{15}BrO_3$: C, 50.19; H, 5.26. Found: C, 50.18; H, 5.26.

Acetic acid 4-bromomethylphenyl ester (2.88)



2.88
M.W. 229.07
$C_9H_9BrO_2$

To a stirred solution of (**2.86**) (548 mg, 3.3 mmol), and methanesulfonyl chloride (331 μL, 4.28mmol) in anhydrous THF (8 mL) was added triethylamine (919 μL, 6.6 mmol) at -40 °C. After 45 minutes a solution of lithium bromide (1.15 g, 13.2 mmol) in THF (10 mL) was added and the resultant reaction mixture stirred at 0 °C for 1.5 h, EtOAc (40 mL) and H_2O (20 mL) was added. The organics were separated and the aqueous further extracted with EtOAc (40 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by a short silica column (SiO_2, 5% EtOAc/Hexane) to give (**2.88**) as a white solid (497 mg, 65%)

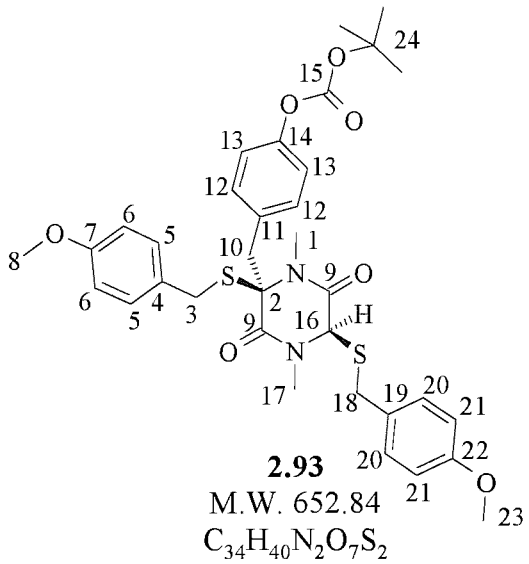
Mp 53.9-54.6 °C (lit. 54-55 °C)[102]; **^{1}H NMR** (270 MHz, $CDCl_3$) δ_H 7.39 (d, 2H, *J* 8.4 Hz, Ar-H), 7.05 (d, 2H, *J* 8.4 Hz, Ar-H), 4.47 (s, 2H, CH_2), 2.29 (s, 3H, CH_3); **FTIR** ν_{max} (neat, cm^{-1}) 3066 (w), 1785 (m), 1428 (m), 1274 (m) cm^{-1}. Data consistent with literature.[102]

***Cis*-Carbonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester *tert*-butyl ester (2.93) and *trans*-carbonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester *tert*-butyl ester (2.94)**

To a solution of (**2.71**) (100 mg, 0.22 mmol) in THF (10 mL) was added a solution of 2.0 M LDA (330 μL, 0.66 mmol) at -78 °C. After stirring at -78 °C for 25 minutes, to the resulting solution was added dropwise a solution of (**2.87**) (83.5 mg, 0.29 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature below -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for 2 h, a solution of acetic acid (19.8 mg, 0.32 mmol) in THF (1 mL) was then added and the resulting reaction mixture allowed to warm to room temperature. The reaction solution was quenched at room temperature with the addition of sat. NaCl (20 mL) and EtOAc

(30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% to 60% EtOAc/Hexane) to give (**2.93**) as a colourless gum and (**2.94**) as a white solid (36 mg and 24 mg, 41% (1.5:1 (**2.93**:**2.94**).

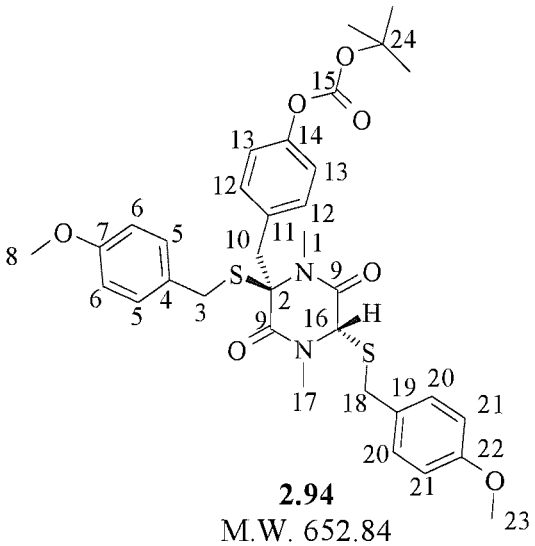
***Cis*-Carbonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester *tert*-butyl ester (2.93)**



2.93
M.W. 652.84
$C_{34}H_{40}N_2O_7S_2$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.28-7.20 (m, 4H, Ar-H), 7.13-6.96 (m, 4H, Ar-H), 6.90-6.77 (m, 4H, Ar-H), 4.17 (s, 1H, H16), 4.07 (d, 1H, *J* 13.5 Hz, H10 or H3 or H18), 3.96 (br d, 1H, *J* 2.9 Hz, H10, H3 or H18), 3.86-3.82 (m, 2H, H10, H3 or H18), 3.79 (s, 3H, H8 or H23), 3.78 (s, 3H, H8 or H23), 3.67 (d, 1H, *J* 14.0 Hz, H10, H3 or H18), 3.23 (s, 3H, H1 or H17), 3.11 (d, 1H, *J* 14.0 Hz, H10, H3 or H18), 2.62 (s, 3H, H1 or H17), 1.54 (s, 9H, H24); **^{13}C NMR** (100 MHz) δ_C 166.2, 164.8 (C9, 2 x Cq), 159.3, 159.2 (C7 & C22, 2 x Cq), 151.6, (C15, Cq), 150.2, (C14, Cq), 131.7, 130.9, 130.2 (C5, C12 & C20, 6 x CH), 129.3, 127.8 (C4, C19 & C11, 3 x Cq), 122.2 (C13, 2 x CH), 114.3, 114.2 (C6 & C21, 4 x CH), 83.8 (C24, Cq), 75.0 (C2, Cq), 61.3 (C16, CH), 55.6, 54.0 (C8 & C23, 2 x CH_3), 41.8 (C10, CH_2), 37.0, 35.1 (C18 & C3, 2 x CH_2), 33.1, 30.8 (C1 & C17, 2 x CH_3), 28.1 (C24, 3 x CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 675.5, $[M+H]^+$ 653.5 peak at 8.82 min; **FTIR** ν_{max} (neat, cm^{-1}) 2915 (w), 1752 (m), 1650 (s), 1609 (s), 1510 (s), 1241 (s) cm^{-1}.

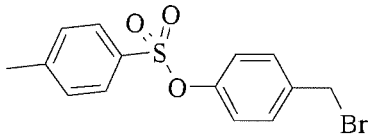
***Trans*-Carbonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester *tert*-butyl ester (2.94)**



2.94
M.W. 652.84
$C_{34}H_{40}N_2O_7S_2$

Mp 131.4-132.6 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.21 (d, 2H, *J* 9.0 Hz, Ar-H), 7.11-7.06 (m, 6H, Ar-H), 6.84 (d, 2H, *J* 8.5 Hz, Ar-H), 6.71 (d, 2H, *J* 8.5 Hz, Ar-H), 3.88 (s, 1H, H16), 3.79 (s, 3H, H8 or H23), 3.75 (d, 1H, *J* 14.0 Hz, H10, H3 or H18), 3.72 (s, 3H, H8 or H23), 3.55-3.43 (m, 4H, H10, H3 or H18), 3.29 (s, 3H, H1 or H17), 3.04 (d, 1H, *J* 14.0 Hz, H10, H3 or H18), 2.20 (s, 3H, H1 or H17), 1.53 (s, 9H, H24); **^{13}C NMR** (100 MHz) δ_C 166.2, 164.8 (C9, 2 x Cq), 159.3 (C7 & C22, 2 x Cq), 151.9 (C15, Cq), 151.2 (C14, Cq), 131.7, 130.9, 130.2 (C5, C12 & C20, 6 x CH), 129.3, 127.8 (C4, C19 & C11, 3 x Cq), 122.2 (C13, 2 x CH), 114.3, 114.2 (C6 & C21, 4 x CH), 83.8 (C24, Cq), 76.5 (C2, Cq), 61.3 (C16, CH), 55.6, 54.0 (C8 & C23, 2 x CH_3), 41.8 (C10, CH_2), 37.0, 35.1 (C18 & C3, 2 x CH_2), 33.1, 30.8 (C1 & C17, 2 x CH_3), 28.1 (C24, 3 x CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 675.50 $[M+H]^+$ 653.5 peak at 8.25 min; **FTIR** ν_{max} (neat, cm^{-1}) 2933 (w), 1752 (m), 1650 (s), 1509 (s), 1243 (m) cm^{-1}; ***Anal.*** Calcd for $C_{34}H_{40}N_2O_7S_2$: C, 62.56; H, 6.18; N, 4.29; S, 9.82. Found: C, 62.26; H, 6.17; N, 4.51; S, 9.58.

Toluene-4-sulfonic acid 4-bromomethylphenyl ester (2.92)

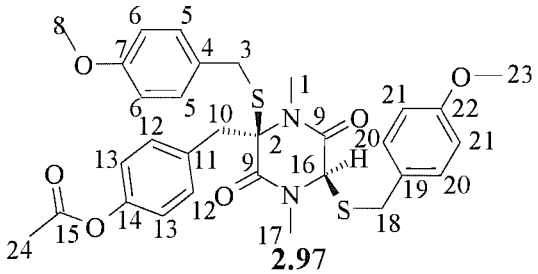


2.92
M.W. 341.23
$C_{14}H_{13}BrO_3S$

To a stirred solution of (**2.91**) (450 mg, 1.6 mmol), and methane sulfonyl chloride (174 µL, 2.2mmol) in anhydrous THF (8 mL) was added triethylamine (464 µL, 3.2 mmol) at -40 °C. After 45 minutes a solution of lithium bromide (559 mg, 13.2 mmol) in THF (10 mL) was added and the resultant reaction mixture stirred at 0 °C for 1.5 hr. EtOAc (40 mL) and H_2O (20 mL) was added. The organics were separated and the aqueous further extracted with EtOAc (40 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by a short silica column (SiO_2, 5% EtOAc/Hexane) to give (**2.92**) as a white solid (340 mg, 62%).

Mp 87.5-88.1 °C; **^{1}H NMR** (270 MHz, $CDCl_3$) δ_H 7.70 (d, 2H, *J* 8.3 Hz, Ar-H), 7.32 (d, 2H, *J* 8.3 Hz, Ar-H), 7.31 (d, 2H, *J* 8.6 Hz, Ar-H), 6.95 (d, 2H, *J* 8.6 Hz, Ar-H), 4.44 (s, 2H, CH_2), 2.45 (s, 3H, CH_3); **FTIR** ν_{max} (neat, cm^{-1}) 2964 (w), 1749 (s), 1270 (s), 1220 (m) cm^{-1}.

***Cis*-Acetic acid 4-[2,5-*bis*-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester (2.97)**



2.97

M.W. 594.75

$C_{31}H_{34}N_2O_6S_2$

To a solution of (**2.71**) (300 mg, 0.7 mmol) in THF (10 mL) was added a solution of 2.0 M LDA (1.0 ml, 2.0 mmol) at -78 °C. After stirring at -78 °C for 25 minutes, to the resulting solution was added dropwise a solution of (**2.88**) (214.8 mg, 0.9 mmol) in THF (2 mL) keeping the internal reaction temperature below -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for 2 h. A solution of acetic acid (59.4 mg, 1.0 mmol) in THF (1 mL) was then added and the resulting reaction mixture allowed to warm to room temperature. The reaction solution was quenched at room temperature with the addition of sat. NaCl (20 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% to 70% EtOAc/Hexane) to give (**2.97**) as a colourless gum (118 mg, 46%)

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.30 (d, 2 H, *J* 8.5 Hz, Ar-H), 7.25 (d, 2 H, *J* 8.5 Hz, Ar-H), 7.05 (d, 2 H, *J* 8.5 Hz, Ar-H), 6.94 (d, 2H, *J* 8.8 Hz, Ar-H), 6.87-6.82 (m, 4 H, Ar-H), 4.15 (s, 1H, H16), 4.06 (d, 1H, *J* 13.6 Hz, H10, H3 or H18), 3.87-3.85 (m, 3 H, H10, H3 or H18), 3.79 (s, 3 H, H8 or H23), 3.78 (s, 3 H, H8 or H23), 3.67 (d, 1H, *J* 14.3 Hz, H10, H3 or H18), 3.23 (s, 3 H, H1 or H17), 3.12 (d, 1H, *J* 14.3 Hz, H10, H3 or H18), 2.62 (s, 3 H, H1 or H17), 2.26 (s, 3 H, H24); **^{13}C NMR** (100 MHz) δ_C 169.6 (C15, Cq), 166.1, 164.5 (C9, 2 x Cq), 159.4, 159.3, (C7 & C22, 2 x Cq), 150.4 (C14, Cq), 132.2 (Cq, C11), 130.9, 130.8, 130.6 (C5, C20 & C12, 6 x CH), 128.9, 128.2 (2 x Cq, C4 & C19), 122.0 (C13, 2 x CH), 114.5, 114.4 (C6 & C21, 4 x CH), 74.8 (C2, Cq), 61.7 (C16, CH), 55.7, 55.6 (C8 & C23, 2 x CH_3), 42.4 (C10, CH_2), 37.7, 35.2 (C18 & C3, 2 x CH_2), 33.3, 30.7 (C1 & C17, CH_3), 21.5 (C24, CH_3); **LCMS ESI+** *m/z* $[M\text{-}SPmb]^+$ 441.2, $[M+Na]^+$ 617.3, $[M+H]^+$ 595.4 at 8.97 min; **FTIR** ν_{max} (neat, cm^{-1}) 2933

(w), 1658 (s), 1608 (s), 1510 (s), 1235 (s) cm^{-1}; ***Anal.*** Calcd for $C_{31}H_{34}N_2O_6S_2$: C, 62.61; H, 5.76; N, 4.71; S, 10.78. Found: C, 61.74; H, 5.85; N, 4.75; S, 10.14.

Cis-(4-Hydroxybenzyl)-3,6-bis-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazine -2,5-dione (2.99) and _Trans_-(4-hydroxybenzyl)-3,6-bis-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.100)

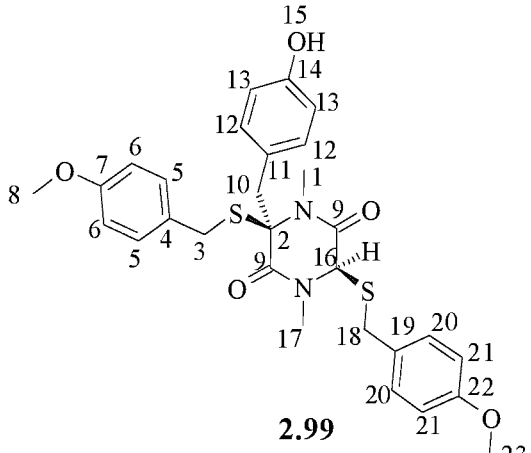
A cold solution of 4 M HCl in dioxin (3 mls) was added to (**2.93**) or (**2.94**) (30 mg, 0.05 mmol) at 0 °C. The solution was stirred at 0 °C while cooled by an ice bath for 18 h, then concentrated and partitioned between EtOAc (20 mL) and saturated $NaHCO_3$ solution (10 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 10 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% EtOAc/ Hexane) to give (**2.99**) or (**2.100**) as a colourless gum (21 mg, 89%).

Alternative method: A solution of (**2.93**) (100 mg, 0.16 mmol) in dichloromethane (5 mL) was cooled to - 78 °C. To the stirred solution was added a solution of 1.0 M boron tribromide (0.23 ml, 0.23 mmol) in dichloromethane. After stirring at -78 °C for 10 min, further dichloromethane (10 mL) and saturated NH_4Cl (aq) solution (5 mL) was added to the reaction mixture at -78 °C. After warming to rt the resultant solution was stirred at room temperature for 20 min. The organics were separated and the aqueous extracted with further dichloromethane (2 x 10 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% EtOAc/ Hexane) to give (**2.99**) as colourless gum (65mg, 77%).

Alternative method: Starting from *cis*-acetic acid 4-[2,5-*bis*-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]-phenyl ester (**2.97**)**:** A solution of (**2.97**) (100 mg, 0.17 mmol) in MeOH (10 mL) was cooled to 0 °C. To the stirred solution was added a solution of 1.0 M KOH (0.20 mL, 0.20 mmol) and the reaction mixture stirred at 0 °C to room temperature for 20 min, then concentrated, and partitioned between EtOAc (20 mL) and 1.0 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 10 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered,

and concentrated. The resulting residue was purified by column chromatography (SiO_2, eluant 50% EtOAc/Hexane to give (**2.99**) and (**2.100**) as a colourless gum (50 mg and 10 mg, 65% (5:1 (**2.99**:**2.100**).

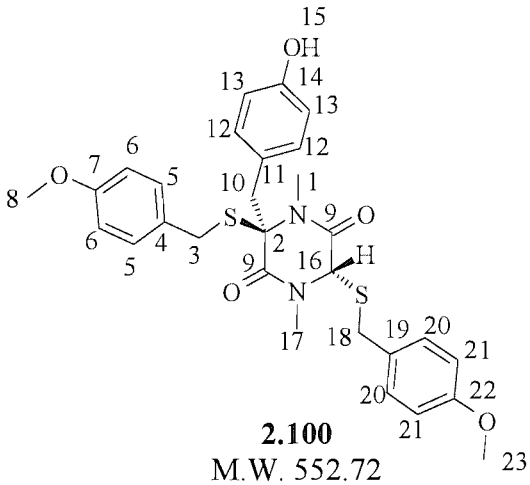
***Cis*-(4-Hydroxybenzyl)-3,6-bis-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.99)**



2.99
M.W. 552.72
$C_{29}H_{32}N_2O_5S_2$

^{1}H NMR (400 MHz, $CDCl_3$, OH not observed) δ_H 7.27 (d, 2H, *J* 8.8 Hz, Ar-H), 7.25 (d, 2H, *J* 8.8 Hz, Ar-H), 6.89 (d, 2H, *J* 8.5 Hz, Ar-H), 6.83 (d, 2H, *J* 8.5 Hz, Ar-H), 6.82 (d, 2H, *J* 8.5 Hz, Ar-H), 6.65 (d, 2H, *J* 8.5 Hz, Ar-H), 4.12 (s, 1H, H16), 4.06 (d, 1H, *J* 13.6 Hz, H10 or H3 or H18), 3.84-3.79 (m, 3H, H10 or H3 or H18), 3.80 (s, 3H, H8 or H23), 3.79 (s, 3H, H8 or H23), 3.56 (d, 1H, *J* 14.3 Hz, H10 or H3 or H18), 3.28 (s, 3H, H1 or H17), 3.06 (d, 1H, *J* 14.1 Hz, H10 or H3 or H18), 2.63 (s, 3H, H1 or H17); **^{13}C NMR** (100 MHz) δ_C 166.4, 164.6 (C9, 2 x Cq), 159.4, 159.4 (C7 & C22, 2 x Cq), 156.1 (C14, Cq), 130.9, 130.7, 130.6, (C5, C12 & C20, 6 x CH), 128.6 (C11, Cq), 127.9, 125.7 (C19 & C4, 2 x Cq), 115.7, 114.4, 114.3 (C13, C5 & C21, 6 x CH), 75.4 (C2, Cq), 61.6 (C16, CH), 55.6, 55.5 (C8 & C23, 2 x CH_3), 42.2 (C10, CH_2), 37.3, 34.9 (C18 & C3, 2 x CH_2), 33.3, 30.7 (C1 & C17, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 399.1, $[M+H]^+$ 553.2, $[M+Na]^+$ 575.2 peak at 7.14 min; **FTIR** ν_{max} (neat, cm^{-1}) 3240 (br), 2925 (w), 1672 (s), 1605 (s), 1509 (s), 1428 (m), 1367 (s), 1236 (s) cm^{-1}.

***Trans*-(4-Hydroxybenzyl)-3,6-bis-(4-methoxybenzylsulfanyl)-1,4-dimethylpiperazi -ne-2,5-dione (2.100)**



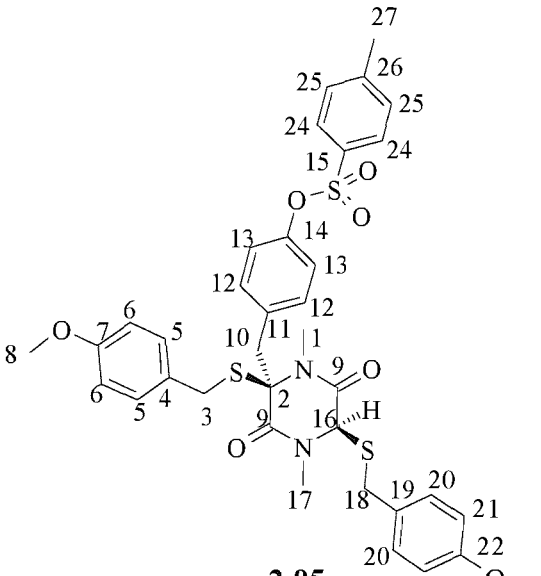
2.100
M.W. 552.72
$C_{29}H_{32}N_2O_5S_2$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.17 (d, 2H, *J* 8.5 Hz, Ar-H), 7.11 (d, 2H, *J* 8.5 Hz, Ar-H), 6.92 (d, 2H, *J* 8.5 Hz, Ar-H), 6.84 (d, 2H, *J* 8.8 Hz, Ar-H), 6.74 (d, 2H, *J* 8.5 Hz, Ar-H), 6.72 (d, 2H, *J* 8.8 Hz, Ar-H), 6.26 (br s, 1H, OH), 3.94 (s, 1H, H16), 3.80 (s, 3H, H8 or H23), 3.71 (s, 3H, H8 or H23), 3.70-3.44 (m, 4H, H10 or H3 or H18), 3.38 (d, 1H, *J* 13.3 Hz, H10 or H3 or H18), 3.30 (s, 3H, H1 or H17), 3.02 (d, 1H, *J* 14.1 Hz, H10 or H3 or H18), 2.23 (s, 3H, H1 or H17); **^{13}C NMR** (100 MHz) δ_C 166.3, 164.9 (C9, Cq), 159.3, 159.2 (C7 & C22, 2 x Cq), 156.3 (C14, Cq), 131.7, 131.0, 130.1 (C5, C12 & C20, 6 x CH), 128.8 (C11, Cq), 127.8, 125.6 (C19, C4, 2 x Cq), 116.0, 114.4, 114.3 (C13, C6 & C21, 6 x CH), 76.4 (C2, Cq), 61.7 (C16, CH), 55.6 (C8 & C23, 2 x CH_3), 41.7 (C10, CH_2), 36.9, 35.1 (C18 & C3, 2 x CH_2), 33.1, 30.9 (C1 & C17, 2 x CH_3); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 399.1, $[M+H]^+$ 553.2 peak at 6.49 min; **FTIR** ν_{max} (neat, cm^{-1}) 3264 (br s), 2935 (w), 1666 (s), 1608 (s), 1509 (s), 1434 (m), 1236 (s) cm^{-1}.

***Cis*-Toluene-4-sulfonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3 ,6-dioxopiperazin-2-ylmethyl]phenyl ester (2.95) and *trans*-toluene-4-sulfonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxopiperazin-2-yl methyl]-phenyl ester (2.96)**

To a solution of (**2.71**) in THF (10 mL) was added a solution of 2.0 M LDA (1.0 mL, 2.0 mmol) at -78 °C. After stirring at -78 °C for 25 minutes, to the resulting solution was added dropwise a solution of (**2.92**) (251 mg, 0.7 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature below -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for 2 hr. A solution of acetic acid (59.4 mg, 1.0 mmol) in THF (1 ml) was then added and the resulting reaction mixture allowed to warm to room temperature. The reaction solution was quenched at room temperature with the addition of sat. NaCl (aq) solution (20 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% to 60% EtOAc/Hexane) to give (**2.95**) as a pale yellow gum and (**2.96**) as a colourless gum (150 mg and 48 mg, 63%, 3:1 (**2.95**:**2.96**).

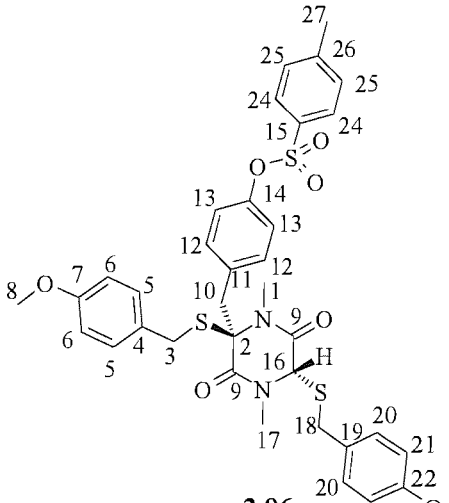
***Cis*-Toluene-4-sulfonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxopiperazin-2-ylmethyl]phenyl ester (2.95)**



2.95
M.W. 706.91
$C_{36}H_{38}N_2O_7S_3$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.63 (d, 2H, *J* 8.3 Hz, Ar-H), 7.32 (d, 2H, *J* 8.5 Hz, Ar-H), 7.24 (d, 2H, *J* 8.8 Hz, Ar-H), 6.96 (d, 2H, *J* 8.8 Hz, Ar-H), 6.88-6.80 (m, 8H, Ar-H), 4.10 (s, 1H, H16), 4.08 (d, 1H, *J* 13.6 Hz, H10 or H3 or H18), 3.89-3.82 (m, 2H, H16 & H10 or H3 or H18), 3.79 (s, 3H, H8 & H23), 3.78 (s, 3H, H8 & H23), 3.65 (d, 1H, *J* 14.3 Hz, H10 or H3 or H18), 3.19 (s, 3H, H1 or H17), 3.08 (d, 1H, *J* 14.3 Hz, H10 or H3 or H18), 2.93 (d, 1H, *J* 14.3 Hz, H10 or H3 or H18), 2.59 (s, 3H, H1 or H17), 2.44 (s, 3H, H27); **^{13}C NMR** (100 MHz) δ_C 166.1, 164.4 (C9, 2 x Cq), 159.5, 159.4 (C7 & C22, 2 x Cq), 149.3, 145.8 (C15 & C26, 2 x Cq) 133.8, 132.7 (C14 & C11, 2 x Cq), 131.2, 130.9, 130.4, 130.2, 128.9 (C5, C20, C12, C24 & C25, 10 x CH), 127.6 (C19 & C4, 2 x Cq), 122.9 (C13, 2 x CH), 114.5 (C21 & C6, 4 x CH), 74.7 (C2, Cq), 61.5 (C16, CH), 55.7, 55.6 (C8 & C23, 2 x CH_3), 42.4 (C10, CH_2), 37.7, 35.2 (C18 & C3, 2 x CH_2), 34.3, 33.3 (C1 & C17, 2 x CH_3), 22.1 (C27, CH_3); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 417.1, $[M+Na]^+$ 729.3, $[M+H]^+$ 707.3 peak at 8.57 min; **FTIR** ν_{max} (neat, cm^{-1}) 2929 (w), 1659 (s), 1608 (m), 1509 (s), 1370 (s), 1300 (m), 1198 (s), 1174 (s) cm^{-1}.

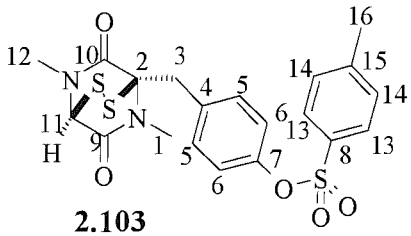
***Trans*-Toluene-4-sulfonic acid 4-[2,5-bis-(4-methoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxopiperazin-2-ylmethyl]phenyl ester (2.96)**



2.96
M.W. 706.91
$C_{36}H_{38}N_2O_7S_3$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.71 (d, 2H, *J* 8.3 Hz, Ar-H), 7.30 (d, 2H, *J* 8.0 Hz, Ar-H), 7.23 (d, 2H, *J* 8.5 Hz, Ar-H), 7.10 (d, 2H, *J* 8.8 Hz, Ar-H), 6.99 (d, 2H, *J* 8.8 Hz, Ar-H), 6.90 - 6.82 (m, 4H, Ar-H), 6.71 (d, 2H, *J* 8.5 Hz, Ar-H), 3.87 (s, 1H, H16), 3.81 (s, 3H, H8 & H23), 3.81 (d, 2H, *J* 13.6 Hz, H10, H3 or H18), 3.72 (s, 3H, H8 & H23), 3.60-3.47 (m, 3H, H10 or H3 or H18), 3.27 (s, 3H, H1 or H17), 3.05 (d, 1H, *J* 13.8 Hz, H10 or H3 or H18),), 2.46 (s, 3H, H11, H17), 2.18 (s, 3H, H27); **^{13}C NMR** (100 MHz) δ_C 166.0, 164.6 (C9, 2 x Cq), 159.3, 160.2, (C7 & C22, 2 x Cq), 149.6, 145.6 (C15 & C26, 2 x Cq), 133.3, 133.0 (C14 & C11, 2 x Cq), 131.9, 131.0, 130.2, 130.1, 129.0 (C5, C20, C12, C24 & C25, 10 x CH), 127.8 (C19 & C4, 2 x Cq), 123.4 (C13, 2 x CH), 114.4, 114.3 (C21 & C6, 4 x CH), 76.3 (C2, Cq), 61.0 (C16, CH), 55.7, 55.6 (C8 & C23, 2 x CH_3), 41.7 (C10, CH_2), 37.2, 35.1 (C18 & C3, 2 x CH_2), 33.0, 30.6 (C1 & C17, 2 x CH_3), 22.0 (C27, CH_3); **LCMS ESI+** *m/z* $[M\text{-SPmb}]^+$ 417.1, $[M+Na]^+$ 729.3, $[M+H]^+$ 707.4 peak at 8.03 min; **FTIR** ν_{max} (neat, cm^{-1}) 2360 (w), 1653 (s), 1540 (s), 1377 (m), 1284 (s), 1243 (s) cm^{-1}.

Toluene-4-sulfonic acid 4-(5,7-dimethyl-6,8-dioxo-2,3-dithia-5,7-diazabicyclo[2.2.2]oct-1-ylmethyl)phenyl ester (2.103)



2.103

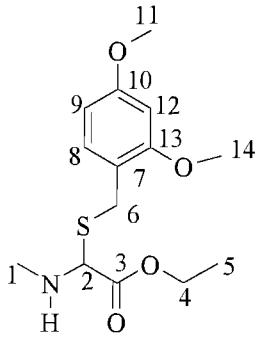
M.W. 464.58

$C_{20}H_{20}N_2O_5S_3$

A solution of **(2.95)** (30 mg, 0.04 mmol) in dichloromethane (2 mL) was cooled to 0 °C. To the stirred solution was added a solution of 1.0 M boron trichloride (0.1 mL, 0.11 mmol) in dichloromethane, and allowed to warm to rt. The resultant dark yellow solution was stirred at r.t for 30 min. The resulting solution was concentrated to give a red residue. The residue was dissolved in a solution of iodine (10.66 mg, 0.08 mmol) in 10% MeOH/DCM (10 mL) and stirred at rt. After 10 minutes the solution was concentrated. The resulting residue was dissolved in EtOAc (20 mL) and partitioned between EtOAc and a mixture of sat $NaHCO_3$ (10 mL) and 1 M $Na_2S_2O_3$ (aq) solution (5 mL), the organics were separated and the aqueous extracted with further EtOAc (4 x 10 mL). The organics were then combined washed with brine (10 mL), dried over ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 35% EtOAc/Hexane) to give **(2.103)** as a colourless gum (14 mg, 71%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.71 (d, 2H, *J* 8.3 Hz, Ar-H), 7.31 (d, 2H, *J* 8.0 Hz, Ar-H), 7.25 (d, 2H, *J* 8.8 Hz, Ar-H), 6.95 (d, 2H, *J* 8.8 Hz, Ar-H), 5.35 (s, 1H, H11), 3.99 (d, 1H, *J* 15.8 Hz, H3), 3.55 (d, 1H, *J* 15.8 Hz, H3), 3.18 (s, 3H, H1 or H12), 2.90 (s, 3H, H1 or H12), 2.46 (s, 3H, H16); **^{13}C NMR** (100 MHz) δ_C 165.8, 165.0 (C10 & C9, 2 x Cq), 149.2, 145.8 (C8 & C15, 2 x Cq), 133.5, 132.8 (C7 & C4, 2 x Cq), 130.8, 130.2, 128.9 (C5, C13 & C14, 6 x CH), 122.9 (C6, 2 x CH), 77.1 (C2, Cq), 67.5 (C11, CH), 36.6 (C3, CH_2), 32.7 (C12, CH_3), 28.5 (C1, CH_3), 22.1 (C16, CH_3); **LCMS ESI+** *m/z* $[M+H-2S]^+$ 401.1, $[M+Na]^+$ 487.1, $[M+H]^+$ 465.1 peak at 6.67 min. **FTIR** ν_{max} (neat, cm^{-1}) 2923 (w), 1686 (s), 1501 (w), 1366 (m), 1151 (m) cm^{-1}; ***Anal.*** Calcd for $C_{20}H_{20}N_2O_5S_2$: C, 51.71; H, 4.34; N, 6.03; S, 20.71. Found: C, 51.54; H, 4.69; N, 5.75; S, 20.40.

2,4-Dimethoxybenzylsulfanyl)methylaminoacetic acid ethyl ester (2.113)

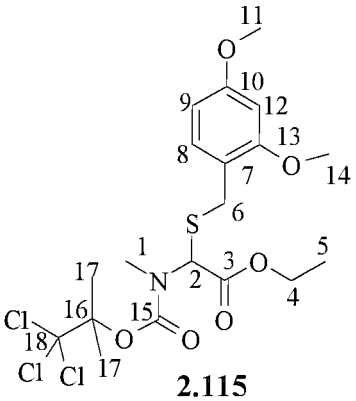


2.113
M.W. 299.39
$C_{14}H_{21}NO_4S$

Methylamine (2.4 mL of a 2.0 M solution in THF, 4.89 mmol) was added to a solution of ethyl glyoxalate (1 mL of a 50% solution in toluene, 4.89 mmol) and sodium sulfate anhydrous (excess) in toluene (5 mL) and the reaction mixture stirred at room temperature for 10 min. **(2.111)** (901 mg, 4.89 mmol) was added and the resulting cloudy solution was stirred at room temperature for 18 h. The crude reaction mixture was concentrated, pre-adsorbed onto silica and purified by column chromatography (SiO_2, 30% EtOAc/ hexane) to give (**2.113**) as a colourless gum (900 mg, 62 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.17 (d, 1H, *J* 8.4 Hz, Ar-H), 6.44-6.38 (m, 2H, Ar-H), 4.32 (s, 1H, H2), 4.24-4.18 (m, 2H, H4), 3.83 (s, 3H, H14), 3.78 (s, 3H, H11), 3.77-3.68 (m, 2H, H6), 2.37 (s, 3H, H1), 2.06 (br s, 1H, NH), 1.29 (t, 3H, *J* 7.2 Hz, H5); **^{13}C NMR** (67.93 MHz) δ_C 170.0 (C3, Cq), 160.1 (C10, Cq), 158.1 (C13, Cq), 130.7 (C8, CH), 118.6 (C7, Cq) 104.0 (C9, CH), 98.5 (C12, CH), 66.3 (C2, CH), 61.2 (C4, CH_2), 55.4 (C14, CH_3), 55.3 (C11, CH_3), 32.0 (C1, CH_3), 27.3 (C6, CH_2), 14.0 (C5, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 300.19 peak at 4.04 min; **FTIR** ν_{max} (neat, cm^{-1}) 3353 (w), 2936 (w), 1725 (s), 1610 (m), 1505 (s), 1289 (m), 1206 (s), 1153 (s) cm^{-1}; ***Anal.*** Calcd for $C_{14}H_{21}NO_4S$: C, 56.17; H, 7.07; N, 4.68; S, 10.71. Found: C, 56.26; H, 6.77; N, 4.48; S, 10.31.

(2,4-Dimethoxybenzylsulfanyl)-[methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]acetic acid ethyl ester (2.115)



2.115

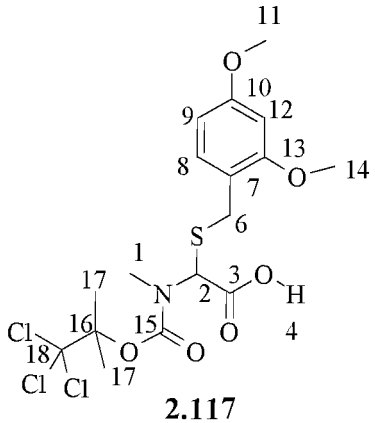
M.W. 502.85

$C_{19}H_{26}Cl_3NO_6S$

2,2,2-Trichloro-1,1-dimethylethyl chloroformate (740 mg, 3.1 mmol) was added portionwise to a solution of (**2.113**) (840 mg, 2.8 mmol), and triethylamine (484 μL, 3.1 mmol) in dichloromethane (20 mL) and the reaction mixture stirred at room temperature for 18 h. The resulting solution was concentrated and the residue purified by column chromatography (SiO_2, 20% EtOAc/ Hexane) to give (**2.115**) as a colourless gum (1.28 g, 91%).

^{1}H NMR (270 MHz, $CDCl_3$, rotamers observed) δ_H 7.18-7.15 (m, 1H, Ar-H), 6.44-6.41 (m, 2H, Ar-H), 6.09 & 6.00 (s, 1H, H2), 4.23-4.18 (m, 2H, H4), 3.82 (s, 3H, H14), 3.79 (s, 3H, H11), 3.73-3.67 (m, 2H, H6), 2.97 (s, 3H, H1), 1.93 (s, 6H, H17), 1.31-1.24 (m, 3H, H5); **^{13}C NMR** (67.93 MHz) δ_C 168.1 (C3, Cq), 160.3 (C10, Cq), 158.2 (C13, Cq), 154.2 (C15, Cq) 130.5 (C8, CH), 117.8 (C7, Cq), 106.2 (C18, Cq), 104.1 (C9, CH), 98.7, (C12, CH), 89.5 (C16, Cq), 63.3 (C2, CH), 62.2 (C4, CH_2) , 55.4 (C14, CH_3), 55.3 (C11, CH_3), 30.7 (C1, CH_3), 30.0 (C6, CH_2), 21.5, 21.4 (C17, 2 x CH_3), 14.0 (C5, CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 524.0, $[M+H]^+$ 504.0 peak at 9.33 min; **FTIR** ν_{max} (neat, cm^{-1}) 2938 (w), 1740 (m), 1703 (m), 1506 (m), 1207 (s), 1154 (s) cm^{-1}; ***Anal.*** Calcd for $C_{19}H_{26}Cl_3NO_6S$: C, 45.38; H, 5.21; N, 2.78; S, 6.38. Found: C, 45.27; H, 5.20; N, 2.24; S, 6.73.

(2,4-Dimethoxybenzylsulfanyl)-[methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]acetic acid (2.117)

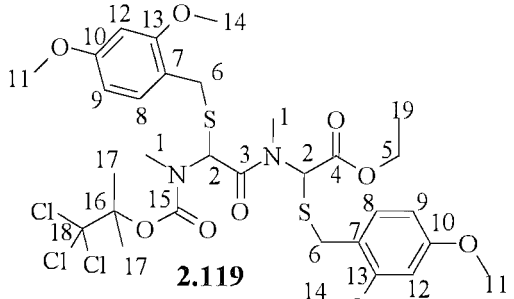


2.117
M.W. 474.79
$C_{17}H_{22}Cl_3NO_6S$

Lithium hydroxide (150 mg, 3.6 mmol) was added to a solution of (**2.115**) (1.2 g, 2.4 mmol) in 1,4-dioxan (20 mL), water (1 mL) and the reaction mixture stirred at room temperature for 4 h. EtOAc (15 mL) and saturated citric acid (aq) solution (1 mL) was added. The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed well with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated to give (**2.117**) as a white solid (830 mg, 73%).

Mp 140.1-140.9 °C; **^{1}H NMR** (270 MHz, $CDCl_3$, rotamers observed, acid OH not observed) δ_H 7.15-7.11 (m, 1H, Ar-H), 6.42-6.38 (m, 2H, Ar-H), 6.03 & 5.95 (s, 1H, H2), 3.82-3.70 (m, 8H, H11, H14, H6), 2.96 (s, 3H, H1), 1.92 (s, 6H, H17); **^{13}C NMR** (67.93 MHz) δ_C 173.2 (C3, Cq), 160.4 (C10, Cq), 158.2 (C13, Cq), 154.2 (C15, Cq) 130.6 (C8, CH), 117.6 (C7, Cq), 106.2 (C18, Cq), 104.0 (C9, CH), 98.7, (C12, CH), 89.5 (C16, Cq), 62.4 (C2, CH), 55.4 (C14, CH_3), 55.3 (C11, CH_3), 30.9 (C1, CH_3), 30.3 (C6, CH_2), 21.4, 21.5 (C17, 2 x CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 497.79, [M+H]+ 497.9 at 7.65 min; **FTIR** ν_{max} (neat, cm^{-1}) 3002 (br), 2952 (w), 1701 (s), 1611 (m), 1506 (m), 1154 (s) cm^{-1}; ***Anal.*** Calcd for $C_{17}H_{21}Cl_3NO_6S$: C, 43.01; H, 4.67; N, 2.95; S, 6.75. Found: C, 43.00; H, 4.68; N, 2.97; S, 6.65.

(2,4-Dimethoxybenzylsulfanyl)-({2-(2,4-dimethoxybenzylsulfanyl)-2-[methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-acetyl}-methylamino)acetic acid (2.119)

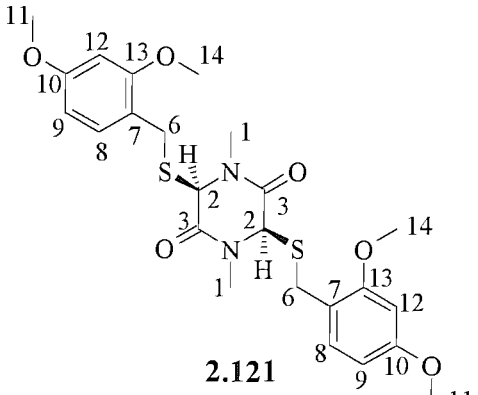


2.119
M.W. 756.17
$C_{31}H_{41}Cl_3N_2O_9S_2$

To a stirring solution of (**2.117**) (4.1 g, 8.6 mmol) and NMM (1.0 g, 10.3 mmol) in THF (10 mL) at -10 °C was added isobutyl chloroformate (1.3 g, 9.5 mmol) in THF (5 mL) dropwise over 5 minutes. The resulting suspension was then stirred at -10 ° C for 40 minutes. The reaction mixture was filtered and the filtrate added to a solution of (**2.113**) (2.8 g, 9.5 mmol) and NMM (1.0 g, 10.3 mmol) in THF (20 mL) at 0 °C. The resulting reaction mixture was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 15 m mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 25% EtOAc/Hexane) to give (**2.119**) as a colourless gum (2.7 g, 41%).

^{1}H NMR (270 MHz, $CDCl_3$, rotamers observed) δ_H 7.17-7.04 (m, 2H, Ar-H), 6.42-6.35 (m, 4H, Ar-H), 6.08-6.04 (m, 2H, H2), 4.19-4.06 (m, 2H, H5), 3.88-3.58 (m, 16H, H6, H11 & H14), 3.14-3.12 (m, 6H, H1), 1.94-1.91 (m, 6H, H17), 1.22-1.17 (m, 3H, H19); **^{13}C NMR** (67.93 MHz) δ_C 169.3 (C4, Cq), 167.8 (C3, Cq), 160.6, 160.5 (C10, 2 x Cq), 158.4 (C13, 2 x Cq), 154.5, 154.2 (C15, Cq) 130.9, 130.7 (C8, 2 x CH), 118.1, 117.7 (C7, 2 x Cq), 106.5 (C18, Cq), 104.3, 104.5 (C9, 2 x CH), 98.9, 98.8, (C12, 2 x CH), 89.5, 89.1 (C16, Cq), 62.3, 61.5 (C2, 2 x CH), 62.2 (C5, CH_2), 55.5 (C14, 2 x CH_3), 55.4 (C11, 2 x CH_3), 31.0, 30.6 (C1, CH_3), 29.6 (C6, 2 x CH_2), 21.7, 21.6 (C17, 2 x CH_3), 14.1 (C19, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 757.2 at 10.22 min; **FTIR** ν_{max} (neat, cm^{-1}) 2937 (w), 1736 (m), 1659 (m), 1506 (m), 1153 (s) cm^{-1}; ***Anal.*** Calcd for $C_{31}H_{41}Cl_3N2O_9S_2$: C, 49.24; H, 5.47; N, 3.70 S, 8.48. Found: C, 49.21; H, 5.59; N, 3.47; S, 8.53.

3,6-Bis-(2,4-Dimethoxybenzylsulfanyl)-1,4-dimethylpiperazine-2,5-dione (2.121)

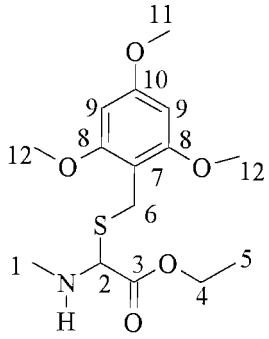


2.121
M.W. 506.64
$C_{24}H_{30}N_2O_6S_2$

Zinc dust (622 mg, 9.5 mmol), was added portionwise to a solution of (**2.119**) (360 mg, 0.5 mmol), in 10% acetic acid, isopropyl alcohol (10 mL) over 1 h. The resultant suspension was stirred at room temperature for 18 h. The zinc was filtered off and washed with EtOAc (3 x 15 mL). The organics were combined and concentrated. The resulting residue was purified by column chromatography (SiO_2, 80% EtOAc/Hexane) to give (**2.121**) as a off white solid (120 mg, 50%).

Mp 80.1-82.4 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.23 (d, 2H, *J* 8.4 Hz, Ar-H), 6.50-6.49 (m, 2H, Ar-H), 6.45 (m, 2H, Ar-H), 4.54 (s, 2H, H2), 3.99 (d, 2H, *J* 13.6 Hz, H6), 3.90 (d, 2H, *J* 13.6 Hz, H6), 3.85 (s, 6H, H14), 3.82 (s, 6H, H11), 2.71 (s, 6H, H1); **^{13}C NMR** (67.93 MHz) δ_C 165.4 (C3, 2 x Cq), 160.4 (C10, 2 x Cq), 158.3 (C13, 2 x Cq), 131.0 (C8, 2 x CH), 117.6 (C7, 2 x Cq) 103.5 (C9, 2 x CH), 98.8, (C12, 2 x CH), 62.4 (C2, 2 x CH), 55.4 (C14, 2 x CH_3), 55.2 (C11, 2 x CH_3), 32.3 (C6, 2 x CH_2), 32.0, 31.2 (C1, 2 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 507.1 peak at 7.0 min; **FTIR** ν_{max} (neat, cm^{-1}) 2926 (w), 1665 (s), 1610 (s), 1506 (s), 1392 (m), 1290 (s), 1206 (s) cm^{-1}.

Methylamino-(2,4,6-trimethoxybenzylsulfanyl)acetic acid ethyl ester (2.114)

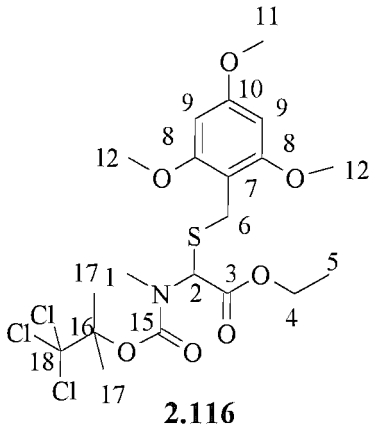


2.114
M.W. 329.42
$C_{15}H_{23}NO_5S$

Methylamine (2.4 mL of a 2.0 M solution in THF, 4.89 mmol) was added to a solution of ethyl glyoxalate (1 mL of a 50 % solution in toluene, 4.89 mmol) and sodium sulfate anhydrous (excess) in toluene (5 mL) and the reaction mixture stirred at room temperature for 10 min. (**2.112**) (1.05 g, 4.89 mmol) was added and the resulting cloudy solution was stirred at room temperature for 18 h. The crude reaction mixture was concentrated, pre-adsorbed onto silica and purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.114**) as a colourless gum (600 mg, 38 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.10 (s, 2H, H9), 4.38 (s, 1H, H2), 4.21 (q, 2H, *J* 7.1 Hz, H4), 3.82 (s, 6H, H12), 3.80 (s, 3H, H11), 3.79-3.74 (m, 2H, H6), 2.33 (s, 3H, H1), 2.00 (br s, 1H, NH), 1.28 (t, 3H, *J* 7.1 Hz, H5); **^{13}C NMR** (67.93 MHz) δ_C 170.1 (C3, Cq), 160.3 (C10, Cq), 158.5 (C8, 2 x Cq), 107.0 (C7, Cq), 90.6 (C9, 2 x CH), 66.5 (C2, CH), 61.0 (C4, CH_2), 55.6 (C12, 2 x CH_3), 55.2 (C11, CH_3), 31.8 (C1, CH_3), 21.0 (C6, CH_2), 14.0 (C5, CH_3) **LCMS ESI+** *m/z* $[M+H]^+$ 330.21 peak at 4.46 min; **FTIR** ν_{max} (neat, cm^{-1}) 3333 (w), 2937 (w), 1727 (s), 1517 (s), 1455 (m), 1146 (s) cm^{-1}; ***Anal.*** Calcd for $C_{15}H_{23}NO_5S$: C, 54.69; H, 7.04; N, 4.25; S, 9.73. Found: C, 54.47; H, 6.94; N, 4.16; S, 9.50.

[Methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-(2,4,6-trimethoxybenzylsulfanyl)acetic acid ethyl ester (2.116)

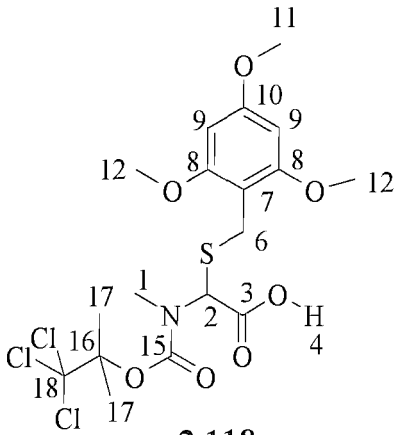


2.116
M.W. 532.87
$C_{20}H_{28}Cl_3NO_7S$

2,2,2-Trichloro-1,1-dimethylethyl chloroformate (1.6 g, 6.1 mmol) was added portion-wise to a solution of (**2.114**) (2.0 g, 6.7 mmol), and triethylamine (1.1 mL, 8.0 mmol) in dichloromethane (30 mL) and the reaction mixture stirred at room temperature for 18 h. The resulting solution was concentrated and the residue purified by column chromatography (SiO_2, 20% EtOAc/Hexane) to give (**2.116**) as a colourless gum (3.17 g, 97%).

^{1}H NMR (400 MHz, $CDCl_3$, rotamers observed) δ_H 6.13 & 6.12 (s, 1H, H2), 6.10 (s, 2H, H9), 4.21 & 4.18 (q, 2H, *J* 7.2 Hz, H4), 3.98 & 3.88 (d, 1H, *J* 11.1 Hz, H6), 3.80 (s, 6H, H12), 3.79 (s, 3H, H11), 3.71-3.68 (m, 1H, H6), 3.03 & 3.01 (s, 3H, H1), 1.95 & 1.93 (s, 6H, H17), 1.27 & 1.24 (t, 3H, *J* 7.2 Hz, H5); **^{13}C NMR** (67.93 MHz) δ_C 168.5 (C3, Cq), 160.6 (C10, Cq), 158.5 (C8, 2 x Cq), 154.2 (C15, Cq), 107.0 (C7, Cq), 106.4 (C18, Cq), 90.3 (C9, 2 x CH), 89.9, (C16, Cq), 63.5 (C2, CH), 61.2 (C4, CH_2), 55.7 (C12, 2 x CH_3), 55.3 (C11, CH_3), 30.6 (C1, CH_3), 23.7 (C6, CH_2), 21.5, 21.4 (C17, 2 x CH_3), 14.0 (C5, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 534.0, $[M+Na]^+$ 556.0 peak at 9.33 min; **FTIR** ν_{max} (neat, cm^{-1}) 2940 (w), 1737 (m), 1703 (s), 1148 (s), 1311 (m) cm^{-1}; ***Anal.*** Calcd for $C_{20}H_{28}Cl_3NO_5S$: C, 45.08; H, 5.30; N, 2.63; S, 6.02. Found: C, 44.94; H, 5.23; N, 2.62; S, 5.92.

[Methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-(2,4,6-trimethoxybenzylsulfanyl)acetic acid (2.118)



2.118

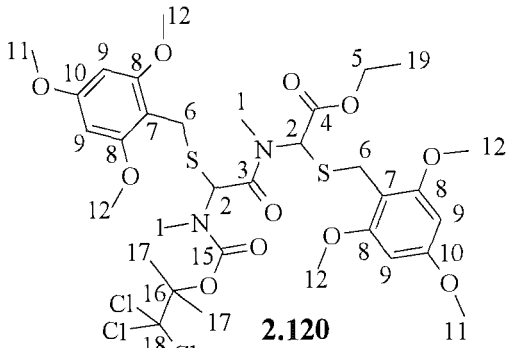
M.W. 504.82

$C_{18}H_{24}Cl_3NO_7S$

Lithium hydroxide (78.1 mg, 1.9 mmol) was added to a solution of (**2.116**) (650 mg, 1.2 mmol) in 1,4-dioxan (20 mL), water (1 mL) and the reaction mixture stirred at room temperature for 4 h. EtOAc (15 ml) and saturated citric acid solution (1 mL) was added. The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed well with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated to give (**2.118**) as a white solid (510 mg, 83%).

Mp 139.3-140.0 °C; **^{1}H NMR** (270 MHz, $CDCl_3$, rotamers observed) δ_H 11.45 (s, 1H, H4), 6.10 (s, 2H, H9), 6.07 & 6.01 (s, 1H, H2), 3.97-2.98 (m, 11H, H11, H12, & H6), 2.98 (s, 3H, H1), 1.93 & 1.90 (s, 6H, H17); **^{13}C NMR** (67.93 MHz) δ_C 173.8 (C3, Cq), 160.6 (C10, Cq), 158.9, 158.8 (C8, 2 x Cq), 154.2 (C15, Cq), 106.4 (C7, Cq), 105.8 (C18, Cq), 90.4 (C9, 2 x CH), 89.5 (C16, Cq), 62.6 (C2, CH), 55.8, 55.7 (C12, 2 x CH_3), 55.2 (C11, CH_3), 31.0 (C1, CH_3), 24.3 (C6, CH_2), 21.6, 21.4 (C17, 2 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 527.9 peak at 7.80 min; **FTIR** ν_{max} (neat, cm^{-1}) 3012 (br), 2972 (w), 1781 (s), 1651 (m), 1556 (m), 1154 (s) cm^{-1}; ***Anal.*** Calcd for $C_{18}H_{24}Cl_3NO_7S$: C, 42.83; H, 4.79; N, 2.77; S, 6.35. Found: C, 42.09; H, 4.66; N, 3.10; S, 6.43.

{Methyl-[2-[methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-2-(2,4,6-trimethoxybenzylsulfanyl)-acetyl]-amino}-(2,4,6-trimethoxybenzylsulfanyl)acetic acid ethyl ester (2.120)

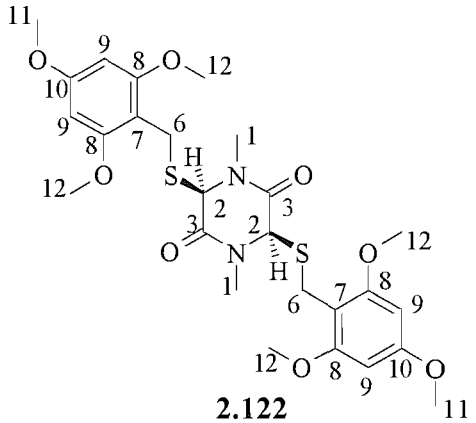


2.120
M.W. 816.22
$C_{33}H_{45}Cl_3N_2O_{11}S_2$

To a stirring solution of (**2.118**) (2.9 g, 5.7 mmol) and NMM (696 mg, 6.9 mmol) in THF (10 mL) at -10 °C was added isobutyl chloroformate (860 mg, 6.3 mmol) in THF (5 mL) dropwise over 5 minutes. The resulting suspension was then stirred at -10 °C for 40 minutes. The reaction mixture was filtered and the filtrate added to a solution of (**2.114**) (2.1 g, 6.3 mmol) and NMM (1.0 g, 10.3 mmol) in THF (20 mL) at 0 °C. The resulting reaction mixture was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.120**) as a colourless gum (2.1 g, 45%).

^{1}H NMR (270 MHz, $CDCl_3$, rotamers observed) δ_H 6.07-6.04 (m, 6H, H9 & H2), 4.14-4.10 (m, 2H, H5), 3.97-2.98 (m, 22H, H11, H12, H6), 3.23-3.02 (m, 6H, H1), 1.94-1.88 (m, 6H, H17), 1.22-1.17 (m, 3H, H19); **^{13}C NMR** (67.93 MHz) δ_C 169.2 (C4, Cq), 168.0 (C3, Cq), 160.5 (C10, 2 x Cq), 158.9, 158.8 (C8, 4 x Cq), 154.2 (C15, Cq), 106.0, 105.9, 105.8 (C7 & C18, 3 x Cq), 90.3, 89.1 (C9, 4 x CH), 88.7 (C16, Cq), 63.3, 62.6 (C2, 2 x CH), 62.0 (C5, CH_2), 55.6 (C12, 4 x CH_3), 55.2 (C11, 2 x CH_3), 30.9, 30.7 (C1, 2 x CH_3), 23.6, 23.4 (C6, 2 x CH_2), 21.4, 21.3 (C17, 2 x CH_3), 14.1, 14.0 (C19, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 817.3, $[M+Na]^+$ 839.4 peak at 10.15 min; **FTIR** ν_{max} (neat, cm^{-1}) 2940 (w), 1736 (m), 1702 (m), 1593 (s), 1417 (m), 1147 (s) cm^{-1}; ***Anal.*** Calcd for $C_{33}H_{45}Cl_3N_2O_{11}S_2$: C, 48.56; H, 5.56; N, 3.43; S, 7.86. Found: C, 47.98; H, 5.49; N, 3.35; S, 7.89.

1,4-Dimethyl-3,6-bis-(2,4,6-trimethoxybenzylsulfanyl)piperazine-2,5-dione (2.122)



2.122

M.W. 566.70

$C_{26}H_{34}N_2O_8S_2$

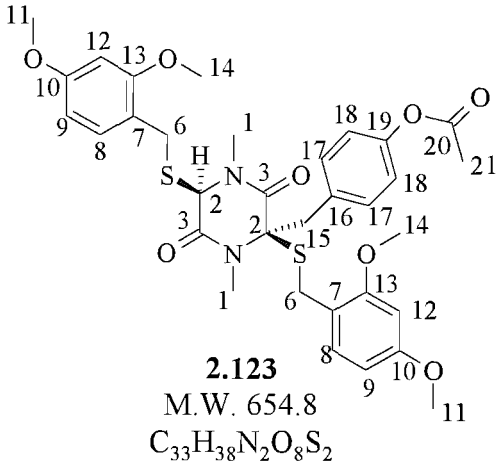
Zinc dust (480 mg, 7.4 mmol), was added portionwise to a solution of (**2.120**) (300 mg, 0.4 mmol), in 10% acetic acid, isopropyl alcohol (10 mL) over 1 h. The resultant suspension was stirred at room temperature for 18 h. The zinc was filtered off and washed with EtOAc (3 x 15 mL). The organics were combined and concentrated. The resulting residue was purified by column chromatography (SiO_2, 80% EtOAc/Hexane) to give (**2.122**) as a pale brown waxy solid (70 mg, 34%).

Mp 78.6-80.2 °C; **^{1}H NMR** (270 MHz, $CDCl_3$) δ_H 6.06 (br s, 4H, H9), 4.85 (s, 2H, H2), 4.41 (d, 2H, *J* 13.4 Hz, H6), 3.85 (d, 2H, *J* 13.4 Hz, H6), 3.79 (br s, 18H, H12 & H11), 2.73 (s, 6H, H1); **^{13}C NMR** (67.93 MHz) δ_C 165.6 (C3, 2 x Cq), 160.5 (C10, 2 x Cq), 158.7 (C8, 4 x Cq), 107.8 (C7, 2 x Cq), 90.4 (C9, 4 x CH), 64.5 (C2, 2 x CH), 55.6 (C12, 4 x CH_3), 55.2 (C11, 2 x CH_3), 31.1 (C1, 2 x CH_3), 26.6 (C6, 2 x CH_2); **LCMS ESI+** *m/z* $[M+H]^+$ 567.1 peak at 7.09 min; **FTIR** ν_{max} (neat, cm^{-1}) 2938 (w), 1674 (m), 1592 (s), 1453 (m), 1203 (m), 1186 (m), 1165 (m) cm^{-1}.

***Cis*-Acetic acid 4-[2,5-bis-(2,4-dimethoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxopiperazin-2-ylmethyl]phenyl ester (2.123) and 3,6-bis-(2,4-dimethoxybenzyl sulfanyl)-3-(4-hydroxybenzyl)-1,4-dimethylpiperazine-2,5-dione (2.125)**

To a solution of 2.0 M LDA (890 µl, 1.78 mmol) in THF (5 ml) at -78 °C was added a solution of (**2.121**) (300 mg, 0.59 mmol) in THF (10 mL) and the reaction mixture stirred at -78 °C for 25 minutes. To the resulting red solution was added dropwise a solution of (**2.88**) (203 mg, 0.89 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature < -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for 2 h. The solution was then allowed to warm to < -10 °C for 2 h. A solution of acetic acid (59 mg, 0.89 mmol) in THF (1 mL) was added and the resulting reaction mixture allowed to warm to room temperature. The reaction solution was quenched at room temperature with the addition of 0.3 M $KHSO_4$ (aq) solution (10 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% to 70% EtOAc/Hexane) to give (**2.123**) and (**2.125**) as colourless gums (60 mg, 30% and 62 mg, 31%).

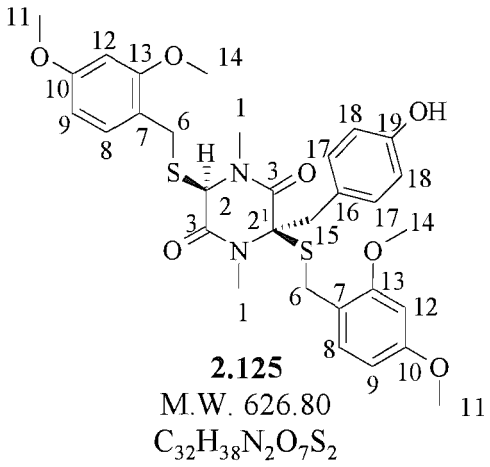
***Cis*-Acetic acid 4-[2,5-bis-(2,4-dimethoxybenzylsulfanyl)-1,4-dimethyl-3,6-dioxo-piperazin-2-ylmethyl]phenyl ester (2.123)**



2.123
M.W. 654.8
$C_{33}H_{38}N_2O_8S_2$

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 7.13 (d, 2H, *J* 8.2 Hz, Ar-H), 7.05 (d, 2H, *J* 8.4 Hz, Ar-H), 6.94 (d, 2H, *J* 8.4 Hz, Ar-H), 6.42-6.38 (m, 4H, Ar-H), 4.31 (s, 1H, H2), 4.02 (d, 1H, *J* 13.3 Hz, H6 or H15), 3.87 (d, 2H, *J* 13.3 Hz, H6 or H15), 3.80-3.77 (m, 14H, H6 or H15 & H11 & H14), 3.60 (d, 1H, *J* 14.1 Hz, H6 or H15), 3.26 (s, 3H, H1), 3.10 (d, 1H, *J* 14.1 Hz, H6 or H15), 2.61 (s, 3H, H1), 2.25 (s, 3H, H21); **^{13}C NMR** (67.93 MHz) δ_C 169.2 (C20, Cq), 166.2, 164.3 (C3, 2 x Cq), 160.6, 160.4 (C10, 2 x Cq), 158.4 (C13, Cq), 150.0 (C19, Cq), 131.3, 130.7 (C17 & C8, 4 x CH), 117.6, 116.6 (C7, 2 x Cq), 115.9 (C18, 2 x CH), 104.2, 103.9 (C9, 2 x CH), 99.0, 98.8 (C12, 2 x CH), 55.4, 55.6 (C14, 2 x CH_3), 55.5 (C11, 2 x CH_3), 42.1 (C15, CH_2), 32.8 (C1, CH_3), 32.7 (C6, CH_2), 30.4 (C1, CH_3), 29.4 (C6, CH_2), 21.2 (C21, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 655.2 peak at 7.77 min; **FTIR** ν_{max} (neat, cm^{-1}) 2933 (w), 1759 (w), 1660 (m), 1505 (s), 1205 (s), 1152 (s) cm^{-1}.

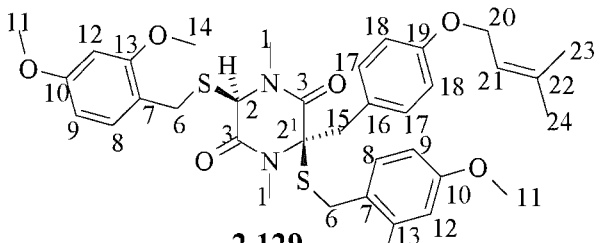
Cis-3,6-Bis-(2,4-Dimethoxybenzylsulfanyl)-3-(4-hydroxybenzyl)-1,4-dimethyl-piperazine-2,5-dione (2.125)



2.125
M.W. 626.80
$C_{32}H_{38}N_2O_7S_2$

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 7.40 (br s, 1H, OH), 7.13 (d, 1H, *J* 8.6 Hz, Ar-H), 7.06 (d, 1H, *J* 8.4 Hz, Ar-H), 6.88 (d, 2H, *J* 8.2 Hz, Ar-H), 6.66 (d, 2H, *J* 8.2 Hz, Ar-H), 6.42-6.35 (m, 4H, Ar-H), 4.25 (s, 1H, H2), 3.99 (d, 1H, *J* 13.3 Hz, H6 or H15), 3.85 (d, 2H, *J* 13.3 Hz, H6 or H15), 3.83-3.74 (m, 14H, H6 or H15 & H11 & H14), 3.45 (d, 1H, *J* 14.1 Hz, H6 or H15), 3.30 (s, 3H, H1), 3.03 (d, 1H, *J* 14.1 Hz, H6 or H15), 2.61 (s, 3H, H1); **^{13}C NMR** (67.93 MHz) δ_C 166.6, 164.1 (C3, 2 x Cq), 160.6, 160.7 (C10, 2 x Cq), 158.5, 158.4 (C13, 2 x Cq), 156.1 (C19, Cq), 131.2, 130.7 (C8 & C17 4 x CH), 125.3 (C16, Cq), 117.6, 116.4 (C7, 2 x Cq), 115.5 (C18, 2 x CH), 104.1, 103.5 (C9, 2 x CH), 99.0, 98.8 (C12, 2 x CH), 76.7 ($C2^1$, Cq), 62.6 (C2, CH), 55.4 (C14, 2 x CH_3), 55.2 (C11, 2 x CH_3), 42.0 (C15, CH_2), 32.9 (C1, CH_3), 32.6 (C6, CH_2), 30.6 (C1, CH_3), 29.4 (C6, CH_2); **LCMS ESI+** *m/z* $[M+H]^+$ 613.2 peak at 7.00 min; **FTIR** ν_{max} (neat, cm^{-1}) 3299 (br w), 2939 (w), 1644 (m), 1609 (s), 1505 (s), 1467 (s), 1236 (m) cm^{-1}.

***Cis*-3,6-Bis-(2,4-Dimethoxy-benzylsulfanyl)-1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]piperazine-2,5-dione (2.129)**



2.129
M.W. 680.89
$C_{36}H_{44}N_2O_7S_2$

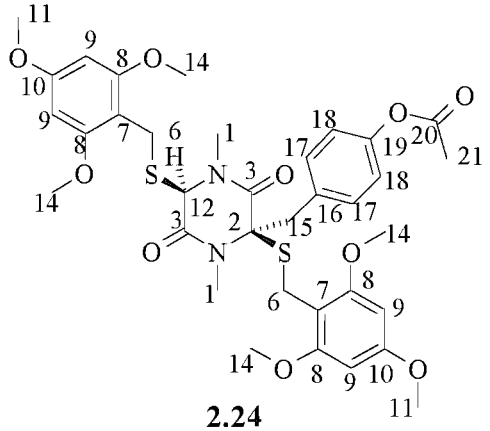
Sodium hydride (60% oil disp, 5.1 mg, 0.15 mmol) was added to a stirring solution of (**2.125**) (90 mg, 0.16 mmol) in DMF (3 mL) at -10 °C. The resultant suspension was stirred at -10 °C for 30 minutes. 3,3-Dimethylallyl bromide (20μL, 0.17 mmol) was added at -10 °C. The resultant suspension was allowed to warm and was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (10 ml) and 1 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 10 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.129**) as a pale yellow gum (41 mg, 41%).

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 7.13 (d, 2H, *J* 8.2 Hz, Ar-H), 6.95 (d, 2H, *J* 8.6 Hz, Ar-H), 6.75 (d, 2H, *J* 8.4 Hz, Ar-H), 6.42-6.37 (m, 4H, Ar-H), 5.45 (t, 1H, 6.7 Hz, H21), 4.44 (d, 2H, *J* 6.7 Hz, H20), 4.26 (s, 1H, H2), 3.99 (d, 1H, *J* 13.3 Hz, H6 or H15), 3.87 (d, 2H, *J* 13.3 Hz, H6 and H15), 3.83-3.74 (m, 14H, H6 or H15 & H11 & H14), 3.53 (d, 1H, *J* 14.1 Hz, H6 or H15), 3.25 (s, 3H, H1), 3.03 (d, 1H, *J* 14.1 Hz, H6 or H15), 2.64 (s, 3H, H1), 1.78 (s, 3H, H23 or H24), 1.72 (s, 3H, H23 or H24); **^{13}C NMR** (67.93 MHz) δ_C 166.0, 164.1 (C3, 2 x Cq), 160.6 (C10, Cq), 158.5 (C13, Cq), 158.2 (C19, Cq), 138.2 (C22, Cq), 131.3, 131.2 (C8, 2 x CH), 130.7, (C17, 2 x CH), 126.3 (C16, Cq), 119.6 (C21, CH), 117.6, 116.4 (C7, 2 x Cq), 114.6 (C18, 2 x CH), 104.1, 103.7 (C9, 2 x CH), 99.1, 98.6 (C12, 2 x CH), 75.4 ($C2^1$, Cq), 64.7 (C20, CH_2), 62.7 (C2, CH), 55.6 (C14, 2 x CH_3), 55.4 (C11, 2 x CH_3), 42.0 (C15, CH_2), 32.9 (C1, CH_3), 32.6 (C6, CH_2), 30.3 (C1, CH_3), 29.5 (C6, CH_2), 25.9, 18.3 (C24 & C23, 2 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 681.28 peak at 11.48 min; **FTIR** ν_{max} (neat, cm^{-1}) 2934 (w), 1660 (s), 1608 (s), 1506 (s), 1384 (m) 1206 (s) cm^{-1}; ***Anal.*** Calcd for $C_{36}H_{44}N_2O_7S_2$: C, 63.51; H, 6.51; N, 4.11 S, 9.42. Found: C, 63.15; H, 6.25; N, 4.18; S, 9.12.

***Cis*-Acetic acid 4-[1,4-dimethyl-3,6-dioxo-2,5-bis-(2,4,6-trimethoxybenzylsulfanyl)-piperazin-2-ylmethyl]phenyl ester (2.124) and 3-(4-Hydroxybenzyl)-1,4-dimethyl-3,6-bis-(2,4,6-trimethoxybenzylsulfanyl)piperazine-2,5-dione (2.126)**

To a solution of solution of 2.0 M LDA (600 µl, 1.19 mmol) in THF (5 ml) at -78 °C was added a solution of (**2.122**) (225 mg, 0.39 mmol) in THF (10 mL) and the reaction mixture stirred at -78 °C for 25 minutes. To the resulting red solution was added dropwise a solution of (**2.88**) (136.5 mg, 0.59 mmol) in THF (2 mL) over a period of 5 minutes keeping the internal reaction temperature < -70 °C. Upon completion of the addition the reaction mixture was stirred at -78 °C for 2 h. The solution was then allowed to warm to < -10 °C for 2 h. A solution of acetic acid (39 mg, 0.59 mmol) in THF (1 mL) was added and the resulting reaction mixture allowed to warm to room temperature. The reaction solution was quenched at room temperature with the addition of 0.3 M $KHSO_4$ (aq) solution (10 mL) and EtOAc (30 mL).The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% to 70% EtOAc/Hexane) to give (**2.124**) and (**2.126**) as colourless gums (53 mg, 21% and 74 mg, 31%).

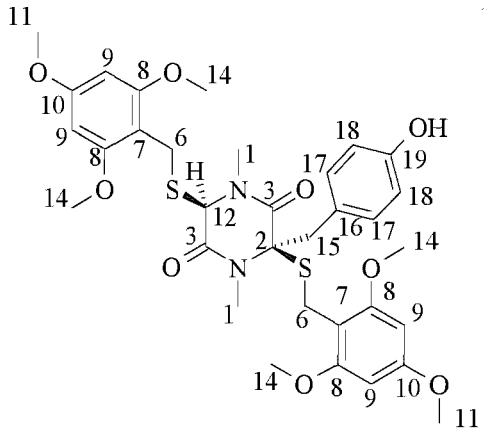
***Cis*-Acetic acid 4-[1,4-dimethyl-3,6-dioxo-2,5-bis-(2,4,6-trimethoxybenzylsulfanyl)-piperazin-2-ylmethyl]phenyl ester (2.124)**



2.24
M.W. 714.86
$C_{35}H_{42}N_2O_{10}S_2$

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 7.07 (d, 2H, *J* 8.6 Hz, Ar-H), 6.94 (d, 2H, *J* 8.6 Hz, Ar-H), 6.07 & 6.08 (s, 4H, H9), 4.58 (s, 1H, H2), 4.14-4.08 (m, 2H, H6 or H15), 3.90-3.69 (m, 20H, H6 & H15 & H11 & H14), 3.31 (s, 3H, H1), 3.21-3.07 (m, 2H, H6 or H15), 2.66 (s, 3H, H1), 2.25 (s, 3H, H21); **^{13}C NMR** (67.93 MHz) δ_C 169.2 (C20, Cq), 166.4, 165.6 (C3, 2 x Cq), 160.8, 160.5 (C10, 2 x Cq), 158.7, 158.5 (C8, 4 x Cq), 149.9 (C19, Cq), 132.4 (C16, Cq), 130.7 (C17, 2 x CH), 115.9 (C18, 2 x CH), 107.8, 104.8 (C7, 2 x Cq), 90.6, 90.4 (C9, 4 x CH), 75.4 (C2, Cq), 64.7 (C12, CH), 55.8, 55.7 (C14, 4 x CH_3), 55.4 (C11, 2 x CH_3), 42.0 (C15, CH_2), 32.6, 30.6 (C1, 2 x CH_3), 26.6, 22.8 (C6, 2 x CH_2), 21.2 (C21, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 715.08, $[M+Na]^+$ 737.06 peak at 9.45min; **FTIR** ν_{max} (neat, cm^{-1}) 2938 (w), 1759 (w), 1663 (m), 1591 (s), 1453 (m), 1203 (s) cm^{-1}.

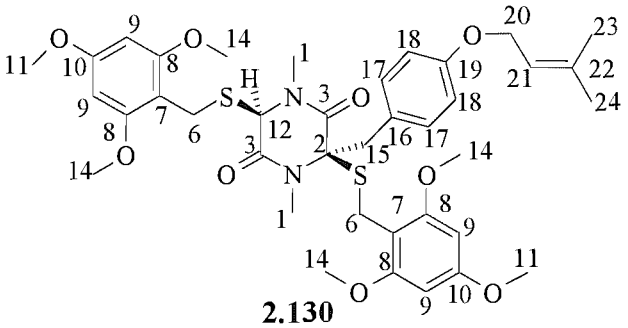
***Cis*-3-(4-Hydroxybenzyl)-1,4-dimethyl-3,6-bis-(2,4,6-trimethoxybenzylsulfanyl)-piperazine-2,5-dione (2.126)**



2.126
M.W. 672.82
$C_{33}H_{40}N_2O_9S_2$

^{1}H NMR (270 MHz, $CDCl_3$, OH not observed) δ_H 6.90 (d, 2H, *J* 8.4 Hz, H17), 6.64 (d, 2H, *J* 8.6 Hz, H18), 6.04 (s, 4H, H9), 4.49 (s, 1H, H2), 4.12-4.08 (m, 2H, H6 or H15), 3.90-3.69 (m, 20H, H6 & H15 & H11 & H14), 3.42 (d, 1H, *J* 13.6 Hz, H15 or H6), 3.35 (s, 3H, H1), 3.00 (d, 1H, *J* 13.6 Hz, H15 or H6), 2.67 (s, 3H, H1); **^{13}C NMR** (67.93 MHz) δ_C 166.7, 164.2 (C3, 2 x Cq), 160.5 (C10, Cq), 158.7, 158.5 (C8, 4 x Cq), 156.1 (C19, Cq), 130.8 (C17, 2 x CH), 125.5 (C16, Cq), 115.4 (C18, 2 x CH), 107.8, 104.8 (C7, 2 x Cq), 90.6, 90.5 (C9, 4 x CH), 75.4 (C2, Cq), 64.8 (C12, CH), 55.8, 55.7 (C14, 4 x CH_3), 55.4 (C11, 2 x CH_3), 42.0 (C15, CH_2), 32.8, 30.6 (C1, 2 x CH_3), 26.3, 22.7 (C6, 2 x CH_2); **LCMS ESI+** *m/z* $[M+H]^+$ 673.1, $[M+Na]^+$ 695.1 peak at 8.56 min; **FTIR** ν_{max} (neat, cm^{-1}) 3295 (br w), 2937 (w), 1591 (s), 1453 (m), 1147 (s) cm^{-1}.

***Cis*-1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3,6-bis-(2,4,6-trimethoxybenzylsulfanyl)piperazine-2,5-dione (2.130)**

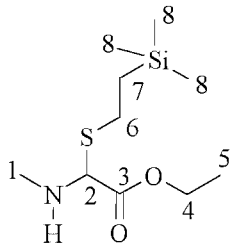


2.130
M.W. 740.94
$C_{38}H_{48}N_2O_9S_2$

Sodium hydride (60% oil disp, 7.3 mg, 0.22 mmol) was added to a stirring solution of (**2.126**) (140 mg, 0.21 mmol) in DMF (3 mL) at -10 °C. The resultant suspension was stirred at -10 °C for 30 minutes. 3,3-Dimethylallyl bromide (28.7µL, 0.25 mmol) was added at -10 °C. The resultant suspension was allowed to warm and was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (10 mL) and 1 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 10 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% EtOAc/Hexane) to give (**2.130**) as a pale yellow glass (70mg, 45%).

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 6.96 (d, 2H, *J* 8.6 Hz, H17), 6.75 (d, 2H, *J* 8.4 Hz, H18), 6.06 & 6.05 (s, 4H, H9), 5.45 (t, 1H, *J* 6.7 Hz, H21), 4.50 (s, 1H, H12), 4.44 (d, 2H, *J* 6.7 Hz, H20), 4.09 (d, 1H, *J* 12.9 Hz, H6 or H15), 3.88-3.83 (m, 2H, H6 or H15), 3.90-3.69 (m, 20H, H6 & H15 & H11 & H14), 3.42 (d, 1H, *J* 14.1 Hz, H15 or H6), 3.30 (s, 3H, H1), 3.00 (d, 1H, *J* 13.6 Hz, H15 or H6), 2.69 (s, 3H, H1), 1.77 (s, 3H, H23 or H24), 1.72 (s, 3H, H23 or H24); **^{13}C NMR** (67.93 MHz) δ_C 166.2, 164.3 (C3, 2 x Cq), 160.8, 160.6 (C10, 2 x Cq), 158.8 (C8, 4 x Cq), 158.1 (C19, Cq), 138.3 (C22, Cq), 130.8 (C17, 4 x CH), 126.6 (C16, Cq), 119.6 (C21, CH), 114.8, 114.5 (C18, 4 x CH), 107.8, 104.9 (C7, 2 x Cq), 90.6, 90.5 (C9, 4 x CH), 75.8 (C2, Cq), 64.9 (C12, CH), 55.8, 55.7, 55.4 (C14 & C11, 6 x CH_3), 42.0 (C15, CH_2), 32.8, 30.4 (C1, 2 x CH_3), 26.4 (C6, CH_2), 25.9 (C24 or C23, CH_3), 22.7 (C6, CH_2), 18.3 (C24 or C23, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 741.10, $[M+Na]^+$ 763.07 peak at 10.80 min; **FTIR** ν_{max} (neat, cm^{-1}) 2935 (w), 1661 (s), 1592 (s), 1454 (m), 1382 (m), 1147 (s) cm^{-1}.

Methylamino-(2-trimethylsilanylethylsulfanyl)acetic acid ethyl ester (2.132)

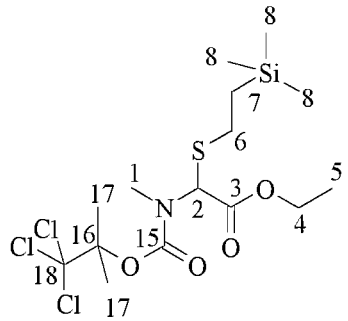


2.132
M.W. 249.45
$C_{10}H_{23}NO_2SSi$

A 2.0 M methylamine solution in THF (15.82 mL, 31.64 mmol) was added to a 50% ethyl glyoxalate, toluene solution (6.33 mL, 31.64 mmol) in toluene (5 mL). The resultant yellow solution was stirred at room temperature for 15 min. 2-(Trimethylsilyl)ethanethiol (5 mL, 31.64 mmol) was added and the resulting cloudy solution was stirred at room temperature for 18 h. The crude reaction mixture was concentrated, pre-adsorbed onto silica and purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.132**) as a pale yellow oil (2.68 g, 35%).

^{1}H NMR (270 MHz, $CDCl_3$) δ_H 4.25 (s, 1H, H2), 4.18 (q, 2H, *J* 7.2 Hz, H4), 2.57-2.51 (m, 2H, H6), 2.46 (s, 3H, H1), 1.27 (t, 3H, *J* 7.2 Hz, H5), 0.86-0.73 (m, 2H, H7), 0.00 (s, 9H, H8); **^{13}C NMR** (67.93 MHz) δ_C 170.2 (C3, Cq), 66.3 (C2, CH), 61.2 (C4, CH_2), 32.4 (C1, CH_3), 24.5 (C6, CH_2), 17.1 (C7, CH_2), 14.2 (C5, CH_3), -1.7 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 250.17 peak at 4.86 min; **FTIR** ν_{max} (neat, cm^{-1}) 2952 (w), 1728 (s), 1247 (s), 1179 (s) cm^{-1}; ***Anal.*** Calcd for $C_{10}H_{23}NO_2SSi$: C, 48.15; H, 9.29; N, 5.61 S, 12.85. Found: C, 48.53; H, 9.09; N, 6.03; S, 12.35.

Methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-(2-trimethylsilanyl ethylsulfanyl)acetic acid ethyl ester (2.133)

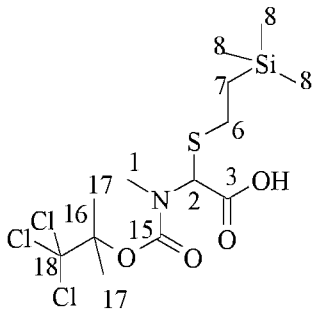


2.133
M.W. 452.90
$C_{15}H_{28}Cl_3NO_4SSi$

2,2,2-Trichloro-1,1-dimethylethyl chloroformate (2.6 g, 10.4 mmol) was added portion-wise to a solution of (**2.132**) (2.6 g, 11.0 mmol), and triethylamine (1.8 ml, 12.0 mmol) in dichloromethane (30 mL) and the reaction mixture stirred at room temperature for 18 h. The resulting solution was concentrated and the residue purified by column chromatography (SiO_2, 20% EtOAc/Hexane) to give (**2.133**) as a colourless gum (4.3 g, 91%).

^{1}H NMR (270 MHz, $CDCl_3$, rotamers observed) δ_H 6.12 & 6.02 (s, 1H, H2), 4.30-4.15 (m, 2H, H4), 2.99 & 2.97 (s, 3H, H1), 2.73-2.45 (m, 2H, H6), 1.92 & 1.91 (s, 6H, H17), 1.28 & 1.12 (t, 3H, *J* 7.2 Hz, H5), 0.86-0.73 (m, 2H, H7), -0.01 & -0.02 (s, 9H, H8); **^{13}C NMR** (67.93 MHz) δ_C 168.1 (C3, Cq), 154.3 (C15, Cq), 106.6 (C18, Cq), 89.2 (C16, Cq), 62.2 (C2, CH), 62.1 (C4, CH_2), 30.6 (C1, CH_3), 27.3 (C6, CH_2), 21.8 (C17, 2 x CH_3), 17.1 (C7, CH_2), 14.2 (C5, CH_3), -1.7 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 475.95, $[M+Na+CH_3CN]^+$ 516.93 peak at 12.9 min; **FTIR** ν_{max} (neat, cm^{-1}) 2953 (w), 1743 (m), 1704 (s), 1384 (m), 1158 (s) cm^{-1}; ***Anal.*** Calcd for $C_{15}H_{28}Cl_3NO_4SSi$: C, 39.78; H, 6.23; N, 3.09 S, 7.08. Found: C, 39.56; H, 6.06; N, 3.12; S, 6.49.

[Methyl-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-amino]-(2-trimethylsilanyl-ethylsulfanyl)acetic acid (2.134)

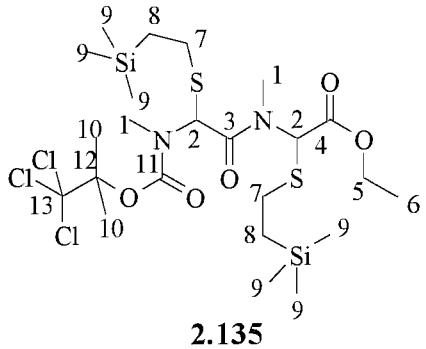


2.134
M.W. 424.85
$C_{13}H_{24}Cl_3NO_4SSi$

Lithium hydroxide (444.0 mg, 10.5 mmol) was added to a solution of (**2.133**) (4.0 g, 8.8 mmol) in 1,4-dioxan (20 mL), water (1 mL) and the reaction mixture stirred at room temperature for 4 h. EtOAc (15 mL) and saturated citric acid solution (1 mL) was added. The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed well with water (2 x 10 mL), dried ($MgSO_4$), filtered, and concentrated to give (**2.134**) as a pale yellow solid (2.6 g, 70%).

Mp 88.7-91 °C; **^{1}H NMR** (270 MHz, $CDCl_3$, rotamers observed) δ_H 6.26 (br s, 1H, OH), 6.14 & 6.05 (s, 1H, H2), 3.00 & 2.98 (s, 3H, H1), 2.65-2.55 (m, 2H, H6), 1.92 & 1.91 (s, 6H, H17), 0.98-0.83 (m, 2H, H7), 0.03 & 0.01 (s, 9H, H8); **^{13}C NMR** (67.93 MHz) δ_C 168.1 (C3, Cq), 154.3 (C15, Cq), 106.3 (C18, Cq), 89.2 (C16, Cq), 62.2 (C2, CH), 30.6 (C1, CH_3), 27.3 (C6, CH_2), 21.8 (C17, 2 x CH_3), 17.1 (C7, CH_2), -1.7 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[M+Na+CH_3CN]^+$ 488.07, $[M-Cl+Na]^+$ 413.02 peak at 9.13 min; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1701 (s), 1379 (m), 1247 (m), 1162 (m) cm^{-1}; ***Anal.*** Calcd for $C_{13}H_{24}Cl_3NO_4SSi$: C, 36.75; H, 5.69; N, 3.30; S, 7.55. Found: C, 36.85; H, 5.73; N, 3.33; S, 7.71.

{Methyl-[2-[methyl-(2,2,2-trichloro-1,1-dimethyl-ethoxycarbonyl)-amino]-2-(2-trimethylsilanylethylsulfanyl)-acetyl]-amino}-(2-trimethylsilanylethylsulfanyl) acetic acid ethyl ester (2.135)

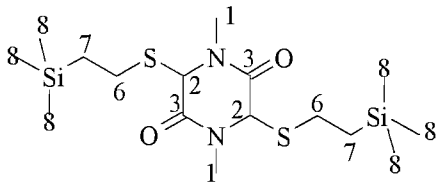


2.135
M.W. 656.28
$C_{23}H_{45}Cl_3N_2O_5S_2Si_2$

To a stirring solution of (**2.134**) (2.2 g, 5.2 mmol) and NMM (628mg, 6.2 mmol) in THF (10 mL) at -10 °C was added isobutyl chloroformate (778 mg, 5.69 mmol) in THF (5 mL) dropwise over 5 minutes. The resulting suspension was then stirred at -10 °C for 40 minutes. The reaction mixture was filtered and the filtrate added to a solution of (**2.132**) (1.6 g, 6.2 mmol) in THF (20 mL) at 0 °C. The resulting reaction mixture was stirred at room temperature for 4 h, then concentrated and partitioned between EtOAc (50 mL) and 1 M $KHSO_4$ (aq) solution (50 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.135**) as a colourless gum (800 mg, 24%).

^{1}H NMR (270 MHz, $CDCl_3$, rotamers observed) δ_H 6.38-6.10 (m, 2H, H2), 4.12-4.5 (m, 2H, H5), 3.13-2.85 (m, 6H, H1), 2.49-2.35 (m, 4H, H7), 1.89 & 1.92 (s, 6H, H10), 1.26 & 1.15 (t, 3H, *J* 7.2 Hz, H6), 0.86-0.73 (m, 4H, H8), -0.01 & -0.07 (s, 18H, H9); **^{13}C NMR** (67.93 MHz) δ_C 168.9, (C4, Cq), 167.7 (C3, Cq), 154.3 (C11, Cq), 106.1 (C13, Cq), 89.2 (C12, Cq), 62.2 (C2, 2 x CH), 62.1 (C5, CH_2), 30.6 (C1, 2 x CH_3), 27.3 (C7, 2 x CH_2), 21.8 (C10, 2 x CH_3), 17.1 (C8, 2 x CH_2), 14.2 (C6, CH_3), -1.7 (C9, 6 x CH_3); **LCMS ESI+** *m/z* $[M+Na+CH_3CN]^+$ 721.05 peak at 11.05 min; **FTIR** ν_{max} (neat, cm^{-1}) 2953 (w), 1742 (m), 1701 (m), 1666 (m), 1142 (s) cm^{-1}; ***Anal.*** Calcd for $C_{23}H_{45}Cl_3N_2O_5S_2Si_2$: C, 42.09; H, 6.91; N, 4.27; S, 9.77. Found: C, 42.49; H, 7.06; N, 4.32; S, 9.02.

1,4-Dimethyl-3,6-bis-(2-trimethylsilanylethylsulfanyl)piperazine-2,5-dione (2.136)



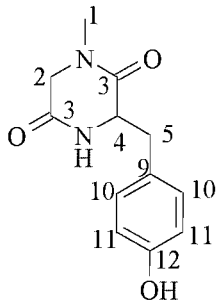
2.136
M.W. 406.76
$C_{16}H_{34}N_2O_2S_2Si_2$

Zinc dust (831 mg, 12.8 mmol), was added portionwise to a solution of (**2.135**) (420 mg, 0.6 mmol), in 10% acetic acid, isopropyl alcohol (10 mL) over 1 h. The resultant suspension was stirred at room temperature for 18 h. The zinc was filtered off and washed with EtOAc (3 x 15 mL). The organics were combined and concentrated. The resulting residue was purified was purified by column chromatography (SiO_2, 80% EtOAc/Hexane) to give (**2.136**) as a waxy solid (35 mg, 14%).

Alternative method: To a stirred suspension of sarcosine anhydride (1 g, 7.0 mmol), in carbon tetrachloride (68 mL), was added AIBN (230 mg, 1.41 mmol) and NBS (2.63 g, 14.8 mmol). The suspension was brought to reflux for 3 h, cooled to room temperature, filtered, and concentrated. The resultant crude dibromide was dissolved in THF (100 mL), a solution of 2-(trimethylsilyl)ethanethiol (2.3 mL, 14.7 mmol) and triethylamine (2.1 mL, 14.7 mmol) in THF (20 mL) was carefully added over 10 min. The resultant red solution was stirred at room temperature for 18 h, then concentrated and partitioned between EtOAc (50 mL) and 0.3 M $KHSO_4$ (aq) solution (20 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 30 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.136**) as a colourless gum (900 mg, 31%).

Mp 60.4 -62.4 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 4.59 (s, 2H, H2), 3.04 (s, 6H, H1), 2.85-2.80 (m, 4H, H6), 0.97-0.81 (m, 4H, H7), -0.00 (s, 18H, H8); **^{13}C NMR** (100 MHz) δ_C 166.2 (C3, 2 x Cq), 65.7 (C2, 2 x CH), 34.0 (C1, 2 x CH_3), 31.4 (C6, 2 x CH_2), 18.6 (C7, 2 x CH_2), -0.01 (C8, 6 x CH_3); **LCMS ESI+** *m/z* $[2M+H]^+$ 813.41, $[M+H]^+$ 407.21 peak at 10.54 min; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1673 (s), 1478 (w), 1245 (m) cm^{-1}.

3-(4-Hydroxybenzyl)-1-methylpiperazine-2,5-dione (2.140)

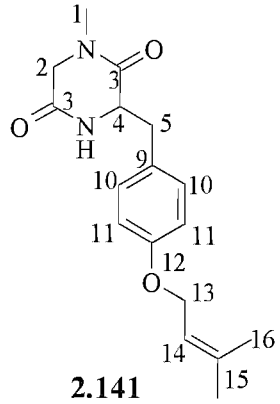


2.140
M.W. 234.26
$C_{12}H_{14}N_2O_3$

Formic acid (20 ml) was added to Boc-Sar-D,L-TyrOMe (1.6 g, 4.4 mmol) and the reaction mixture stirred at room temperature for 1 h. The solution was concentrated and the residue azeotroped with toluene (2 x 10 mL). The resultant white solid was dissolved in a mixture of *sec*-butanol (40 mL), toluene (10 mL) and stirred at reflux for 54 h. The reaction mixture was concentrated. The resultant crude residue was treated with acetonitrile (20 mL) to give (**2.140**) as an off-white solid (840 mg, 83%).

Mp 226.5-228.1 °C; **^{1}H NMR** (400 MHz, DMSO-d6) δ_H 9.33 (s, 1H, OH), 8.20 (br s, 1H, NH), 6.84 (d, 2H, *J* 8.4 Hz, H10), 6.64 (d, 2H, *J* 8.4 Hz, H11), 4.05-4.04 (m, 1H, H4), 3.43 (d, 1H, *J* 17.2 Hz, H2), 2.98 (1H, dd, *J* 13.5, 3.8 Hz, H5), 2.70 (1H, dd, *J* 13.5, 4.5 Hz, H5), 2.62 (s, 3H, H1), 2.52 (d, 1H, *J* 17.2 Hz, H2); **^{13}C NMR** (100 MHz) δ_C 165.5, 164.9 (C3, 2 x Cq), 156.3 (C12, Cq), 130.9 (C10, 2 x CH), 125.4 (C9, Cq), 114.9 (C11, 2 x CH), 55.7 (C4, CH), 50.2 (C2, CH_2), 38.9 (C5, CH_2), 32.6 (C1, CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 235.25, $[M+Na+CH_3CN]^+$ 276.26 peak at 0.86 min; **FTIR** ν_{max} (neat, cm^{-1}) 3276 (br), 1650 (s), 1513 (m), 1336 (m), 1213 (s) cm^{-1}.

1-Methyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]piperazine-2,5-dione (2.141)

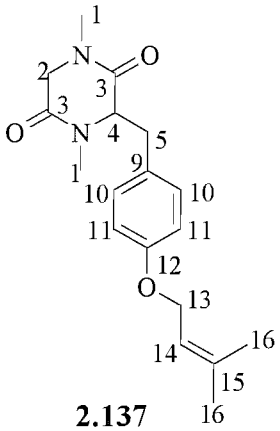


2.141
M.W. 302.38
$C_{17}H_{22}N_2O_3$

To a solution of (**2.140**) (780 mg, 3.3 mmol) in DMF (3 mL) were added with stirring NaH (60% oil disp, 123.1 mg, 3.7 mmol) at -10 °C for 30 min and then 3,3-dimethylallyl bromide (384 μL, 3.3 mmol). After stirring at -10 °C for 30 min and then room temperature for 18 h, the reaction mixture was poured into ice water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with brine (2 x 5 mL), dried ($MgSO_4$) filtered, and concentrated to give crude crystals, which were recrystallised from a mixture of ethyl acetate and 60-80 pet ether to give (**2.141**) as an off-white solid (900 mg , 89%).

Mp 120.4-120.9 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.08 (d, 2H, *J* 8.6 Hz, H10), 6.96 (br s, 1H, NH), 6.87 (d, 2H, *J* 8.6 Hz, H11), 5.47 (t, 1H, *J* 6.7 Hz, H14), 4.49 (d, 2H, *J* 6.7 Hz, H13), 4.26-4.25 (m, 1H, H4), 3.52 (d, 1H, *J* 17.5 Hz, H2), 3.14 (dd, *J* 13.8, 5.5 Hz, H5), 3.02 (dd, *J* 13.8, 4.0 Hz, H5), 2.93 (d, 1H, *J* 17.5 Hz, H2), 2.82 (s, 3H, H1), 1.79 (s, 3H, H16), 1.74 (s, 3H, H16); **^{13}C NMR** (100 MHz) δ_C 165.9, 164.5 (C3, 2 x Cq), 156.4 (C12, Cq), 138.4 (C15, Cq), 131.0 (C10, 2 x CH), 126.6 (C9, Cq), 119.4 (C14, CH), 114.9 (C11, 2 x CH), 64.8 (C13, CH_2), 56.6 (C4, CH), 50.9 (C2, CH_2), 40.1 (C5, CH_2), 33.6 (C1, CH_3), 25.8, 18.2 (C16, 2 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 303.26, $[M+H+CH_3CN]^+$ 344.27, $[2M+H]^+$ 605.34 peak at 4.99 min; **FTIR** ν_{max} (neat, cm^{-1}) 2917 (w), 1656 (s), 1605 (m), 1507 (s), 1439 (m), 1336 (m), 1219 (s) cm^{-1}.

1,4-Dimethyl-3-[4-(3-methyl-but-2-enyloxy)-benzyl]piperazine-2,5-dione (2.137)

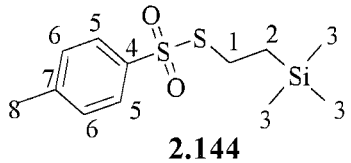


2.137
M.W. 316.40
$C_{18}H_{24}N_2O_3$

To a solution of (**2.141**) (750 mg, 2.5 mmol) in DMF (3 mL) were added with stirring NaH (60% oil disp, 91.6 mg, 2.8 mmol) at -10 °C for 30 min and then iodomethane (185 µL, 3.0 mmol). After stirring at -10 °C for 30 min and then room temperature for 18 h, the reaction mixture was poured into ice water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined extracts were washed with brine (2 x 5 mL), dried ($MgSO_4$) filtered, and concentrated to give crude crystals, which were recrystallised from ethyl acetate and 60-80 pet ether to give (**2.137**) as an off-white solid (670 mg , 86%).

Mp 115.2-116.6 °C (lit 121-123 °C);[94] **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 6.94 (d, 2H, *J* 8.6 Hz, H10), 6.82 (d, 2H, *J* 8.6 Hz, H11), 5.47 (m, 1H, *J* 6.7 Hz, H14), 4.49 (d, 2H, *J* 6.7 Hz, H13), 4.14 (apparent t, 1H, *J* 3.6 Hz, H4), 3.32 (d, 1H, *J* 17.2 Hz, H2), 3.21 (dd, 1H, *J* 14.0 Hz, 3.3 Hz, H5), 3.04 (dd, 1H, *J* 14.0, 4.4 Hz, H5), 3.05 (s, 3H, H1), 2.72 (s, 3H, H1), 2.36 (d, 1H, *J* 17.2 Hz, H2), 1.79 (s, 3H, H16), 1.74 (s, 3H, H16); **^{13}C NMR** (100 MHz) δ_C 165.8, 164.4 (C3, 2 x Cq), 158.6 (C12, Cq), 138.4 (C15, Cq), 130.8 (C10, 2 x CH), 126.6 (C9, Cq), 119.4 (C14, CH), 115.0 (C11, 2 x CH), 64.8 (C13, CH_2), 56.7 (C4, CH), 50.9 (C2, CH_2), 36.19 (C5, CH_2), 33.0, 32.2 (C1, 2 x CH_3), 25.8, 18.3 (C16, 2 x CH_3); **LCMS ESI+** *m/z* $[M+H]^+$ 317.25, $[M+H+CH_3CN]^+$ 358.29, $[2M+H]^+$ 633.39 peak at 5.29 min; **FTIR** ν_{max} (neat, cm^{-1}) 2918 (w), 1658 (s), 1510 (m), 1441 (w), 1234 (s), 1187 (m) cm^{-1}. Data consistent with literature.[94]

Toluene-4-thiosulfonic acid S-(2-trimethylsilanylethyl) ester (2.144)



2.144
M.W. 288.51
$C_{12}H_{20}O_2S_2Si$

2-(Trimethylsilyl)bromoethane (3 g, 0.02 mol), was added dropwise to a solution of potassium thiotosylate (4.48 g, 0.02 mol), in acetone (20 mL) at room temperature over 10 min. The resultant solution was stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (100 mL) and water (20 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 15 mL). The organics were combined and washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 2% EtOAc/Hexane) to give (**2.144**) as a colourless oil (2.2 g, 48%).

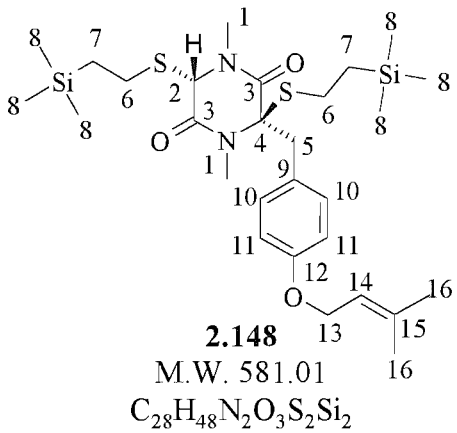
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.78 (d, 2H, *J* 8.2 Hz, Ar-H), 7.33 (d, 2H, *J* 8.2 Hz, Ar-H), 3.03-2.98 (m, 2H, H1), 2.43 (s, 3H, H8), 0.87-0.83 (m, 2H, H2), -0.02 (s, 9H, H3); **^{13}C NMR** (100 MHz) δ_C 144.8, 142.2 (C4 & C7, 2 x Cq), 130.0, 127.1 (C5 & C6, 4 x CH), 32.7 (C1, CH_2), 21.8 (C8, CH_3), 16.6 (C2, CH_2), -1.73 (C3, 3 x CH_3); **LCMS ESI+** *m/z* $[2M+Na]^+$ 599.0 at 9.88 min; **FTIR** ν_{max} (neat, cm^{-1}) 2952 (w), 1736 (w), 1593 (w), 1323 (m), 1138 (s) cm^{-1}. Data consistent with literature.[95]

1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3,6-bis-(2-trimethylsilanyl-ethylsulfanyl)piperazine-2,5-dione (2.148), 1,4-dimethyl-6-[4-(3-methylbut-2-enyloxy)-benzyl]-3,3-bis-(2-trimethylsilanylethylsulfanyl)piperazine-2,5-dione (2.147) 1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-6-(2-trimethylsilanyl-ethylsulfanyl)piperazine-2,5-dione (2.146) and 1,4-dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3-(2-trimethylsilanylethylsulfanyl)piperazine-2,5-dione (2.145)

To a solution of *N*, *N*-diisopropylamine (602 µL, 4.26 mmol) in THF (10 ml) was added a solution of 2.5 M *n*-butyllithuim in hexanes (1.70 mL, 4.26 mmol) at -78 °C for 30 min. A solution of (**2.137**) (450 mg, 1.42 mmol) in THF (5 mL) and HMPA (741 µL, 4.26 mmol) was added to the resulting solution. After stirring for 1 h, (**2.144**) (2.45 g,

8.52 mmol) was added drop by drop at -78 °C for 1 h. The reaction mixture was stirred at -78 °C for a further 3 h. The reaction mixture was treated with the addition of 0.3 M $KHSO_4$ (aq) solution (10 mL) and EtOAc (30 mL) and was stirred at room temperature for 20 min. The organics were separated and the aqueous extracted with further EtOAc (2 x 15 mL). The organics were combined and washed with brine (10 ml), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 10% to 90% EtOAc/Hexane) to give (**2.148,** 67 mg, 8%), (**2.147,** 120mg, 14%), (**2.146,** 112 mg, 18%) and (**2.145,** 125 mg, 20%) as colourless gums.

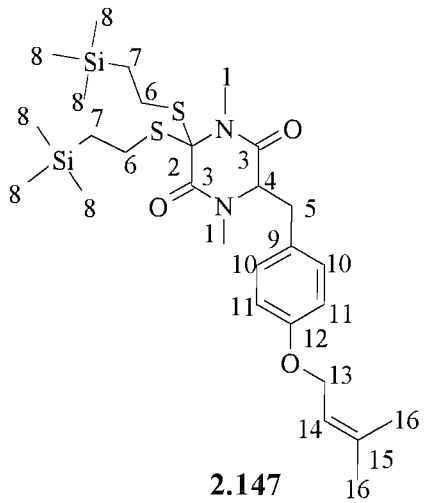
***Trans*-1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3,6-bis-(2-trimethylsilanylethylsulfanyl)piperazine-2,5-dione (2.148)**



2.148
M.W. 581.01
$C_{28}H_{48}N_2O_3S_2Si_2$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.01 (d, 2H, *J* 8.7 Hz, H10), 6.82 (d, 2H, *J* 8.7 Hz, H11), 5.49-5.45 (m, 1H, H14), 4.51 (s, 1H, H2), 4.45 (2H, *J* 6.7 Hz, H13), 3.56 (d, 1H, *J* 14.0 Hz, H5), 3.23 (s, 3H, H1), 3.03 (d, 1H, *J* 14.0 Hz, H5), 3.02 (s, 3H, H1), 2.64-2.51 (m, 2H, H6), 2.47-2.32 (m, 2H, H6), 1.79 (s, 3H, H16), 1.72 (s, 3H, H16), 0.81-0.71 (m, 4H, H7), -0.00 & -0.01 (2 x s, 18H, H8); **^{13}C NMR** (100 MHz) δ_C 165.7, 165.5 (C3, 2 x Cq), 158.7 (C12, Cq), 138.3 (C15, Cq), 131.5 (C10, 2 x CH), 126.3 (C9, Cq), 119.9 (C14, CH), 115.3 (C11, 2 x CH), 76.9 (C4, Cq), 64.9 (C13, CH_2), 64.6 (C2, CH), 41.4 (C5, CH_2), 33.6, 30.9 (C1, 2 x CH_3), 30.2 (C6, CH_2), 26.1 (C16, 1 x CH_3), 25.6 (C6, CH_2), 18.4 (C16, 1 x CH_3), 16.9, 16.0 (C7, 2 x CH_2), -1.5 & -1.6 (C8, 6 x CH_3); **LCMS ESI+** *m/z* $[M+Na+CH_3CN]^+$ 644.65, $[2M+Na]^+$ 1185.01, $[M+Na]^+$ 603.59 peak at 7.69 min (alternative gradient; 10% to 90% 0.1% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O over 6 min, 7 to 11.0 min, 90% to 100% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O, flow rate 1.5 mL/min, using a Chromolith Speedrod RP-18e column); **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1659 (m), 1509 (m), 1379 (m), 1243 (s) cm^{-1}.

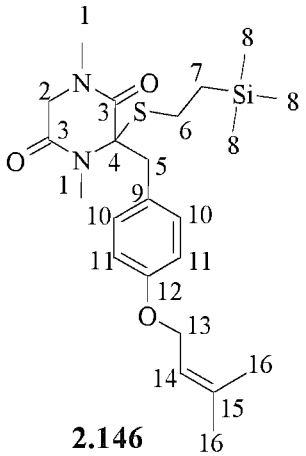
1,4-Dimethyl-6-[4-(3-methylbut-2-enyloxy)-benzyl]-3,3-bis-(2-trimethylsilanyl-ethylsulfanyl)piperazine-2,5-dione (2.147)



2.147
M.W. 581.01
$C_{28}H_{48}N_2O_3S_2Si_2$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.06 (d, 2H, *J* 8.7 Hz, H10), 6.82 (d, 2H, *J* 8.7 Hz, H11), 5.51-5.47 (m, 1H, H14), 4.45 (d, 2H, *J* 6.7 Hz, H13), 4.17-4.15 (m, 1H, H4), 3.37-3.25 (m, 2H, H5), 3.20 (s, 3H, H1), 2.90 (s, 3H, H1), 2.70-2.54 (m, 2H, H6), 2.41-2.37 (m, 2H, H6), 1.78 (s, 3H, H16), 1.72 (s, 3H, H16), 0.83-0.65 (m, 4H, H7), -0.02 & -0.03 (2 x s, 18H, H8); **^{13}C NMR** (100 MHz) δ_C 166.6, 162.9 (C3, 2 x Cq), 158.6 (C12, Cq), 138.4 (C15, Cq), 130.9 (C10, 2 x CH), 127.8 (C9, Cq), 119.8 (C14, CH), 115.2 (C11, 2 x CH), 78.9 (C2, Cq), 64.9 (C13, CH_2), 64.2 (C4, CH), 38.5 (C5, CH_2), 35.5, 37.8 (C1, 2 x CH_3), 28.0, 27.4 (C6, 2 x CH_2), 26.1, 18.5 (C16, 2 x CH_3), 16.9, 15.9 (C7, 2 x CH_2), -1.5 & -1.6 (C8, 6 x CH_3); **LCMS ESI+** *m/z* $[M+Na+CH_3CN]^+$ 644.37, $[2M+Na]^+$ 1183.95, $[M+Na]^+$ 603.59 peak at 8.28 min (alternative gradient; 10% to 90% 0.1% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O over 6 min, 7 to 11.0 min, 90% to 100% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O, flow rate 1.5 mL/min, using a Chromolith Speedrod RP-18e column); **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1660 (s), 1510 (m), 1379 (m), 1244 (s) cm^{-1}.

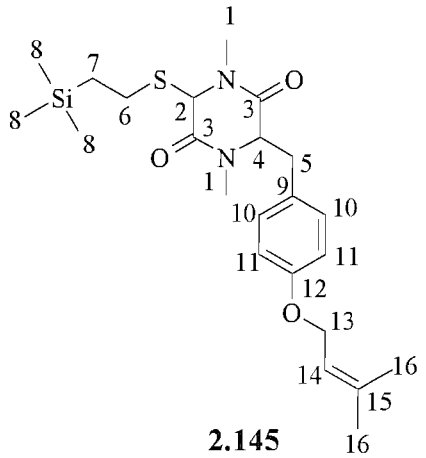
1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3-(2-trimethylsilanylethyl-sulfanyl)piperazine-2,5-dione (2.146)



2.146
M.W. 448.70
$C_{23}H_{36}N_2O_3SSi$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.94 (d, 2H, *J* 8.7 Hz, H10), 6.77 (d, 2H, *J* 8.7 Hz, H11), 5.44-5.41 (m, 1H, H14), 4.43 (d, 2H, *J* 6.7 Hz, H13), 3.61 (d, 1H, *J* 17.6 Hz, H2), 3.41 (d, 1H, *J* 13.9 Hz, H5), 3.22 (s, 3H, H1), 2.97-2.90 (m, 2H, H5 & H2), 2.80 (s, 3H, H1), 2.49-2.34 (m, 2H, H6), 1.76 (s, 3H, H16), 1.71 (s, 3H, H16), 0.83-0.65 (m, 2H, H7), -0.04 (s, 9H, H8); **^{13}C NMR** (100 MHz) δ_C 166.6, 162.9 (C3, 2 x Cq), 158.6 (C12, Cq), 138.4 (C15, Cq), 130.9 (C10, 2 x CH), 127.8 (C9, Cq), 119.6 (C14, CH), 114.8 (C11, 2 x CH), 64.9 (C13, CH_2), 50.9 (C2, CH_2), 41.7 (C5, CH_2), 34.3, 30.3 (C1, 2 x CH_3), 25.6 (C6, CH_2), 26.1, 18.5 (C16, 2 x CH_3), 16.1 (C7, CH_2), -1.6 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[2M+H]^+$ 897.22, $[M+CH_3CN+H]^+$ 490.35, $[M+H]^+$ 449.35 peak at 9.39; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1662 (s), 1508 (m), 1443 (m), 1387 (m), 1241 (s) cm^{-1}.

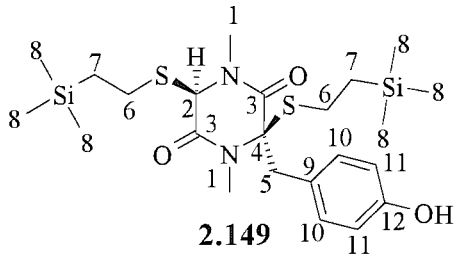
1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-6-(2-trimethylsilanylethyl-sulfanyl)piperazine-2,5-dione (2.145)



2.145
M.W. 448.70
$C_{23}H_{36}N_2O_3SSi$

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.16 (d, 2H, *J* 8.7 Hz, H10), 6.85 (d, 2H, *J* 8.7 Hz, H11), 5.49-5.45 (m, 1H, H14), 4.63 (s, 1H, H2), 4.48 (d, 2H, *J* 6.7 Hz, H13), 4.01-3.98 (m, 1H, H4), 3.22-3.20 (m, 2H, H5), 3.05 (s, 3H, H1), 3.00-2.86 (m, 2H, H6), 2.52 (s, 3H, H1), 1.78 (s, 3H, H16), 1.73 (s, 3H, H16), 1.03-0.85 (m, 2H, H7), 0.05 (s, 9H, H8); **^{13}C NMR** (100 MHz) δ_C 167.3, 165.1 (C3, 2 x Cq), 158.5 (C12, Cq), 138.4 (C15, Cq), 130.7 (C10, 2 x CH), 127.6 (C9, Cq), 119.8 (C14, CH), 115.4 (C11, 2 x CH), 65.7 (C2, CH), 65.0 (C13, CH_2), 64.5 (C4, CH), 39.8 (C5, CH_2), 34.8, 32.4 (C1, 2 x CH_3), 30.3 (C6, CH_2), 26.1, 18.4 (C16, 2 x CH_3), 17.5 (C7, CH_2), -1.6 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[2M+H]^+$ 897.22, $[M+H]^+$ 449.35, $[M+Na]^+$ 490.35 peak at 9.95; **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1665 (s), 1509 (m), 1440 (w), 1397 (w), 1238 (s) cm^{-1}.

***Cis*-3-(4-Hydroxybenzyl)-1,4-dimethyl-3,6-bis-(2-trimethylsilanylethylsulfanyl)-piperazine-2,5-dione (2.149)**

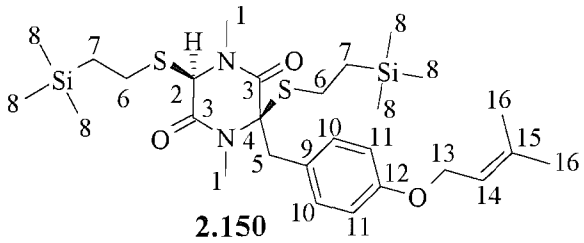


2.149
M.W. 512.89
$C_{23}H_{40}N_2O_3S_2Si_2$

To a solution of *N*, *N*-diisopropylamine (320 µL, 2.21 mmol) in THF (10 mL) was added a solution of 2.5 M *n*-butyllithuim in hexanes (0.88 mL, 2.21 mmol) at -78 °C for 30 min. (**2.136**) (300 mg, 0.74 mmol) in THF (5 mL) was then added dropwise over 10 min. After stirring for 1 h, a solution of (**2.88**) (136.5 mg, 0.59 mmol) in THF (2 mL) was added dropwise, keeping the internal reaction temperature < -70 °C. The reaction mixture was then stirred at -78 °C for a further 4 h. Acetic acid (59 mg, 0.89 mmol) in THF (1 mL) was added and the resulting reaction mixture allowed to warm and stirred at room temperature for 10 min. The reaction mixture was cooled to 0 °C and 1.0 M KOH (aq) solution (10 mL) was added and stirred at 0 °C to room temperature for 20 min, EtOAc (30 ml) and 1 M $KHSO_4$ (aq) solution (20 mL) was added and the organics were separated. The aqueous was extracted with further EtOAc (2 x 10 mL). The organics were then combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane to give (**2.149**) as a colourless gum (149 mg, 40%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.03 (s, 1H, OH), 6.90 (d, 2H, *J* 8.5 Hz, H10), 6.65 (d, 2H, *J* 8.5 Hz, H11), 4.28 (s, 1H, H2), 3.47 (d, 1H, *J* 14.1 Hz, H5), 3.27 (s, 3H, H1), 3.04 (d, 1H, *J* 14.1 Hz, H5), 2.94 (s, 3H, H1), 2.89-2.79 & 2.64-2.60 (m, 4H, H6), 1.04-0.78 (m, 4 H, H7), 0.02 & -0.01 (2 x s, 18H, H8); **^{13}C NMR** (100 MHz) δ_C 166.2, 164.8 (C3, 2 x Cq), 156.2 (C12, Cq), 130.9 (C10, 2 x CH), 125.4 (C9, Cq), 115.6 (C11, 2 x CH), 75.9 (C4, Cq), 63.6 (C2, CH), 42.4 (C5, CH_2), 33.7, 30.8 (C1, 2 x CH_3), 30.4, 27.2 (C6, 2 x CH_2), 17.2, 15.9 (C7, 2 x CH_2), -1.51, (C8, 3 x CH_3), -1.56 (C8, 3 x CH_3); **LCMS ESI+** *m/z* $[M\text{-}S(CH_2)_2Si(CH_3)_3]^+$ 379.47, $[M+Na+CH_3CN]^+$ 576.6, $[M+H]^+$ 513.51 peak at 10.85 min; **FTIR** ν_{max} (neat, cm^{-1}) 3316 (br w), 2950 (w), 1643 (s), 1514 (m), 1245 (s), 1162 (m) cm^{-1}.

***Cis*-1,4-Dimethyl-3-[4-(3-methylbut-2-enyloxy)-benzyl]-3,6-bis-(2-trimethylsilanyl-ethylsulfanyl)piperazine-2,5-dione (2.150),**



2.150

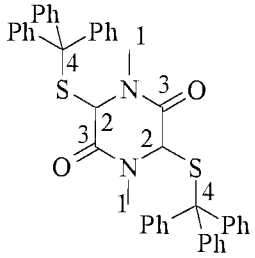
M.W. 581.01

$C_{28}H_{48}N_2O_3S_2Si_2$

Sodium hydride (60% oil disp, 8.4 mg, 0.25 mmol) was added to a stirring solution of (**2.149**) (120 mg, 0.23 mmol) in DMF (3 mL) at -10 °C. The resultant suspension was stirred at -10 °C for 30 minutes. 3,3-dimethylallyl bromide (30.5 μL, 0.26 mmol) was added at -10 °C. The resultant suspension was allowed to warm and was stirred at room temperature for 4 h, concentrated and partitioned between EtOAc (10 mL) and 1 M $KHSO_4$ (aq) solution (10 mL). The organics were separated and the aqueous extracted with EtOAc (2 x 10 mL). The organics were combined and washed with brine (10 mL), dried over ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 30% EtOAc/Hexane) to give (**2.150**) as a pale yellow gum (88 mg, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.97 (d, 2H, *J* 8.4 Hz, H10), 6.76 (d, 2H, *J* 8.4 Hz, H11), 5.48-5.44 (m, 1H, H14), 4.44 (d, 1H, *J* 6.8 Hz, H13), 4.31 (s, 1H, H2), 3.53 (d, 1H, *J* 14.1 Hz, H5), 3.23 (s, 3H, H1), 3.05 (d, 1H, *J* 14.1 Hz, H5), 2.96 (s, 3H, H1), 2.89-2.83 & 2.65-2.61 (m, 4H, H6), 1.79 & 1.73 (s, 6H, H16), 0.91-0.78 (m, 4 H, H7), 0.02 & 0.01 (2 x s, 18H, H8); **^{13}C NMR** (100 MHz) δ_C 165.6, 164.8 (C3, 2 x Cq), 158.3 (C12, Cq), 138.5 (C15, Cq), 130.8 (C10, 2 x CH), 126.2 (C9, Cq), 119.7 (C14, CH), 114.8 (C11, 2 x CH), 75.4 (C4, Cq), 64.9 (C13, CH_2), 63.6 (C2, CH), 42.3 (C5, CH_2), 33.6, 30.5 (C1, 2 x CH_3), 30.4, 27.4 (C6, 2 x CH_2), 26.5 & 18.4 (C16, 2 x CH_3), 17.1, 15.9 (C7, 2 x CH_2), -1.41, -1.54 (C8, 6 x CH_3); **LCMS ESI+** *m/z* $[M\text{-}S(CH_2)_2Si(CH_3)_3]^+$ 447.56, $[M+Na+CH_3CN]^+$ 644.68, $[M+Na]^+$ 603.64, $[M+H]^+$ 581.65 peak at 8.05 min (alternative gradient; 10% to 90% 0.1% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O over 6 min, 7 to 11.0 min, 90% to 100% HCO_2H/CH_3CN into 0.1% HCO_2H/H_2O, flow rate 1.5 mL/min, using a Chromolith Speedrod RP-18e column); **FTIR** ν_{max} (neat, cm^{-1}) 2950 (w), 1663 (s), 1509 (m), 1382 (w), 1243 (s) cm^{-1}.

1,4-Dimethyl-3,6-bis-tritylsulfanylpiperazine-2,5-dione (2.154)



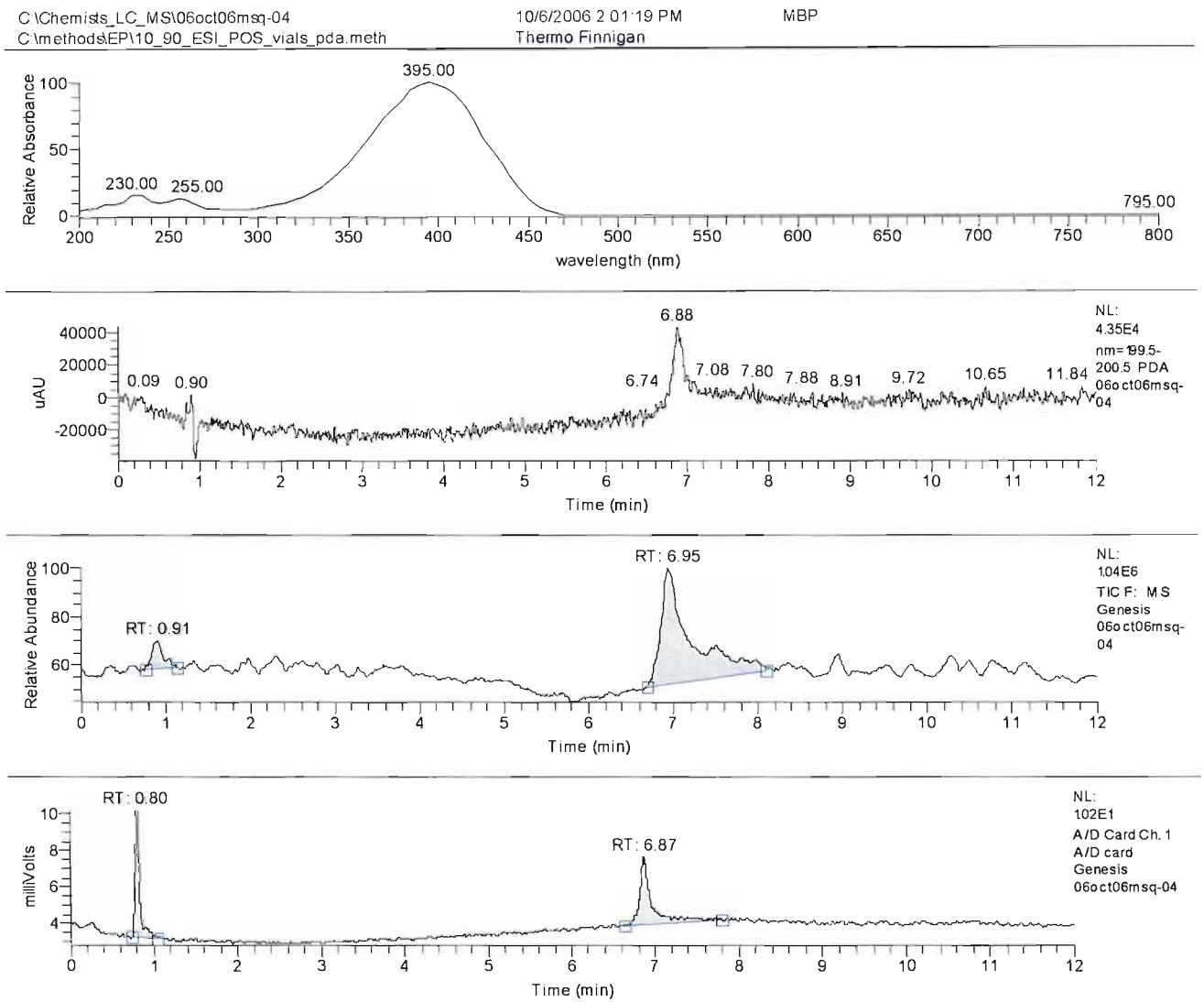
2.154
M.W. 690.93
$C_{44}H_{38}N_2O_2S_2$

To a stirred suspension of sarcosine anhydride (1 g, 7.0 mmol), in carbon tetrachloride (68 mL), was added AIBN (230 mg, 1.41 mmol) and NBS (2.63 g, 14.8 mmol). The suspension was brought to reflux for 3 h, cooled to room temperature, filtered, and concentrated. The resultant crude dibromide was dissolved in THF (100 mL), a solution of triphenylmethyl mercaptan (4.1 g, 14.7 mmol) and triethylamine (2.1 ml, 14.7 mmol) in THF (20 mL) was carefully added over 10 min. The resultant red solution was stirred at room temperature for 18 h. Concentrated and partitioned between EtOAc (50 mL) and 0.3 M $KHSO_4$ (aq) solution (20 mL). The organics were separated and the aqueous extracted with further EtOAc (2 x 30 mL). The organics were combined and washed with brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was purified by column chromatography (SiO_2, 50% EtOAc/Hexane) to give (**2.154**) as a white solid, crystallised from EtOAc / Hexane (820 mg, 17%).

Mp 173.9-174.9 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.43-7.28 (m, 30H, Ar-H), 3.86 (s, 2H, H2), 2.37 (s, 6H, H1); **^{13}C NMR** (MHz) δ_C 163.2 (C3, 2 x Cq), 143.7, (6 x Cq), 130.6, 129.5, 128.2, 127.6 (30 x CH), 72.4 (C4, 2 x Cq), 65.3 (C2, 2 x CH), 32.7 (C1, 2 x CH_3); **LCMS ESI+** *m/z* $[M+Na]^+$ 713.4 peak at 12.09 min; **FTIR** ν_{max} (neat, cm^{-1}) 1687 (s), 1441 (m), 1390 (m), 1240 (m) cm^{-1}.

Appendix

UV PDA scan of MBP039-06 (**1.83**)



CD spectrum of MBP039-06 (**1.83**) in methanol

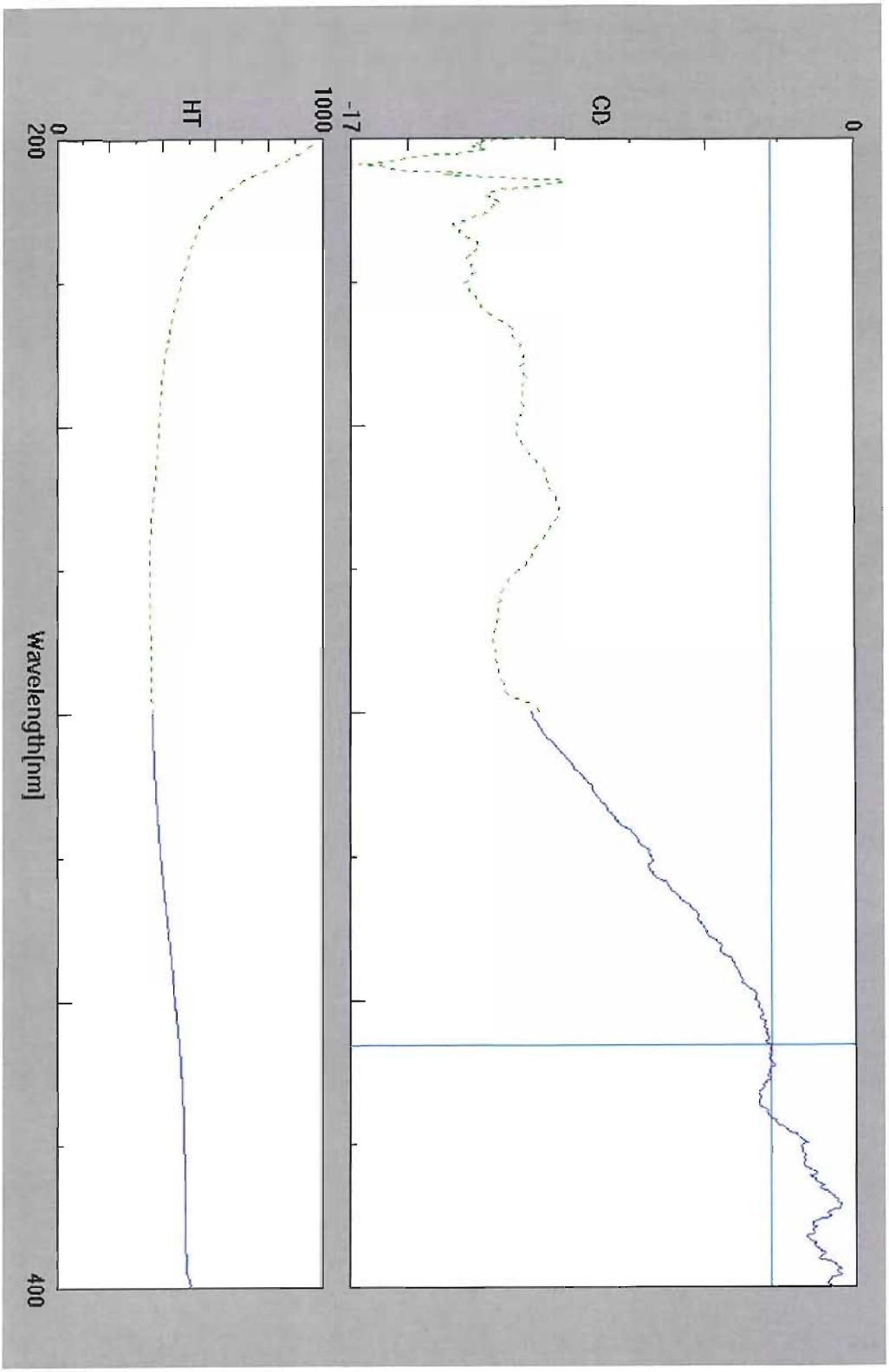


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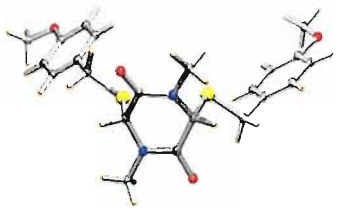
Table 1. Crystal data and structure refinement details.



Identification code	**(2.71)**
Empirical formula	$C_{22}H_{26}N_2O_4S_2$
Formula weight	446.57
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*–1
Unit cell dimensions	a = 8.8403(6) Å α = 80.093(3)°
	b = 12.0480(11) Å β = 78.621(6)°
	c = 21.218(2) Å γ = 89.624(5)°
Volume	2181.6(3) Å^3
Z	4
Density (calculated)	1.360 Mg / m^3
Absorption coefficient	0.275 mm^{-1}
F(000)	944
Crystal	Lath; Colourless
Crystal size	0.3 × 0.06 × 0.02 mm^3
θ range for data collection	2.98 – 26.21°
Index ranges	$-10 \le h \le 10$, $-14 \le k \le 14$, $-26 \le l \le 26$
Reflections collected	26197
Independent reflections	8549 [R_{int} = 0.1347]
Completeness to θ = 26.21°	97.7 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9945 and 0.9119
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	8549 / 336 / 550
Goodness-of-fit on F^2	1.030
Final *R* indices [$F^2 > 2\sigma(F^2)$]	*R1* = 0.1434, *wR2* = 0.3805
R indices (all data)	*R1* = 0.2437, *wR2* = 0.4295
Extinction coefficient	0.032(4)
Largest diff. peak and hole	0.897 and –0.711 e Å^{-3}

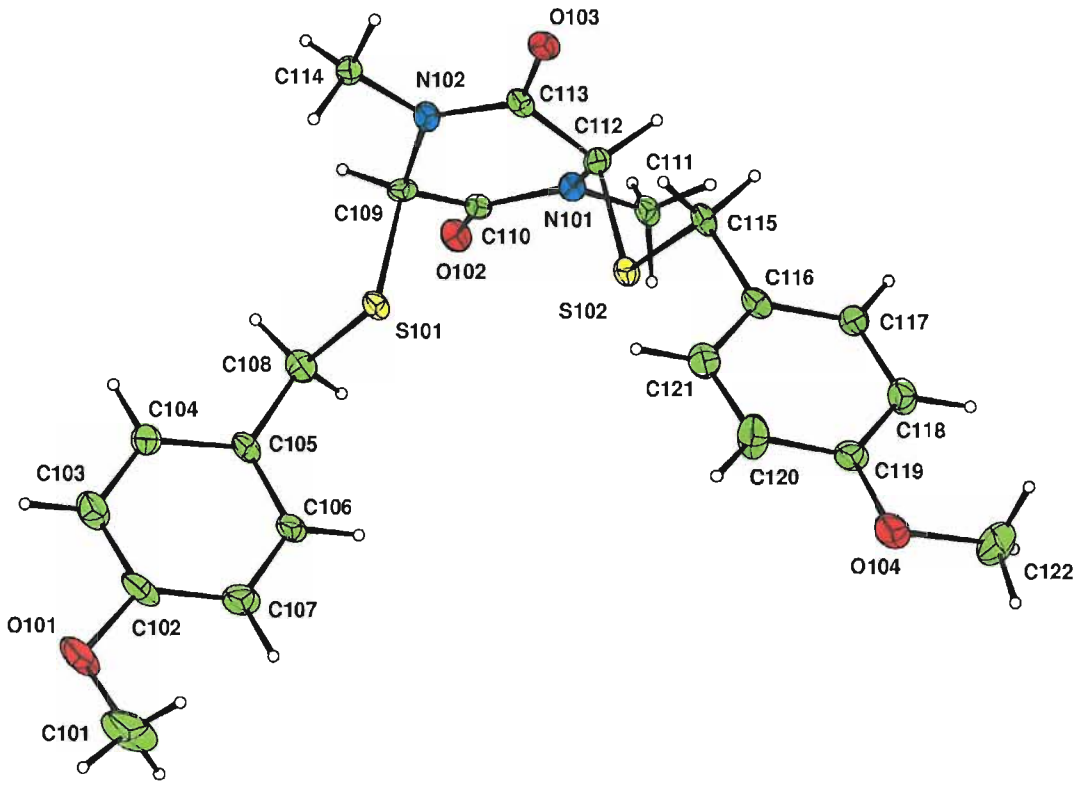
Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit*). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.10 **Structure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). **Structure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model.

Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
S101	13891(3)	11948(2)	1857(1)	30(1)	1
S102	12340(3)	9235(2)	1814(1)	31(1)	1
N101	12067(10)	10794(7)	769(4)	28(2)	1
N102	15188(10)	11149(7)	771(5)	31(2)	1
O101	14510(15)	14395(9)	4281(5)	75(3)	1
O102	11724(9)	12679(6)	711(4)	36(2)	1
O103	15589(9)	9309(6)	659(4)	36(2)	1
O104	10227(13)	5192(8)	4254(4)	64(3)	1
C101	13830(30)	13717(16)	4874(7)	117(9)	1
C102	14124(19)	14074(11)	3730(6)	53(4)	1
C103	14947(17)	14639(12)	3157(7)	52(3)	1
C104	14629(14)	14401(10)	2582(6)	39(3)	1
C105	13488(13)	13610(9)	2571(6)	32(3)	1
C106	12668(16)	13073(10)	3154(6)	44(3)	1
C107	12949(19)	13294(12)	3736(7)	57(4)	1
C108	13224(14)	13358(10)	1922(6)	39(3)	1
C109	14091(12)	11985(9)	971(5)	29(2)	1
C110	12538(13)	11852(10)	813(5)	31(3)	1
C111	10527(13)	10623(9)	681(6)	32(3)	1
C112	12971(12)	9826(9)	942(5)	29(2)	1
C113	14696(13)	10066(9)	782(5)	30(2)	1
C114	16787(12)	11437(10)	673(6)	32(3)	1
C115	12949(14)	7806(9)	1807(6)	32(3)	1
C116	12206(14)	7108(10)	2457(6)	36(3)	1
C117	10981(14)	6372(10)	2497(6)	38(3)	1
C118	10290(16)	5707(10)	3094(6)	45(3)	1
C119	10823(16)	5813(11)	3653(6)	46(3)	1
C120	12060(18)	6556(12)	3604(7)	59(4)	1
C121	12744(15)	7199(11)	3013(6)	45(3)	1
C122	9070(20)	4343(16)	4292(8)	90(6)	1
S201	8897(3)	4215(2)	1895(1)	32(1)	1
S202	7216(3)	6985(2)	1812(1)	31(1)	1
N201	6996(10)	6024(7)	784(5)	31(2)	1
N202	10079(10)	5696(8)	796(4)	32(2)	1
O201	4853(14)	9475(8)	4263(4)	65(3)	1
O202	10492(8)	7571(6)	689(4)	31(2)	1
O203	6583(9)	4154(7)	824(4)	38(2)	1
O204	9220(14)	59(9)	4328(4)	66(3)	1
C201	4000(20)	10495(14)	4273(7)	66(4)	1
C202	5547(18)	9265(12)	3652(7)	54(3)	1
C203	5277(16)	9865(10)	3069(6)	44(3)	1
C204	6032(14)	9572(10)	2494(6)	39(3)	1
C205	7054(13)	8707(9)	2487(6)	32(3)	1
C206	7344(17)	8129(11)	3057(7)	50(3)	1
C207	6581(19)	8420(12)	3651(7)	58(4)	1
C208	7860(13)	8392(8)	1838(5)	31(3)	1
C209	7864(12)	6893(8)	939(5)	28(2)	1
C210	5452(13)	6271(10)	672(6)	38(3)	1
C211	7417(13)	4942(9)	870(5)	31(2)	1
C212	8995(12)	4716(9)	1019(5)	28(2)	1
C213	11708(12)	5461(9)	744(5)	29(2)	1
C214	9583(13)	6739(9)	788(5)	29(2)	1
C215	8284(14)	2766(9)	1963(7)	41(3)	1
C216	8603(13)	2068(9)	2600(6)	34(3)	1
C217	9623(14)	1204(9)	2576(6)	38(3)	1

C218	9896(15)	509(10)	3143(6)	42(3)	1
C219	9080(16)	687(11)	3745(6)	48(3)	1
C220	8088(17)	1576(11)	3775(6)	48(3)	1
C221	7839(15)	2251(11)	3204(6)	45(3)	1
C222	10060(20)	−1011(12)	4326(8)	68(4)	1



One of the 2 chemically identical molecules in the asymmetric unit. Thermal ellipsoids drawn at the 35% probability level

Departmental Single Crystal X-Ray Diffraction Service
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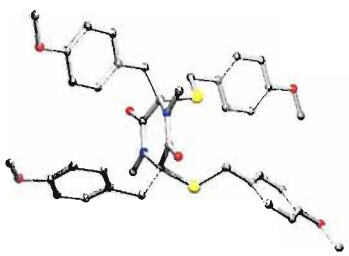
Table 1. Crystal data and structure refinement details.

Identification code	**(2.73)**
Empirical formula	$C_{38}H_{42}N_2O_6S_2$
Formula weight	686.86
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 22.7880(7) Å
	b = 7.6256(2) Å β = 114.4190(10)°
	c = 21.2046(7) Å
Volume	3355.15(18) Å^3
Z	4
Density (calculated)	1.360 Mg / m^3
Absorption coefficient	0.210 mm^{-1}
$F(000)$	1456
Crystal	Lath; Colourless
Crystal size	0.2 × 0.07 × 0.02 mm^3
θ range for data collection	3.17 – 27.16°
Index ranges	$-29 \le h \le 29$, $-9 \le k \le 9$, $-27 \le l \le 27$
Reflections collected	41412
Independent reflections	7392 [R_{int} = 0.1711]
Completeness to θ = 27.16°	99.2 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9958 and 0.9492
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7392 / 0 / 439
Goodness-of-fit on F^2	1.006
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0813, $wR2$ = 0.1228
R indices (all data)	$R1$ = 0.2121, $wR2$ = 0.1598
Largest diff. peak and hole	0.330 and –0.431 e Å^{-3}

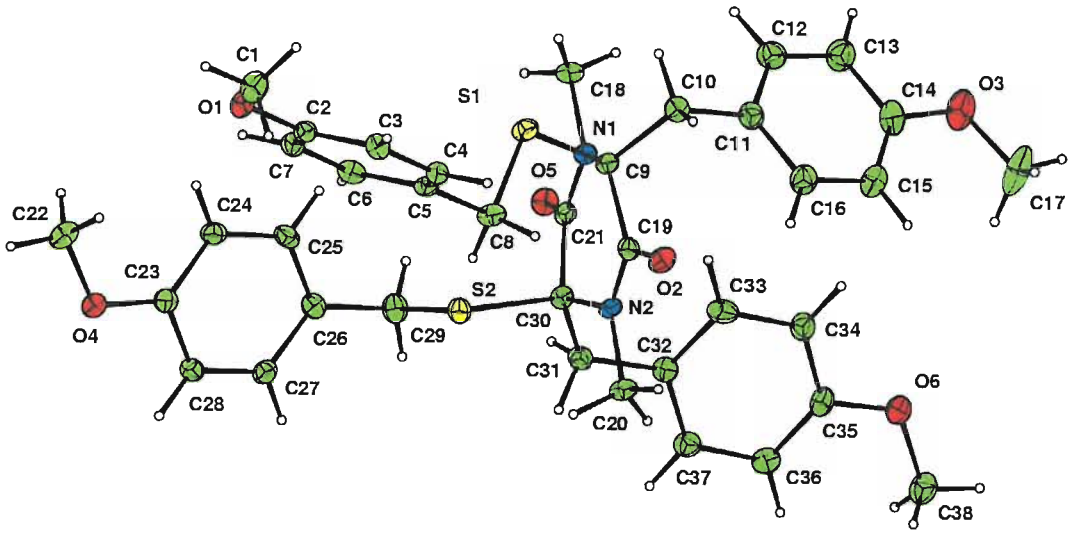


Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit*). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.10 **Structure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). **Structure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model.

Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
C1	−1540(2)	9969(5)	−1611(2)	42(1)	1
C2	−552(2)	8664(5)	−823(2)	26(1)	1
C3	−220(2)	9264(5)	−1198(2)	30(1)	1
C4	423(2)	8828(5)	−983(2)	27(1)	1
C5	746(2)	7822(4)	−403(2)	25(1)	1
C6	403(2)	7237(5)	−27(2)	32(1)	1
C7	−238(2)	7657(5)	−241(2)	31(1)	1
C8	1442(2)	7353(4)	−182(2)	33(1)	1
C9	2326(2)	4425(4)	−153(2)	25(1)	1
C10	2370(2)	3768(5)	−820(2)	32(1)	1
C11	3002(2)	2911(5)	−735(2)	33(1)	1
C12	3036(2)	1125(5)	−830(2)	41(1)	1
C13	3578(2)	333(6)	−827(2)	47(1)	1
C14	4113(2)	1361(6)	−728(2)	42(1)	1
C15	4111(2)	3121(6)	−600(2)	44(1)	1
C16	3552(2)	3885(6)	−608(2)	41(1)	1
C17	5166(2)	1464(7)	−708(3)	74(2)	1
C18	2151(2)	1361(4)	147(2)	32(1)	1
C19	2748(2)	6062(5)	99(2)	26(1)	1
C20	3469(2)	7922(4)	1015(2)	33(1)	1
C21	2863(2)	3240(5)	1048(2)	26(1)	1
C22	−409(2)	6999(5)	2000(2)	41(1)	1
C23	737(2)	6740(5)	2415(2)	27(1)	1
C24	690(2)	5566(5)	1895(2)	32(1)	1
C25	1250(2)	4921(5)	1865(2)	32(1)	1
C26	1861(2)	5412(5)	2351(2)	30(1)	1
C27	1894(2)	6564(5)	2869(2)	31(1)	1
C28	1343(2)	7215(5)	2904(2)	32(1)	1
C29	2453(2)	4783(5)	2269(2)	37(1)	1
C30	3104(2)	5072(5)	1317(2)	26(1)	1
C31	3746(2)	4970(5)	1959(2)	30(1)	1
C32	4354(2)	4652(5)	1848(2)	31(1)	1
C33	4382(2)	3669(5)	1315(2)	35(1)	1
C34	4965(2)	3372(5)	1263(2)	34(1)	1
C35	5531(2)	4073(5)	1762(2)	34(1)	1
C36	5514(2)	5080(5)	2288(2)	34(1)	1
C37	4932(2)	5355(5)	2330(2)	35(1)	1
C38	6661(2)	4566(6)	2122(2)	45(1)	1
N1	2496(1)	3029(3)	363(2)	25(1)	1
N2	3130(1)	6241(4)	785(2)	25(1)	1
O1	−1195(1)	8982(3)	−995(1)	33(1)	1
O2	2728(1)	7165(3)	−329(1)	33(1)	1
O3	4618(1)	465(4)	−769(2)	60(1)	1
O4	225(1)	7508(3)	2482(1)	35(1)	1
O5	2972(1)	2022(3)	1459(1)	33(1)	1
O6	6084(1)	3693(3)	1674(1)	39(1)	1
S1	1463(1)	5065(1)	−421(1)	31(1)	1
S2	2463(1)	6030(1)	1543(1)	30(1)	1



Thermal ellipsoids drawn at the 35% probability level

Departmental Single Crystal X-Ray Diffraction Service
School of Chemistry - University of Southampton
Contact: Dr Mark E Light, light@soton.ac.uk, ex 26722

Table 1. Crystal data and structure refinement details.

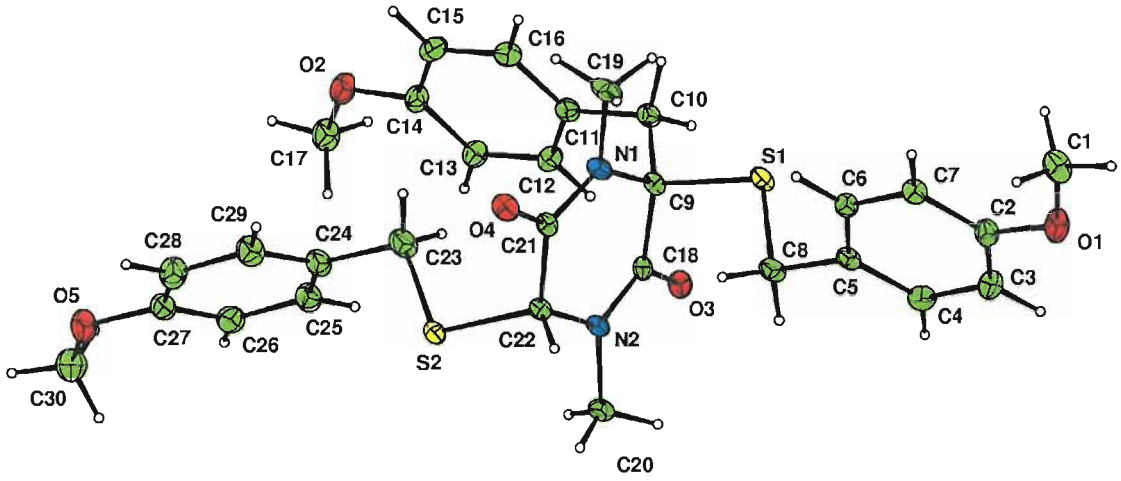
Identification code	**(2.75)**
Empirical formula	$C_{30}H_{34}N_2O_5S_2$
Formula weight	566.71
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 18.0166(5) Å
	b = 10.1044(2) Å β = 106.1060(10)°
	c = 16.4767(4) Å
Volume	2881.80(12) Å^3
Z	4
Density (calculated)	1.306 Mg / m^3
Absorption coefficient	0.227 mm^{-1}
$F(000)$	1200
Crystal	Plate; Colourless
Crystal size	0.28 × 0.13 × 0.02 mm^3
θ range for data collection	2.97 – 27.48°
Index ranges	$-23 \le h \le 23$, $-13 \le k \le 12$, $-21 \le l \le 21$
Reflections collected	35332
Independent reflections	6587 [R_{int} = 0.0960]
Completeness to θ = 27.48°	99.8 %
Absorption correction	Semi–empirical from equivalents
Max. and min. transmission	0.9955 and 0.9293
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6587 / 0 / 357
Goodness-of-fit on F^2	1.014
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0555, $wR2$ = 0.1069
R indices (all data)	$R1$ = 0.1113, $wR2$ = 0.1249
Largest diff. peak and hole	0.271 and –0.315 e Å^{-3}

Diffractometer: *Nonius KappaCCD* area detector (ϕ scans and ω scans to fill *asymmetric unit*). **Cell determination:** DirAx (Duisenberg, A.J.M.(1992). J. Appl. Cryst. 25, 92-96.) **Data collection:** Collect (Collect: Data collection software, R. Hooft, Nonius B.V., 1998). **Data reduction and cell refinement**: *Denzo* (Z. Otwinowski & W. Minor, *Methods in Enzymology* (1997) Vol. **276**: *Macromolecular Crystallography*, part A, pp. 307–326; C. W. Carter, Jr. & R. M. Sweet, Eds., Academic Press). **Absorption correction**: Sheldrick, G. M. SADABS - Bruker Nonius area detector scaling and absorption correction - V2.10 **Structure solution**: *SHELXS97* (G. M. Sheldrick, Acta Cryst. (1990) A**46** 467–473). **Structure refinement**: *SHELXL97* (G. M. Sheldrick (1997), University of Göttingen, Germany). **Graphics:** Cameron - A Molecular Graphics Package. (D. M. Watkin, L. Pearce and C. K. Prout, Chemical Crystallography Laboratory, University of Oxford, 1993).

Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model.

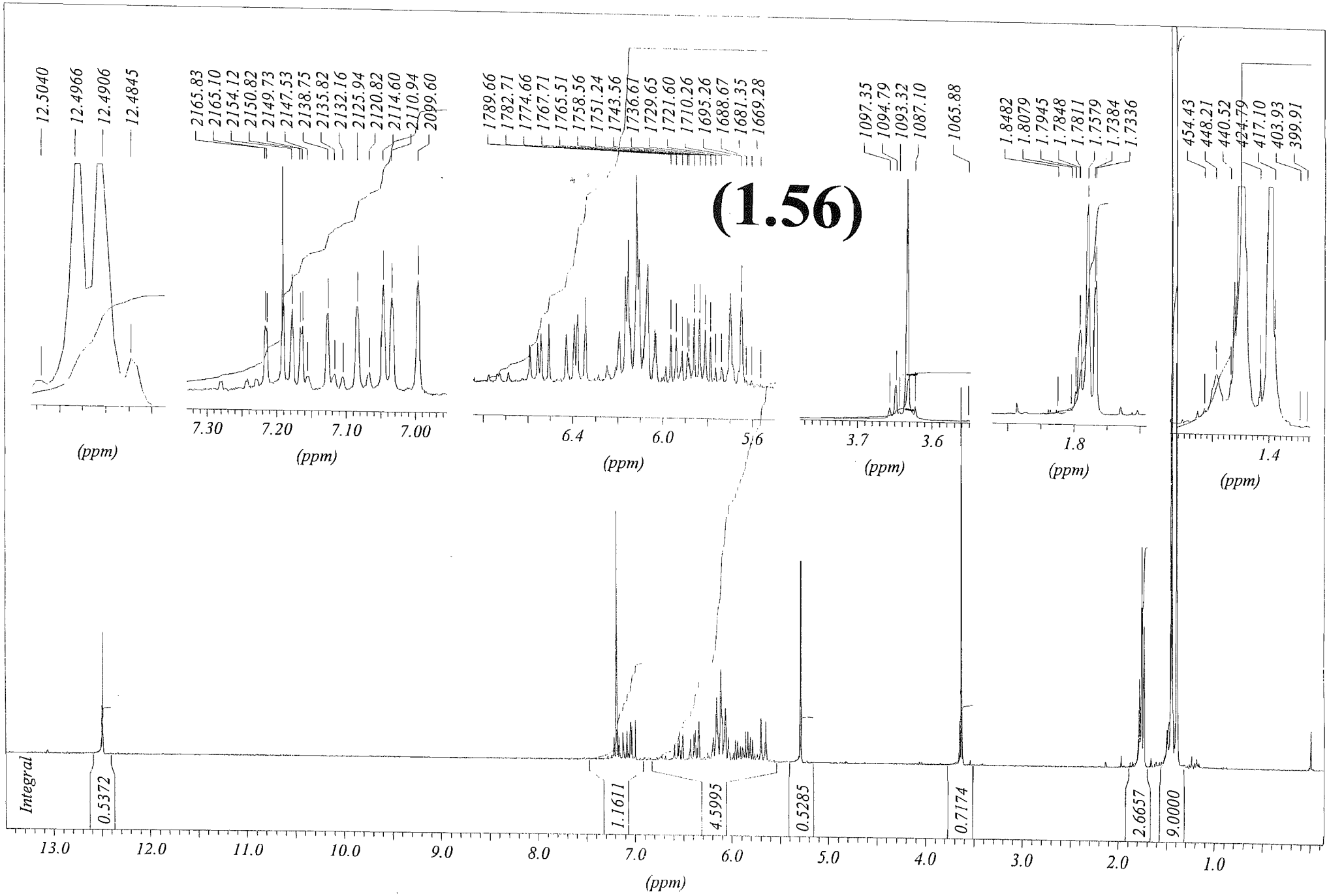
Table 2. Atomic coordinates [$\times 10^4$], equivalent isotropic displacement parameters [$Å^2 \times 10^3$] and site occupancy factors. U_{eq} is defined as one third of the trace of the orthogonalized U^{ij} tensor.

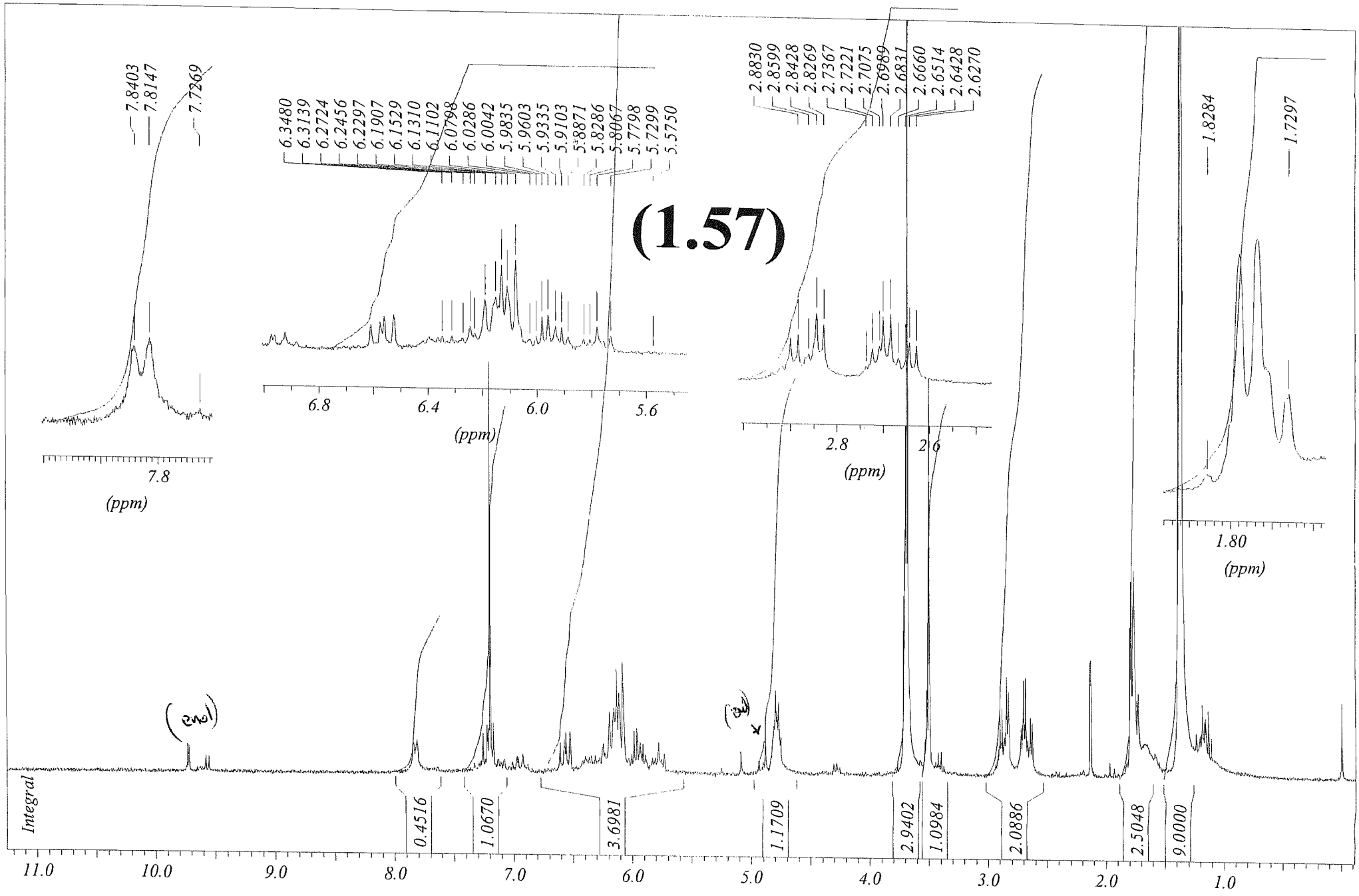
Atom	*x*	*y*	*z*	U_{eq}	*S.o.f.*
C1	−3105(2)	6040(3)	−2348(2)	43(1)	1
C2	−2586(2)	7111(3)	−1021(2)	30(1)	1
C3	−2694(2)	8030(3)	−440(2)	33(1)	1
C4	−2103(2)	8298(2)	283(2)	30(1)	1
C5	−1393(1)	7674(2)	434(2)	25(1)	1
C6	−1293(2)	6774(2)	−162(2)	28(1)	1
C7	−1880(1)	6486(2)	−889(2)	28(1)	1
C8	−741(1)	7984(3)	1208(2)	29(1)	1
C9	838(1)	8887(2)	1754(1)	24(1)	1
C10	1380(1)	9962(2)	1560(2)	28(1)	1
C11	2145(1)	10093(2)	2221(2)	27(1)	1
C12	2223(2)	10902(2)	2920(2)	29(1)	1
C13	2927(1)	11054(3)	3533(2)	31(1)	1
C14	3570(1)	10402(3)	3439(2)	31(1)	1
C15	3507(2)	9592(3)	2741(2)	34(1)	1
C16	2806(2)	9446(3)	2141(2)	32(1)	1
C17	4383(2)	11398(3)	4691(2)	43(1)	1
C18	624(1)	9245(2)	2560(1)	22(1)	1
C19	1367(2)	7091(3)	1018(2)	39(1)	1
C20	245(2)	8616(3)	3793(2)	33(1)	1
C21	1208(1)	6641(2)	2383(1)	23(1)	1
C22	953(1)	6999(2)	3151(1)	22(1)	1
C23	2545(2)	7568(3)	3919(2)	41(1)	1
C24	3191(2)	7477(3)	4730(2)	37(1)	1
C25	3303(2)	8495(3)	5319(2)	40(1)	1
C26	3875(2)	8411(3)	6092(2)	39(1)	1
C27	4328(2)	7282(3)	6270(2)	38(1)	1
C28	4217(2)	6270(3)	5692(2)	43(1)	1
C29	3653(2)	6367(3)	4926(2)	42(1)	1
C30	5012(2)	8107(3)	7636(2)	53(1)	1
N1	1161(1)	7560(2)	1772(1)	26(1)	1
N2	601(1)	8291(2)	3116(1)	23(1)	1
O1	−3212(1)	6888(2)	−1702(1)	40(1)	1
O2	4292(1)	10500(2)	3996(1)	40(1)	1
O3	428(1)	10388(2)	2654(1)	28(1)	1
O4	1452(1)	5523(2)	2323(1)	31(1)	1
O5	4908(1)	7098(2)	7006(1)	47(1)	1
S1	−61(1)	9054(1)	878(1)	28(1)	1
S2	1725(1)	6724(1)	4114(1)	26(1)	1



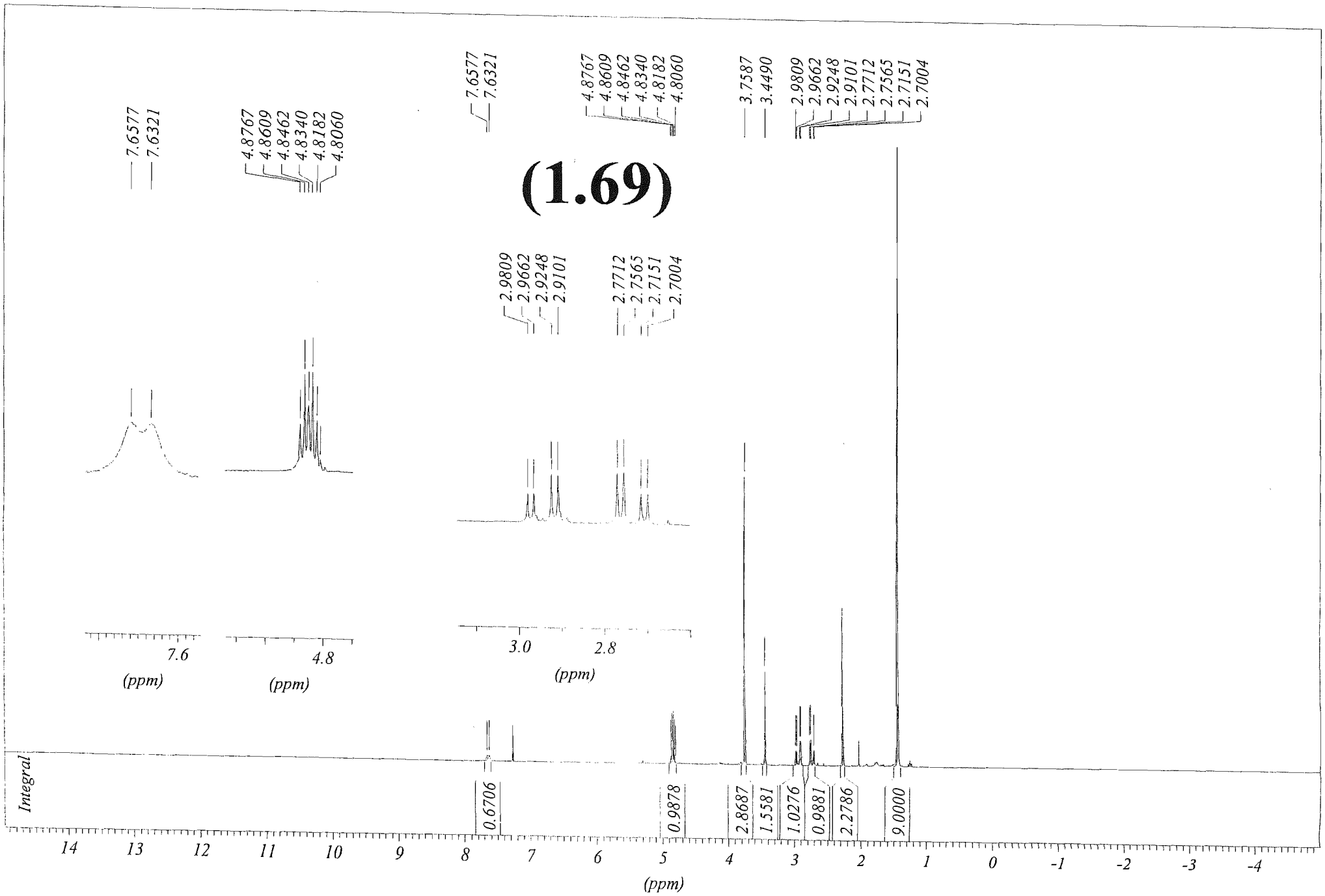
Thermal ellipsoids drawn at the 35% probability level

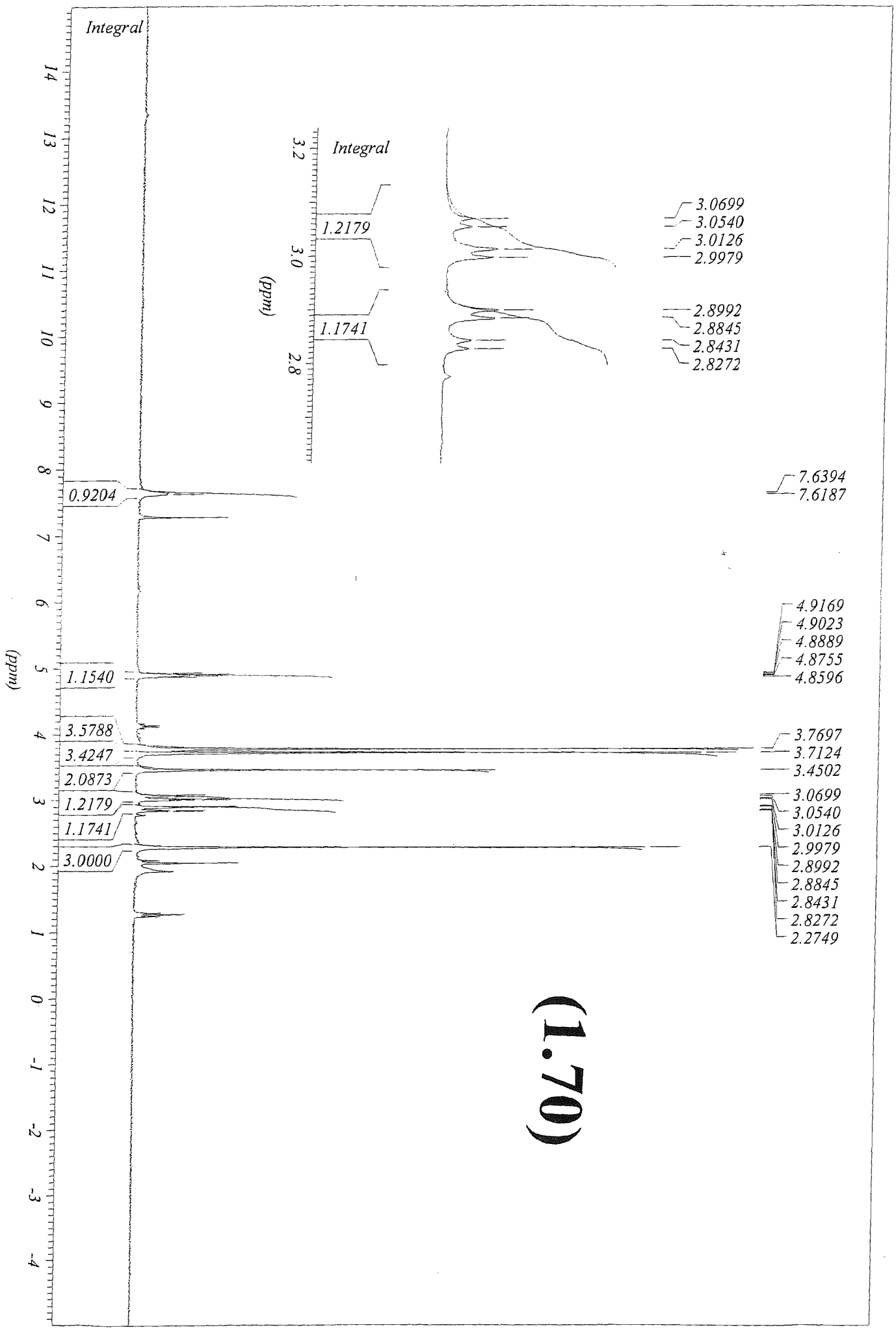
grp4226-14


(1.56)
12.5040
12.4966
12.4906
12.4845
2165.83
2165.10
2154.12
2150.82
2149.73
2147.53
2138.75
2135.82
2132.16
2125.94
2120.82
2114.60
2110.94
2099.60
1789.66
1782.71
1774.66
1767.71
1765.51
1758.56
1751.24
1743.56
1736.61
1729.65
1721.60
1710.26
1695.26
1688.67
1681.35
1669.28
1097.35
1094.79
1093.32
1087.10
1065.88
1.8482
1.8079
1.7945
1.7848
1.7811
1.7579
1.7384
1.7336
454.43
448.21
440.52
424.79
417.10
403.93
399.91
7.30
7.20
7.10
7.00
6.4
6.0
5.6
3.7
3.6
1.8
1.4
(ppm)
Integral
0.5372
1.1611
4.5995
0.5285
0.7174
2.6657
9.0000
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

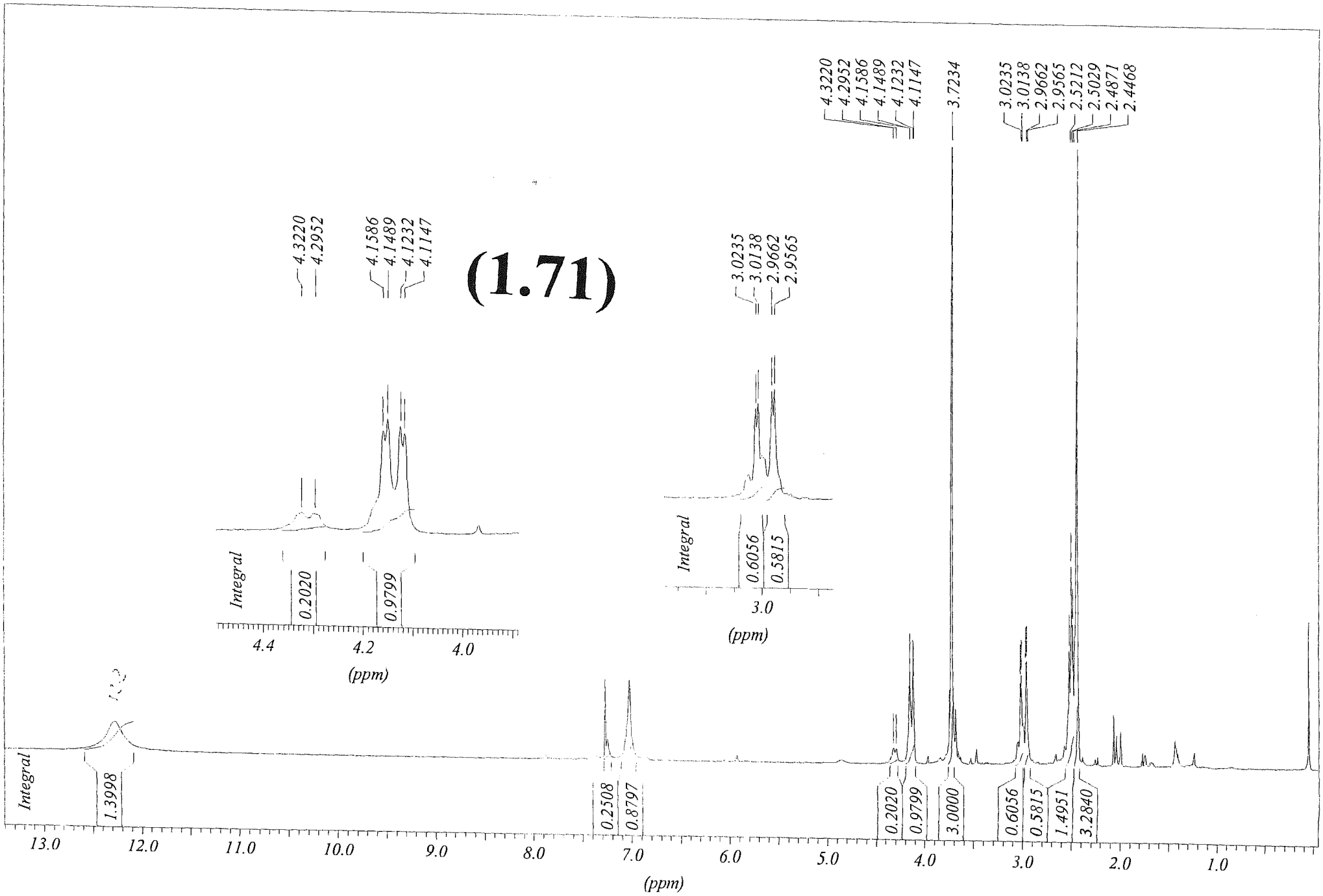

(1.57)
7.8403
7.8147
7.7269
7.8
(ppm)
6.3480
6.3139
6.2724
6.2456
6.2297
6.1907
6.1529
6.1310
6.1102
6.0798
6.0286
6.0042
5.9835
5.9603
5.9335
5.9103
5.8871
5.8286
5.8067
5.7798
5.7299
5.5750
6.8
6.4
6.0
5.6
(ppm)
2.8830
2.8599
2.8428
2.8269
2.7367
2.7221
2.7075
2.6989
2.6831
2.6660
2.6514
2.6428
2.6270
2.8
2.6
(ppm)
1.8284
1.7297
1.80
(ppm)
Integral
0.4516
1.0670
3.6981
1.1709
2.9402
1.0984
2.0886
2.5048
9.0000
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

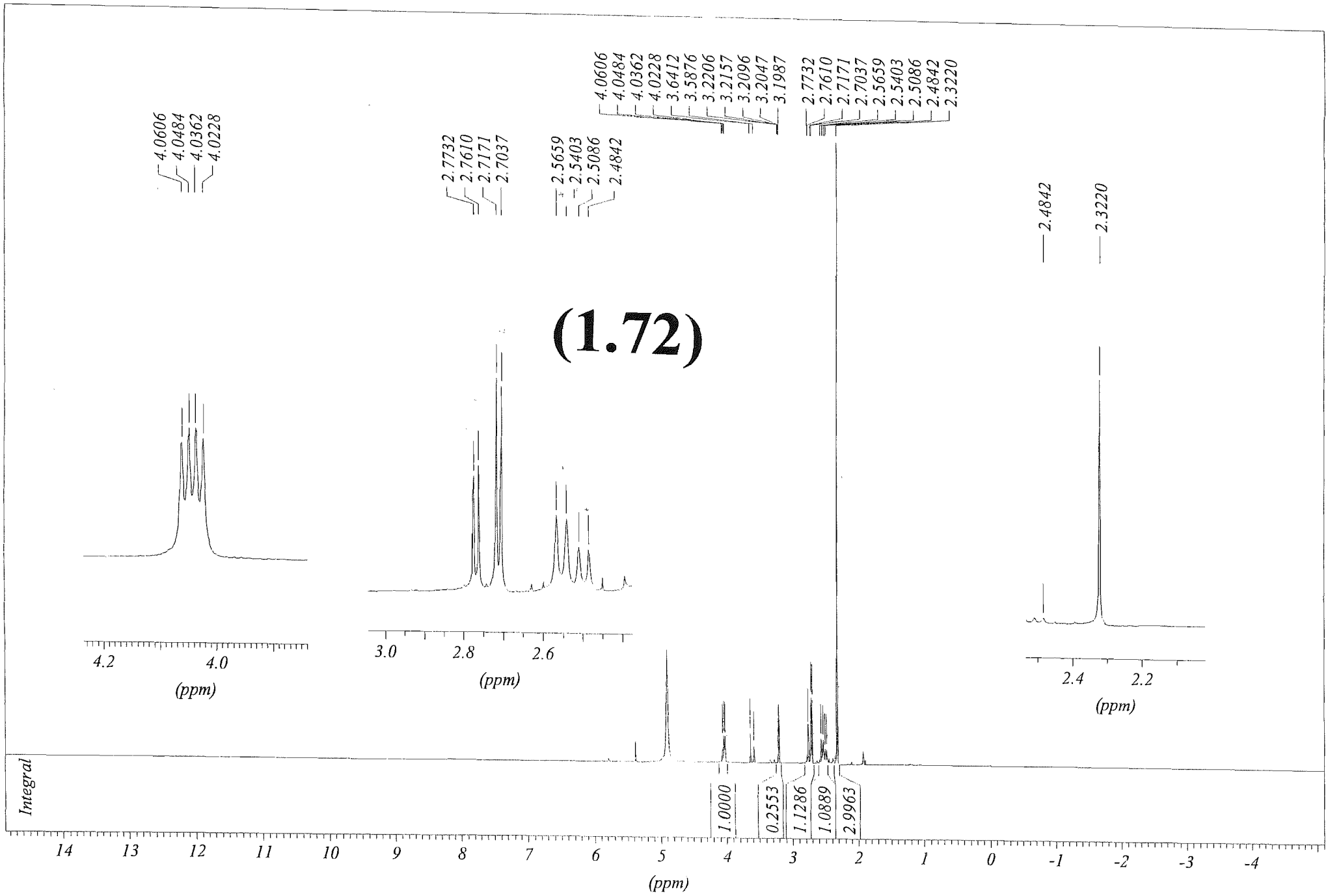
grp-4225-37

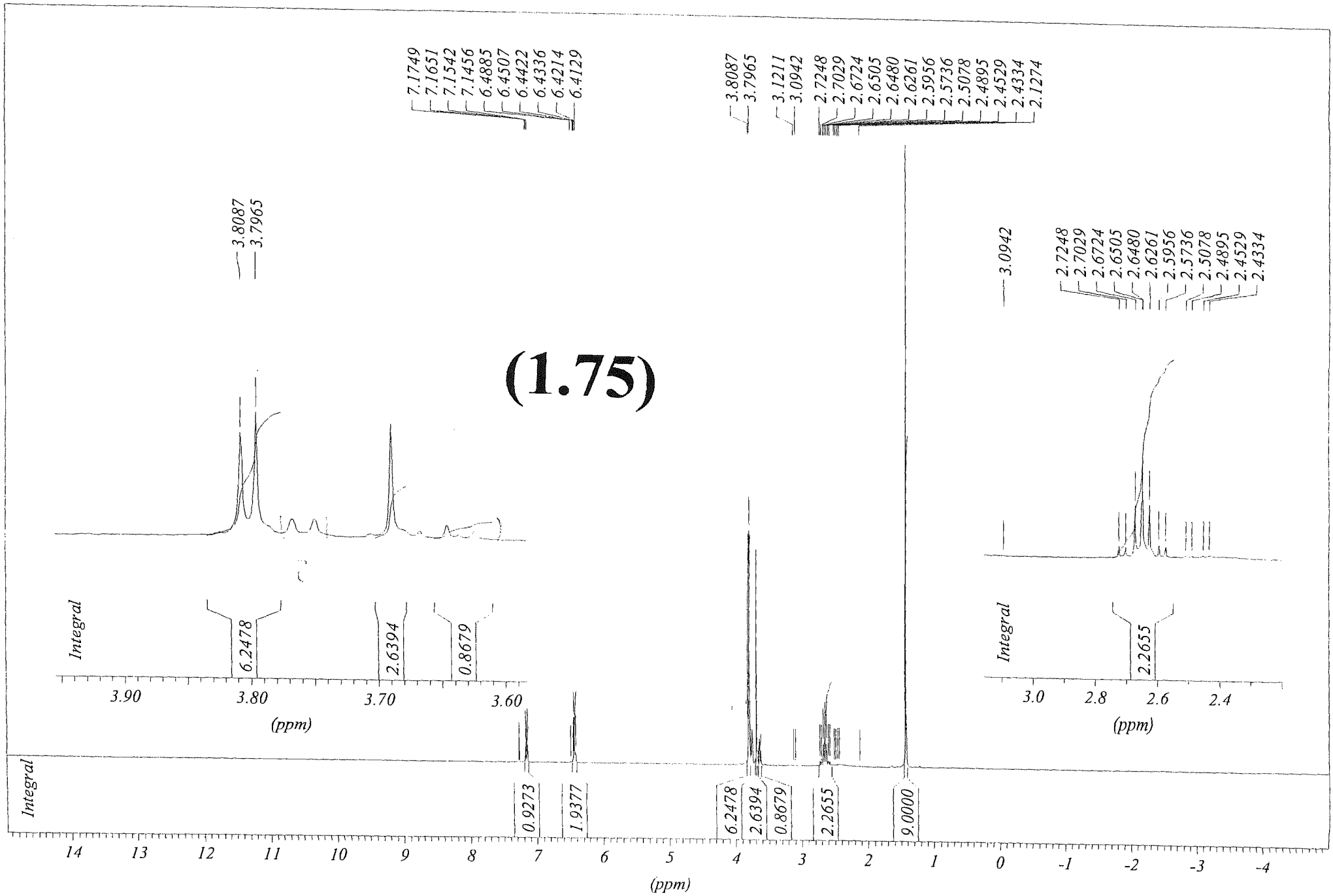

(1.69)
7.6577
7.6321
4.8767
4.8609
4.8462
4.8340
4.8182
4.8060
3.7587
3.4490
2.9809
2.9662
2.9248
2.9101
2.7712
2.7565
2.7151
2.7004
7.6
4.8
3.0
2.8
(ppm)
Integral
0.6706
0.9878
2.8687
1.5581
1.0276
0.9881
2.2786
9.0000
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4


Integral
Integral
3.2
3.0
2.8
(ppm)
1.2179
1.1741
3.0699
3.0540
3.0126
2.9979
2.8992
2.8845
2.8431
2.8272
7.6394
7.6187
4.9169
4.9023
4.8889
4.8755
4.8596
3.7697
3.7124
3.4502
3.0699
3.0540
3.0126
2.9979
2.8992
2.8845
2.8431
2.8272
2.2749
0.9204
1.1540
3.5788
3.4247
2.0873
1.2179
1.1741
3.0000
(ppm)

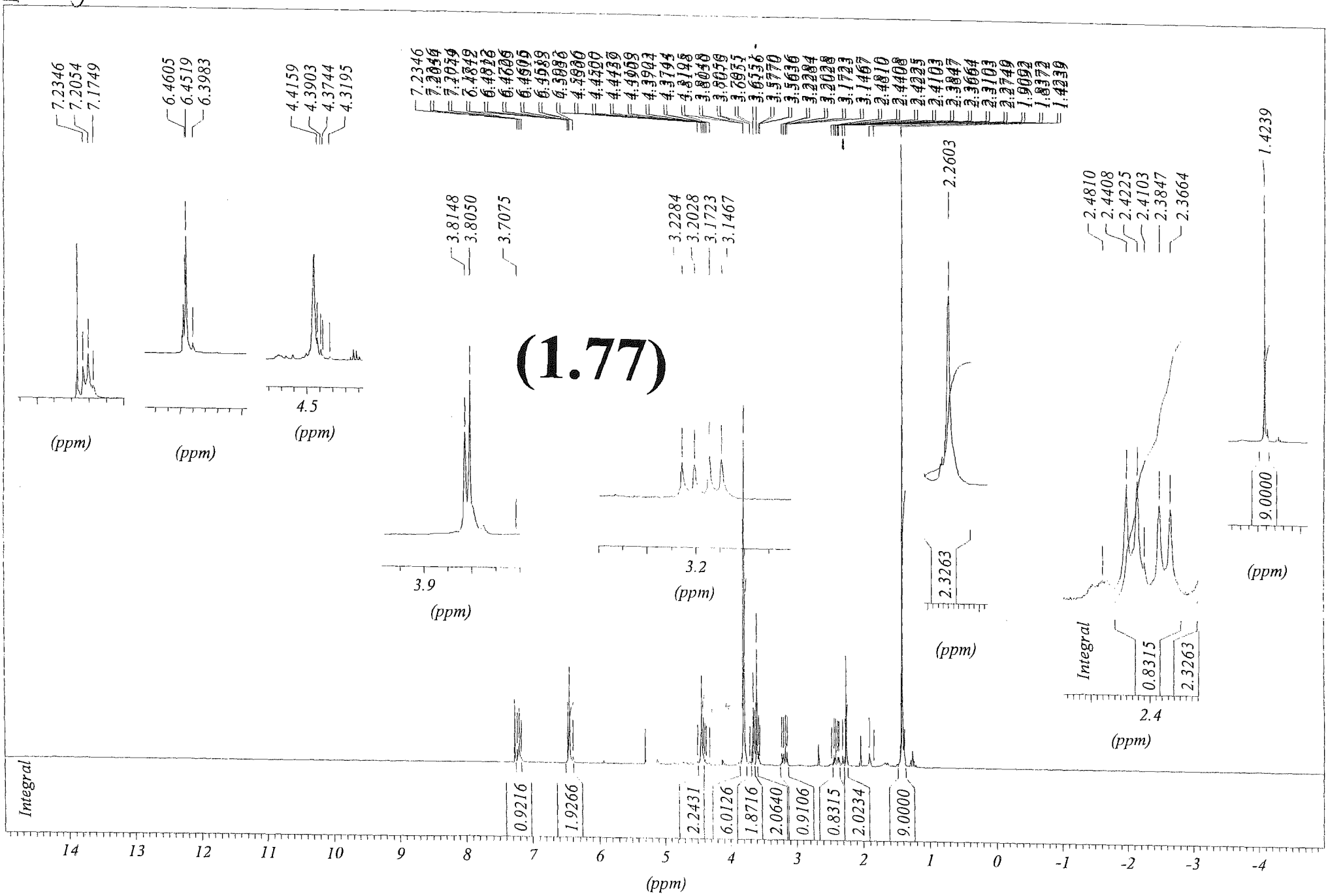
(1.70)

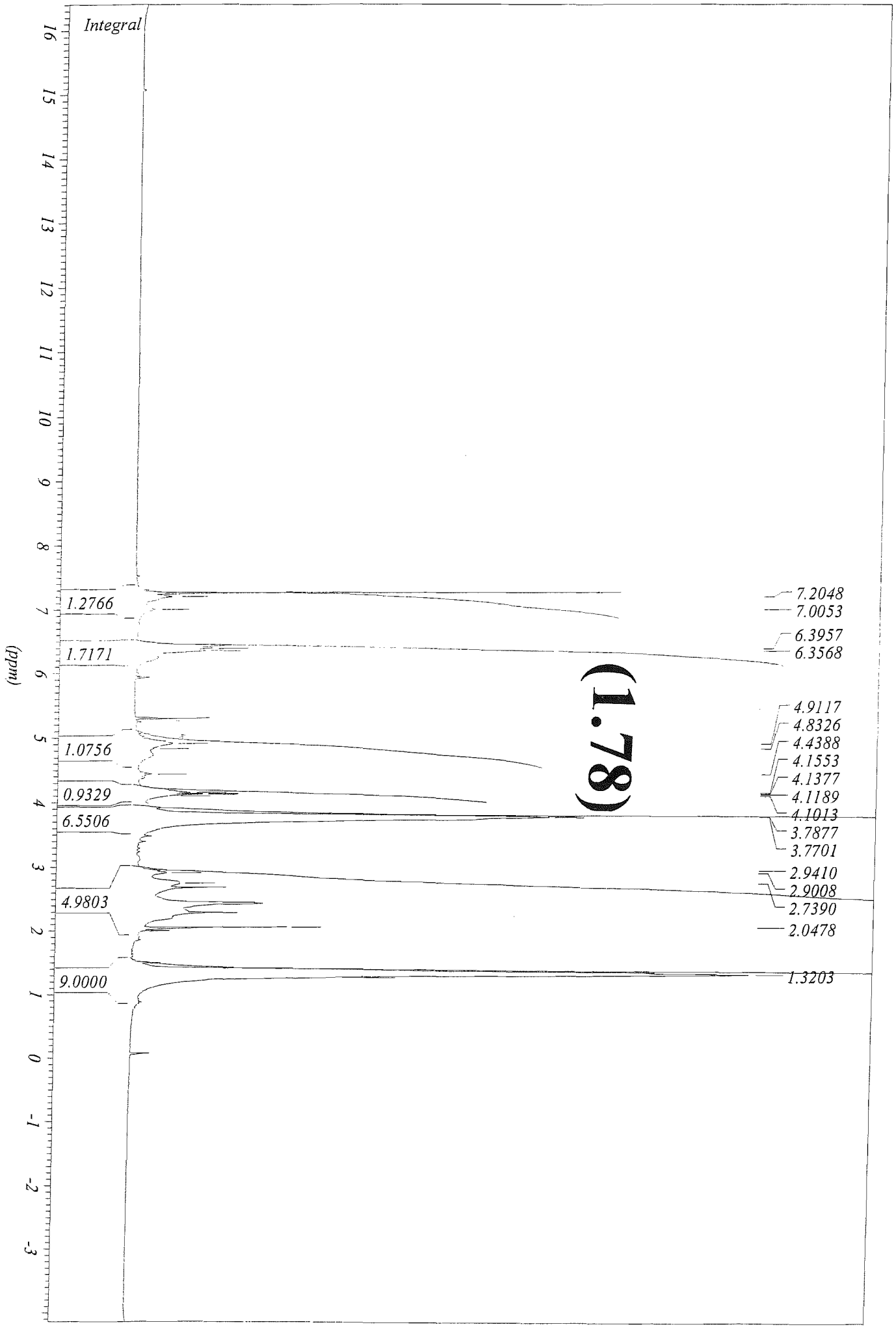

(1.71)
4.3220
4.2952
4.1586
4.1489
4.1232
4.1147
3.7234
3.0235
3.0138
2.9662
2.9565
2.5212
2.5029
2.4871
2.4468
Integral
1.3998
0.2508
0.8797
0.2020
0.9799
3.0000
0.6056
0.5815
1.4951
3.2840
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)
Integral
0.2020
0.9799
4.4
4.2
4.0
(ppm)
Integral
0.6056
0.5815
3.0
(ppm)


(1.72)
4.0606
4.0484
4.0362
4.0228
2.7732
2.7610
2.7171
2.7037
2.5659
2.5403
2.5086
2.4842
3.6412
3.5876
3.2206
3.2157
3.2096
3.2047
3.1987
2.3220
4.2
4.0
3.0
2.8
2.6
2.4
2.2
(ppm)
Integral
1.0000
0.2553
1.1286
1.0889
2.9963
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4

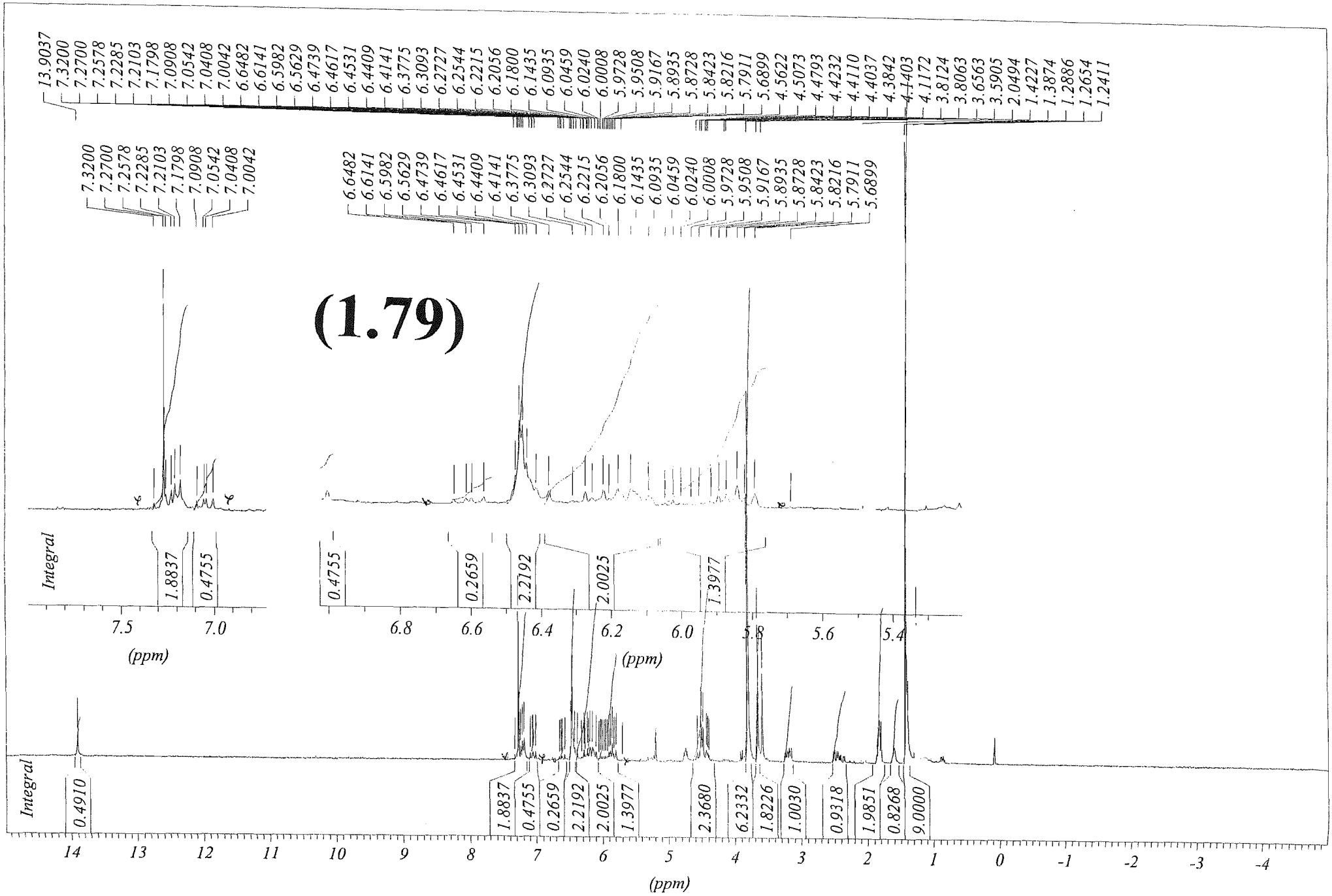

(1.75)
7.1749
7.1651
7.1542
7.1456
6.4885
6.4507
6.4422
6.4336
6.4214
6.4129
3.8087
3.7965
3.1211
3.0942
2.7248
2.7029
2.6724
2.6505
2.6480
2.6261
2.5956
2.5736
2.5078
2.4895
2.4529
2.4334
2.1274
Integral
0.9273
1.9377
6.2478
2.6394
0.8679
2.2655
9.0000
(ppm)
Integral
6.2478
2.6394
0.8679
(ppm)
Integral
2.2655
(ppm)

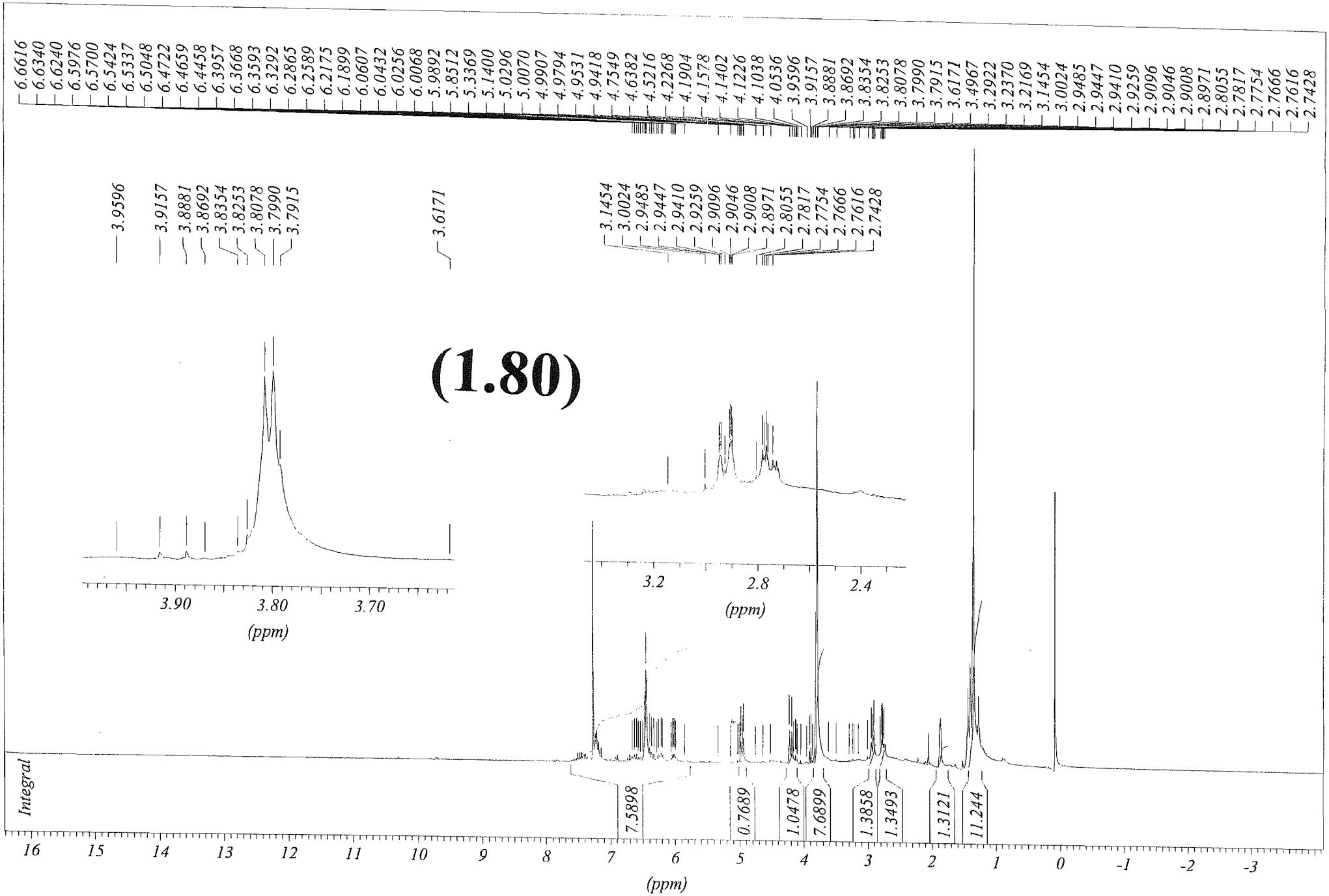
grp-4226-44

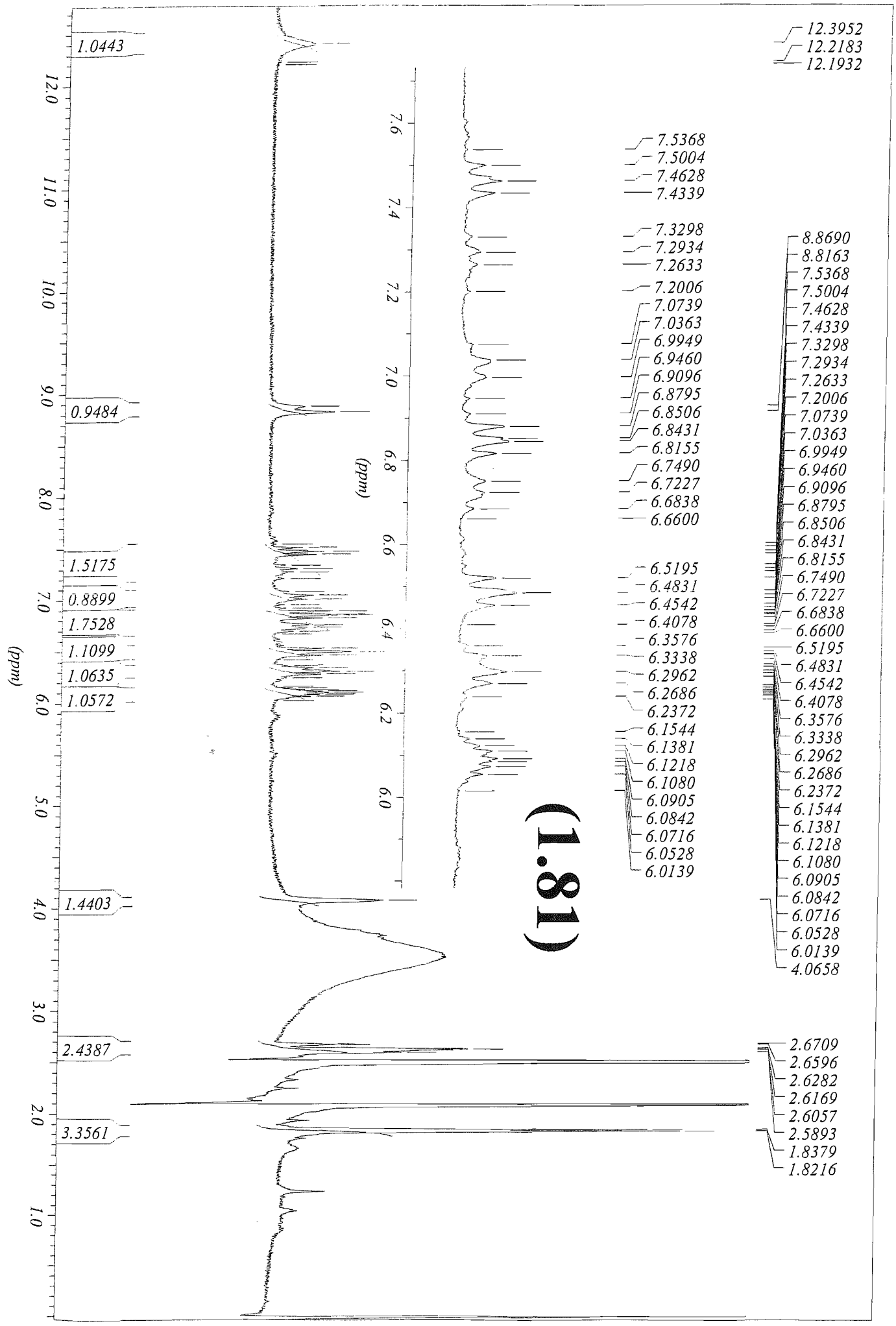

(1.77)
7.2346
7.2054
7.1749
6.4605
6.4519
6.3983
4.4159
4.3903
4.3744
4.3195
3.8148
3.8050
3.7075
3.2284
3.2028
3.1723
3.1467
2.2603
2.4810
2.4408
2.4225
2.4103
2.3847
2.3664
1.4239
(ppm)
Integral
0.9216
1.9266
2.2431
6.0126
1.8716
2.0640
0.9106
0.8315
2.0234
9.0000
2.3263

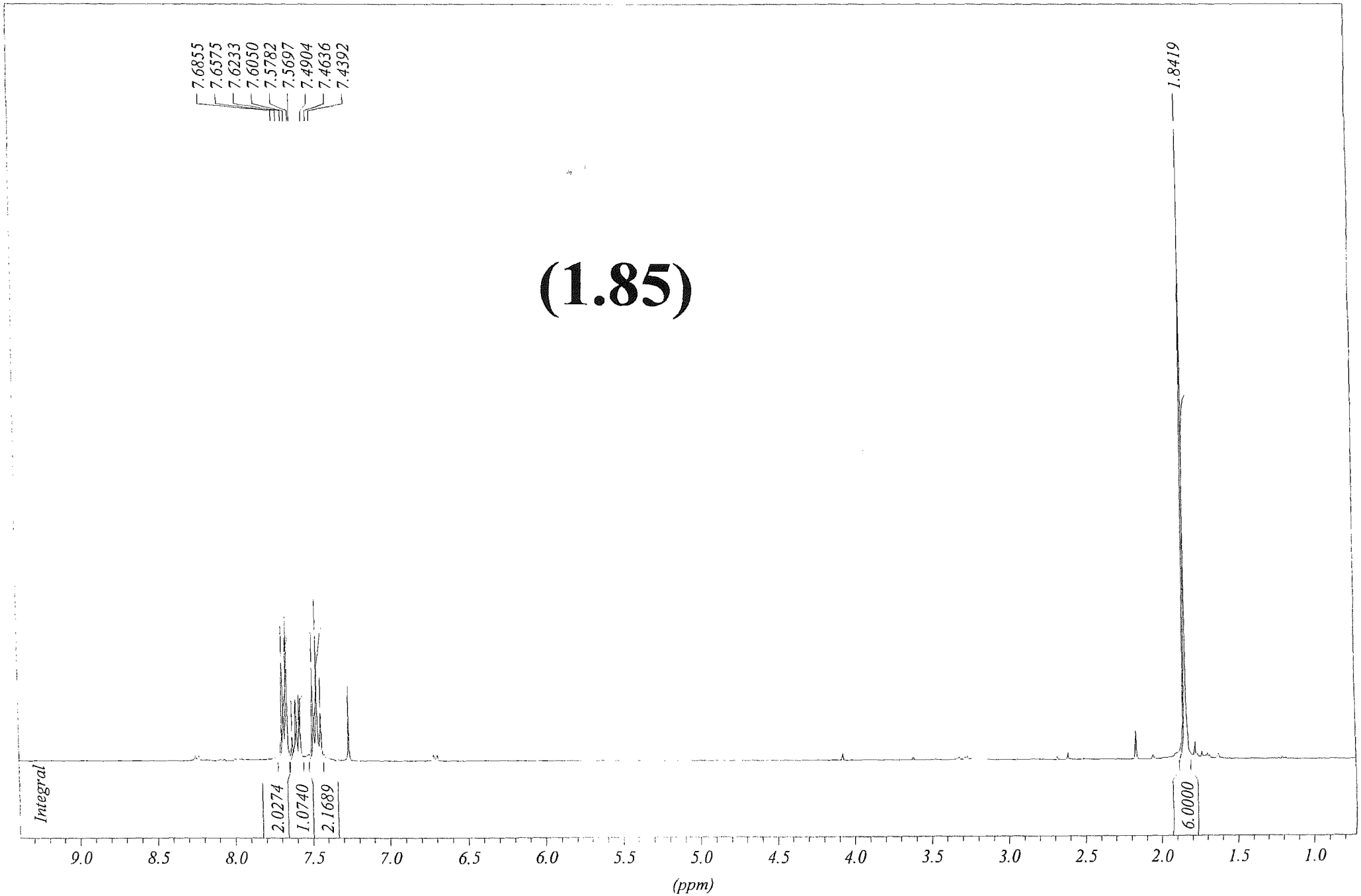

Integral
(ppm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
1.2766
1.7171
1.0756
0.9329
6.5506
4.9803
9.0000
7.2048
7.0053
6.3957
6.3568
4.9117
4.8326
4.4388
4.1553
4.1377
4.1189
4.1013
3.7877
3.7701
2.9410
2.9008
2.7390
2.0478
1.3203
(1.78)

grp-4226-50


(1.79)
Integral
1.8837
0.4755
7.5
7.0
(ppm)
0.4755
0.2659
2.2192
2.0025
1.3977
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
(ppm)
Integral
0.4910
1.8837
0.4755
0.2659
2.2192
2.0025
1.3977
2.3680
6.2332
1.8226
1.0030
0.9318
1.9851
0.8268
9.0000
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)


(1.80)
Integral
(ppm)


(1.81)
(ppm)
12.3952
12.2183
12.1932
8.8690
8.8163
7.5368
7.5004
7.4628
7.4339
7.3298
7.2934
7.2633
7.2006
7.0739
7.0363
6.9949
6.9460
6.9096
6.8795
6.8506
6.8431
6.8155
6.7490
6.7227
6.6838
6.6600
6.5195
6.4831
6.4542
6.4078
6.3576
6.3338
6.2962
6.2686
6.2372
6.1544
6.1381
6.1218
6.1080
6.0905
6.0842
6.0716
6.0528
6.0139
4.0658
2.6709
2.6596
2.6282
2.6169
2.6057
2.5893
1.8379
1.8216
1.0443
0.9484
1.5175
0.8899
1.7528
1.1099
1.0635
1.0572
1.4403
2.4387
3.3561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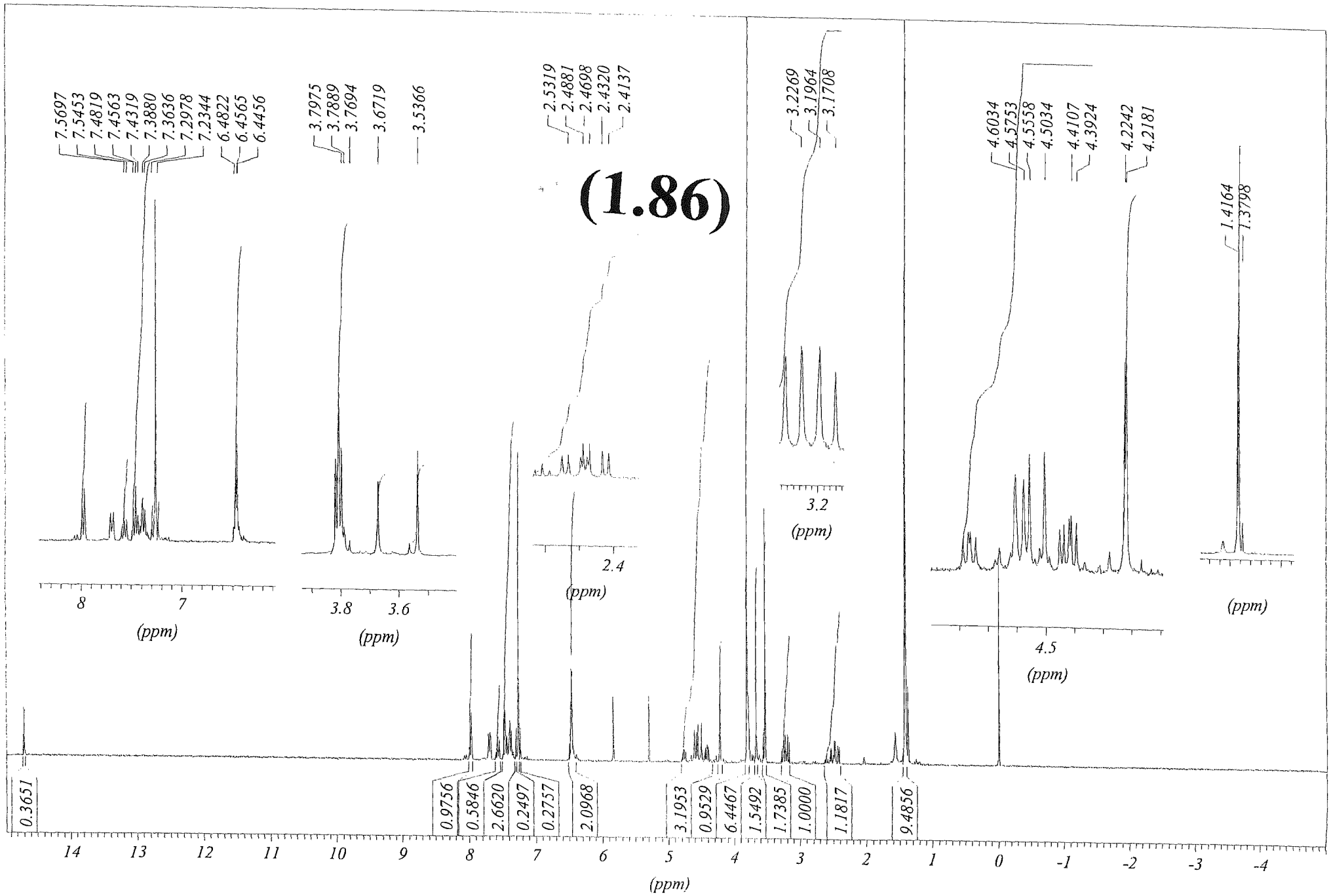
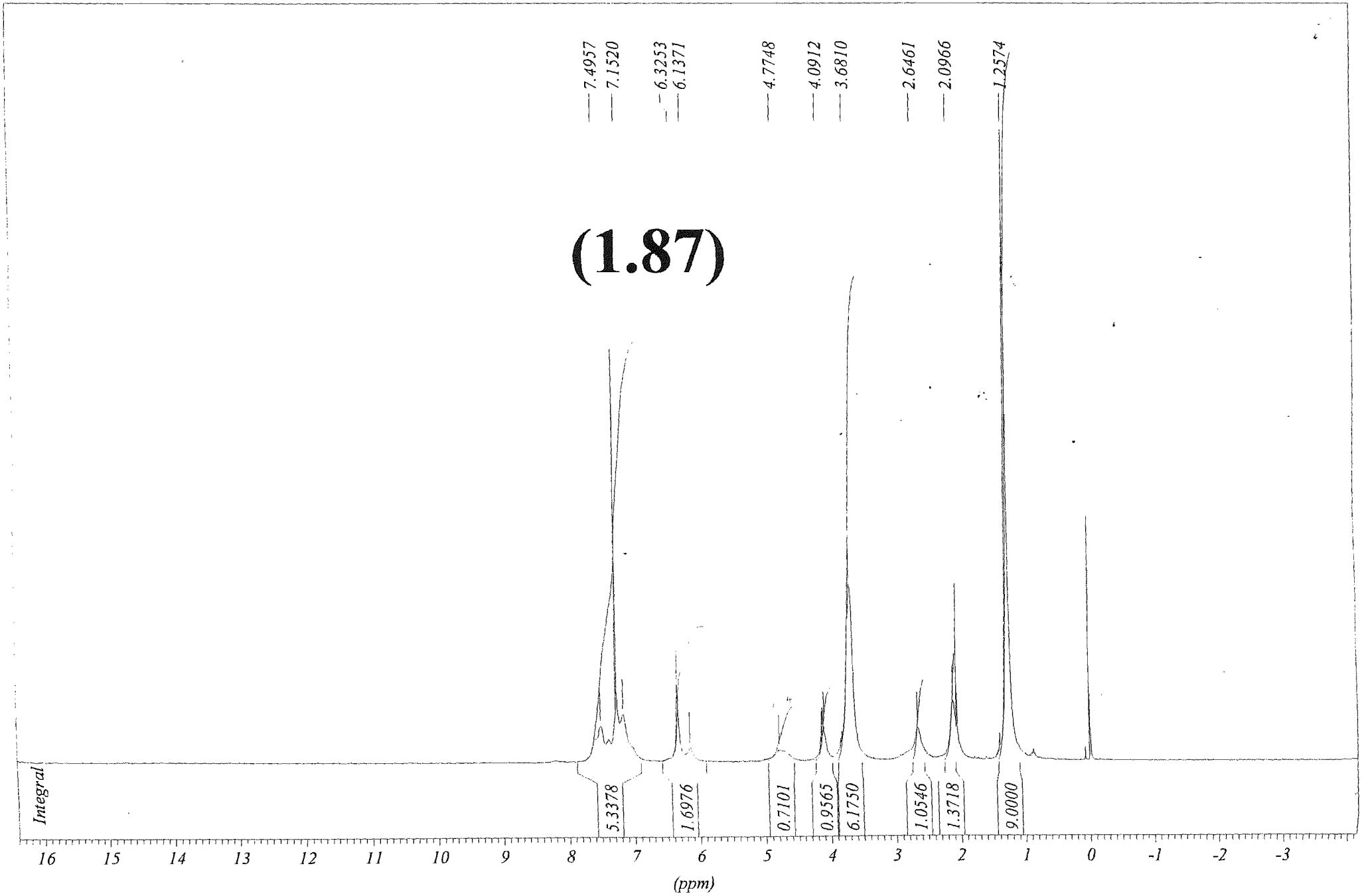
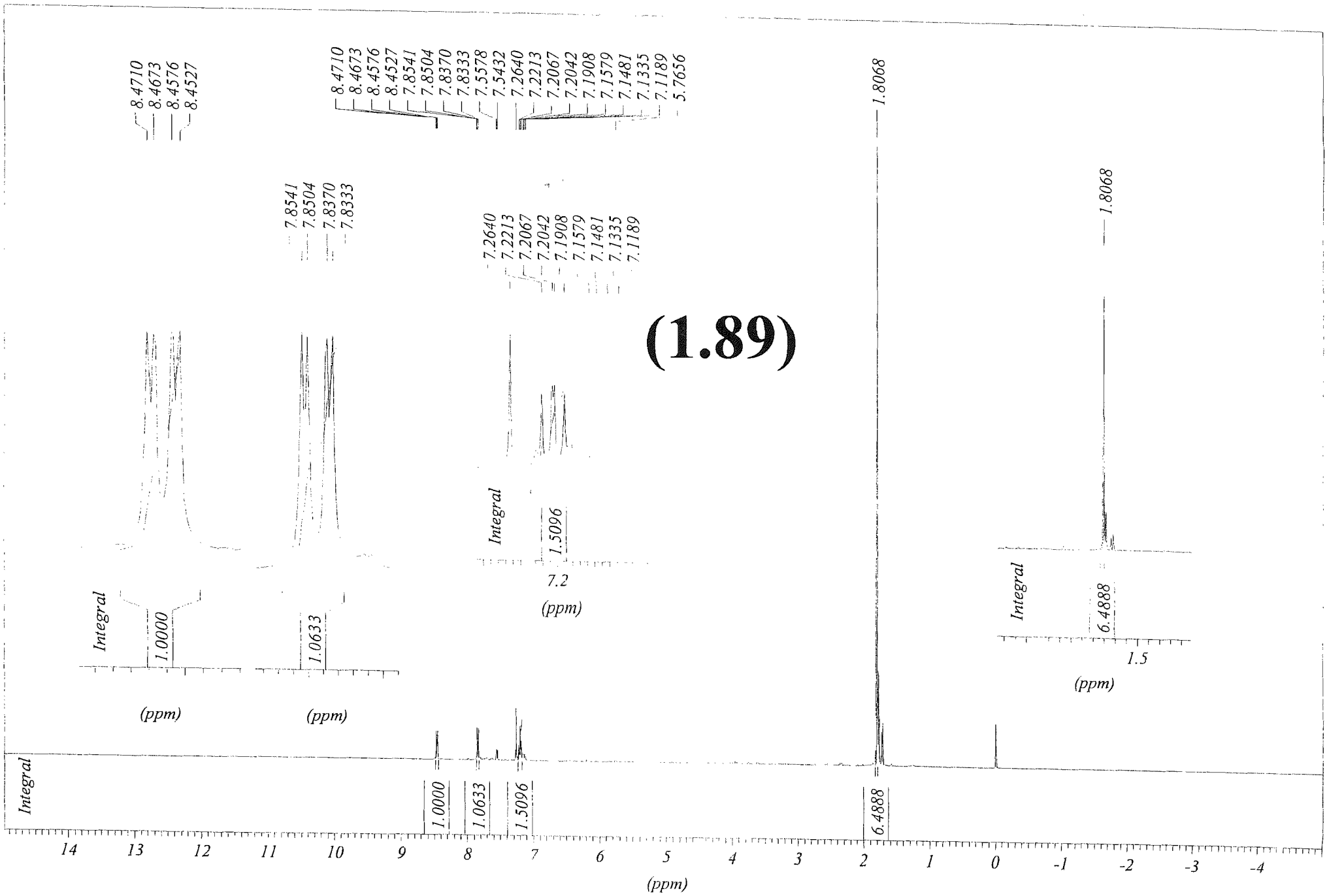
7.6575
7.6233
7.6050
7.5782
7.5697
7.4904
7.4636
7.4392
1.8419
(1.85)
Integral
2.0274
1.0740
2.1689
6.0000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(ppm)

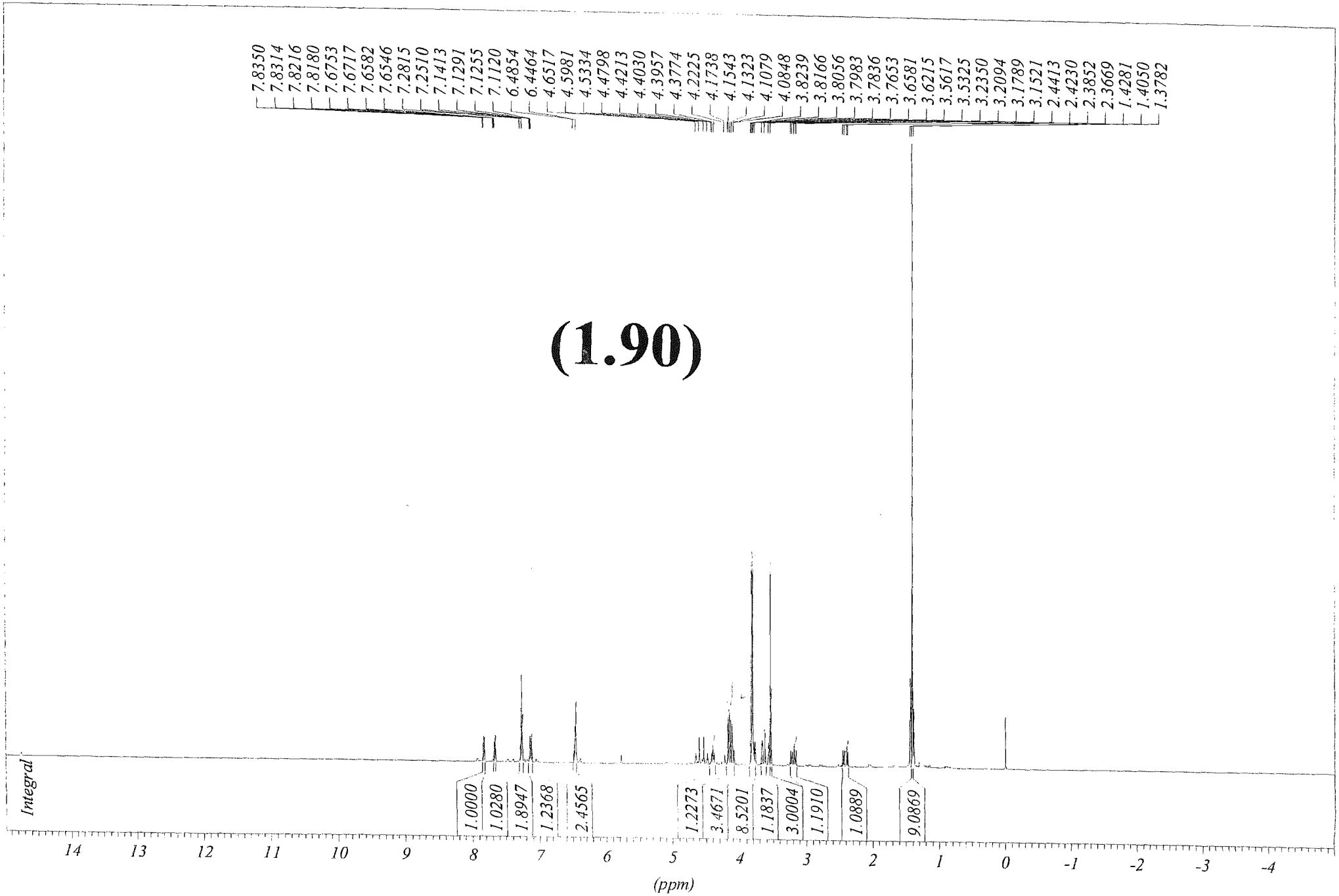

(1.86)
7.5697
7.5453
7.4819
7.4563
7.4319
7.3880
7.3636
7.2978
7.2344
6.4822
6.4565
6.4456
3.7975
3.7889
3.7694
3.6719
3.5366
2.5319
2.4881
2.4698
2.4320
2.4137
3.2269
3.1964
3.1708
4.6034
4.5753
4.5558
4.5034
4.4107
4.3924
4.2242
4.2181
1.4164
1.3798
0.3651
0.9756
0.5846
2.6620
0.2497
0.2757
2.0968
3.1953
0.9529
6.4467
1.5492
1.7385
1.0000
1.1817
9.4856
(ppm)

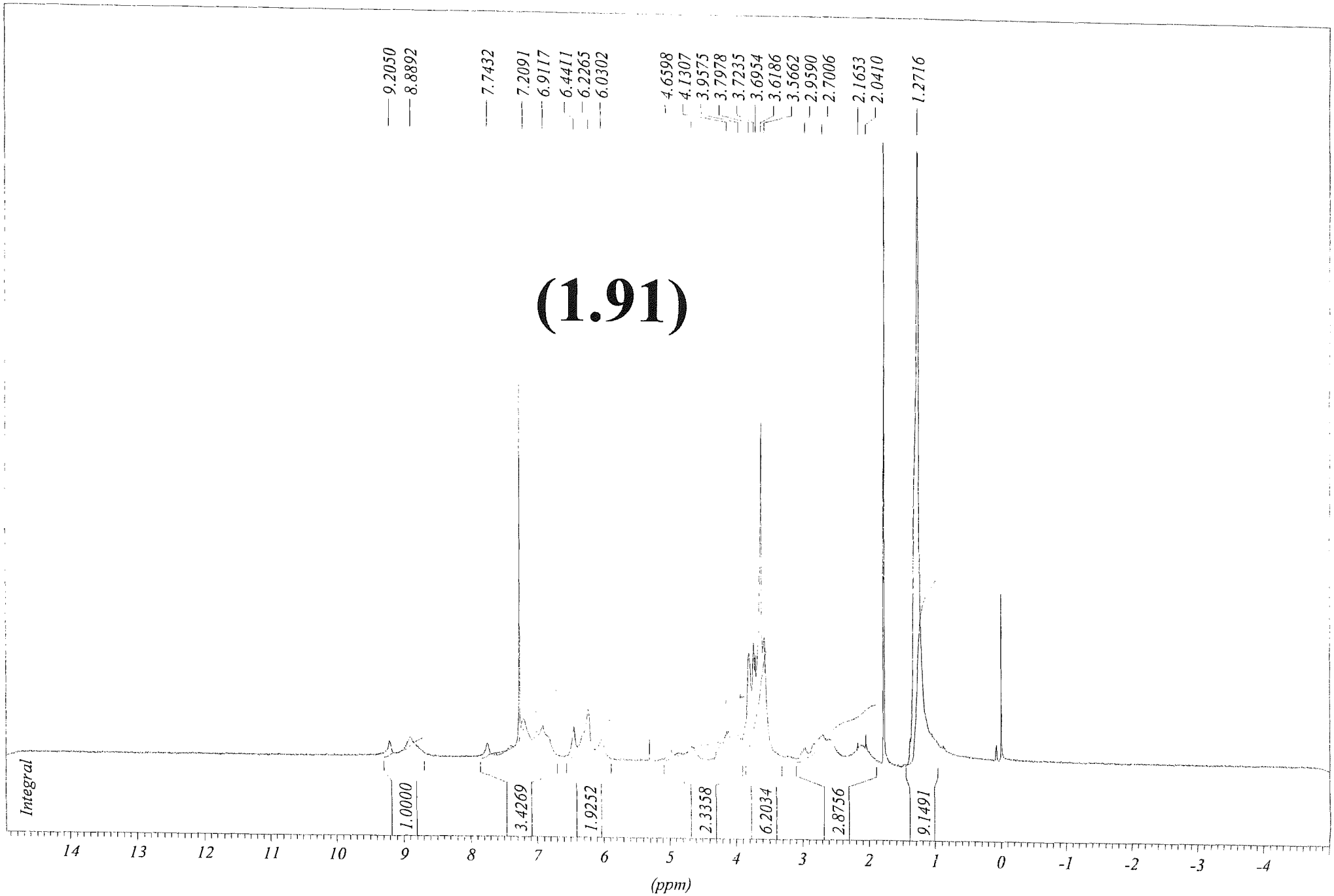

(1.87)
7.4957
7.1520
6.3253
6.1371
4.7748
4.0912
3.6810
2.6461
2.0966
1.2574
Integral
5.3378
1.6976
0.7101
0.9565
6.1750
1.0546
1.3718
9.0000
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
(ppm)

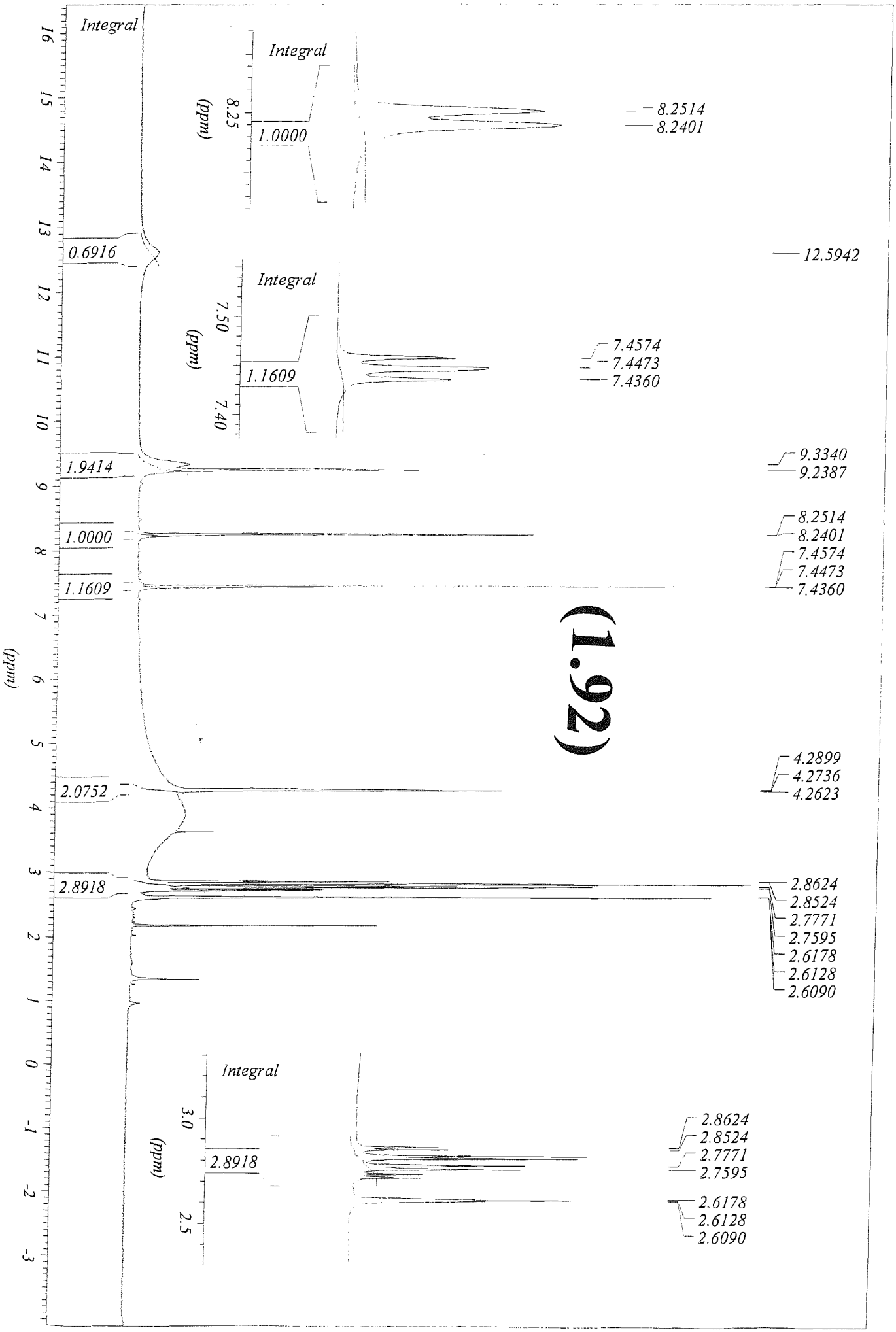
Integral
0.4854
0.7829
1.7165
0.8500
1.8113
2.8024
2.0000
8.2100
8.1912
12.6131
7.7722
7.7534
7.7359
7.6581
7.6204
9.2587
4.2573
4.2297
2.8361
2.8248
2.7934
2.7821
2.7758
2.7595
2.7332
2.7169
(ppm)

(1.88)

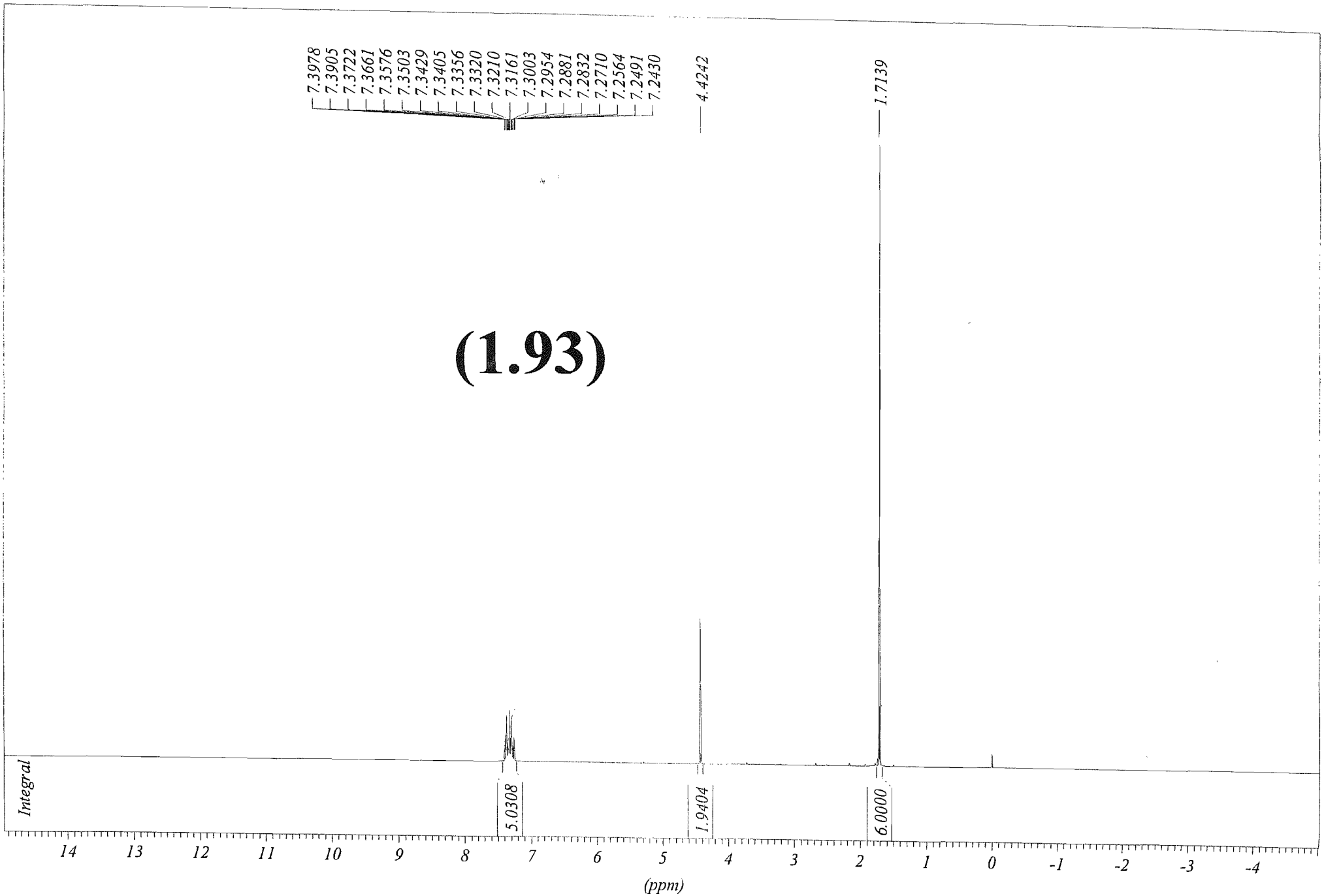

8.4710
8.4673
8.4576
8.4527
7.8541
7.8504
7.8370
7.8333
7.5578
7.5432
7.2640
7.2213
7.2067
7.2042
7.1908
7.1579
7.1481
7.1335
7.1189
5.7656
1.8068
(1.89)
Integral
1.0000
1.0633
1.5096
6.4888
(ppm)
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4

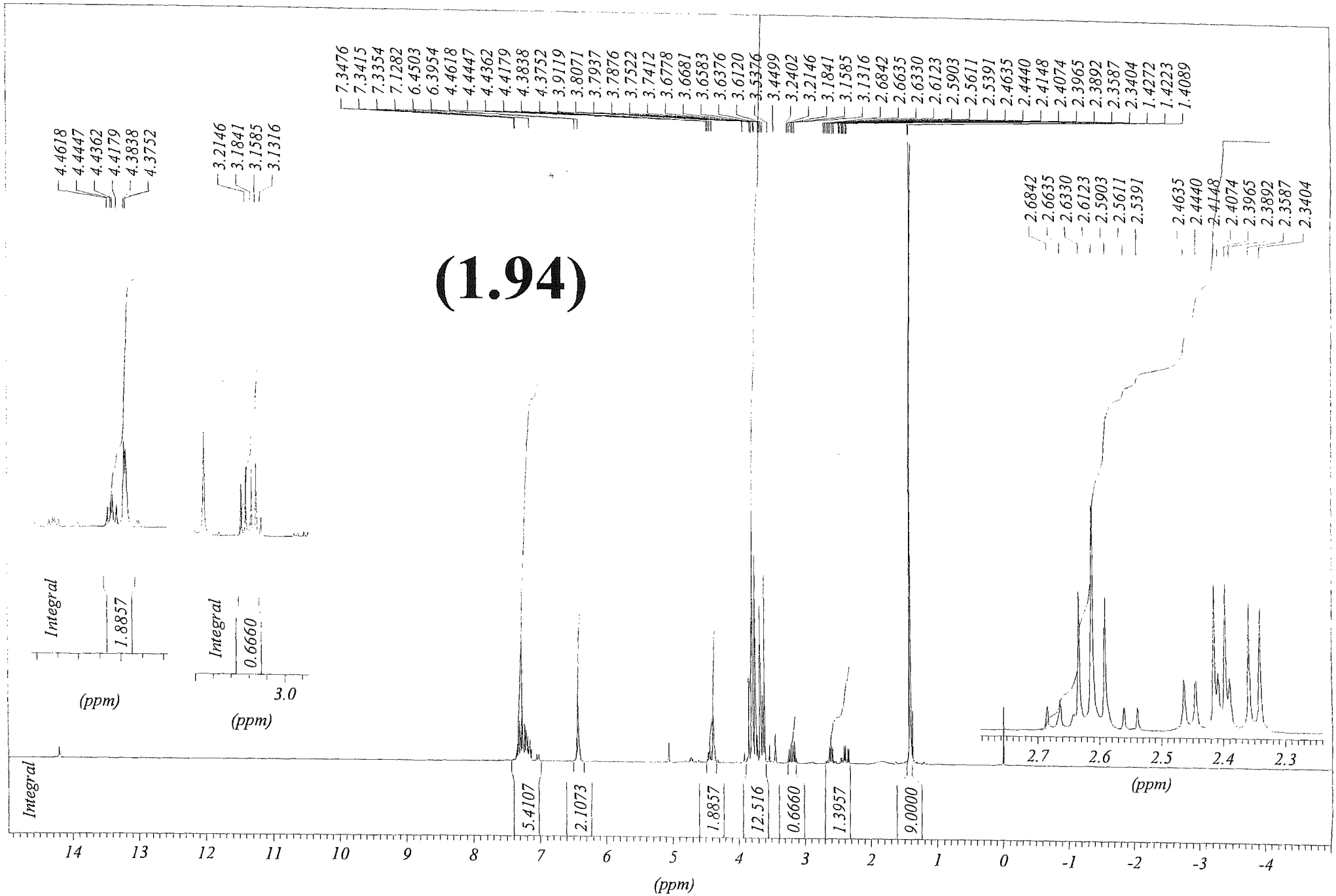

7.8350
7.8314
7.8216
7.8180
7.6753
7.6717
7.6582
7.6546
7.2815
7.2510
7.1413
7.1291
7.1255
7.1120
6.4854
6.4464
4.6517
4.5981
4.5334
4.4798
4.4213
4.4030
4.3957
4.3774
4.2225
4.1738
4.1543
4.1323
4.1079
4.0848
3.8239
3.8166
3.8056
3.7983
3.7836
3.7653
3.6581
3.6215
3.5617
3.5325
3.2350
3.2094
3.1789
3.1521
2.4413
2.4230
2.3852
2.3669
1.4281
1.4050
1.3782
(1.90)
Integral
1.0000
1.0280
1.8947
1.2368
2.4565
1.2273
3.4671
8.5201
1.1837
3.0004
1.1910
1.0889
9.0869
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)

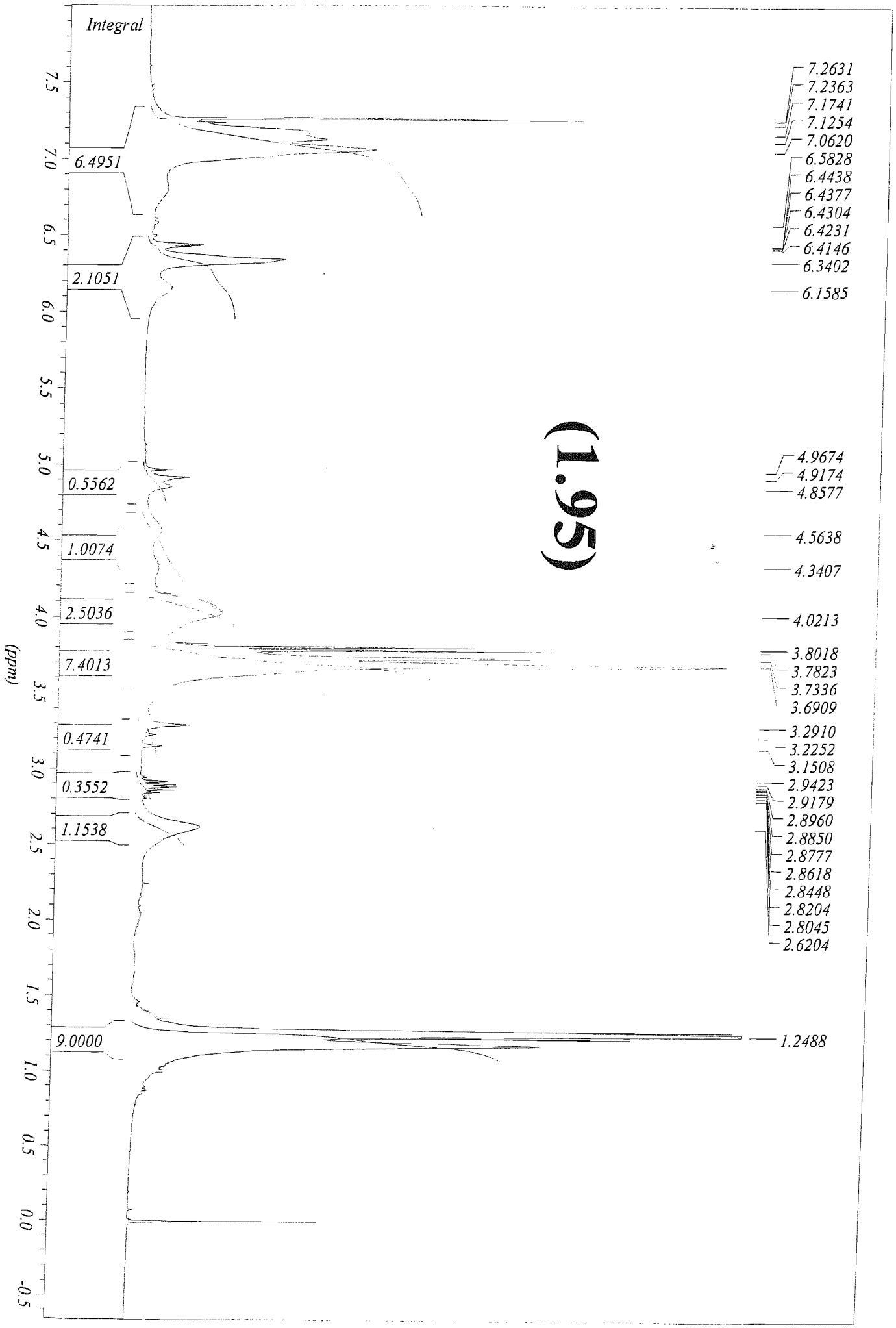

9.2050
8.8892
7.7432
7.2091
6.9117
6.4411
6.2265
6.0302
4.6598
4.1307
3.9575
3.7978
3.7235
3.6954
3.6186
3.5662
2.9590
2.7006
2.1653
2.0410
1.2716
(1.91)
Integral
1.0000
3.4269
1.9252
2.3358
6.2034
2.8756
9.1491
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)


Integral
Integral
8.25
(ppm)
1.0000
8.2514
8.2401
0.6916
12.5942
Integral
7.50
(ppm)
7.40
1.1609
7.4574
7.4473
7.4360
1.9414
9.3340
9.2387
1.0000
8.2514
8.2401
1.1609
7.4574
7.4473
7.4360
(1.92)
(ppm)
2.0752
4.2899
4.2736
4.2623
2.8918
2.8624
2.8524
2.7771
2.7595
2.6178
2.6128
2.6090
Integral
3.0
(ppm)
2.5
2.8918
2.8624
2.8524
2.7771
2.7595
2.6178
2.6128
2.6090

grp-4226-82

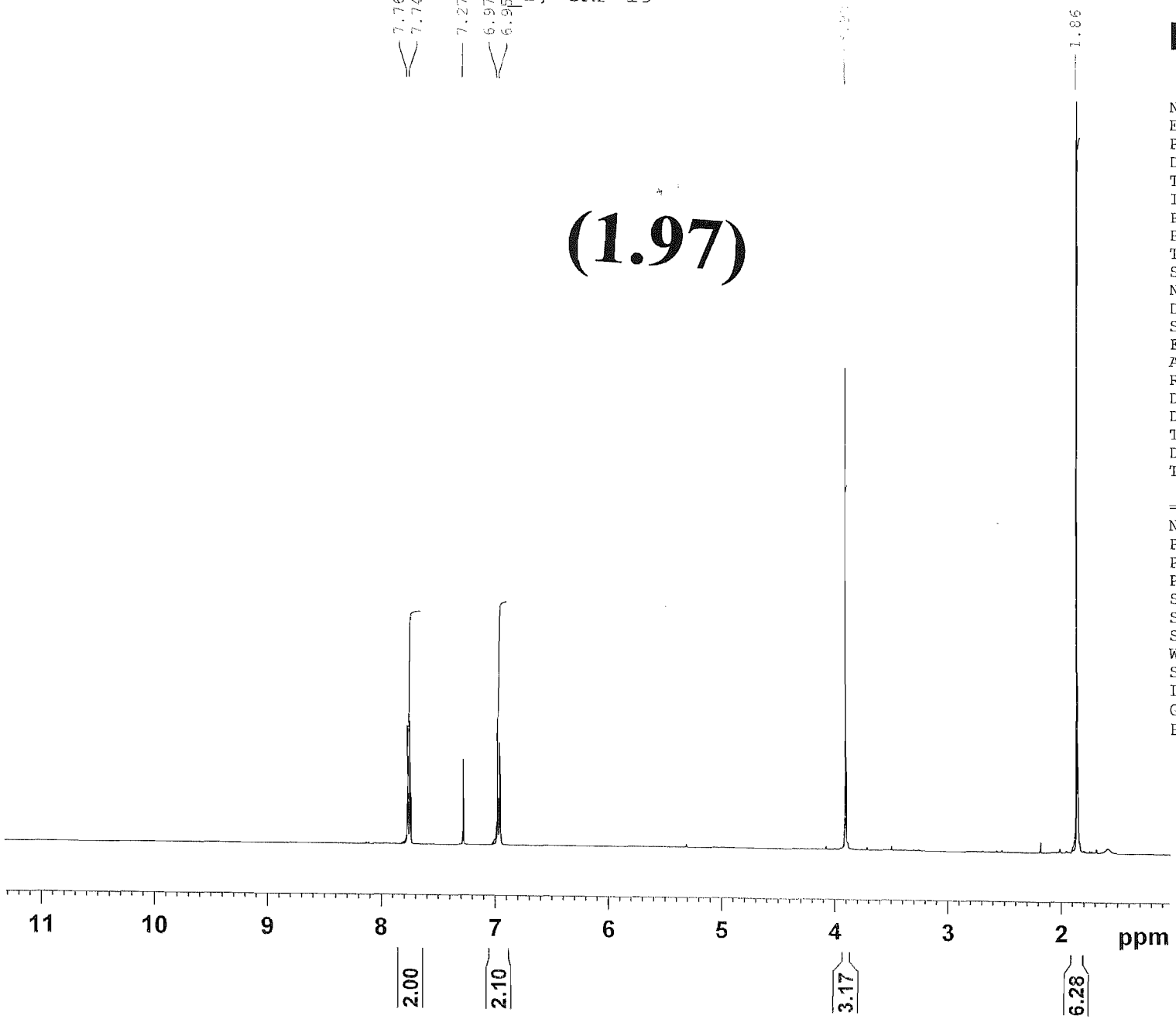

7.3978
7.3905
7.3722
7.3661
7.3576
7.3503
7.3429
7.3405
7.3356
7.3320
7.3210
7.3161
7.3003
7.2954
7.2881
7.2832
7.2710
7.2564
7.2491
7.2430
4.4242
1.7139
(1.93)
Integral
5.0308
1.9404
6.0000
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)


(1.94)
7.3476
7.3415
7.3354
7.1282
6.4503
6.3954
4.4618
4.4447
4.4362
4.4179
4.3838
4.3752
3.9119
3.8071
3.7937
3.7876
3.7522
3.7412
3.6778
3.6681
3.6583
3.6376
3.6120
3.5376
3.4499
3.2402
3.2146
3.1841
3.1585
3.1316
2.6842
2.6635
2.6330
2.6123
2.5903
2.5611
2.5391
2.4635
2.4440
2.4148
2.4074
2.3965
2.3892
2.3587
2.3404
1.4272
1.4223
1.4089
Integral
5.4107
2.1073
1.8857
12.516
0.6660
1.3957
9.0000
(ppm)
Integral
1.8857
(ppm)
Integral
0.6660
3.0
(ppm)
2.7
2.6
2.5
2.4
2.3
(ppm)


Integral
6.4951
2.1051
0.5562
1.0074
2.5036
7.4013
0.4741
0.3552
1.1538
9.0000
7.2631
7.2363
7.1741
7.1254
7.0620
6.5828
6.4438
6.4377
6.4304
6.4231
6.4146
6.3402
6.1585
4.9674
4.9174
4.8577
4.5638
4.3407
4.0213
3.8018
3.7823
3.7336
3.6909
3.2910
3.2252
3.1508
2.9423
2.9179
2.8960
2.8850
2.8777
2.8618
2.8448
2.8204
2.8045
2.6204
1.2488
(1.95)
(ppm)

(1.96)
Integral
0.6967
0.9125
5.0000
1.1921
1.5968
2.2261
4.24
4.20
(ppm)
4.2573
4.2297
4.2071
4.1883
12.5917
8.9878
7.4386
7.3671
7.3495
7.3432
4.5747
4.2573
4.2297
4.2071
4.1883
4.1532
2.8273
2.8147
2.7846
2.7733
2.7533
2.7269
2.7119
2.6178
2.6090
2.80
2.72
(ppm)
2.8273
2.8147
2.7846
2.7733
2.7533
2.7269
2.7119
(ppm)

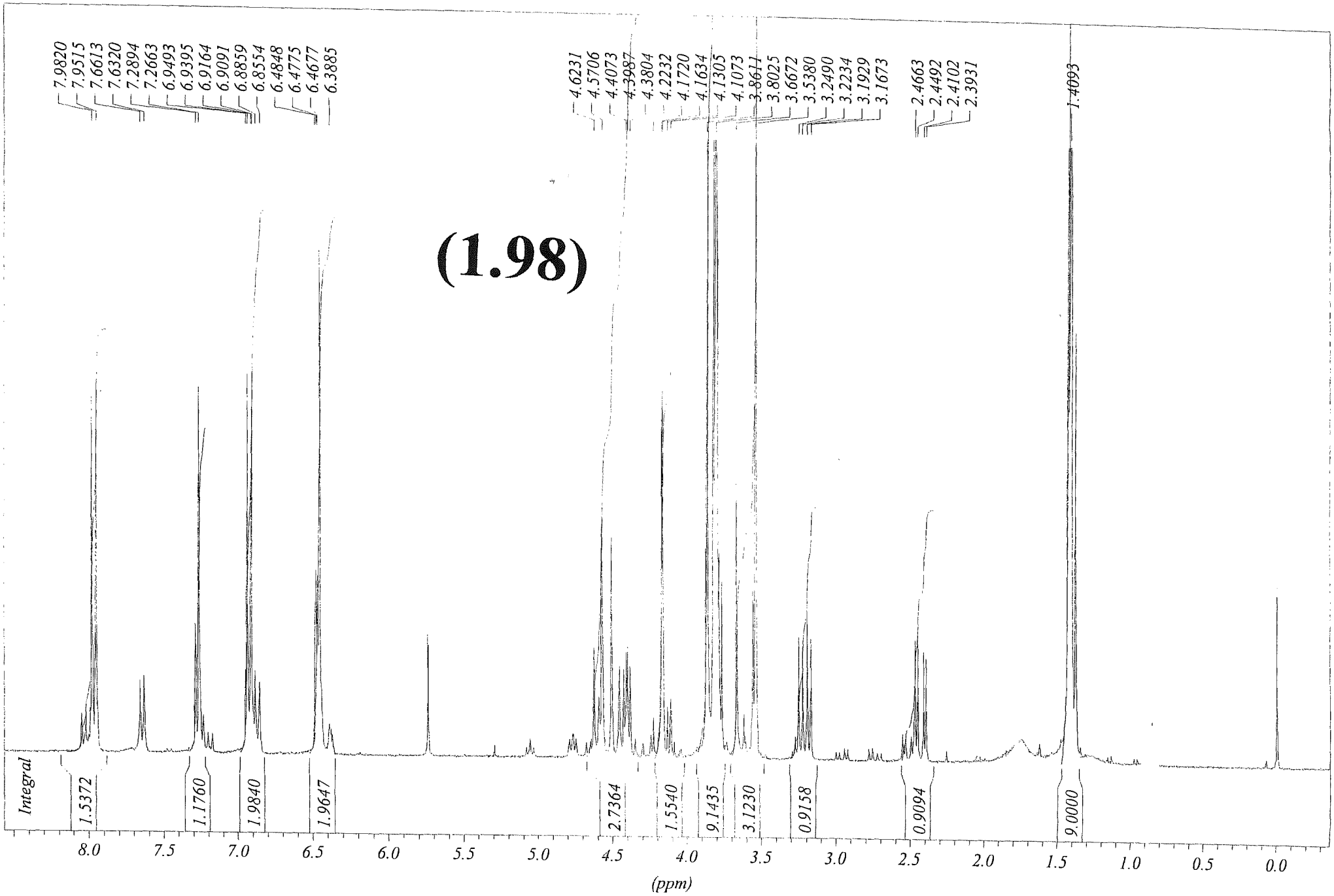
GRP-4226-95
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2_1} GRP 19

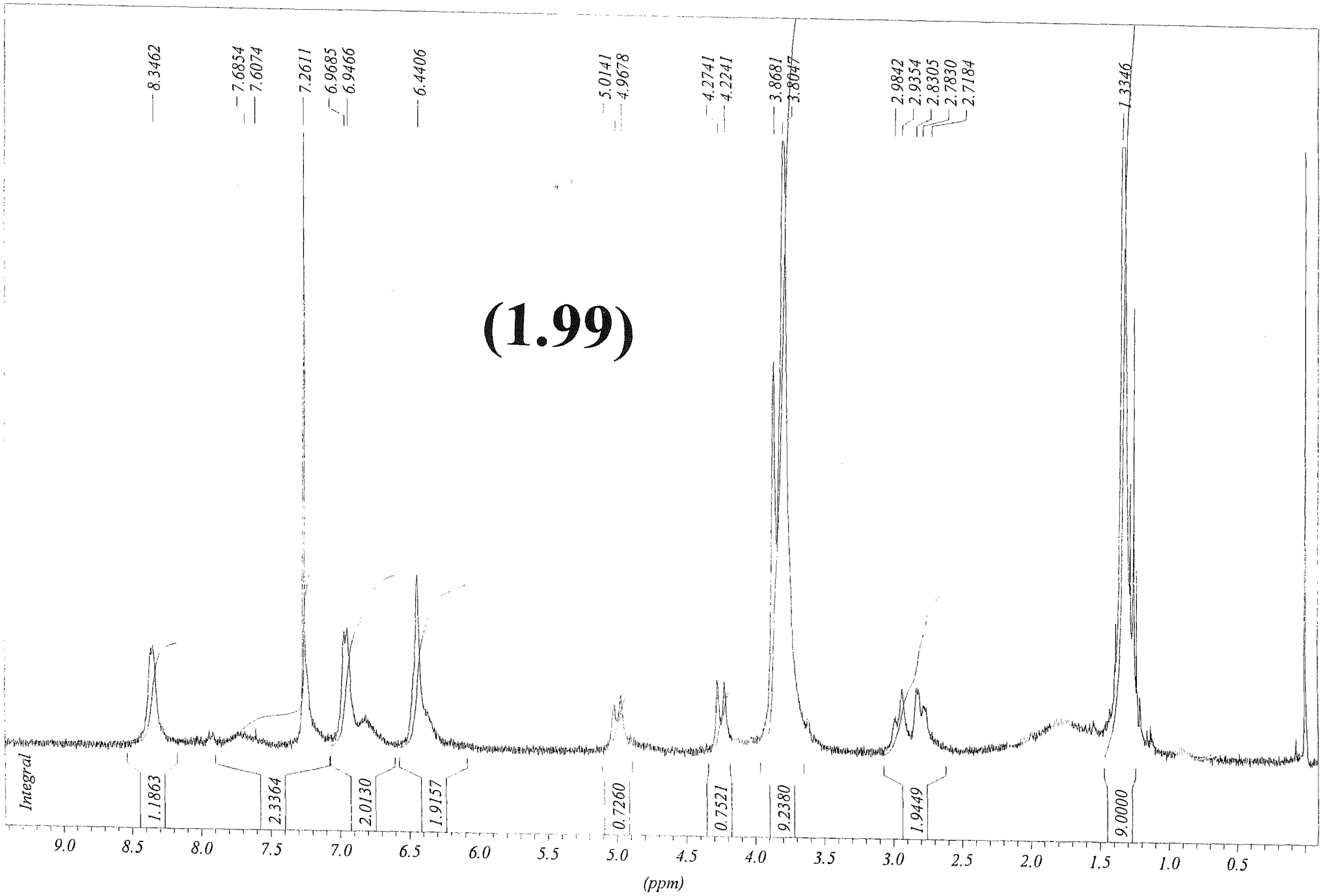


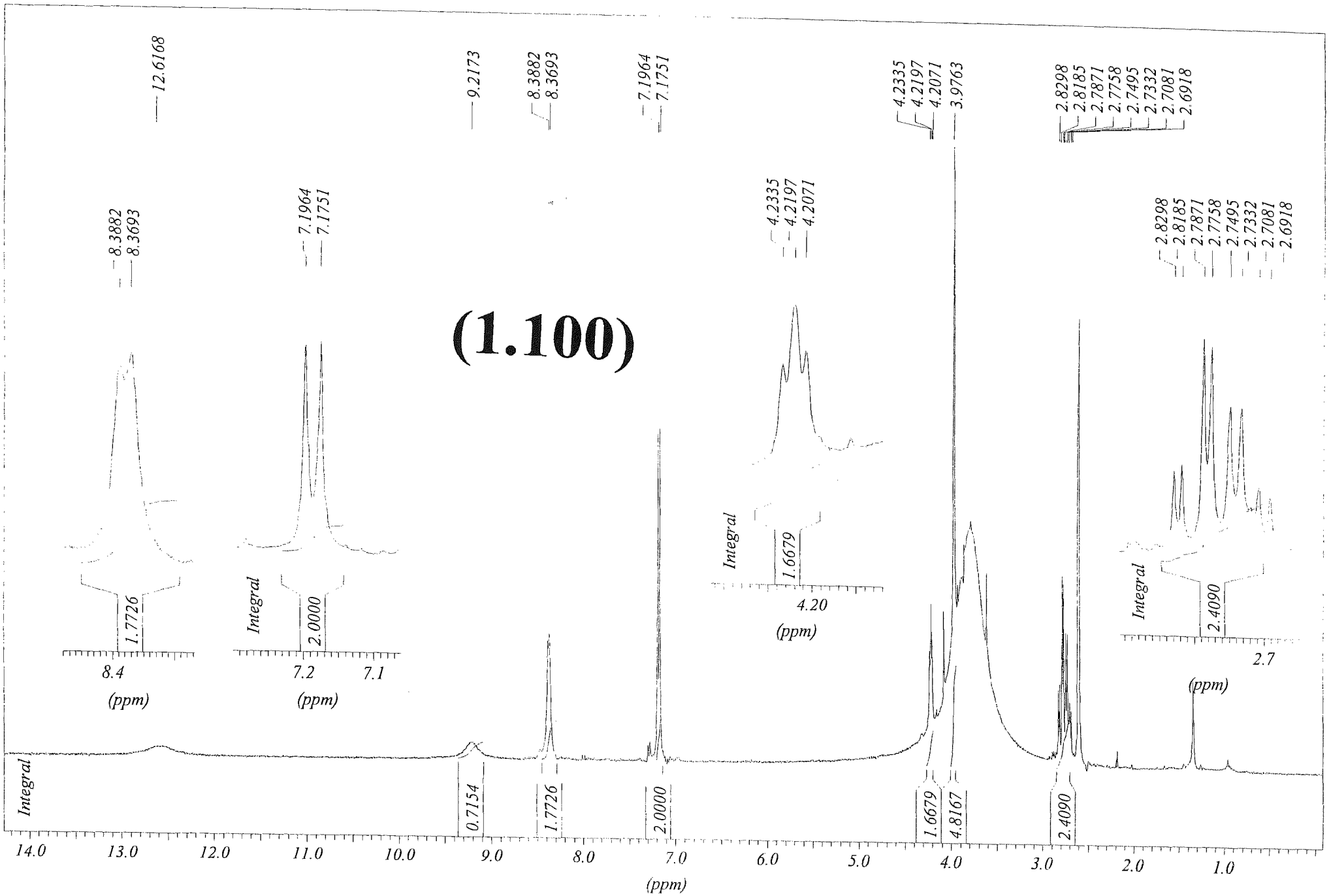
(1.97)

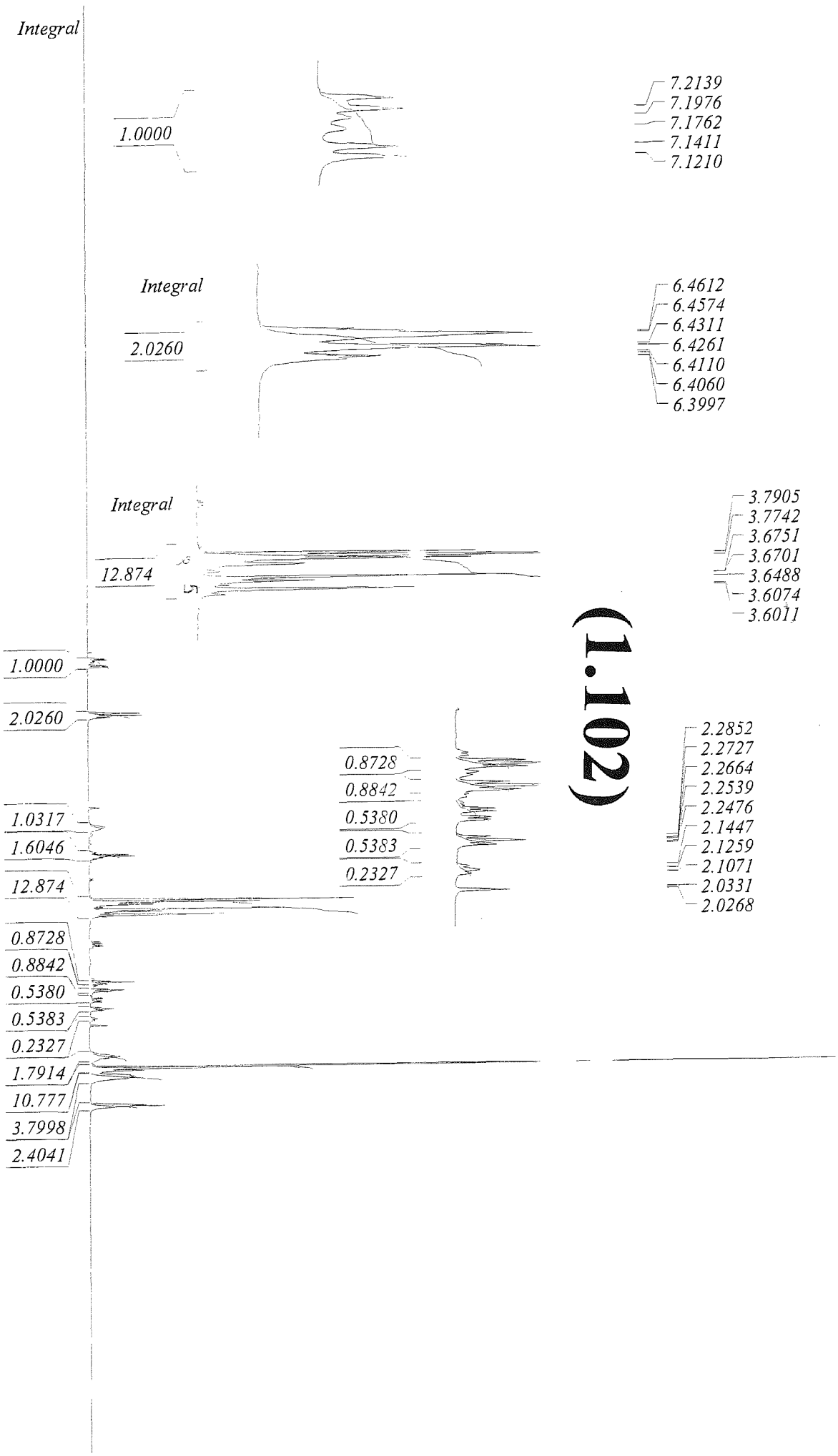
NAME	GRP-4226-95
EXPNO	20
PROCNO	1
Date_	20080220
Time	12.13
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	16
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	575
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1299996 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

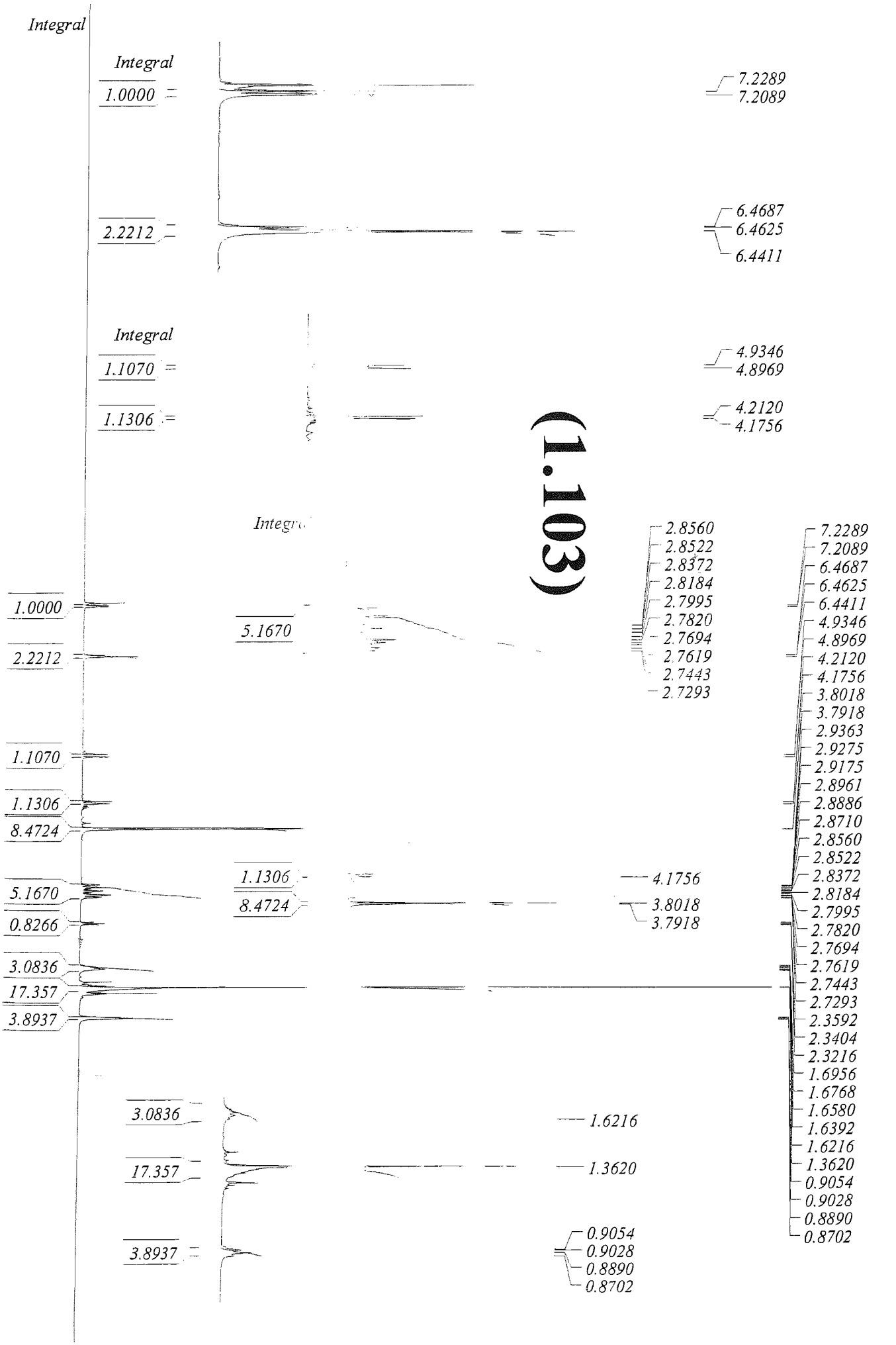
grp-4226-96

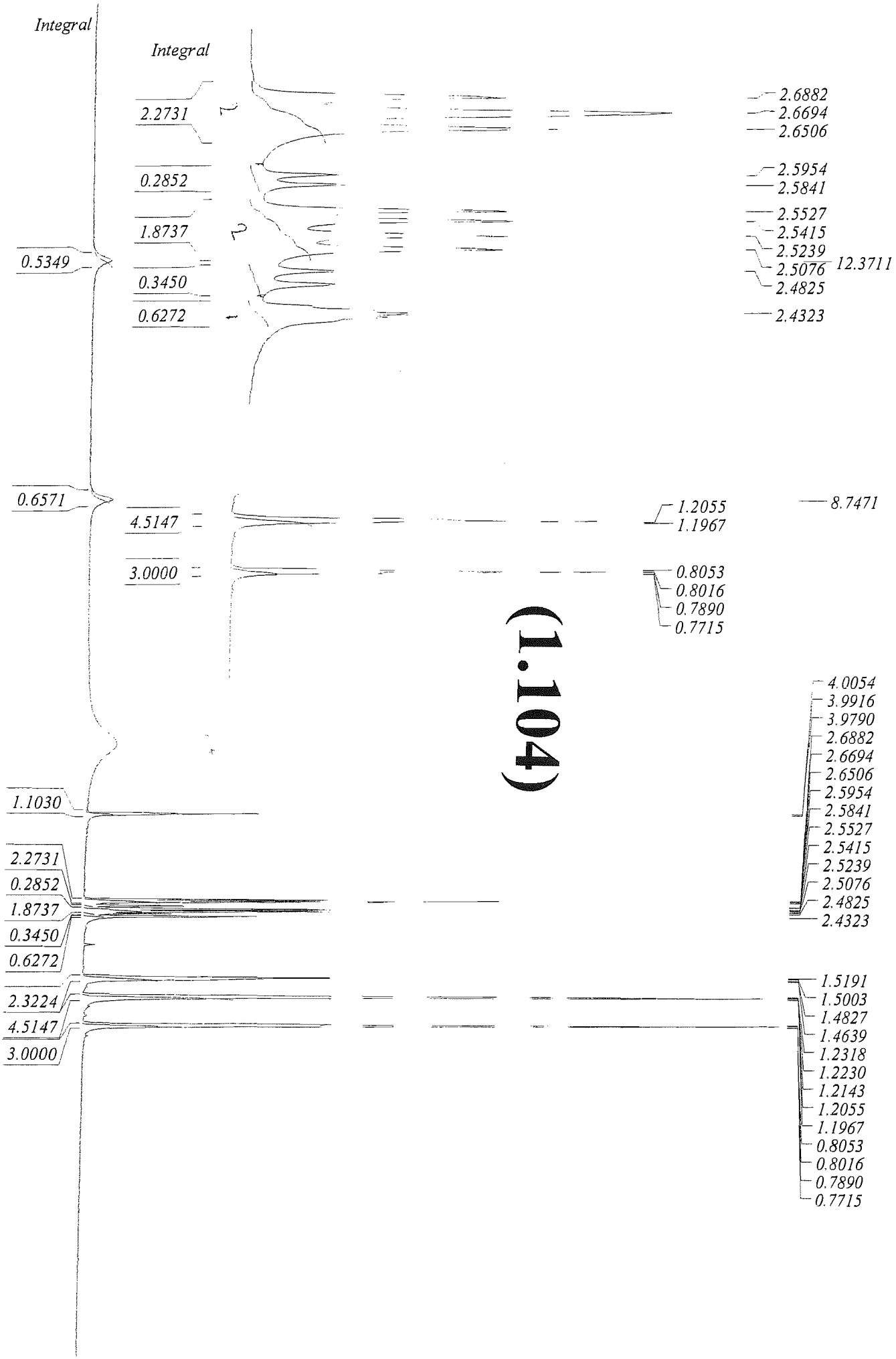

(1.98)
7.9820
7.9515
7.6613
7.6320
7.2894
7.2663
6.9493
6.9395
6.9164
6.9091
6.8859
6.8554
6.4848
6.4775
6.4677
6.3885
4.6231
4.5706
4.4073
4.3987
4.3804
4.2232
4.1720
4.1634
4.1305
4.1073
3.8611
3.8025
3.6672
3.5380
3.2490
3.2234
3.1929
3.1673
2.4663
2.4492
2.4102
2.3931
1.4093
Integral
1.5372
1.1760
1.9840
1.9647
2.7364
1.5540
9.1435
3.1230
0.9158
0.9094
9.0000
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
(ppm)


8.3462
7.6854
7.6074
7.2611
6.9685
6.9466
6.4406
5.0141
4.9678
4.2741
4.2241
3.8681
3.8047
2.9842
2.9354
2.8305
2.7830
2.7184
1.3346
(1.99)
Integral
1.1863
2.3364
2.0130
1.9157
0.7260
0.7521
9.2380
1.9449
9.0000
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
(ppm)

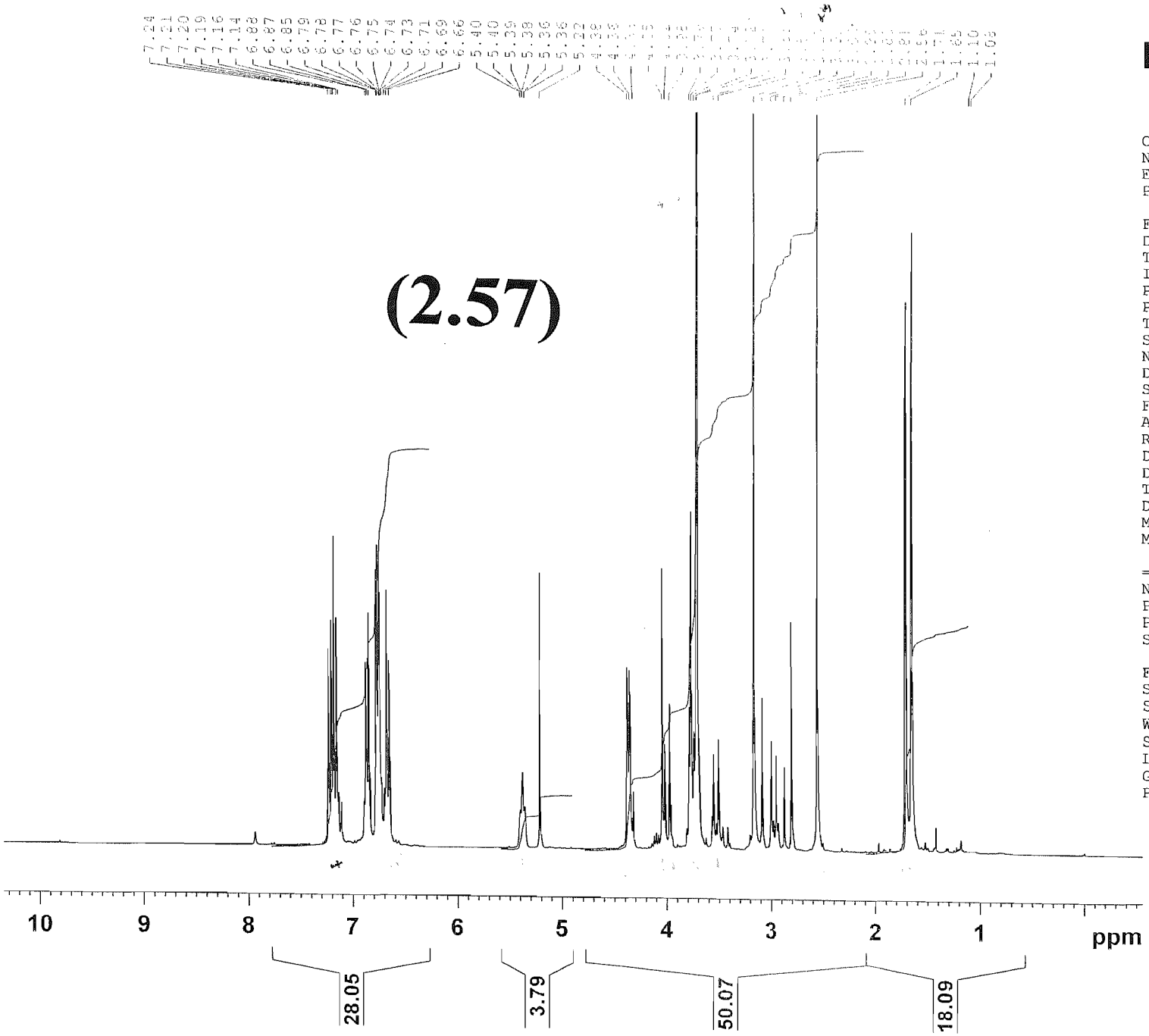

(1.100)
12.6168
9.2173
8.3882
8.3693
7.1964
7.1751
4.2335
4.2197
4.2071
3.9763
2.8298
2.8185
2.7871
2.7758
2.7495
2.7332
2.7081
2.6918
Integral
0.7154
1.7726
2.0000
1.6679
4.8167
2.4090
(ppm)


Integral
1.0000
7.2139
7.1976
7.1762
7.1411
7.1210
Integral
2.0260
6.4612
6.4574
6.4311
6.4261
6.4110
6.4060
6.3997
Integral
12.874
3.7905
3.7742
3.6751
3.6701
3.6488
3.6074
3.6011
(1.102)
1.0000
2.0260
1.0317
1.6046
12.874
0.8728
0.8842
0.5380
0.5383
0.2327
1.7914
10.777
3.7998
2.4041
0.8728
0.8842
0.5380
0.5383
0.2327
2.2852
2.2727
2.2664
2.2539
2.2476
2.1447
2.1259
2.1071
2.0331
2.0268
3.7742
3.6751
3.6701
3.6488
3.6074
3.6011
3.2198
3.2060
3.2010
3.1834
3.1771
3.1633
3.1583
2.7004
2.6967
2.6841
2.6804
2.6616
2.6565
2.6452
2.6415
2.6302
2.6252
2.6026
2.5913
2.5863
2.5499
2.5336
2.5135
2.4935
2.4797
2.4521
2.4370
2.4282
2.4232
2.4144
2.4094
2.3856
2.3806
2.3718
2.3680
2.2915
2.2852
2.2727
2.2664
2.2539
2.2476
2.1447
2.1259
2.1071
2.0331
2.0268
1.6279
1.6154
1.6103
1.5991
1.5815
1.5627
1.4272
1.3168
1.3105
1.3030
1.2917
1.2880
1.2716
1.2666
1.2528
0.8991
0.8941
0.8878
0.8828
0.8702
0.8665


Integral
Integral
1.0000
2.2212
7.2289
7.2089
6.4687
6.4625
6.4411
Integral
1.1070
1.1306
4.9346
4.8969
4.2120
4.1756
(1.103)
Integral
1.0000
2.2212
1.1070
1.1306
8.4724
5.1670
0.8266
3.0836
17.357
3.8937
5.1670
2.8560
2.8522
2.8372
2.8184
2.7995
2.7820
2.7694
2.7619
2.7443
2.7293
1.1306
8.4724
4.1756
3.8018
3.7918
3.0836
17.357
3.8937
1.6216
1.3620
0.9054
0.9028
0.8890
0.8702
7.2289
7.2089
6.4687
6.4625
6.4411
4.9346
4.8969
4.2120
4.1756
3.8018
3.7918
2.9363
2.9275
2.9175
2.8961
2.8886
2.8710
2.8560
2.8522
2.8372
2.8184
2.7995
2.7820
2.7694
2.7619
2.7443
2.7293
2.3592
2.3404
2.3216
1.6956
1.6768
1.6580
1.6392
1.6216
1.3620
0.9054
0.9028
0.8890
0.8702


Integral
Integral
2.2731
0.2852
1.8737
0.5349
0.3450
0.6272
2.6882
2.6694
2.6506
2.5954
2.5841
2.5527
2.5415
2.5239
2.5076
2.4825
2.4323
12.3711
0.6571
4.5147
3.0000
1.2055
1.1967
0.8053
0.8016
0.7890
0.7715
8.7471
(1.104)
1.1030
2.2731
0.2852
1.8737
0.3450
0.6272
2.3224
4.5147
3.0000
4.0054
3.9916
3.9790
2.6882
2.6694
2.6506
2.5954
2.5841
2.5527
2.5415
2.5239
2.5076
2.4825
2.4323
1.5191
1.5003
1.4827
1.4639
1.2318
1.2230
1.2143
1.2055
1.1967
0.8053
0.8016
0.7890
0.7715

GRP4744-51/1



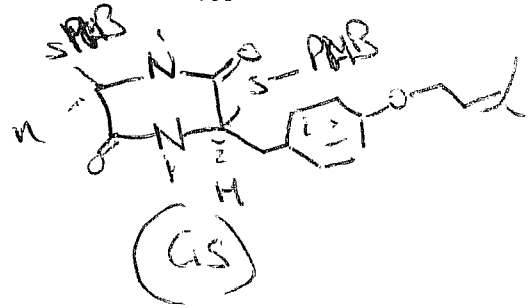
BRUKER

Current Data Parameters
NAME NMR6579GRP4744-51.1.1
EXPNO 1
PROCNO 1

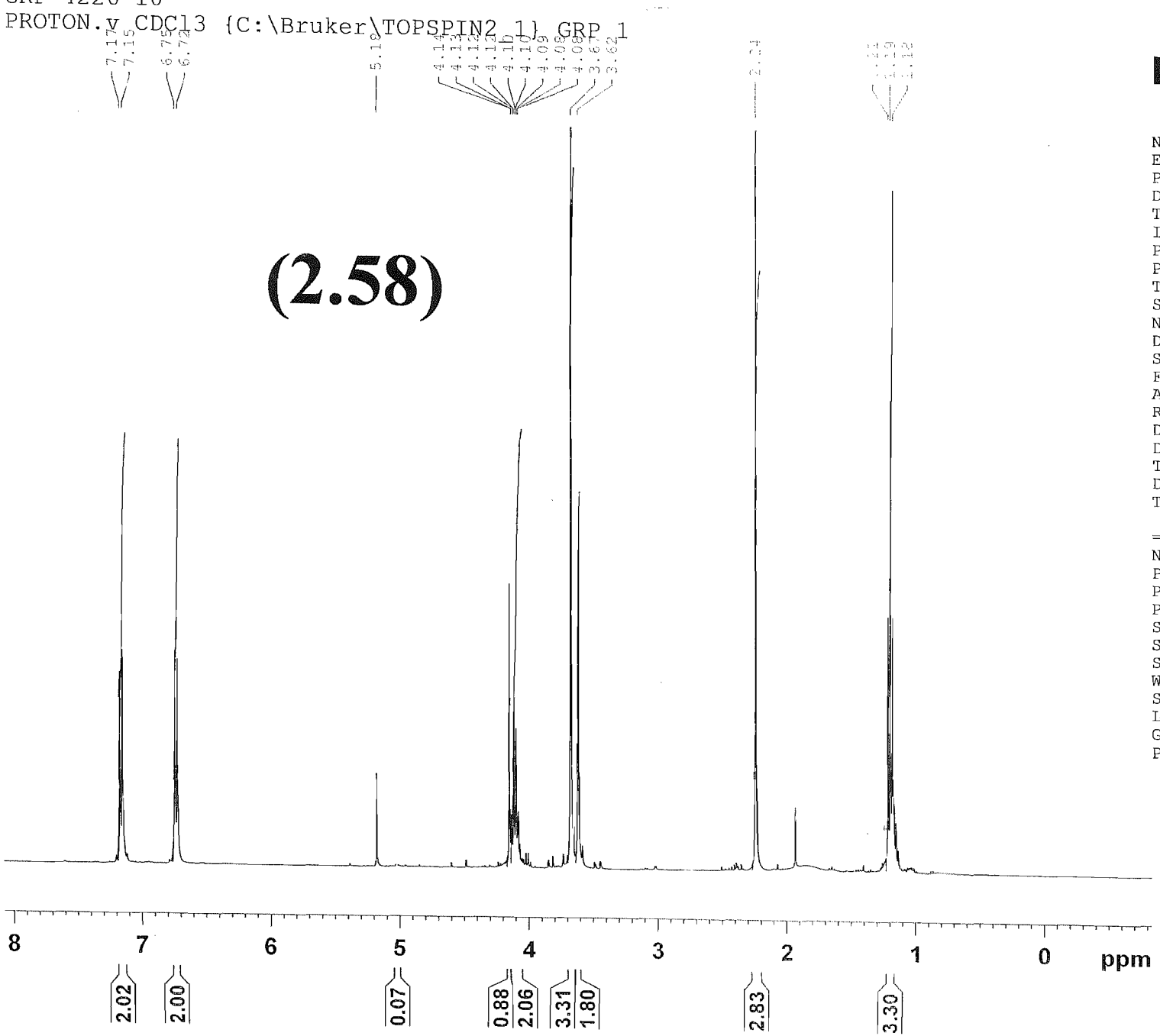
F2 - Acquisition Parameters
Date_ 20071130
Time 9.44
INSTRUM av300
PROBHD 5 mm QNP 1H/15
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 64
DS 2
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 143.7
DW 81.000 usec
DE 6.00 usec
TE 292.7 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 8.88 usec
PL1 2.00 dB
SFO1 300.1318534 MHz

F2 - Processing parameters
SI 32768
SF 300.1300281 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



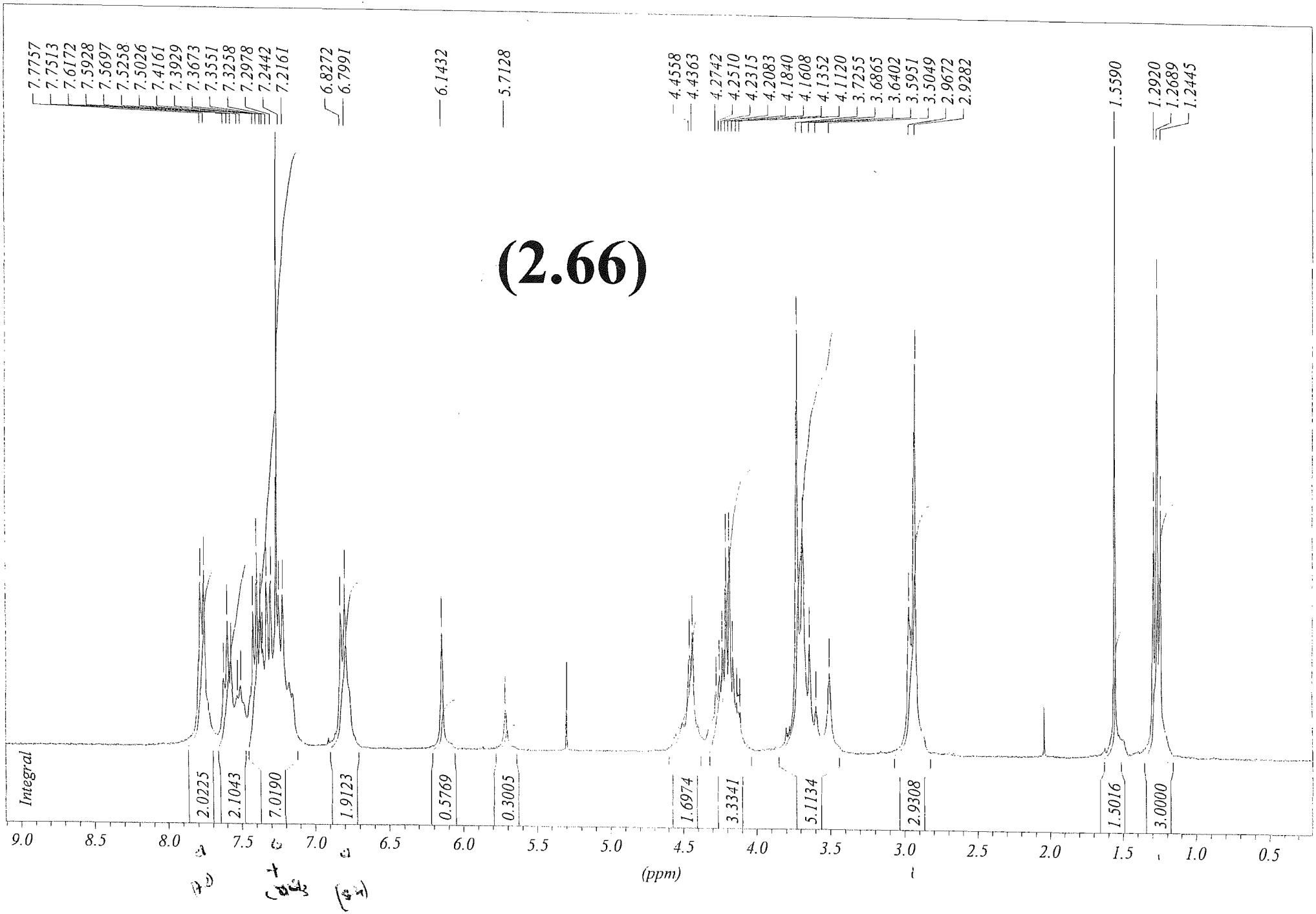
GRP-4226-10
PROTON.y CDCl3 {C:\Bruker\TOPSPIN2_1} GRP 1



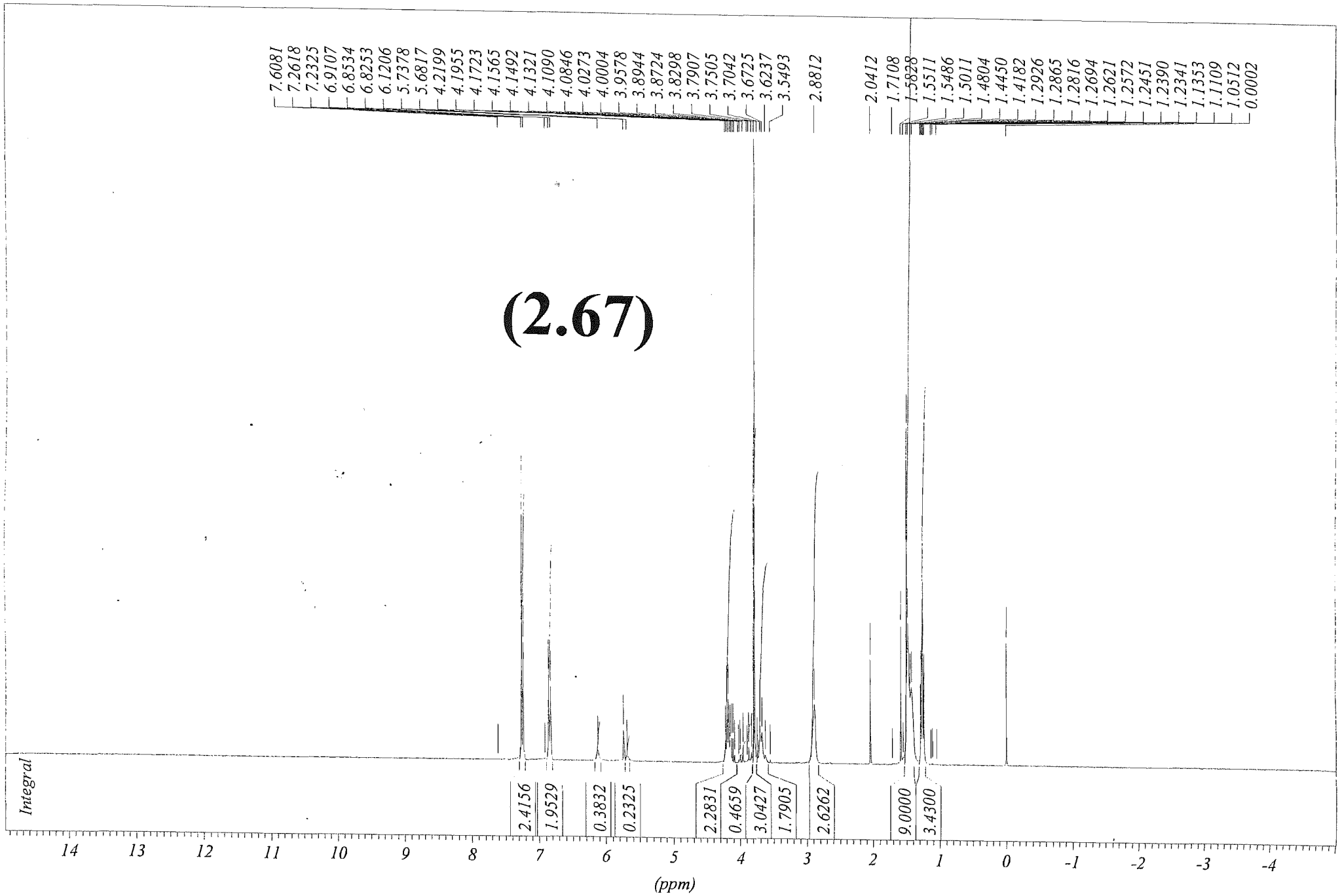
BRUKER

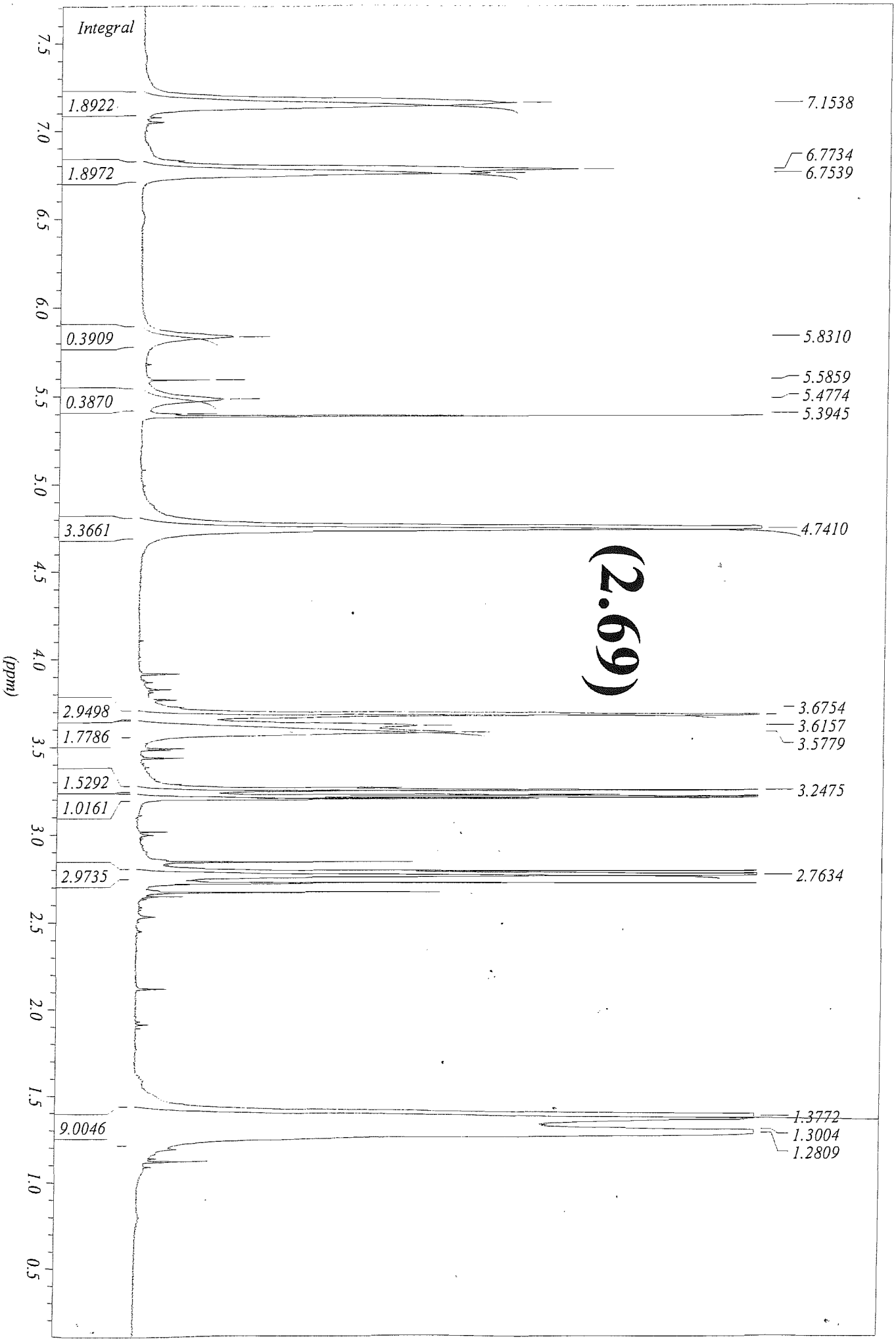
NAME	GRP-4226-10
EXPNO	10
PROCNO	1
Date_	20080214
Time	17.16
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	22.6
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300282 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

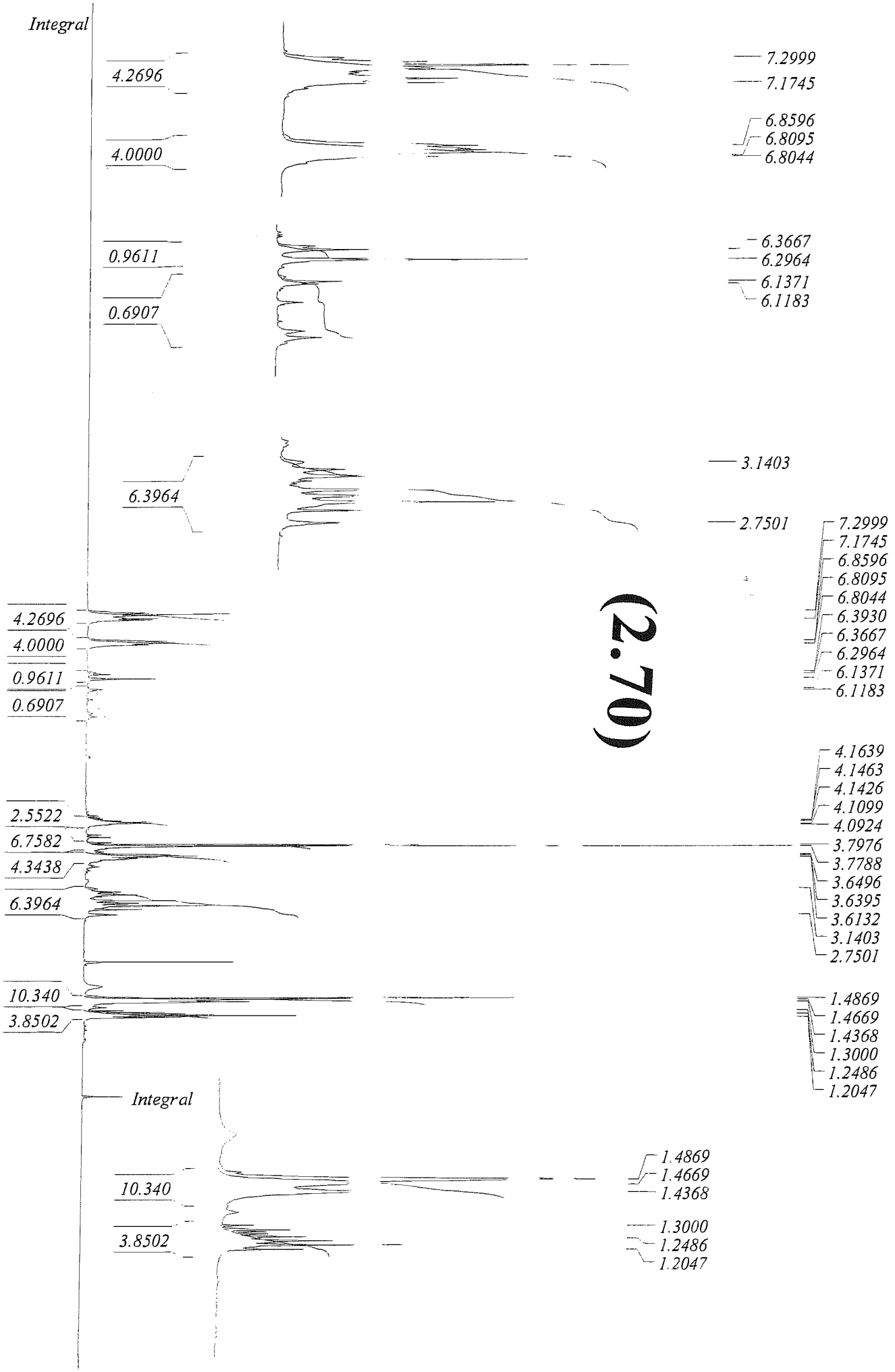
grp-4226-65


7.7757
7.7513
7.6172
7.5928
7.5697
7.5258
7.5026
7.4161
7.3929
7.3673
7.3551
7.3258
7.2978
7.2442
7.2161
6.8272
6.7991
6.1432
5.7128
4.4558
4.4363
4.2742
4.2510
4.2315
4.2083
4.1840
4.1608
4.1352
4.1120
3.7255
3.6865
3.6402
3.5951
3.5049
2.9672
2.9282
1.5590
1.2920
1.2689
1.2445
(2.66)
Integral
2.0225
2.1043
7.0190
1.9123
0.5769
0.3005
1.6974
3.3341
5.1134
2.9308
1.5016
3.0000
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
(ppm)

grp-4226-78

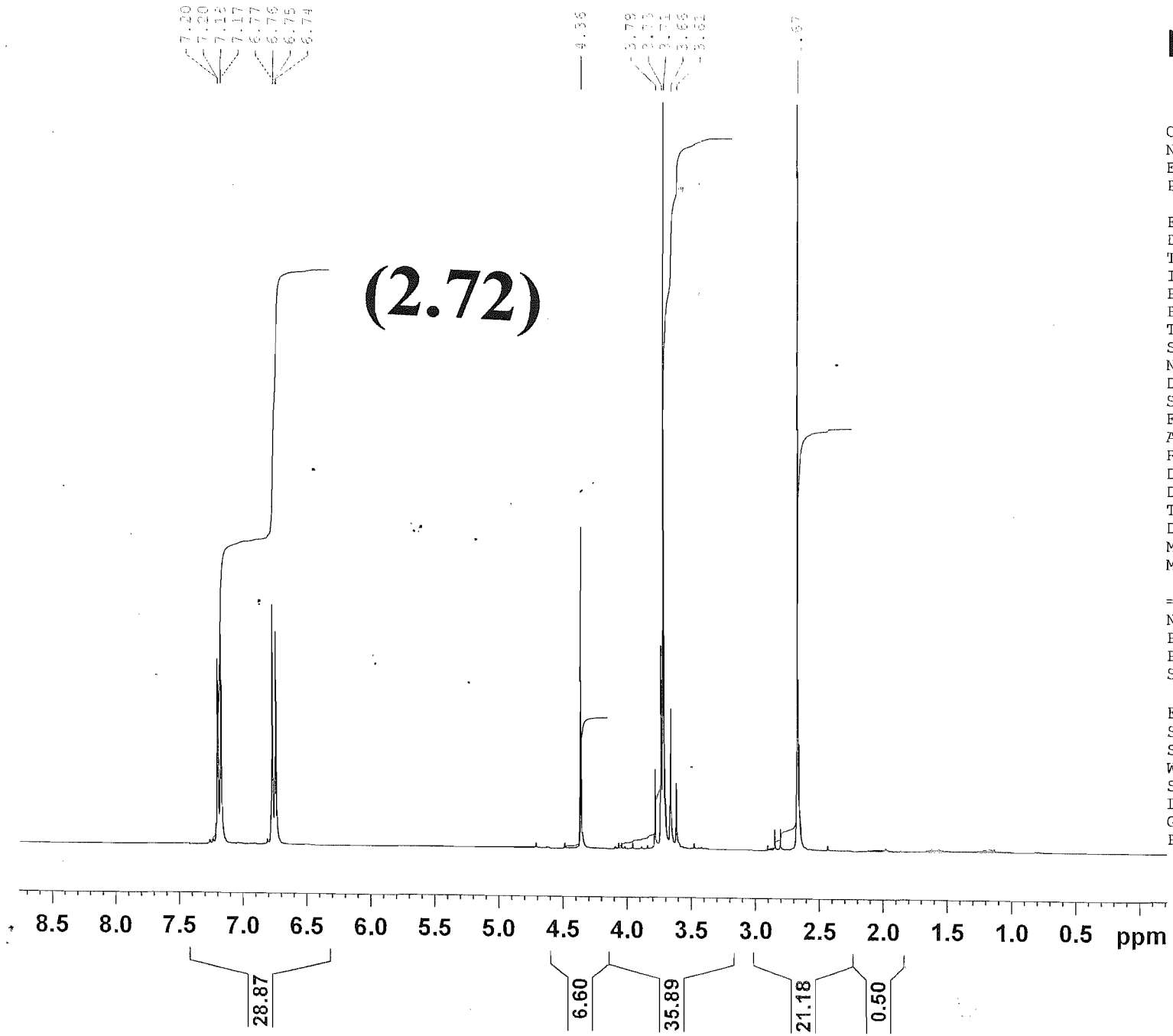

7.6081
7.2618
7.2325
6.9107
6.8534
6.8253
6.1206
5.7378
5.6817
4.2199
4.1955
4.1723
4.1565
4.1492
4.1321
4.1090
4.0846
4.0273
4.0004
3.9578
3.8944
3.8724
3.8298
3.7907
3.7505
3.7042
3.6725
3.6237
3.5493
2.8812
2.0412
1.7108
1.5828
1.5511
1.5486
1.5011
1.4804
1.4450
1.4182
1.2926
1.2865
1.2816
1.2694
1.2621
1.2572
1.2451
1.2390
1.2341
1.1353
1.1109
1.0512
0.0002
(2.67)
Integral
2.4156
1.9529
0.3832
0.2325
2.2831
0.4659
3.0427
1.7905
2.6262
9.0000
3.4300
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)


Integral
1.8922
1.8972
0.3909
0.3870
3.3661
2.9498
1.7786
1.5292
1.0161
2.9735
9.0046
7.1538
6.7734
6.7539
5.8310
5.5859
5.4774
5.3945
4.7410
3.6754
3.6157
3.5779
3.2475
2.7634
1.3772
1.3004
1.2809
(ppm)
(2.69)


Integral
4.2696
4.0000
0.9611
0.6907
6.3964
7.2999
7.1745
6.8596
6.8095
6.8044
6.3667
6.2964
6.1371
6.1183
3.1403
2.7501
(2.70)
2.5522
6.7582
4.3438
10.340
3.8502
4.1639
4.1463
4.1426
4.1099
4.0924
3.7976
3.7788
3.6496
3.6395
3.6132
1.4869
1.4669
1.4368
1.3000
1.2486
1.2047

(2.71)
Integral
1.7444
1.7227
0.7614
1.1310
1.1583
3.0915
3.0000
7.3709
7.3496
6.8817
6.8604
3.8034
4.3365
4.0066
3.9727
3.8673
3.8034
2.6794
(ppm)

GRP5019-9/11



BRUKER

Current Data Parameters

NAME	NMR6575GRP5019-.1.1
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

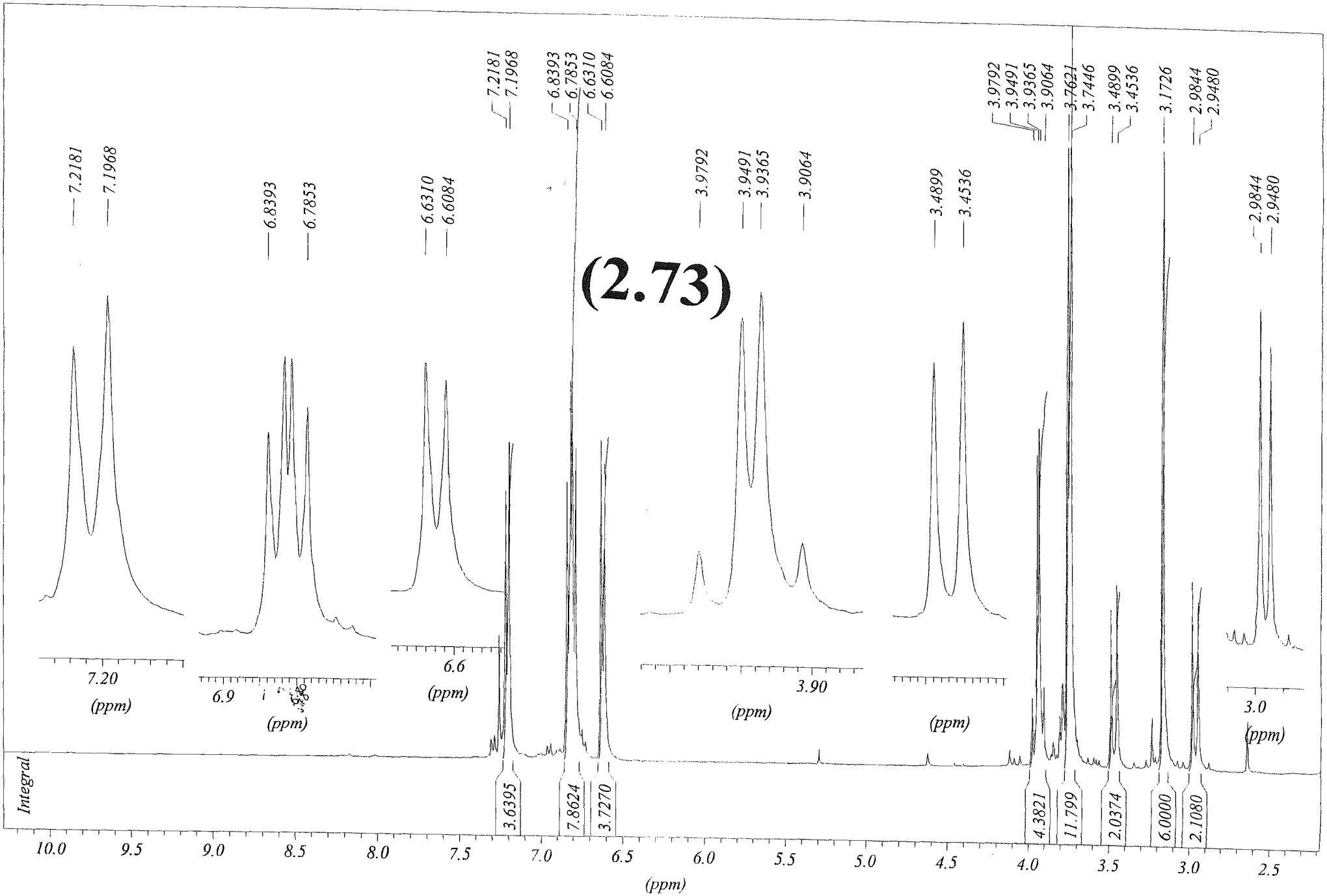
Date_	20071130
Time	8.31
INSTRUM	av300
PROBHD	5 mm QNP 1H/15
PULPROG	zg
TD	65536
SOLVENT	CDCl3
NS	32
DS	0
SWH	4990.020 Hz
FIDRES	0.076142 Hz
AQ	6.5667572 sec
RG	228.1
DW	100.200 usec
DE	6.00 usec
TE	292.6 K
D1	1.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	8.88 usec
PL1	2.00 dB
SFO1	300.1318534 MHz

F2 - Processing parameters

SI	32768
SF	300.1300261 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


(2.73)
7.2181
7.1968
6.8393
6.7853
6.6310
6.6084
3.9792
3.9491
3.9365
3.9064
3.4899
3.4536
2.9844
2.9480
7.20
(ppm)
6.9
(ppm)
6.6
(ppm)
3.90
(ppm)
3.0
(ppm)
Integral
3.6395
7.8624
3.7270
4.3821
11.799
2.0374
6.0000
2.1080
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
(ppm)

Acquisition Time (sec)	1.9923	Comment	grp4429/20/2
Date Stamp	19 Nov 2005 04:50:08		
Frequency (MHz)	400.13	Nucleus	1H
Owner	jms	Points Count	16384
Solvent	CHLOROFORM-d		

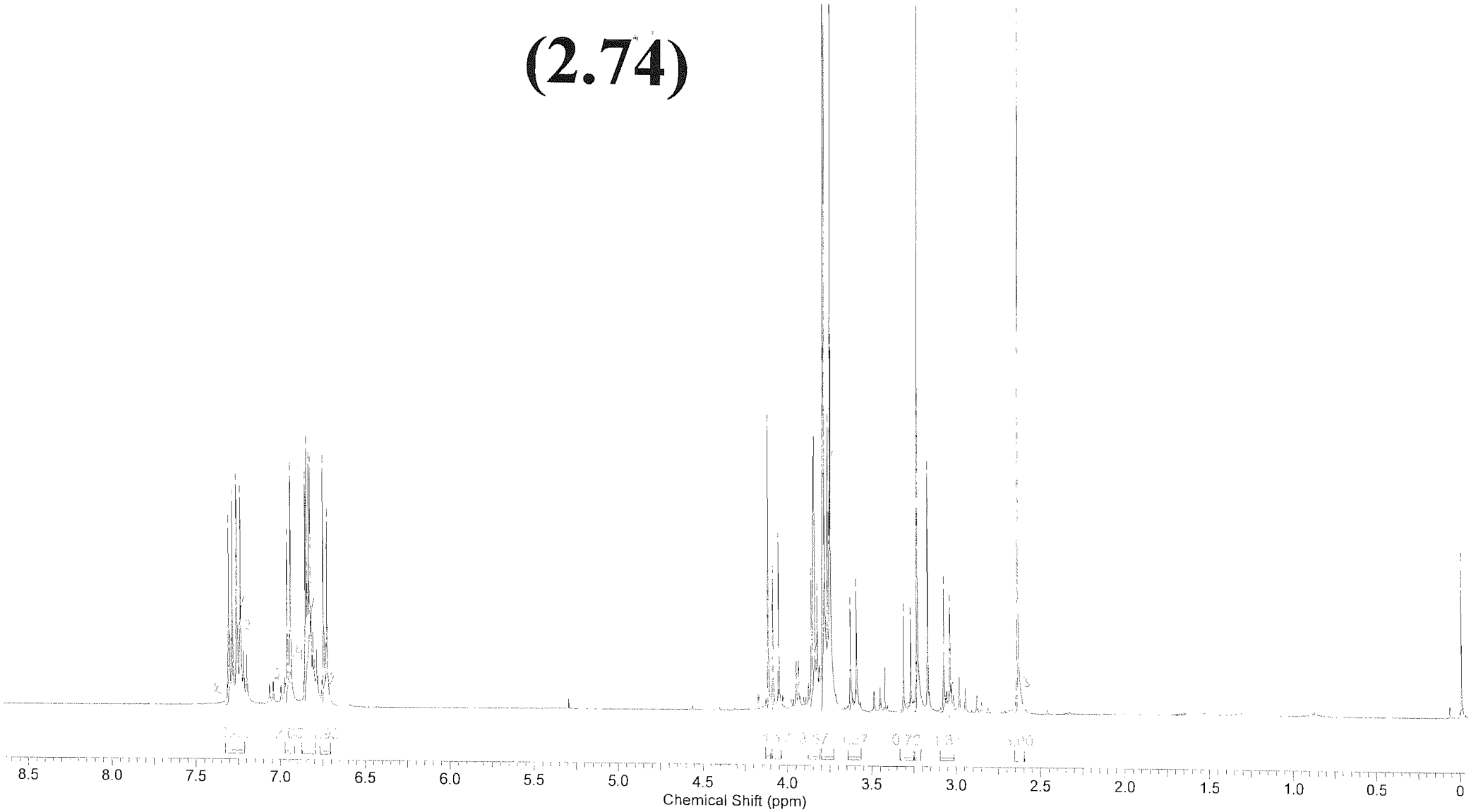
Date	19 Nov 2005 04:50:08				
File Name	C:\Documents and Settings\A Ganesan\My Documents\gary\gary_001000fid				
Number of Transients	32	Origin	spect	Original Points Count	16384
Pulse Sequence	zg	Receiver Gain	181.00	SW(cyclical) (Hz)	8223.68
Spectrum Offset (Hz)	2450.1104	Sweep Width (Hz)	8223.18	Temperature (degree C)	27.000

gary_001000fid

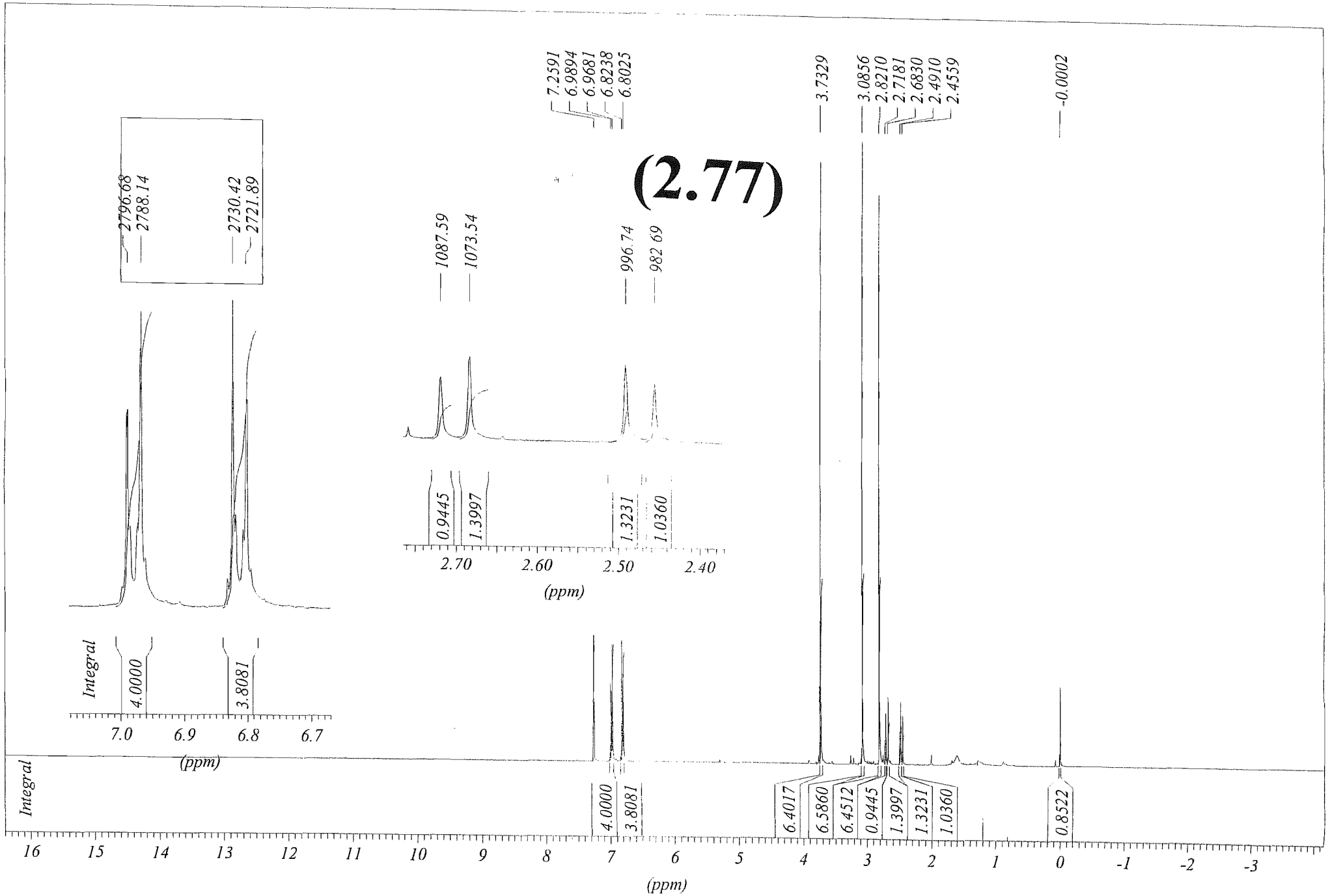
7.31 7.28 7.26 7.24 6.94 6.85 6.85 6.83 6.82 6.75 6.72

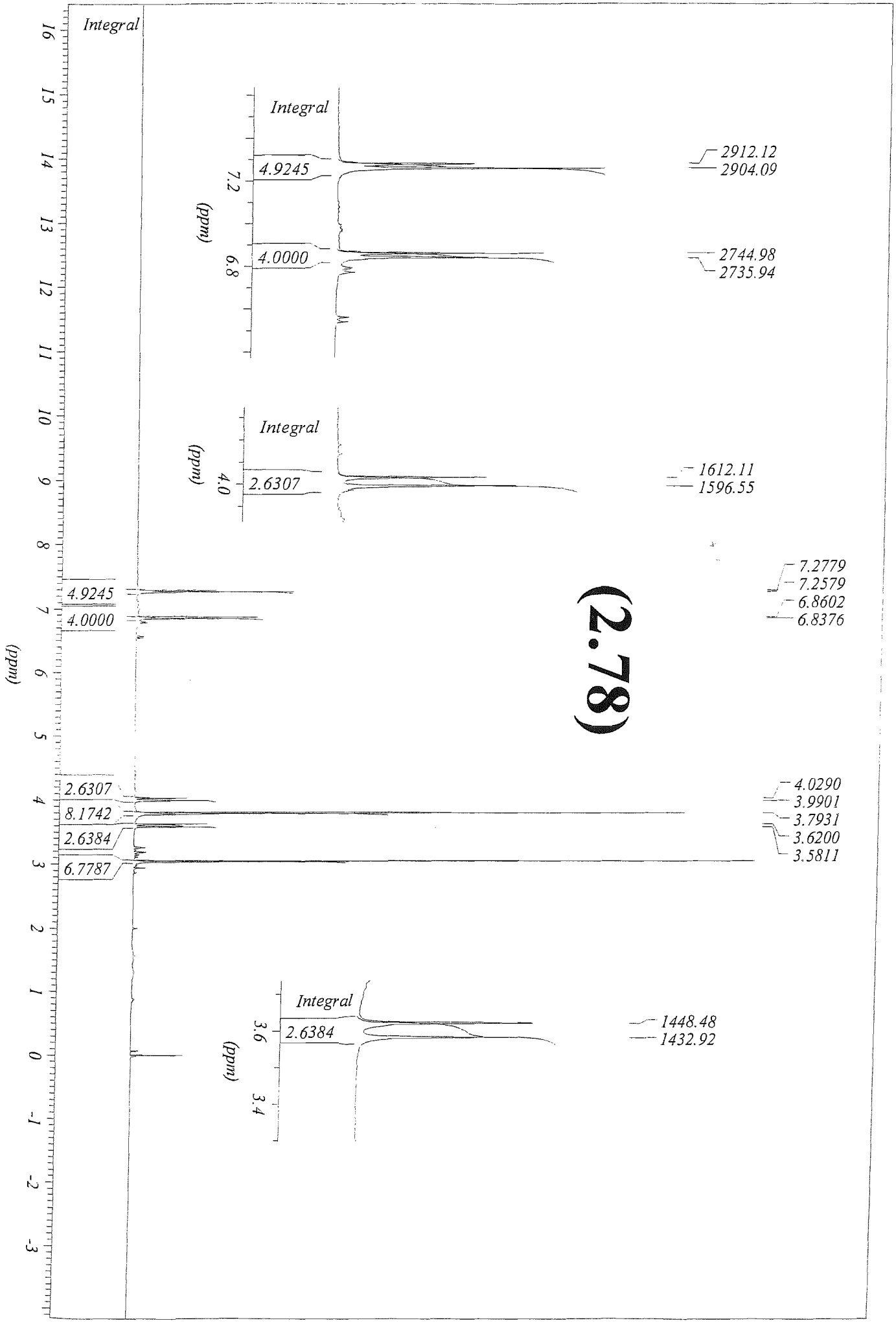
4.12 4.05 3.85 3.84 3.79 3.79 3.78 3.76 3.75 3.75 3.63 3.59 3.31 3.23 3.17 3.08 3.04 2.64

(2.74)

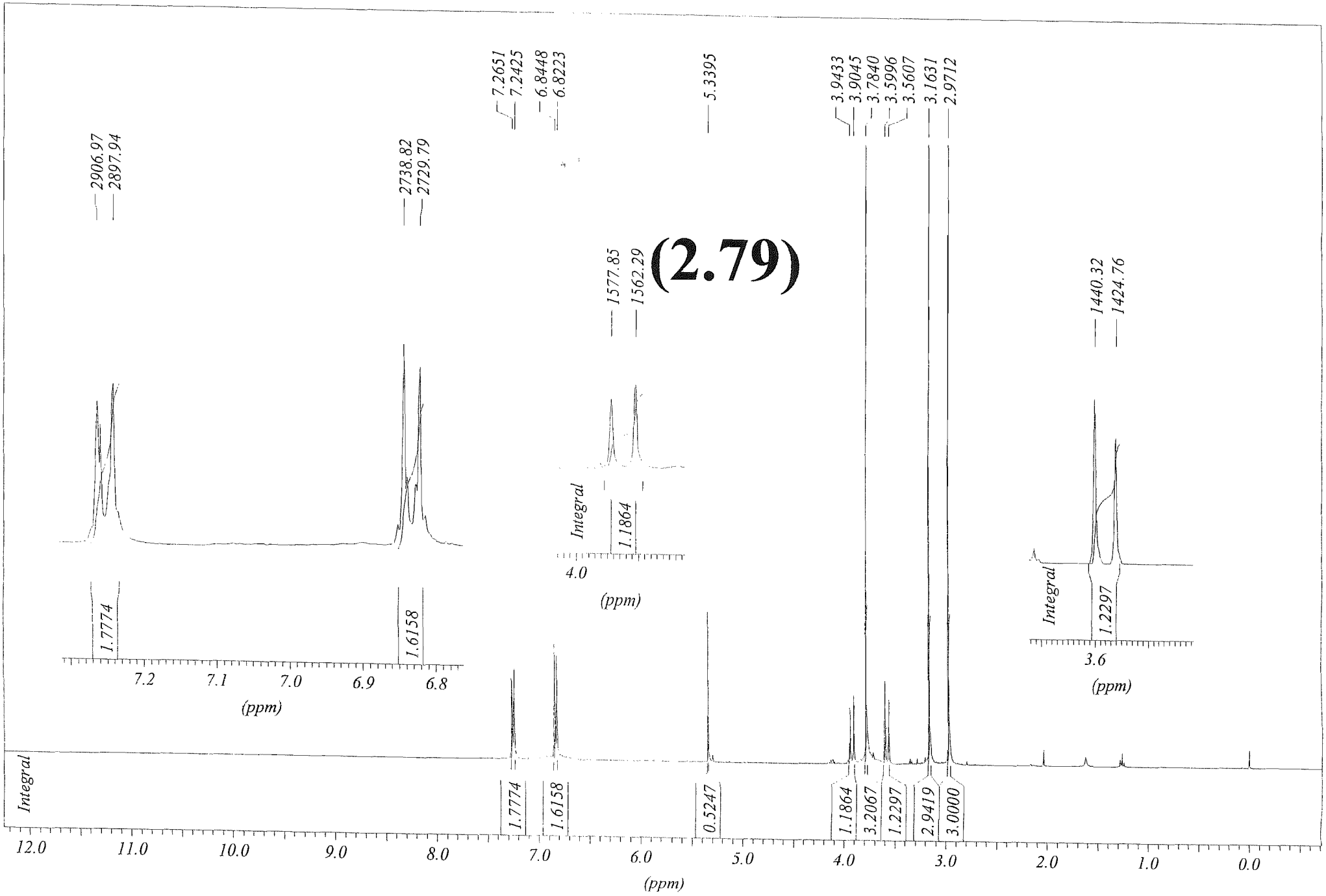


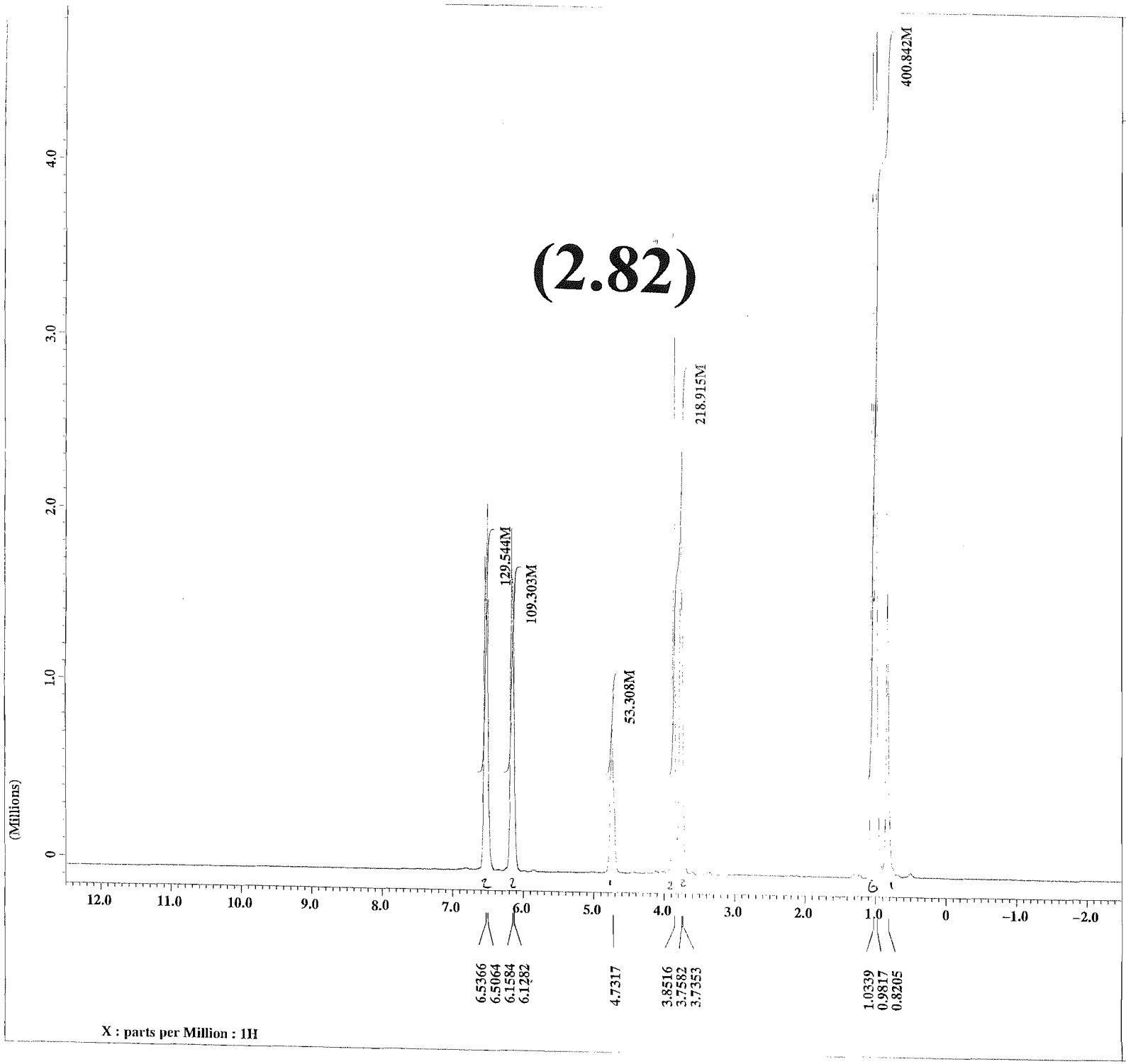
Integral
(ppm)
7.2
7.0
6.8
6.6
7.1735
7.1171
7.0004
6.9778
6.8449
6.8236
6.7608
6.7169
4.0
3.8
3.6
3.9923
3.7966
3.7188
3.6335
3.6160
3.6072
3.5821
3.5733
3.5370
3.5269
3.5018
3.4918
3.9944
2.1517
2.2775
3.9703
0.9524
3.7528
3.2460
2.9817
1.0734
1.4631
1.3198
0.9419
4.2729
1.1515
3.0000
(2.75)
3.3187
3.3074
3.2848
3.0615
3.0264
2.2963
3.2
2.8
2.4


(2.77)
7.2591
6.9894
6.9681
6.8238
6.8025
3.7329
3.0856
2.8210
2.7181
2.6830
2.4910
2.4559
-0.0002
2796.68
2788.14
2730.42
2721.89
1087.59
1073.54
996.74
982.69
Integral
4.0000
3.8081
7.0
6.9
6.8
6.7
(ppm)
0.9445
1.3997
1.3231
1.0360
2.70
2.60
2.50
2.40
(ppm)
Integral
4.0000
3.8081
6.4017
6.5860
6.4512
0.9445
1.3997
1.3231
1.0360
0.8522
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
(ppm)


Integral
4.9245
4.0000
2.6307
8.1742
2.6384
6.7787
(ppm)
Integral
4.9245
4.0000
7.2
6.8
(ppm)
2912.12
2904.09
2744.98
2735.94
Integral
2.6307
4.0
(ppm)
1612.11
1596.55
7.2779
7.2579
6.8602
6.8376
4.0290
3.9901
3.7931
3.6200
3.5811
Integral
2.6384
3.6
3.4
(ppm)
1448.48
1432.92

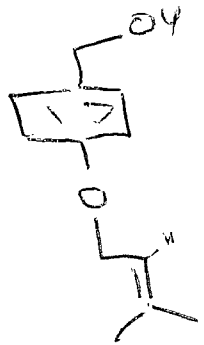
(2.78)

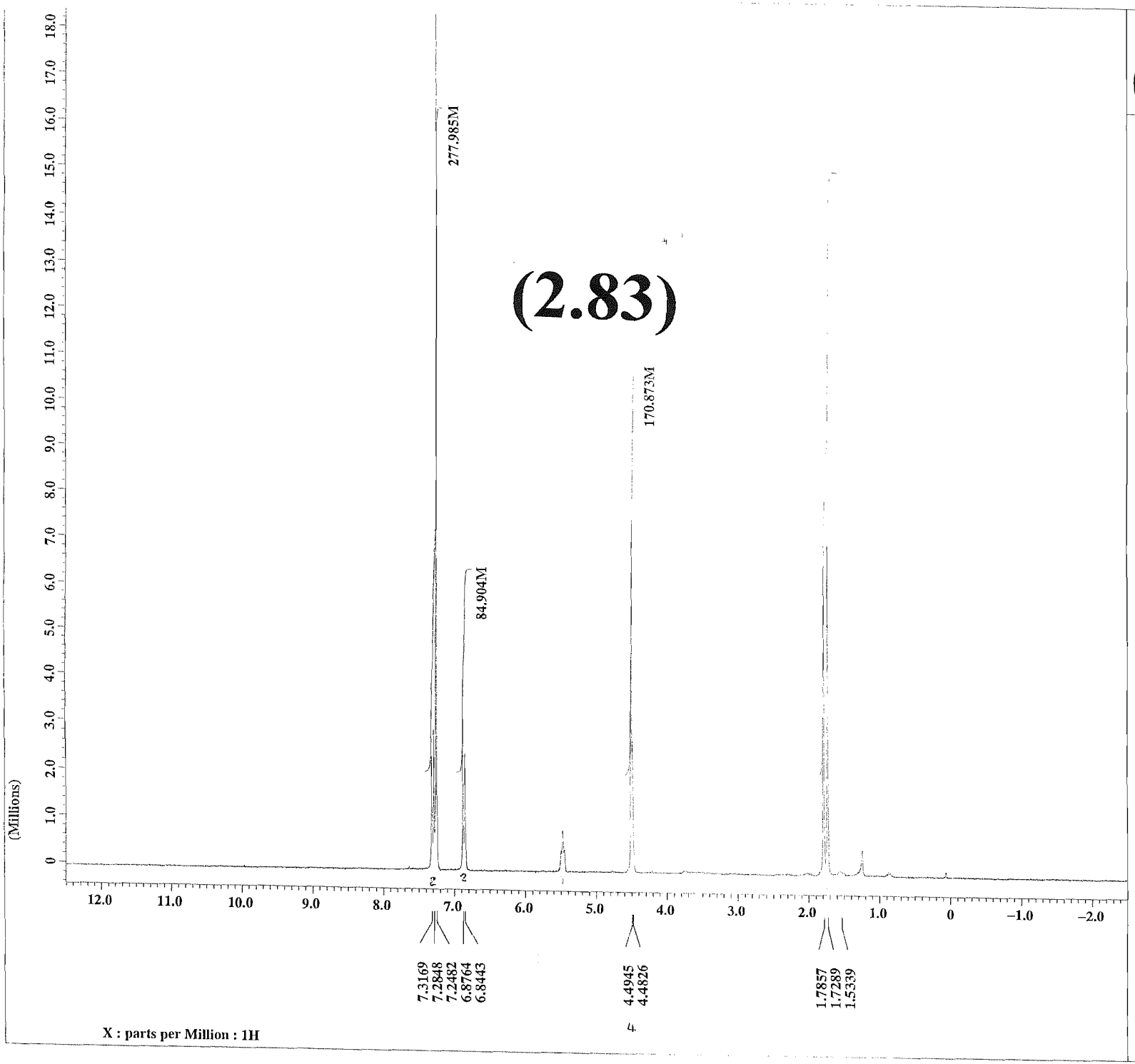

(2.79)
2906.97
2897.94
2738.82
2729.79
7.2651
7.2425
6.8448
6.8223
5.3395
3.9433
3.9045
3.7840
3.5996
3.5607
3.1631
2.9712
1577.85
1562.29
1440.32
1424.76
Integral
1.7774
1.6158
0.5247
1.1864
3.2067
1.2297
2.9419
3.0000
(ppm)



JEOL

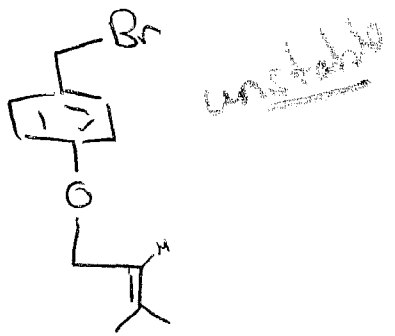
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 48[us]
Irr_pwidth	= 48[us]
Lock_status	= IDLE
Recvr_gain	= 21
Relaxation_delay	= 1[s]
Scans	= 64
Solvent	= CHLOROFORM-D
Spin_get	= 0[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 0[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN OFF
Temp_get	= 19.7[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 48[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]

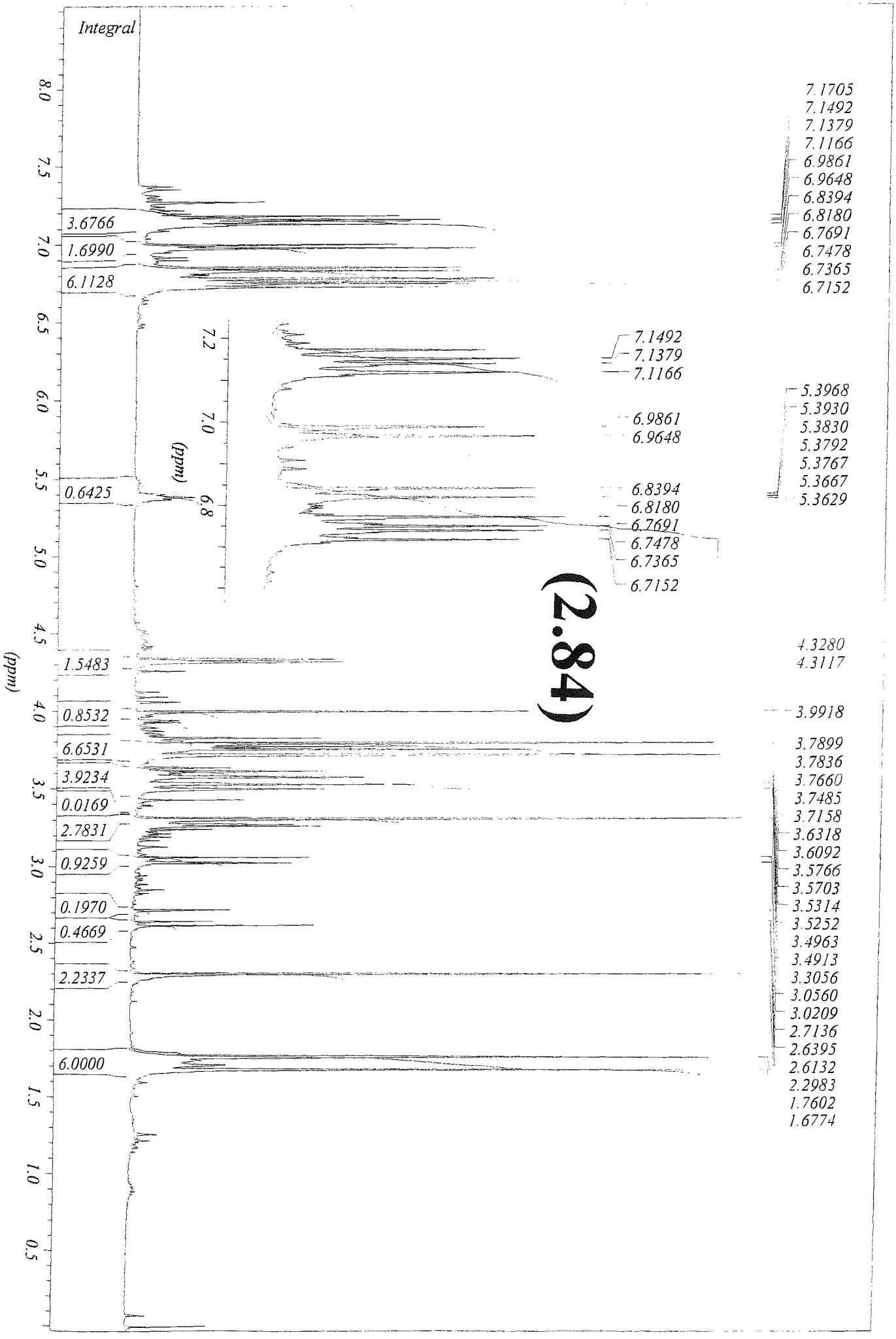


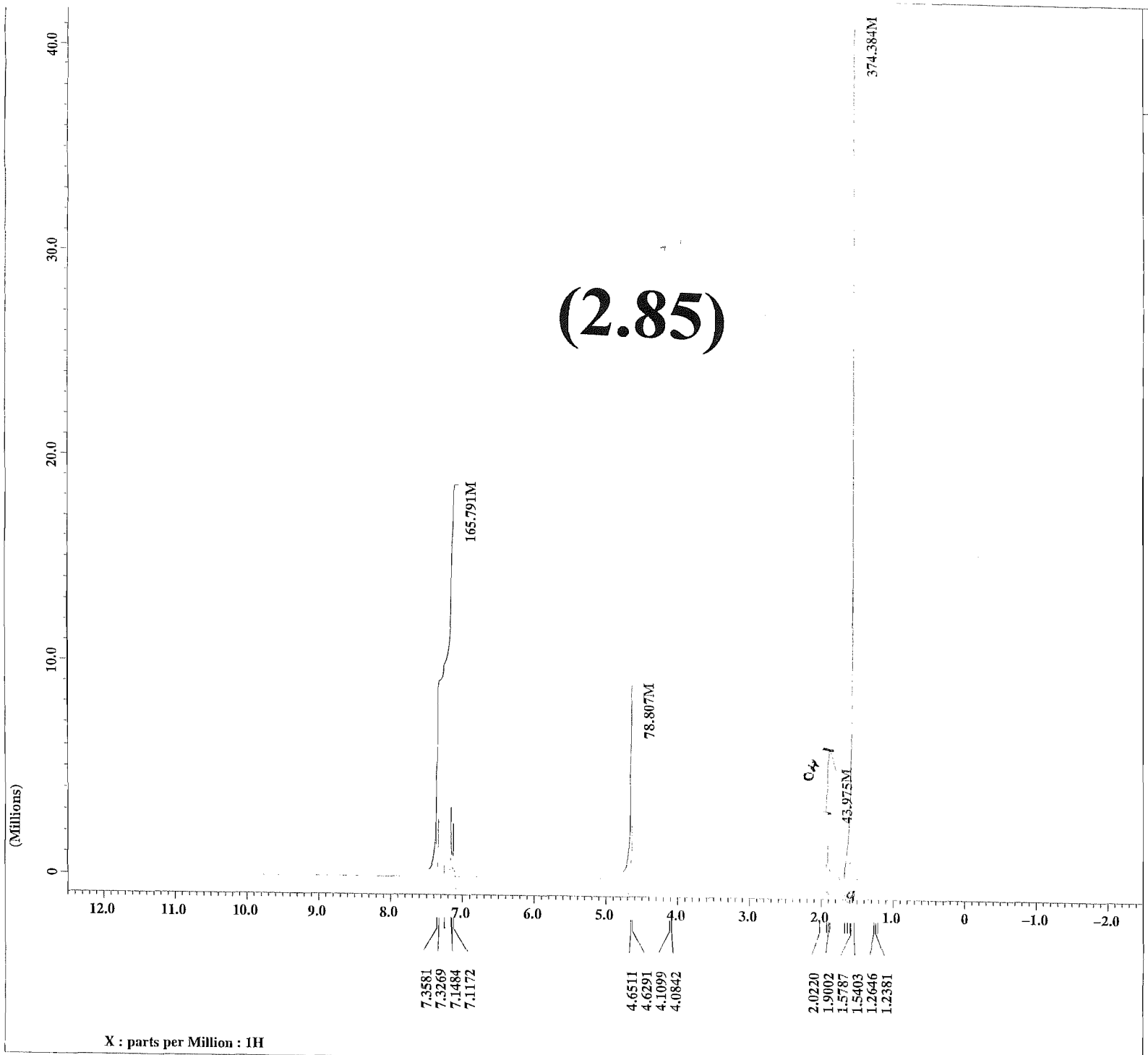


JEOL

Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 28
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 0[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN OFF
Spin_status	= SPIN OFF
Temp_get	= 19.3[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]

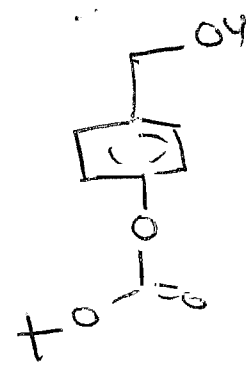


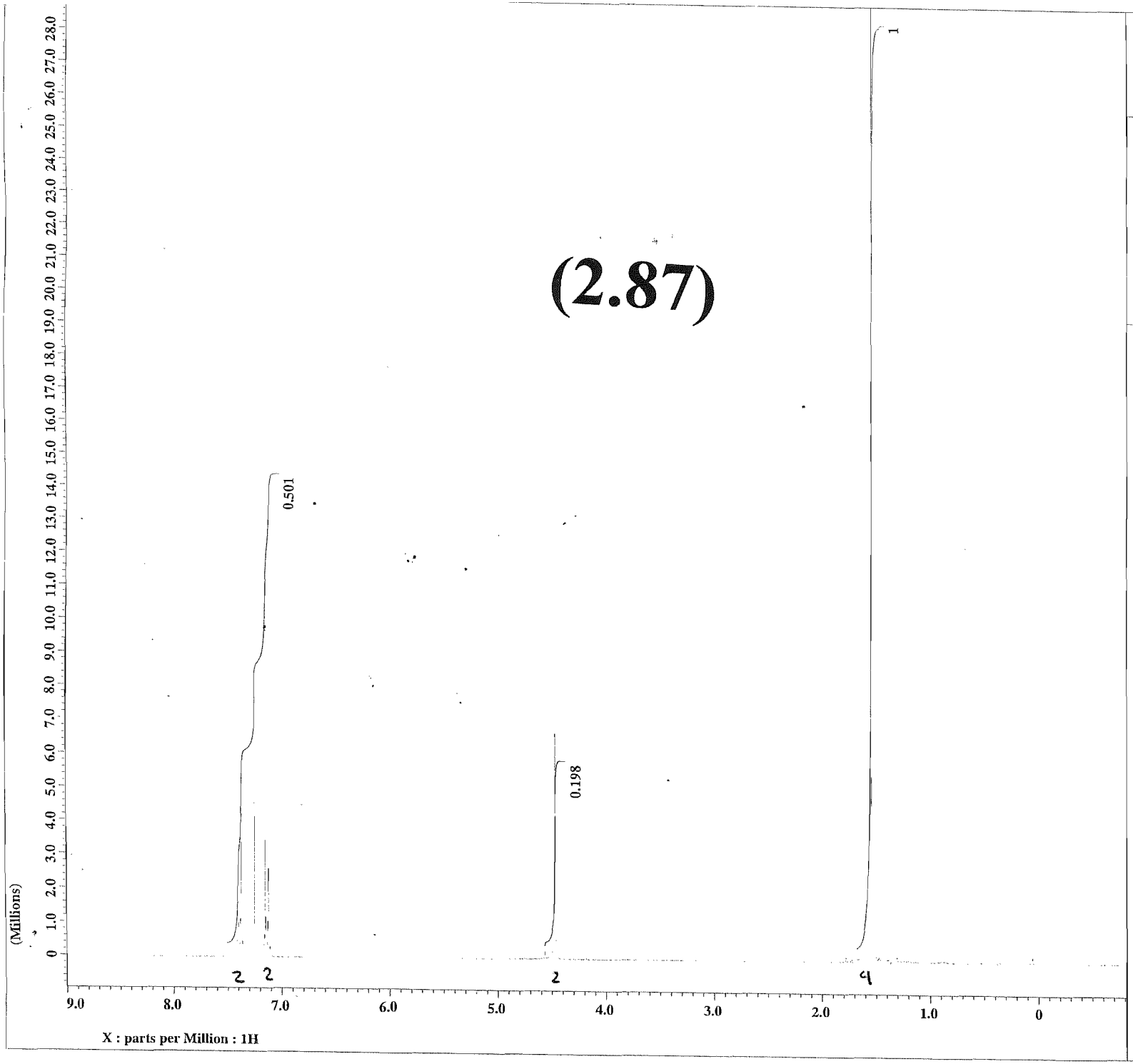

Integral
3.6766
1.6990
6.1128
0.6425
1.5483
0.8532
6.6531
3.9234
0.0169
2.7831
0.9259
0.1970
0.4669
2.2337
6.0000
(ppm)
7.2
7.0
6.8
7.1705
7.1492
7.1379
7.1166
6.9861
6.9648
6.8394
6.8180
6.7691
6.7478
6.7365
6.7152
5.3968
5.3930
5.3830
5.3792
5.3767
5.3667
5.3629
4.3280
4.3117
3.9918
3.7899
3.7836
3.7660
3.7485
3.7158
3.6318
3.6092
3.5766
3.5703
3.5314
3.5252
3.4963
3.4913
3.3056
3.0560
3.0209
2.7136
2.6395
2.6132
2.2983
1.7602
1.6774
(2.84)



JEOL

Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 17
Relaxation_delay	= 1[s]
Scans	= 16
Solvent	= CHLOROFORM-D
Spin_get	= 15[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 21.3[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]



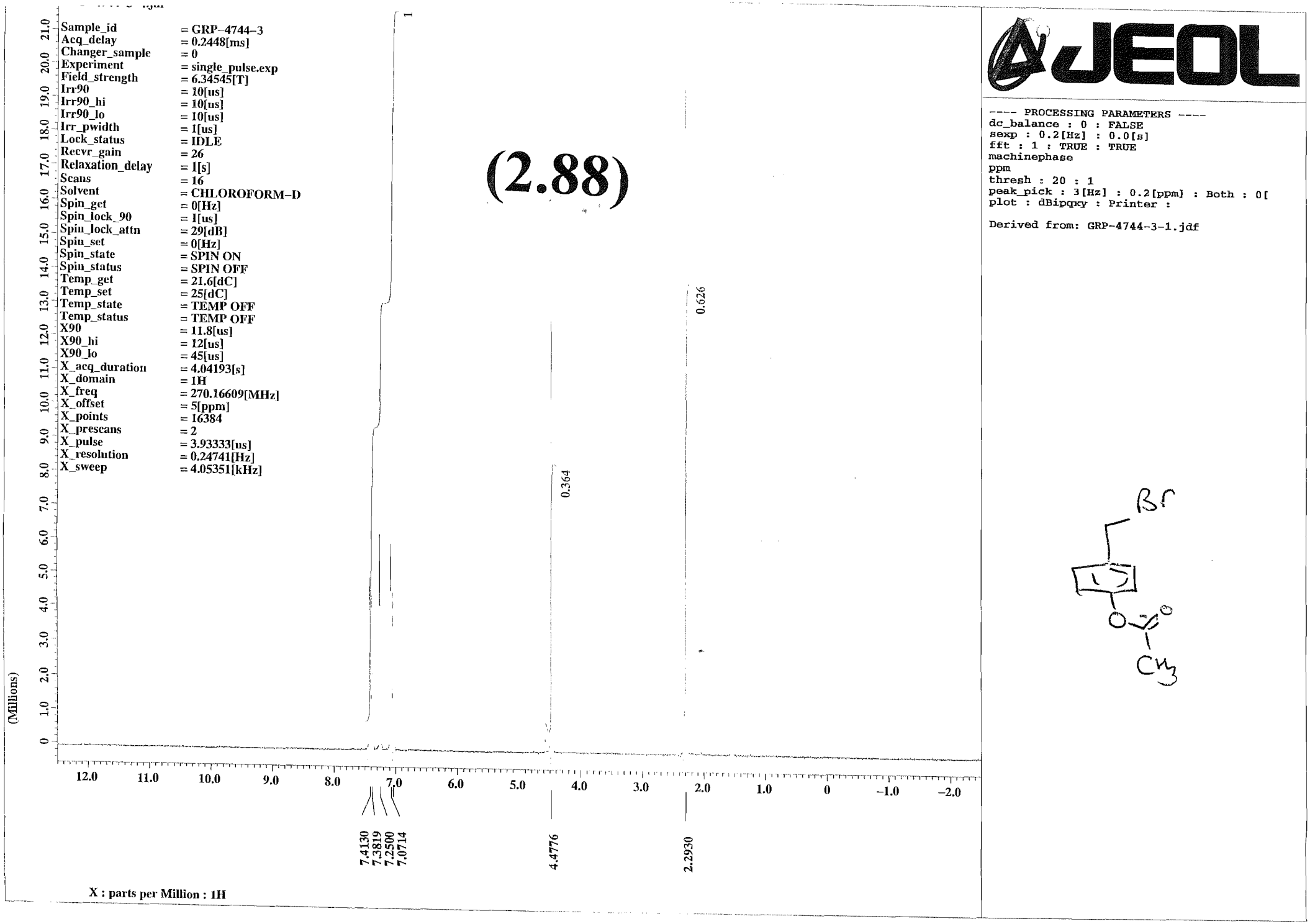


JEOL

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4429-~~76-1~~.jdf
```

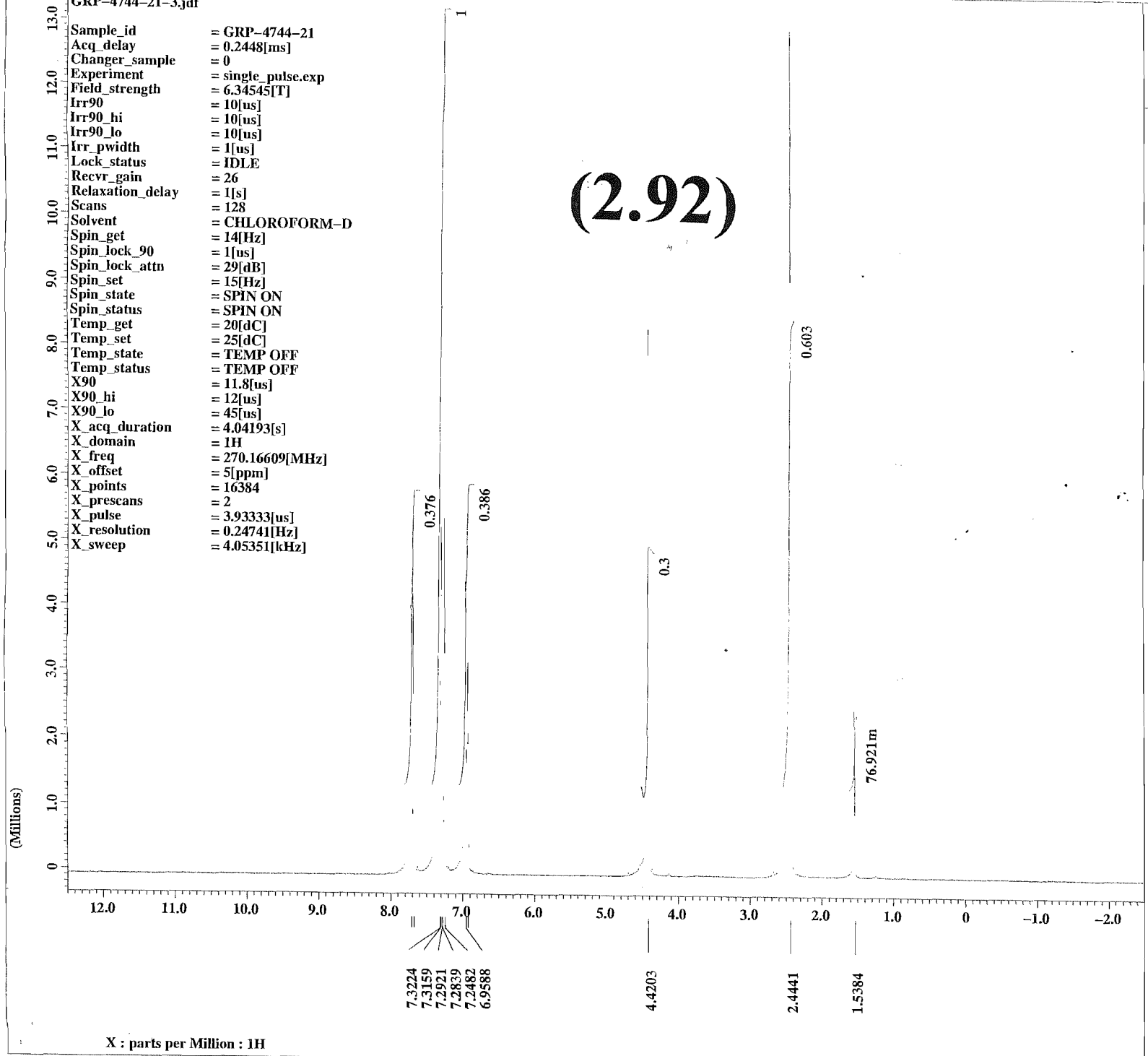
75

Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 23
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 16[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 26.5[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]


Sample_id = GRP-4744-3
Acq_delay = 0.2448[ms]
Changer_sample = 0
Experiment = single_pulse.exp
Field_strength = 6.34545[T]
Irr90 = 10[us]
Irr90_hi = 10[us]
Irr90_lo = 10[us]
Irr_pwidth = 1[us]
Lock_status = IDLE
Recvr_gain = 26
Relaxation_delay = 1[s]
Scans = 16
Solvent = CHLOROFORM-D
Spin_get = 0[Hz]
Spin_lock_90 = 1[us]
Spin_lock_attn = 29[dB]
Spin_set = 0[Hz]
Spin_state = SPIN ON
Spin_status = SPIN OFF
Temp_get = 21.6[dC]
Temp_set = 25[dC]
Temp_state = TEMP OFF
Temp_status = TEMP OFF
X90 = 11.8[us]
X90_hi = 12[us]
X90_lo = 45[us]
X_acq_duration = 4.04193[s]
X_domain = 1H
X_freq = 270.16609[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 2
X_pulse = 3.93333[us]
X_resolution = 0.24741[Hz]
X_sweep = 4.05351[kHz]
(2.88)
0.626
0.364
(Millions)
21.0
20.0
19.0
18.0
17.0
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
7.4130
7.3819
7.2500
7.0714
4.4776
2.2930
X : parts per Million : 1H
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqqy : Printer :
Derived from: GRP-4744-3-1.jdf
Br
O
O
CH3

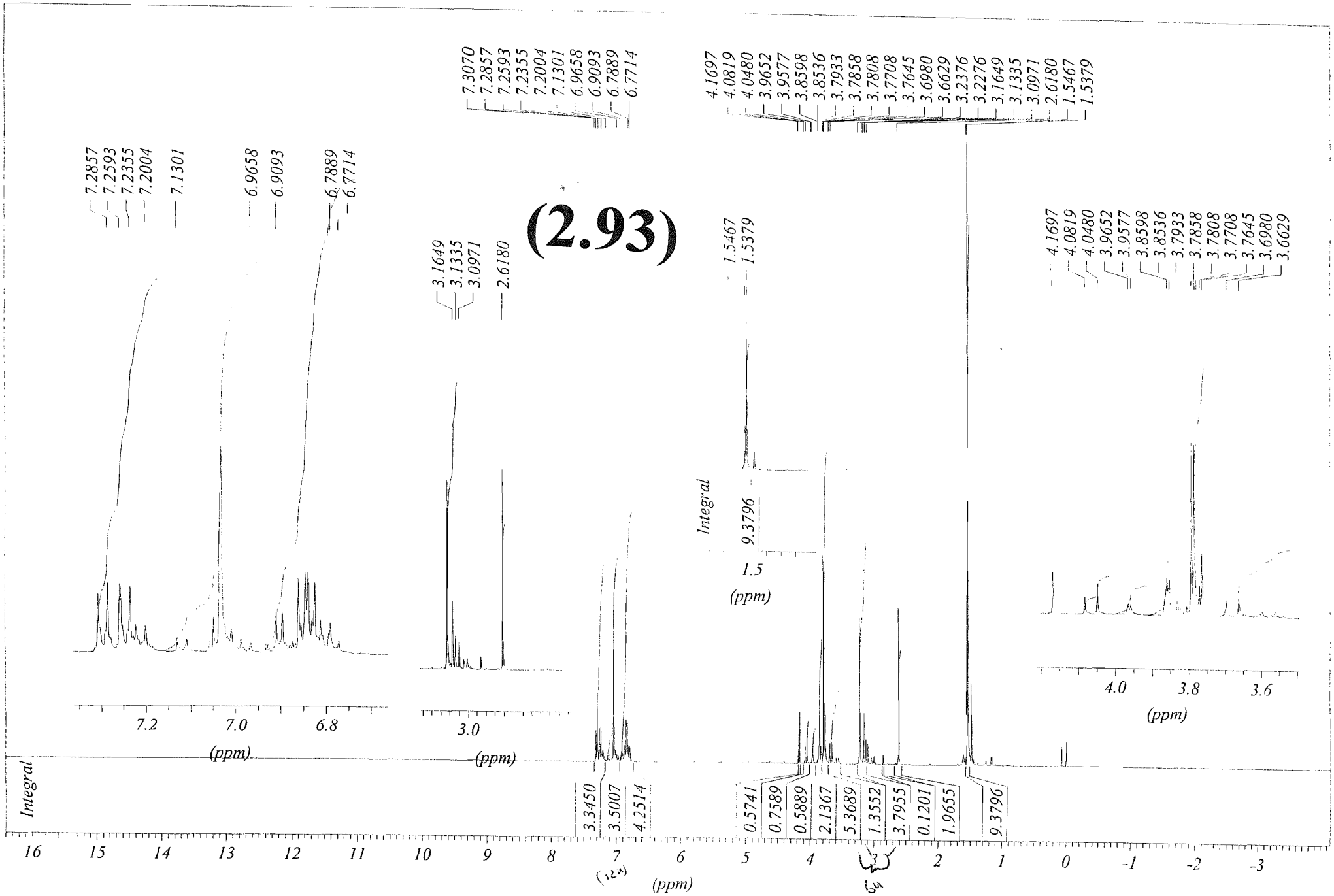
GRP-4744-21-3.jdf

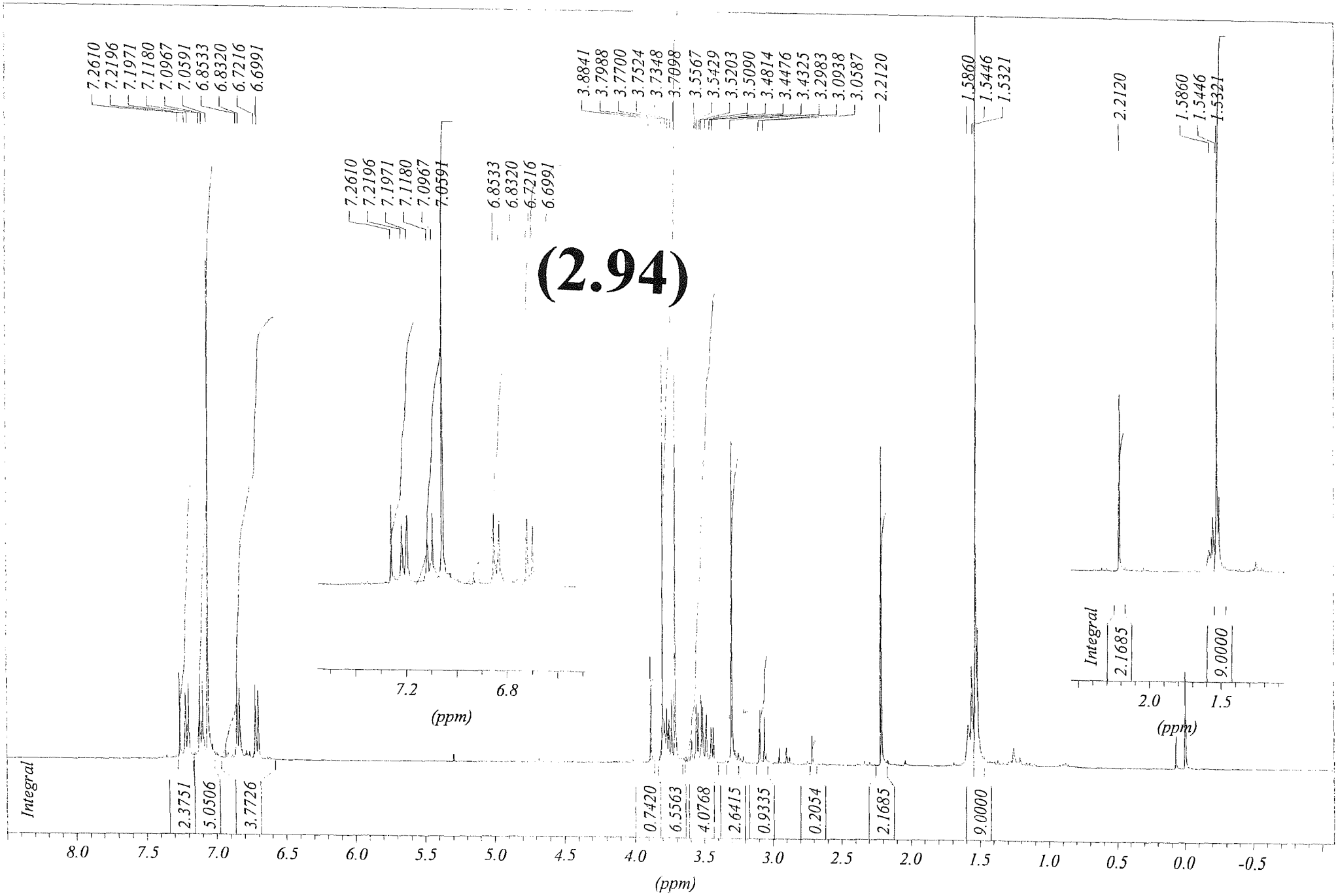
Sample_id	= GRP-4744-21
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 10[us]
Irr90_hi	= 10[us]
Irr90_lo	= 10[us]
Irr_pwidth	= 1[us]
Lock_status	= IDLE
Recvr_gain	= 26
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 14[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 20[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]




```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4744-21-1.jdf
```

grp4429/78/1□


(2.93)
7.3070
7.2857
7.2593
7.2355
7.2004
7.1301
6.9658
6.9093
6.7889
6.7714
4.1697
4.0819
4.0480
3.9652
3.9577
3.8598
3.8536
3.7933
3.7858
3.7808
3.7708
3.7645
3.6980
3.6629
3.2376
3.2276
3.1649
3.1335
3.0971
2.6180
1.5467
1.5379
Integral
9.3796
1.5
(ppm)
7.2
7.0
6.8
3.0
4.0
3.8
3.6
3.3450
3.5007
4.2514
0.5741
0.7589
0.5889
2.1367
5.3689
1.3552
3.7955
0.1201
1.9655
9.3796
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3


(2.94)
7.2610
7.2196
7.1971
7.1180
7.0967
7.0591
6.8533
6.8320
6.7216
6.6991
3.8841
3.7988
3.7700
3.7524
3.7348
3.7098
3.5567
3.5429
3.5203
3.5090
3.4814
3.4476
3.4325
3.2983
3.0938
3.0587
2.2120
1.5860
1.5446
1.5321
Integral
2.3751
5.0506
3.7726
0.7420
6.5563
4.0768
2.6415
0.9335
0.2054
2.1685
9.0000
(ppm)

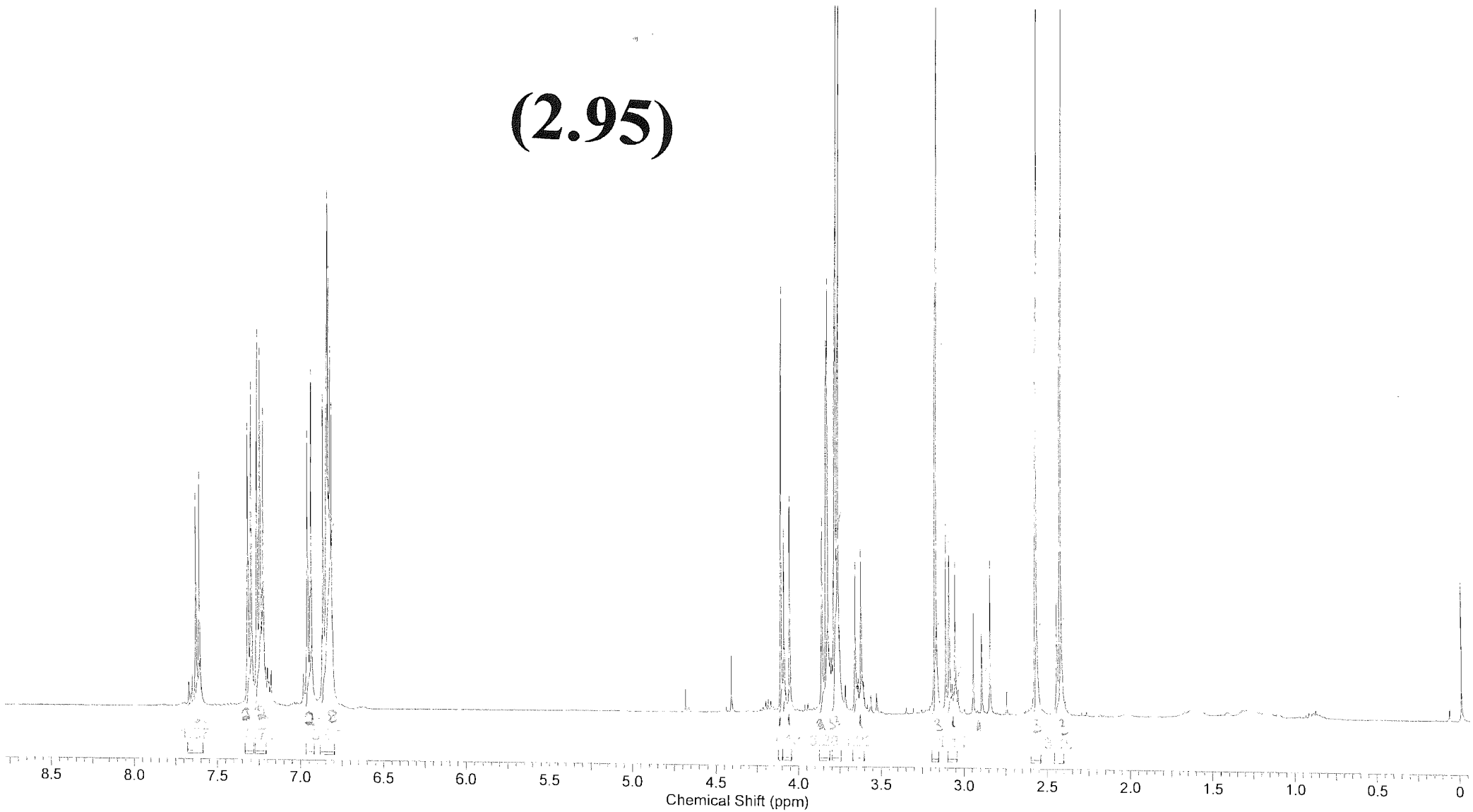
Acquisition Time (sec)	1.9923	Comment	grp4744/26/1	Date	22 Nov 2006 18:08:00				
Date Stamp	22 Nov 2006 18:08:00			File Name	C:\ACD10\Nmr400\NMR400Z\gan\nmr\nv2206ac1\nv2206ac1_001000fid				
Frequency (MHz)	400.13	Nucleus	1H	Number of Transients	32	Origin	spect	Original Points Count	16384
Owner	jms	Points Count	16384	Pulse Sequence	zg	Receiver Gain	114.00	SW(cyclical) (Hz)	8223.68
Solvent	CHLOROFORM-d			Spectrum Offset (Hz)	2450.9744	Sweep Width (Hz)	8223.18	Temperature (degree C)	27.000

nv2206ac1_001000fid

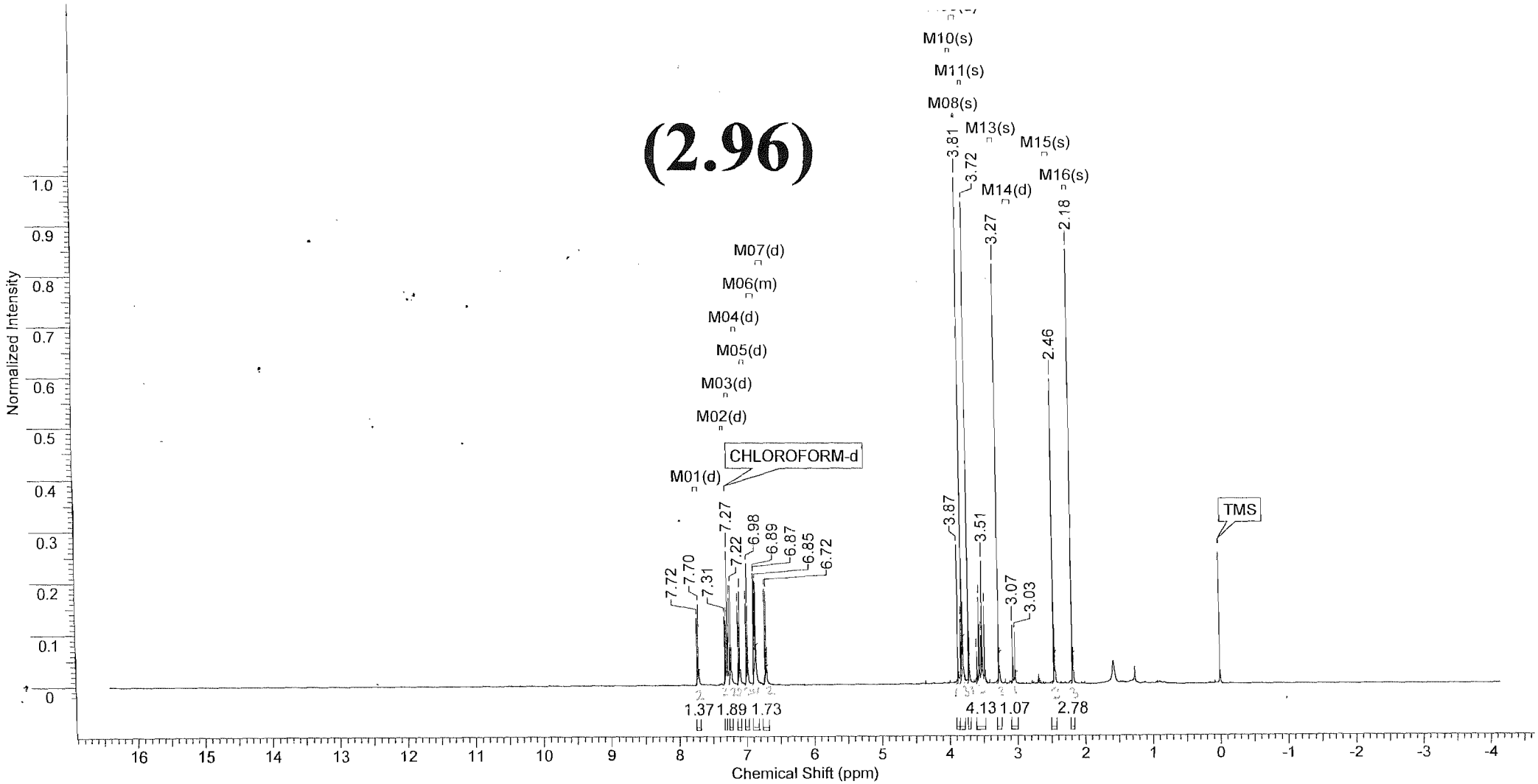
7.63 7.61 7.32 7.30 7.26 7.25 7.24 7.22 6.94 6.86 6.84 6.83 6.82 6.81

4.11 4.09 4.06 3.86 3.84 3.83 3.79 3.77 3.66 3.62 3.18 3.11 3.09 3.06 2.85 2.58 2.43

(2.95)

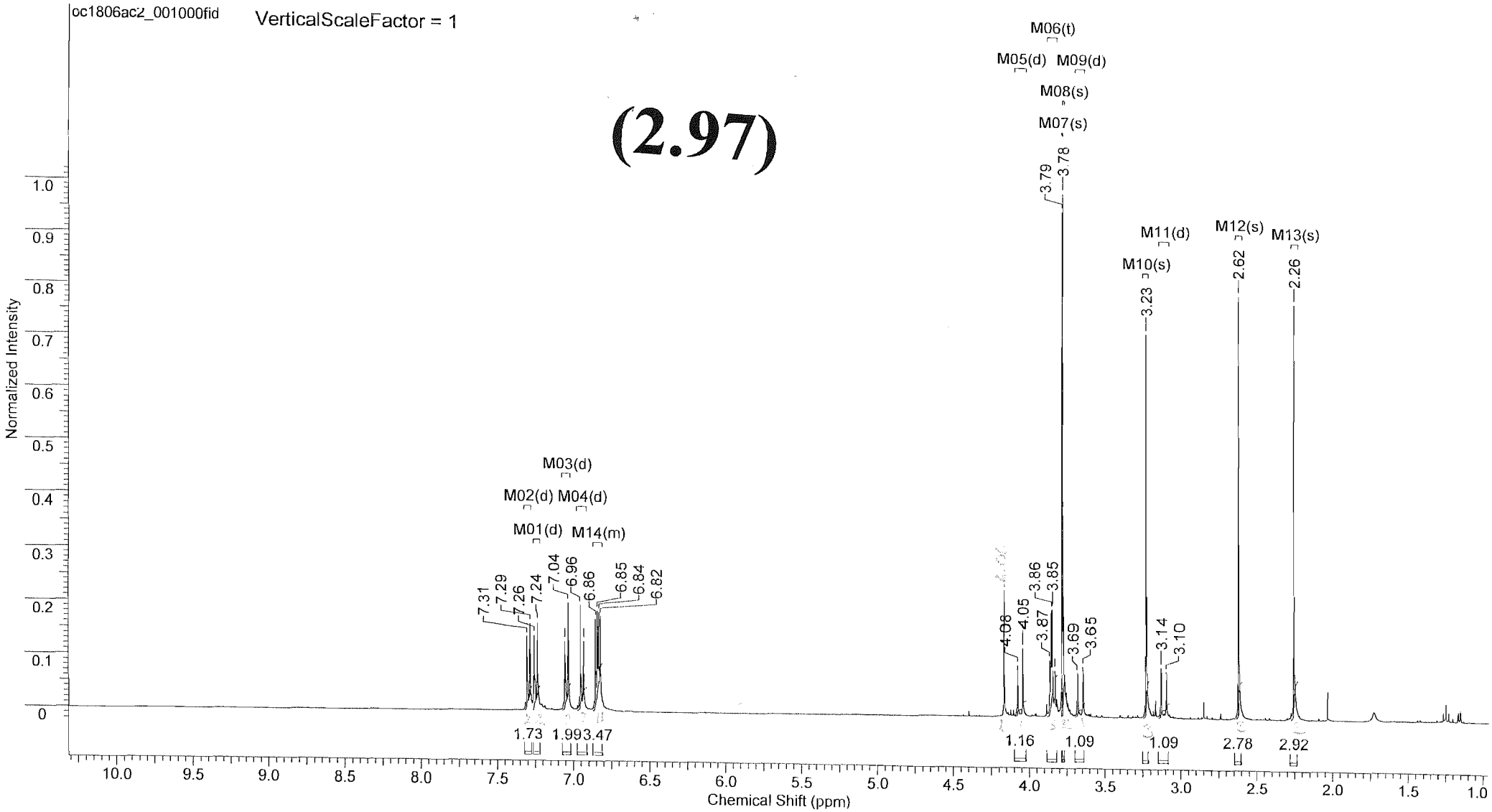


Acquisition Time (sec)	1.9923	***Comment***	grp4744/26/2	***Date***	22 Nov 2006 19:44:00
Date Stamp	22 Nov 2006 19:44:00			***File Name***	C:\Documents and Settings\grp\Desktop\NMR\nv2206ac2\nv2206ac2_001000fid



Acquisition Time (sec) 1.9923	Comment	grp4744/4	Date	18 Oct 2006 15:17:20	26/03/2007 16:33:01

C:\Documents and Settings\grp\Desktop\NMR\oc1806ac2\oc1806ac2_001000fid



GRP-4744-11(S.M)-3.jdf

Sample_id	= GRP-4744-11(S.M)
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 10[us]
Irr90_hi	= 10[us]
Irr90_lo	= 10[us]
Irr_pwidth	= 1[us]
Lock_status	= IDLE
Recvr_gain	= 17
Relaxation_delay	= 1[s]
Scans	= 16
Solvent	= CHLOROFORM-D
Spin_get	= 16[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 20.3[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

(Millions)

0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0

1

(2.99)

0.258 0.248

79.911m 81.306m 58.007m

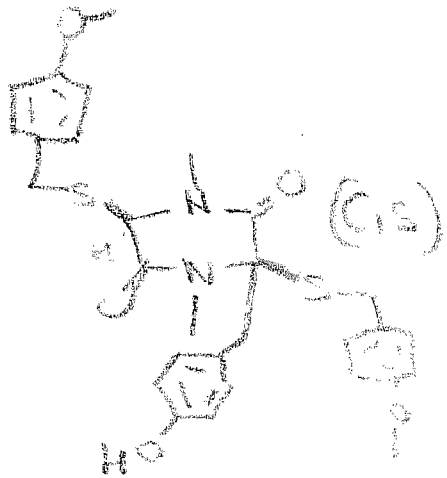
7.0 6.0 5.0 4.0 3.0 2.0 1.0

7.2464 7.2354 7.2143 6.8462 6.8407 6.8095 6.7775 6.6420 4.0870 4.0037 3.8489 3.8123 3.7747 3.7628 3.5439 3.4917 3.2738 3.2518 3.0604 3.0082 2.6035 2.0366 1.2729 1.2463 1.2198

X : parts per Million : 1H

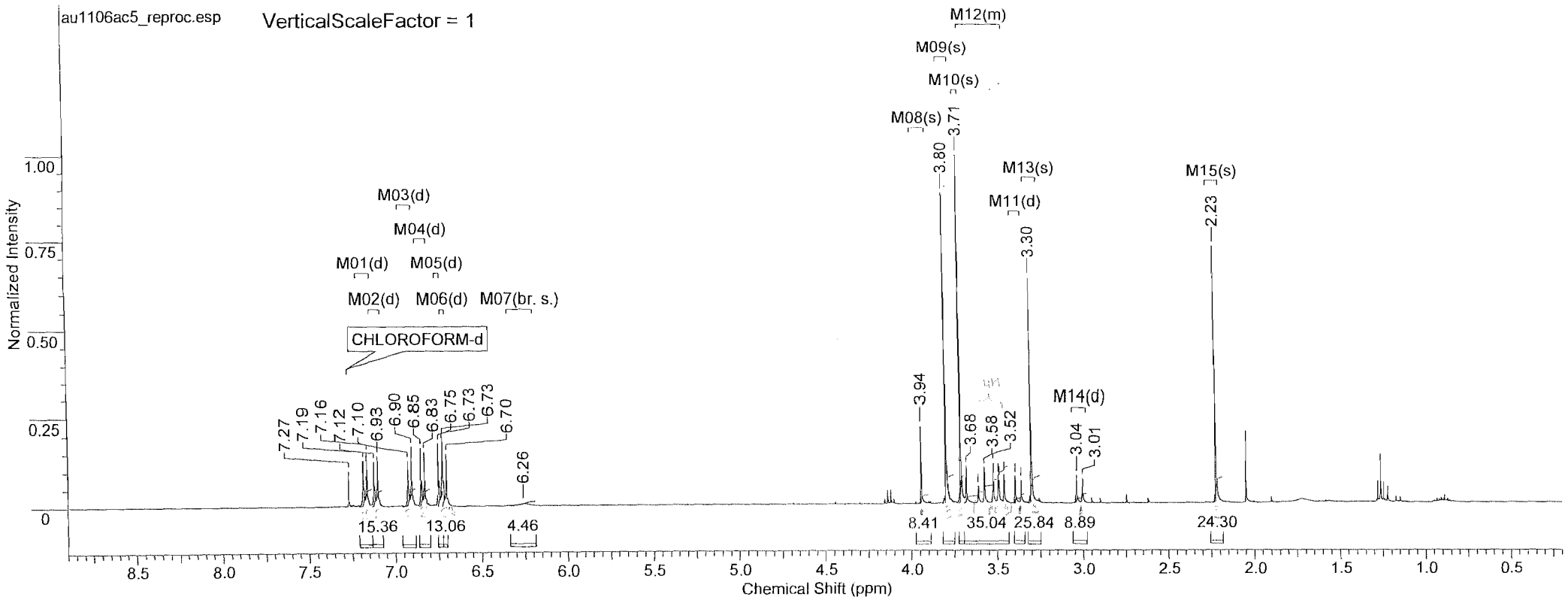
JEOL

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4744-11(S.M)-1.jdf
```



Formula $C_{29}H_{32}N_2O_5S_2$	**FW** 552.7048		
Acquisition Time (sec) 1.9923	**Comment** grp4229/90/1	**Date** 12 Aug 2006 04:54:24	
Date Stamp 12 Aug 2006 04:54:24		**File Name** C:\Documents and Settings\grp\Desktop\NMR\au1106ac5\au1106ac5_001000fid	
Frequency (MHz) 400.13	**Nucleus** 1H	**Number of Transients** 16	**Origin** spect **Original Points Count** 16384

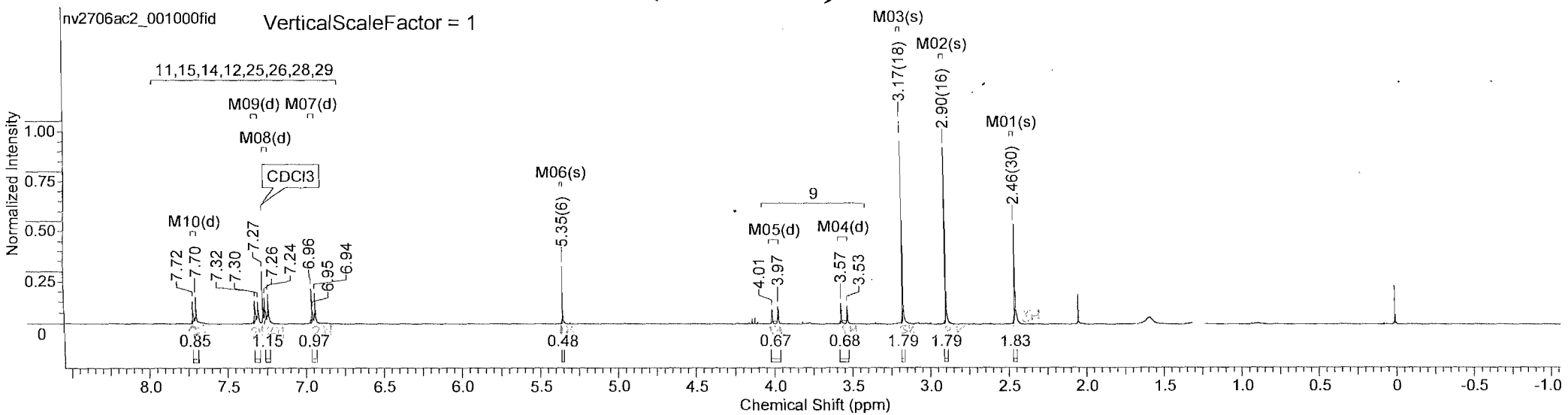
(2.100)



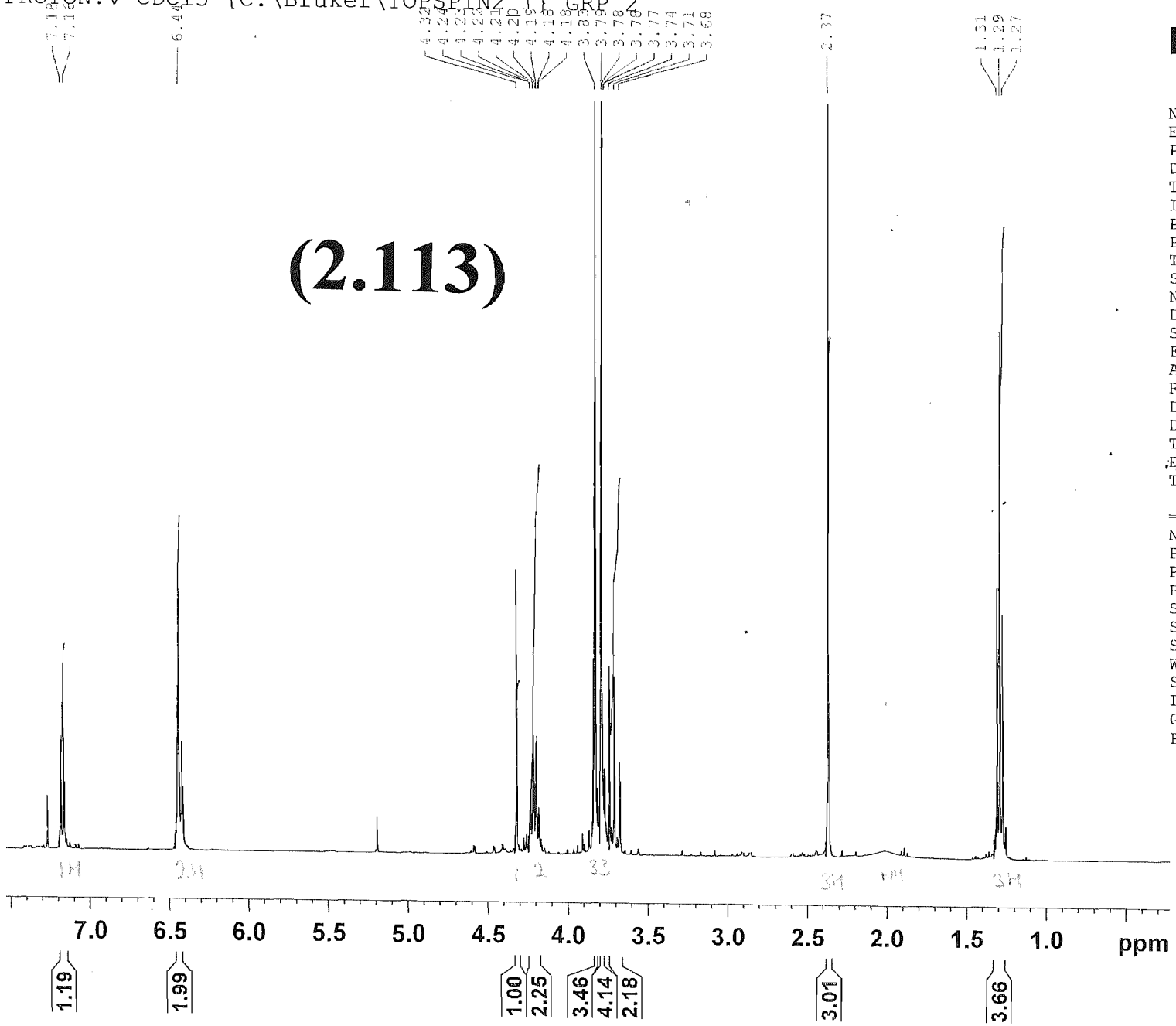
Formula $C_{20}H_{20}N_2O_5S_3$		**FW**	464.5782

Acquisition Time (sec)	1.9923	***Comment***	grp4744/31	***Date***	27 Nov 2006 20:58:40		
Date Stamp	27 Nov 2006 20:58:40			***File Name***	C:\Documents and Settings\grp\Desktop\NMR\nv2706ac2\nv2706ac2_001000fid		
Frequency (MHz)	400.13	***Nucleus***	1H	***Number of Transients***	16	***Origin***	spect

(2.103)



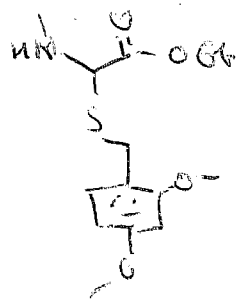
GRP-4744-57
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2 1} GRP 2



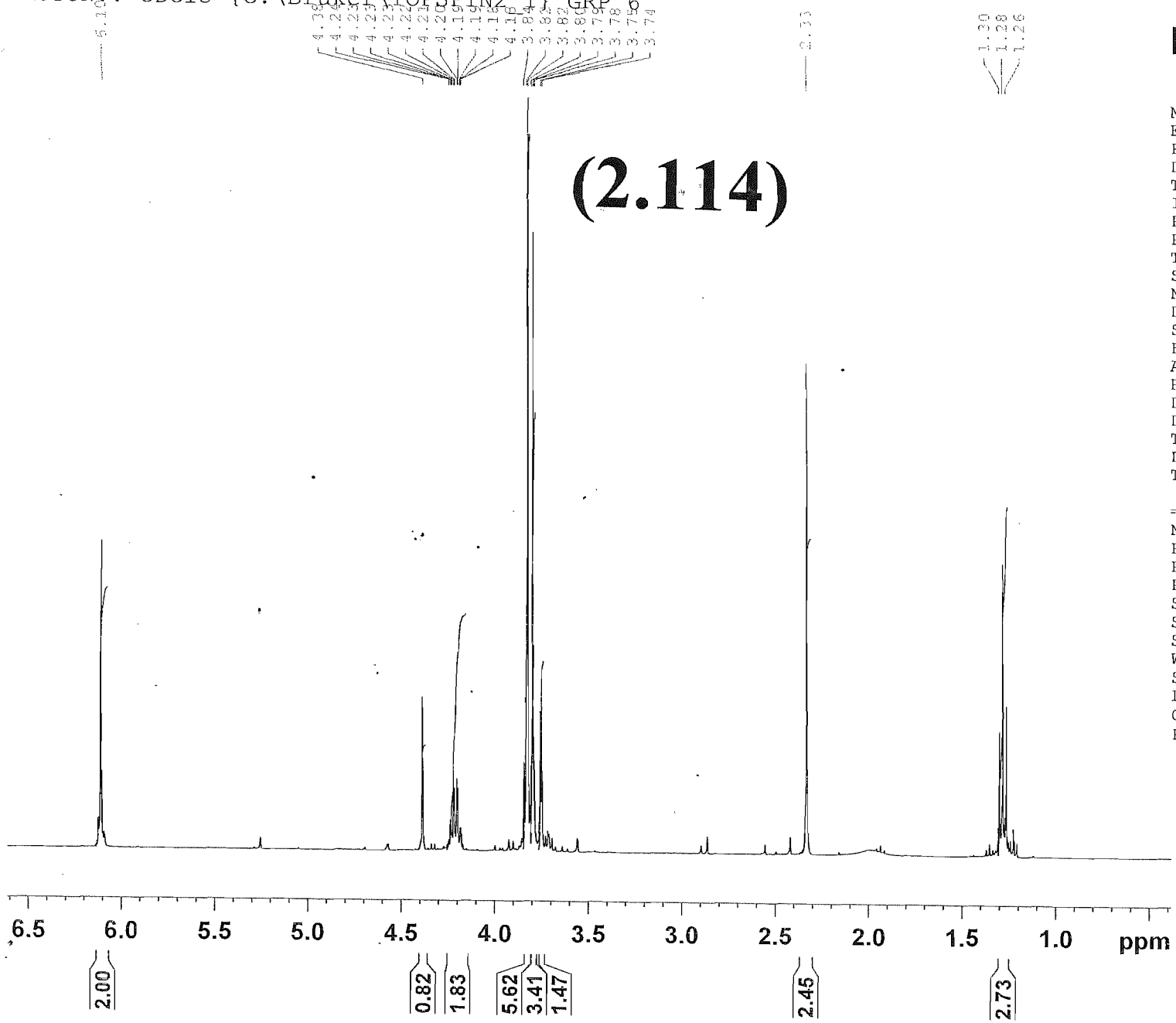
NAME	GRP-4744-57
EXPNO	10
PROCNO	1
Date_	20080211
Time	11.11
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	114
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1

======== CHANNEL f1 ========

NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300022 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



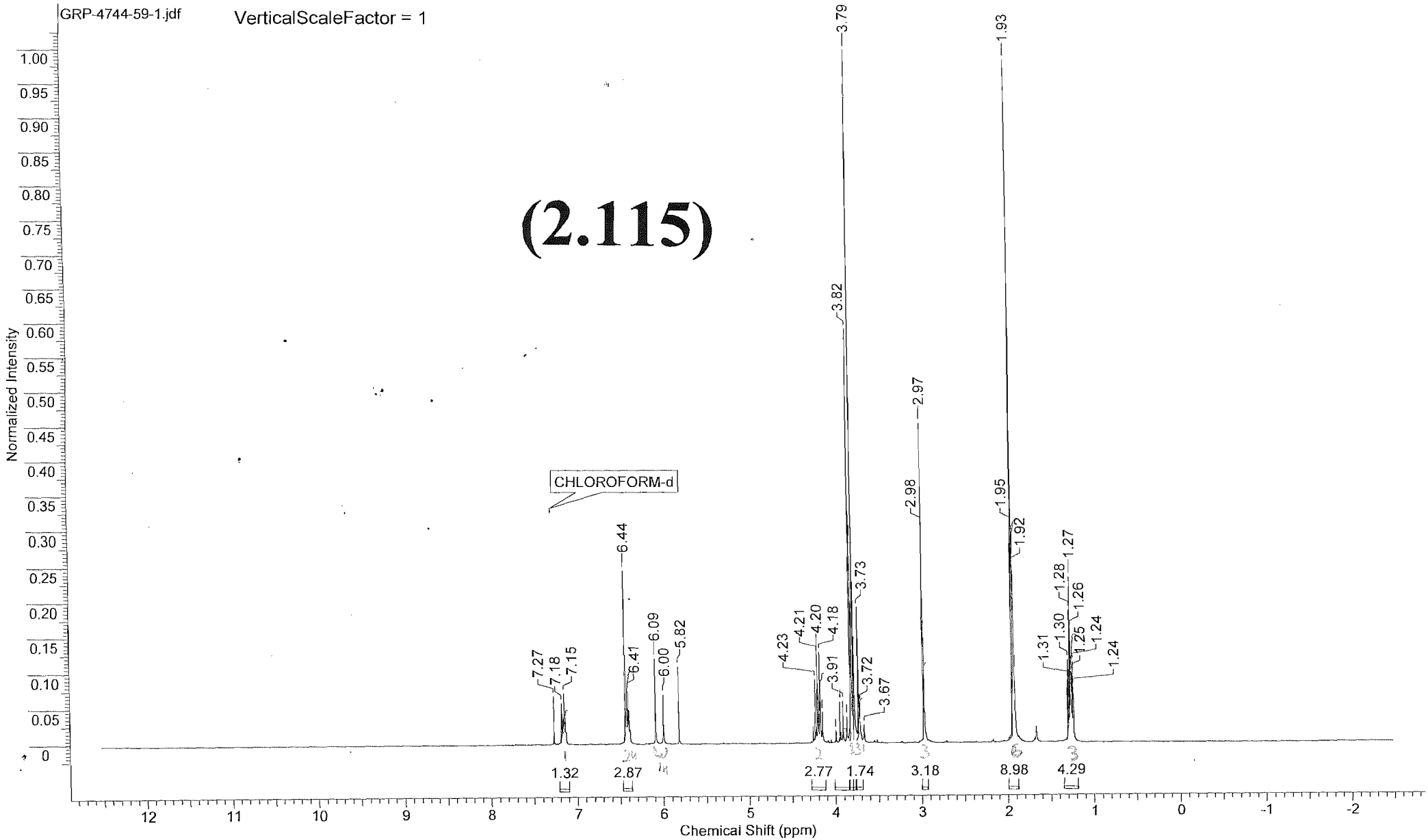
GRP-4744-58-1
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2_1} GRP 6



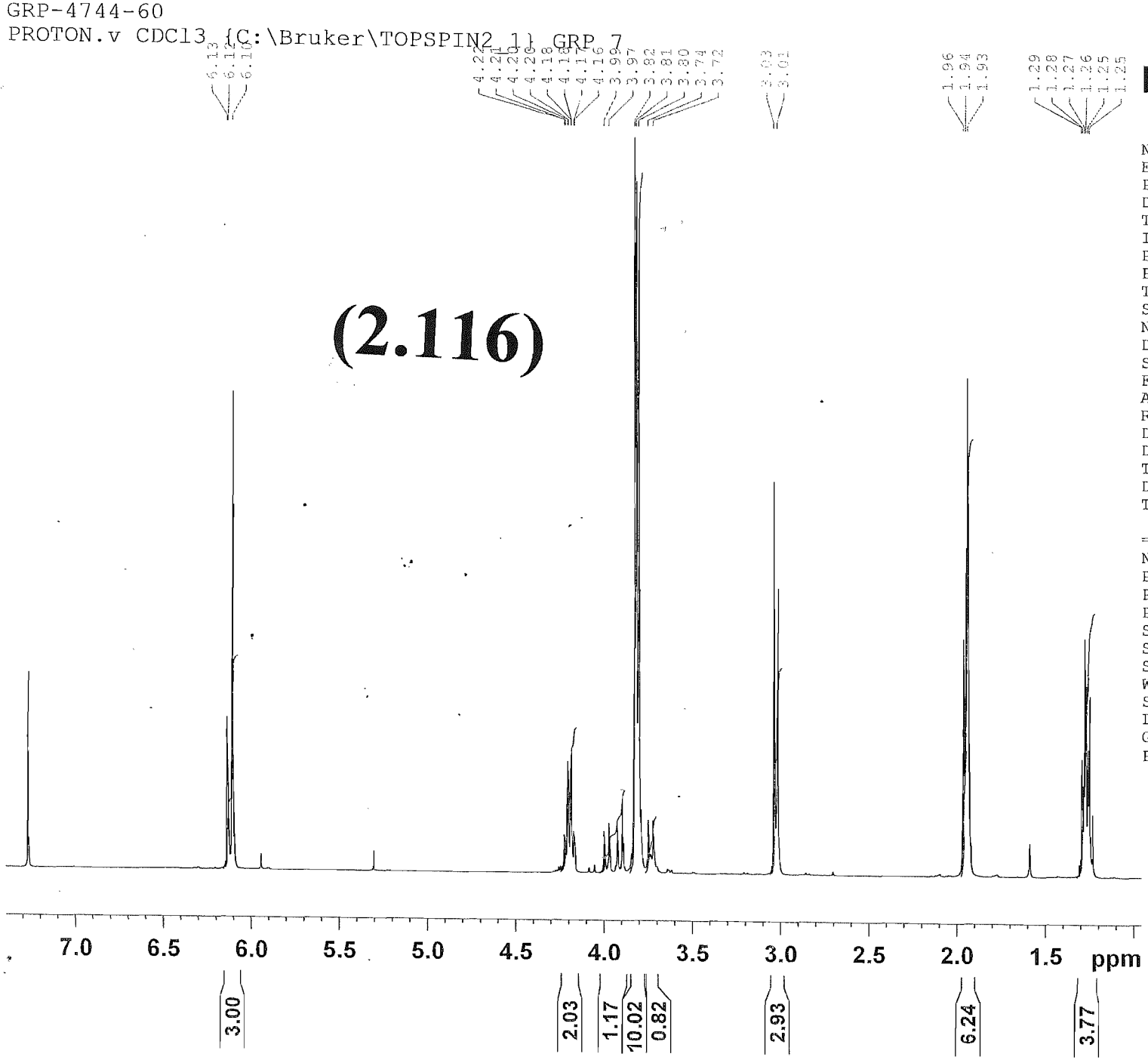
(2.114)

NAME	GRP-4744-58-1
EXPNO	10
PROCNO	1
Date_	20080211
Time	15.29
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	90.5
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300050 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Acquisition Time (sec)	4.0419	***Date***	03 Apr 2007 10:32:39			***Date Stamp***	03 Apr 2007 12:33:05
File Name	\\Fegb1lrc005\data\grp\GRP-4744-59-1.jdf			***Frequency (MHz)***	270.17	***Nucleus***	1H
Number of Transients	128	***Origin***	EX-Delta 270	***Original Points Count***	16384	***Owner***	APPL-GB1-LAB
Points Count	32768	***Pulse Sequence***	single_pulse.exp			***Receiver Gain***	17.00
Solvent	CHLOROFORM-d	***Spectrum Offset (Hz)***	1356.8917	***Sweep Width (Hz)***	4053.51	***Temperature (degree C)***	28.200

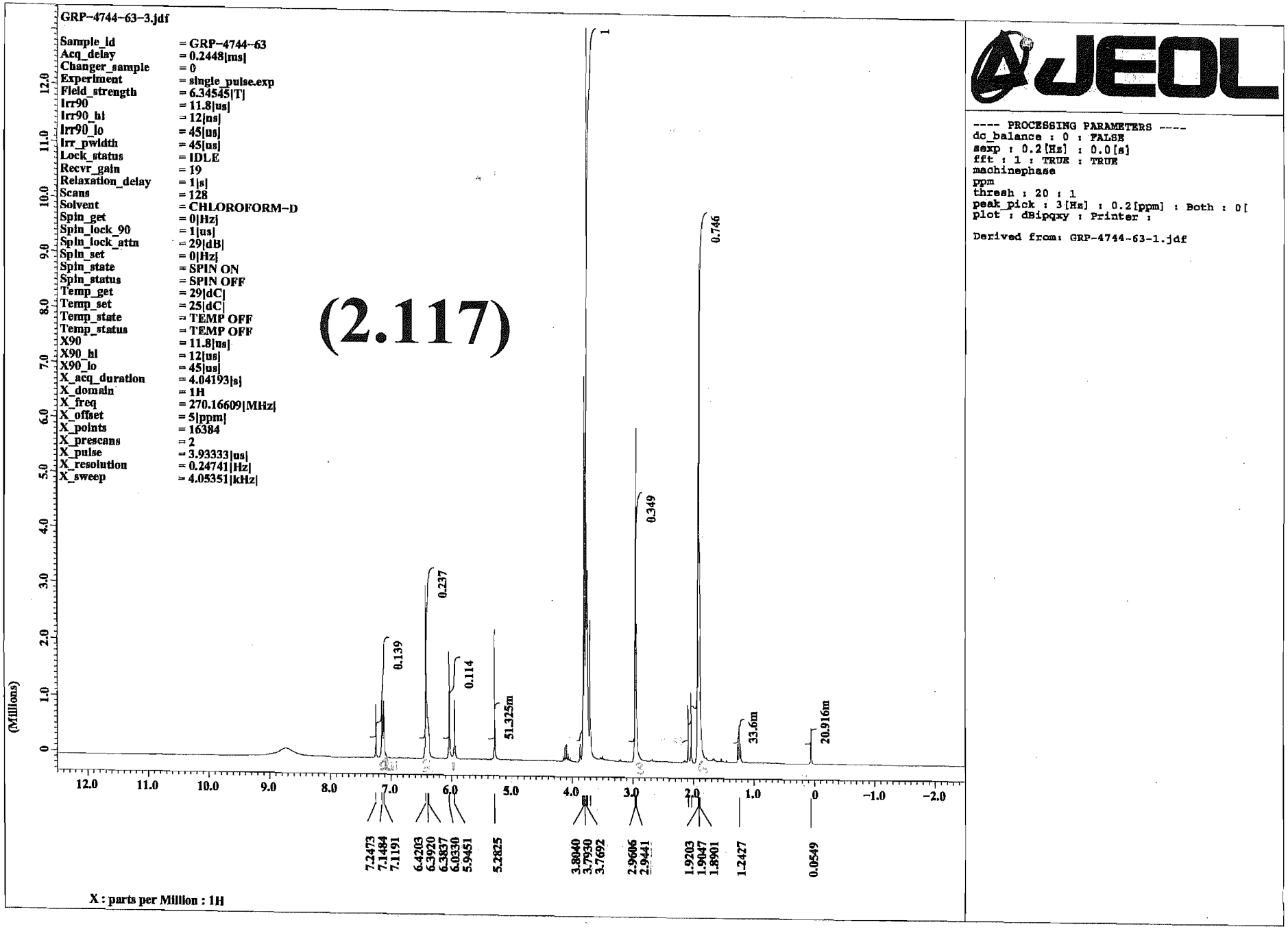


GRP-4744-60
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2_1} GRP_7



BRUKER

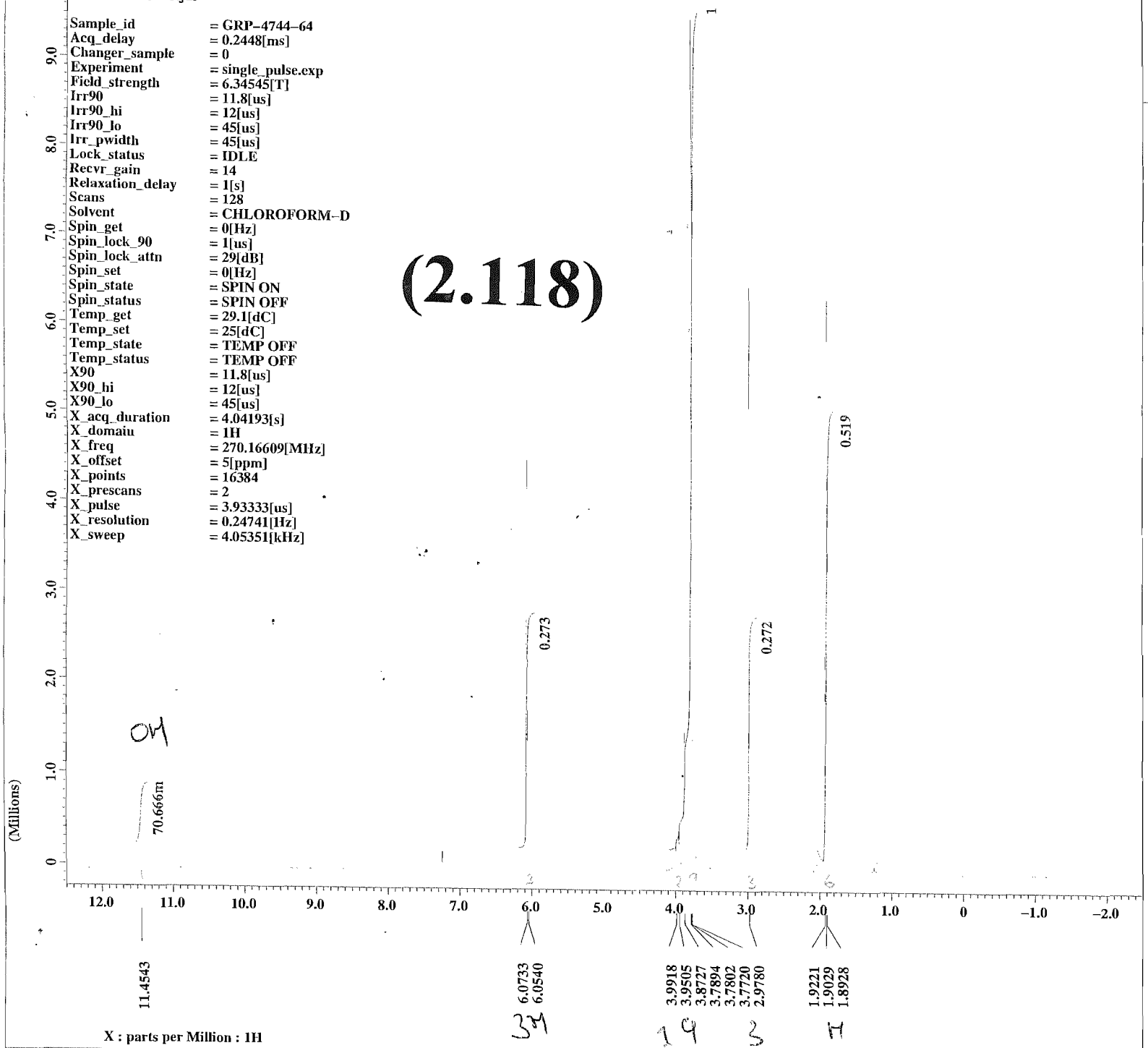
NAME	GRP-4744-60
EXPNO	10
PROCNO	1
Date_	20080211
Time	15.39
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	287
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300027 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00


GRP-4744-63-3.jdf
Sample_id = GRP-4744-63
Acq_delay = 0.2448|ms|
Changer_sample = 0
Experiment = single_pulse.exp
Field_strength = 6.34545|T|
Irr90 = 11.8|us|
Irr90_hi = 12|ns|
Irr90_lo = 45|us|
Irr_pwidth = 45|us|
Lock_status = IDLE
Recvr_gain = 19
Relaxation_delay = 1|s|
Scans = 128
Solvent = CHLOROFORM-D
Spin_get = 0|Hz|
Spin_lock_90 = 1|us|
Spin_lock_attn = 29|dB|
Spin_set = 0|Hz|
Spin_state = SPIN ON
Spin_status = SPIN OFF
Temp_get = 29|dC|
Temp_set = 25|dC|
Temp_state = TEMP OFF
Temp_status = TEMP OFF
X90 = 11.8|us|
X90_hi = 12|us|
X90_lo = 45|us|
X_acq_duration = 4.04193|s|
X_domain = 1H
X_freq = 270.16609|MHz|
X_offset = 5|ppm|
X_points = 16384
X_prescans = 2
X_pulse = 3.93333|us|
X_resolution = 0.24741|Hz|
X_sweep = 4.05351|kHz|
(2.117)
(Millions)
0.139
0.237
0.114
51.325m
0.349
0.746
33.6m
20.916m
7.2473
7.1484
7.1191
6.4203
6.3920
6.3837
6.0330
5.9451
5.2825
3.8040
3.7930
3.7692
2.9606
2.9441
1.9203
1.9047
1.8901
1.2427
0.0549
X : parts per Million : 1H
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :
Derived from: GRP-4744-63-1.jdf

GRP-4744-64-3.jdf

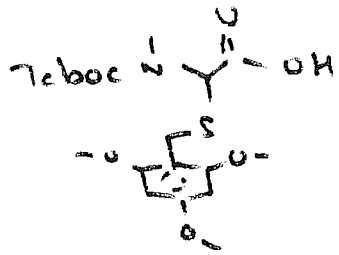
Sample_id	= GRP-4744-64
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 14
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 0[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 0[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN OFF
Temp_get	= 29.1[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

(2.118)



JEOL

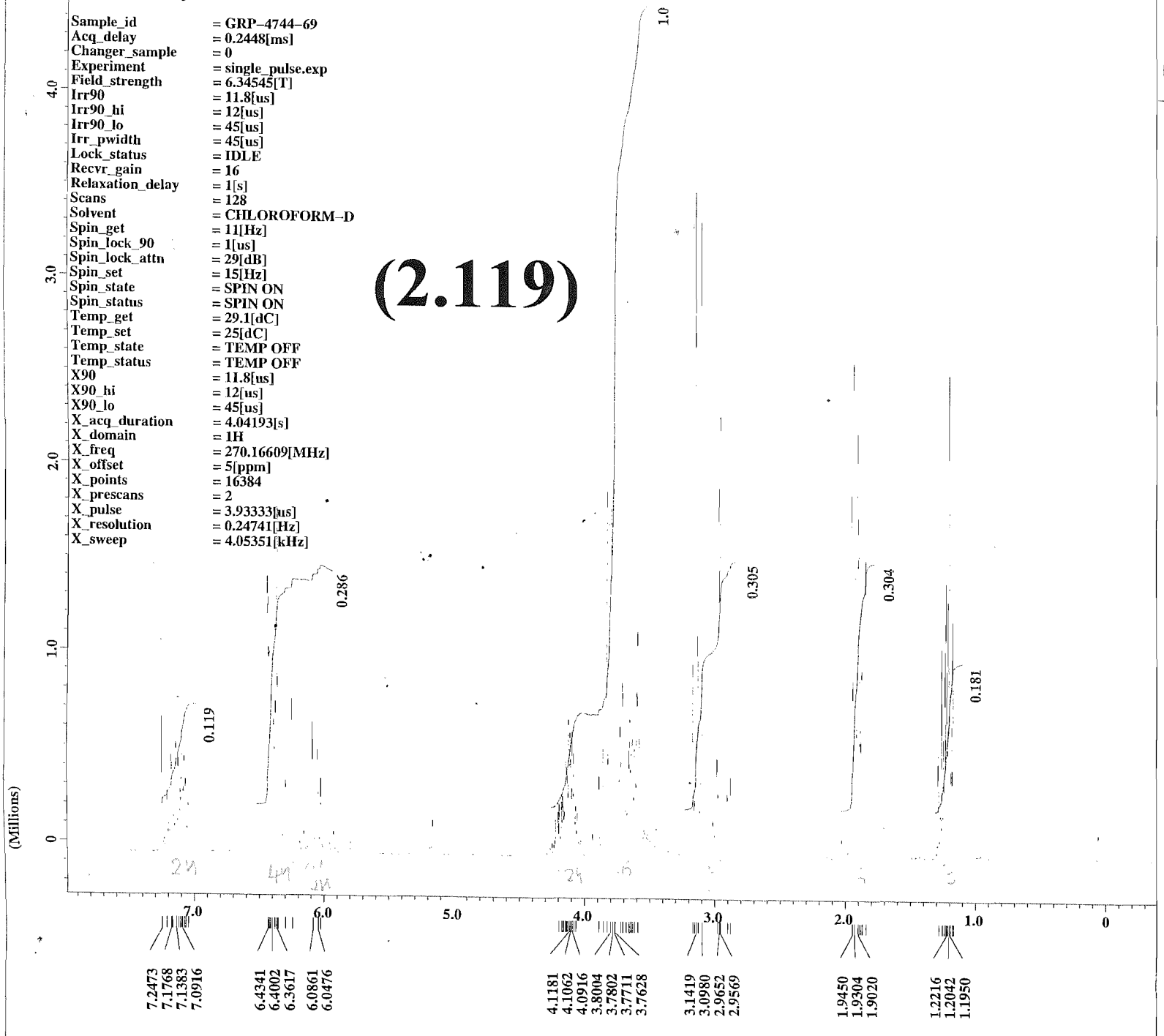
```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqpy : Printer :

Derived from: GRP-4744-64-1.jdf
```



GRP-4744-69-3.jdf

Sample_id	= GRP-4744-69
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 16
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 11[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 29.1[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

(2.119)



X : parts per Million : 1H

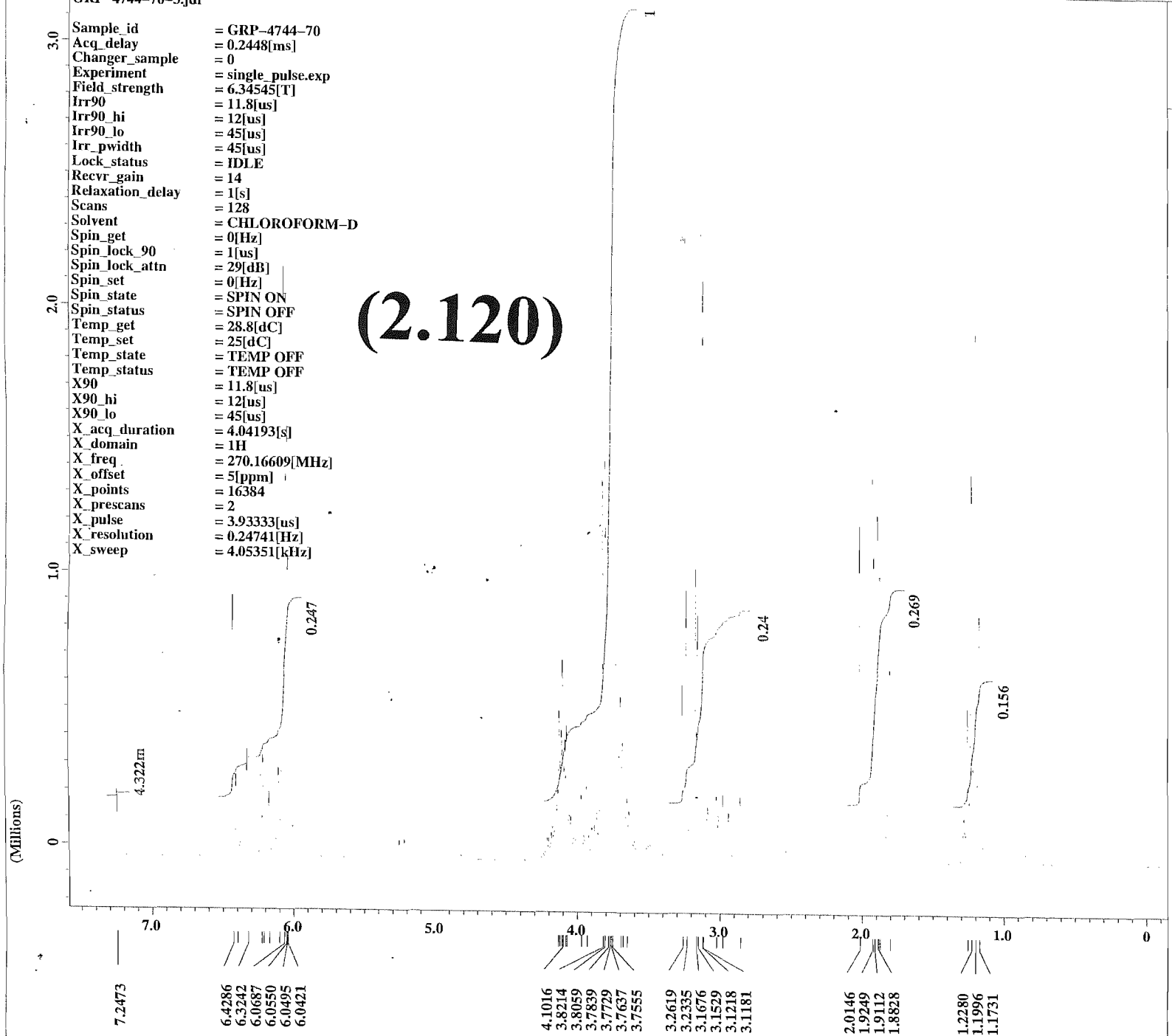
JEOL

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4744-69-1.jdf
```

GRP-4744-70-3.jdf

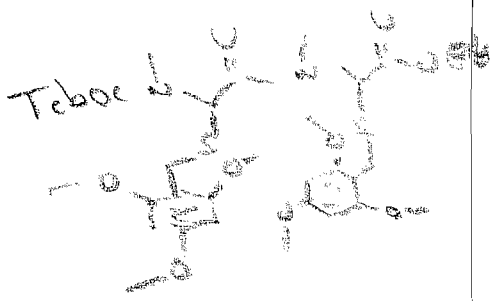
Sample_id	= GRP-4744-70
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 14
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 0[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 0[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN OFF
Temp_get	= 28.8[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

(2.120)



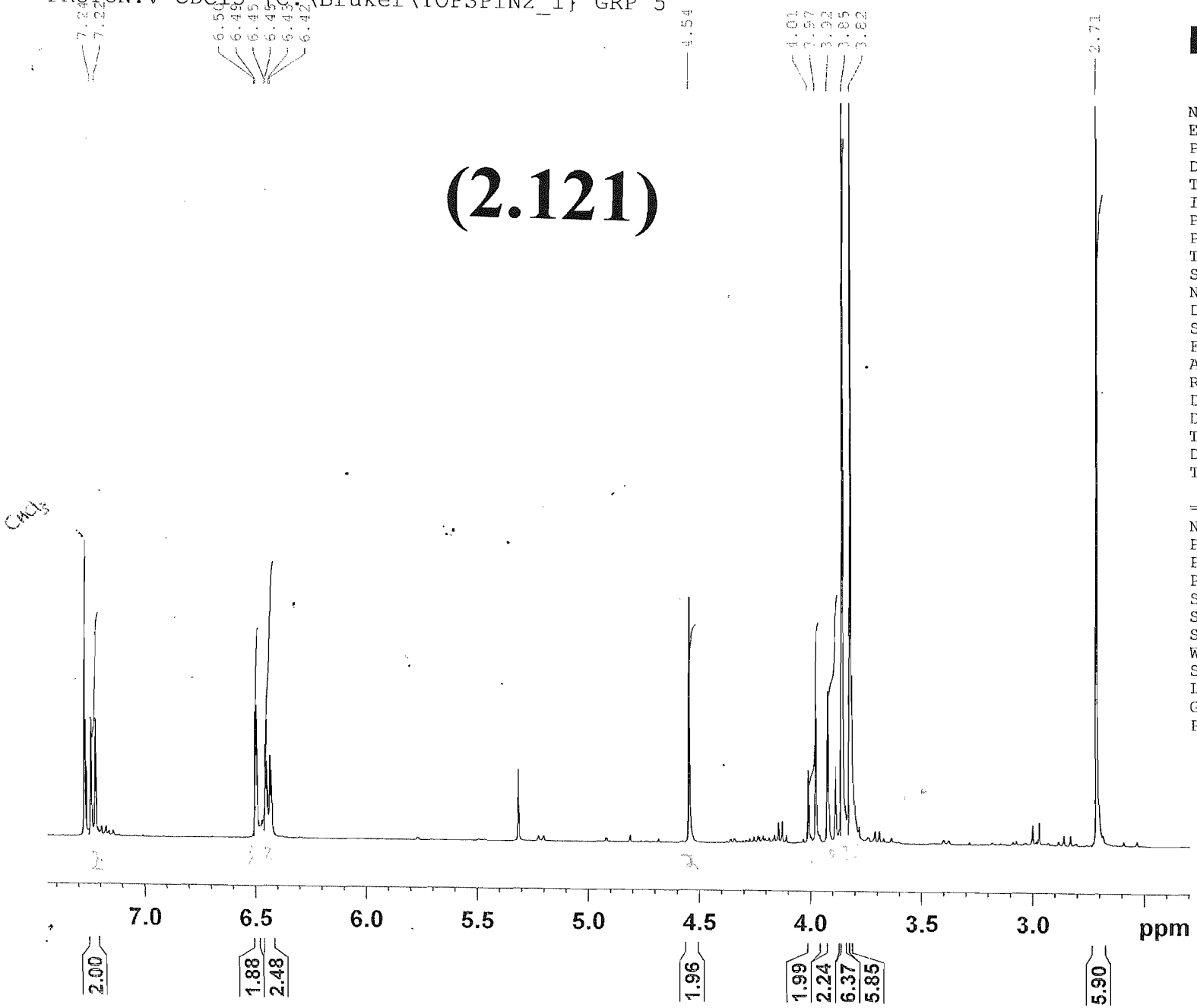
JEOL

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4744-70-1.jdf
```

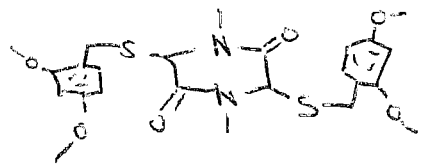


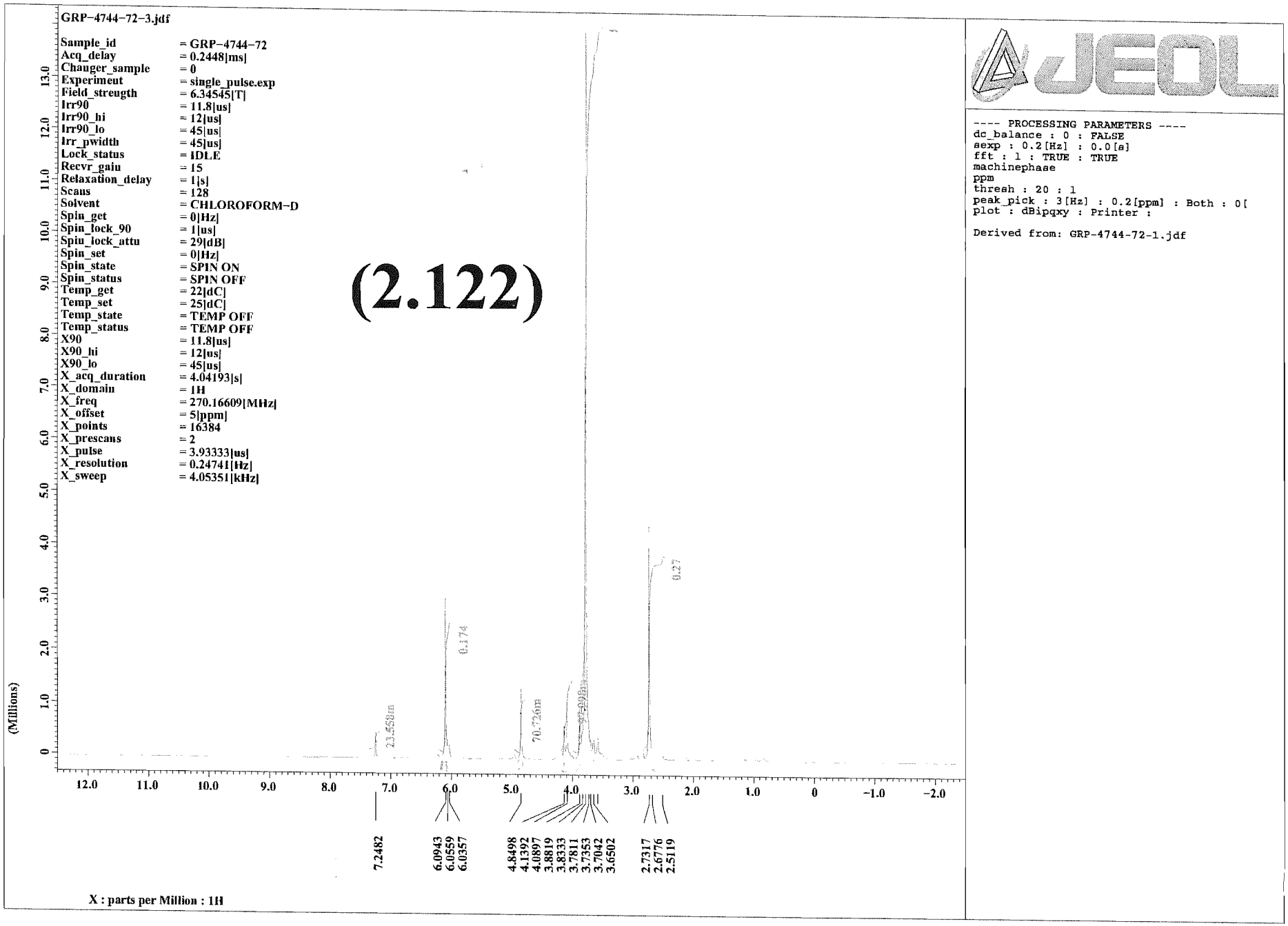
GRP-4744-71
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2_1} GRP 5

(2.121)



NAME	GRP-4744-71
EXPNO	10
PROCNO	1
Date_	20080211
Time	11.41
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	724
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1299998 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

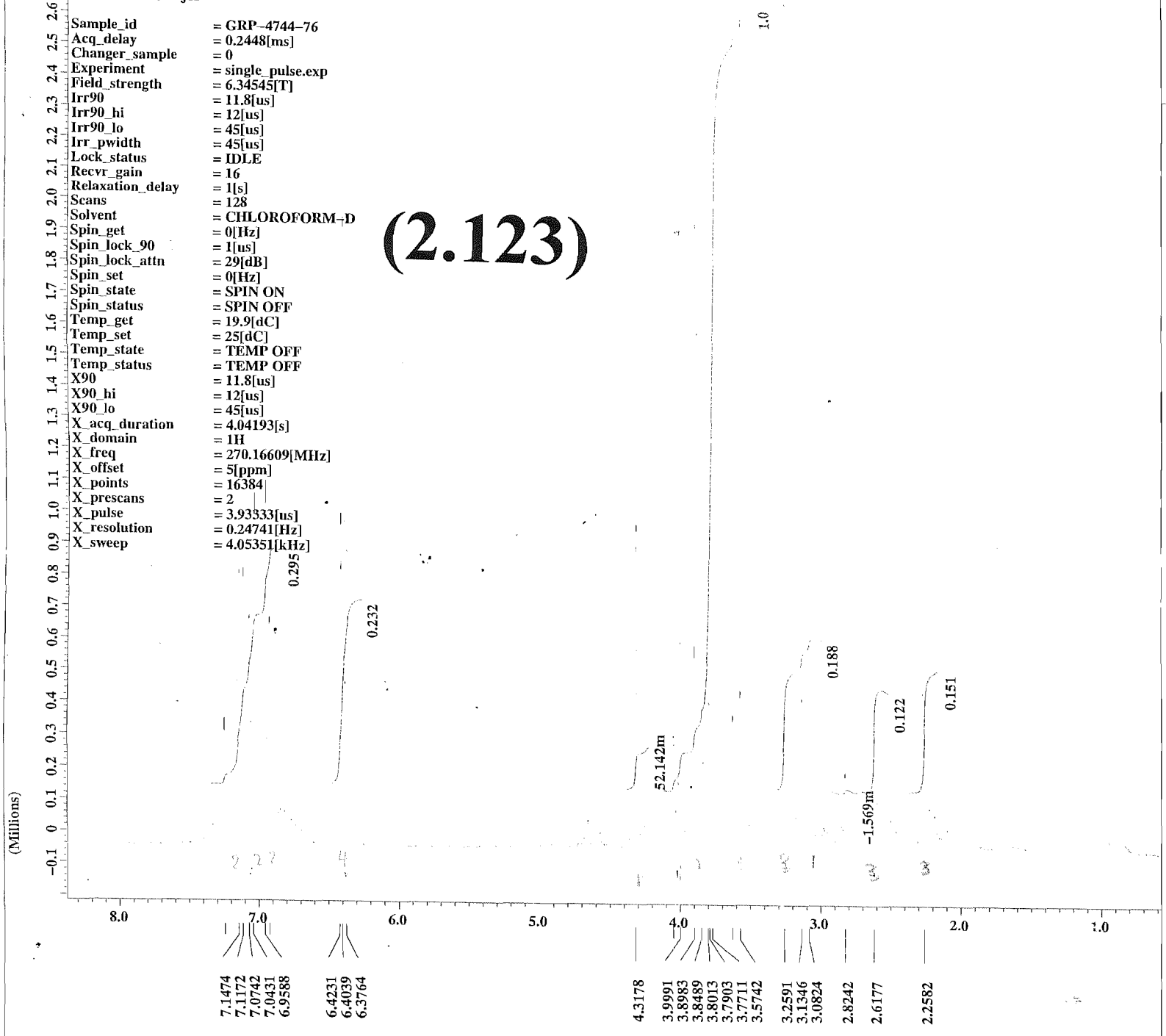



GRP-4744-72-3.jdf
Sample_id = GRP-4744-72
Acq_delay = 0.2448[ms]
Changer_sample = 0
Experiment = single_pulse.exp
Field_strength = 6.34545[T]
Irr90 = 11.8[us]
Irr90_hi = 12[us]
Irr90_lo = 45[us]
Irr_pwidth = 45[us]
Lock_status = IDLE
Recvr_gain = 15
Relaxation_delay = 1[s]
Scans = 128
Solvent = CHLOROFORM-D
Spin_get = 0[Hz]
Spin_lock_90 = 1[us]
Spin_lock_attn = 29[dB]
Spin_set = 0[Hz]
Spin_state = SPIN ON
Spin_status = SPIN OFF
Temp_get = 22[dC]
Temp_set = 25[dC]
Temp_state = TEMP OFF
Temp_status = TEMP OFF
X90 = 11.8[us]
X90_hi = 12[us]
X90_lo = 45[us]
X_acq_duration = 4.04193[s]
X_domain = 1H
X_freq = 270.16609[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 2
X_pulse = 3.93333[us]
X_resolution = 0.24741[Hz]
X_sweep = 4.05351[kHz]
(2.122)
(Millions)
0.174
0.27
7.2482
6.0943
6.0559
6.0357
4.8498
4.1392
4.0897
3.8819
3.8333
3.7811
3.7353
3.7042
3.6502
2.7317
2.6776
2.5119
X : parts per Million : 1H
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
aexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :
Derived from: GRP-4744-72-1.jdf

GRP-4744-76-2.jdf

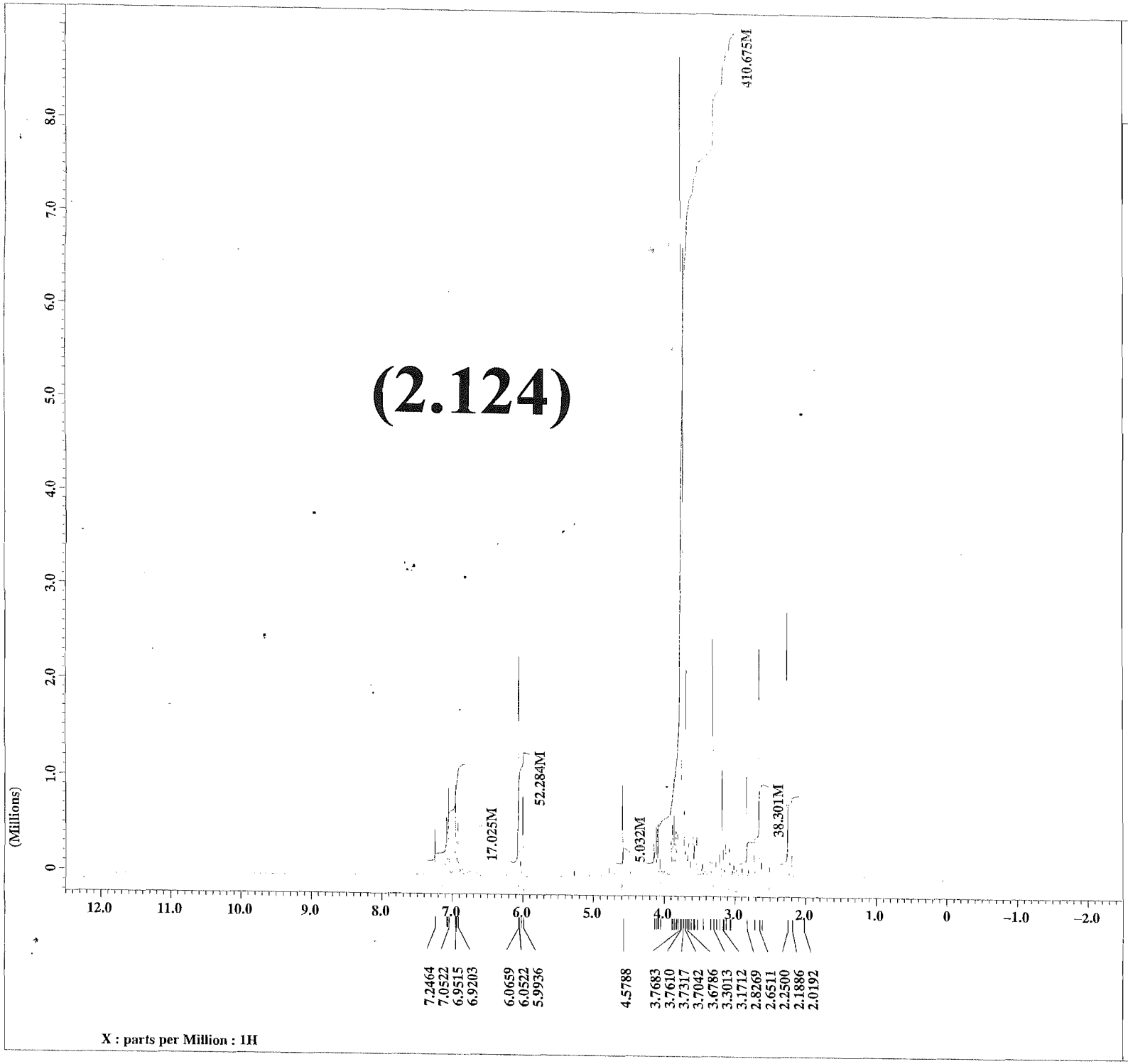
Sample_id	= GRP-4744-76
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 16
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 0[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 0[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN OFF
Temp_get	= 19.9[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

(2.123)

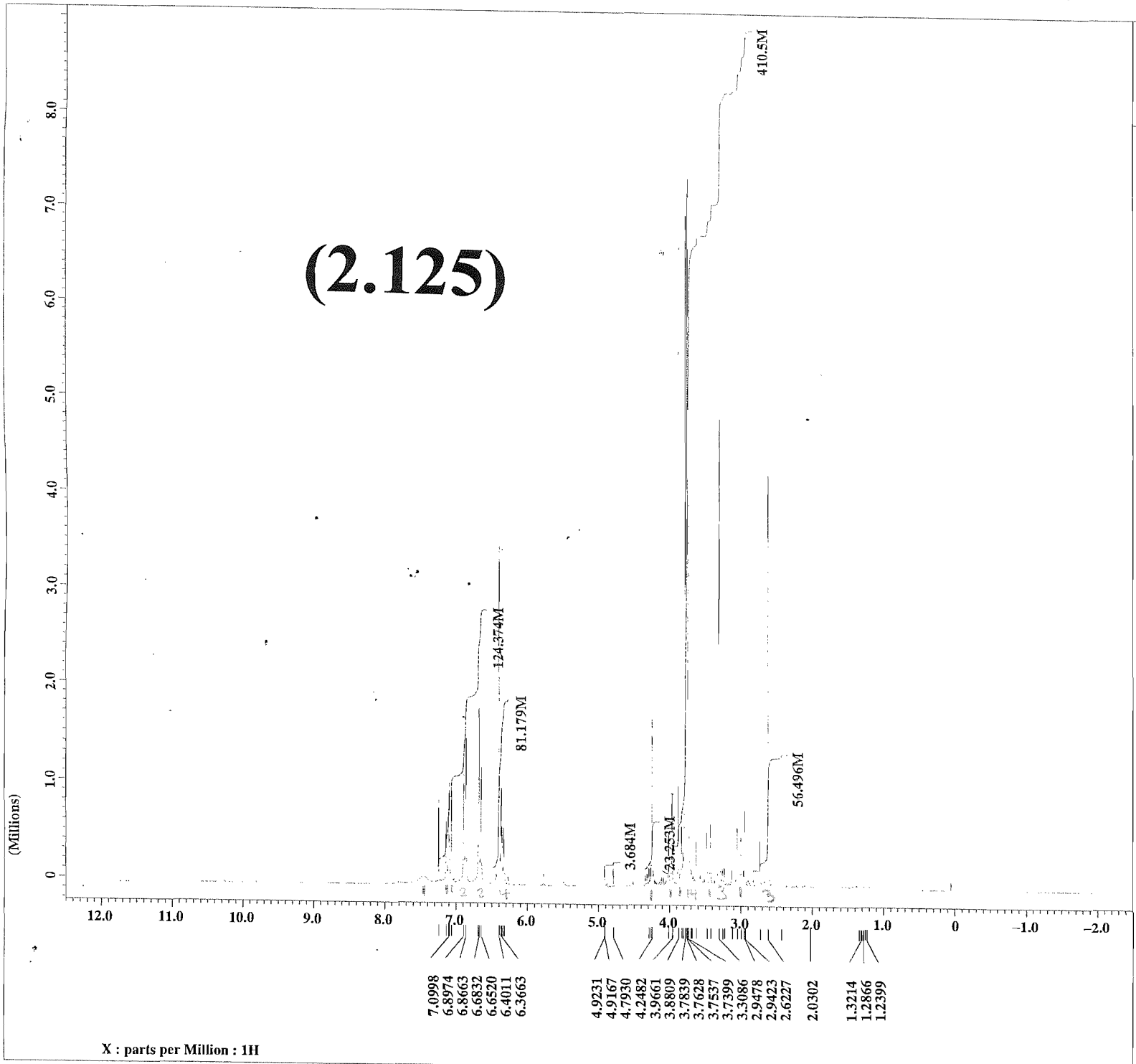


X : parts per Million : 1H

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqqy : Printer :

Derived from: GRP-4744-76-1.jdf
```

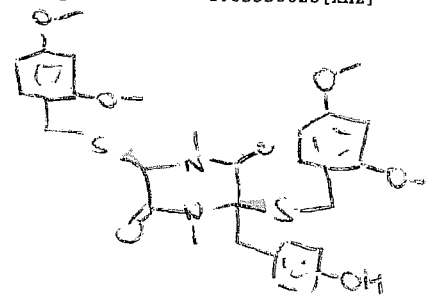


Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 15
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 19[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 20.7[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]



JEOL

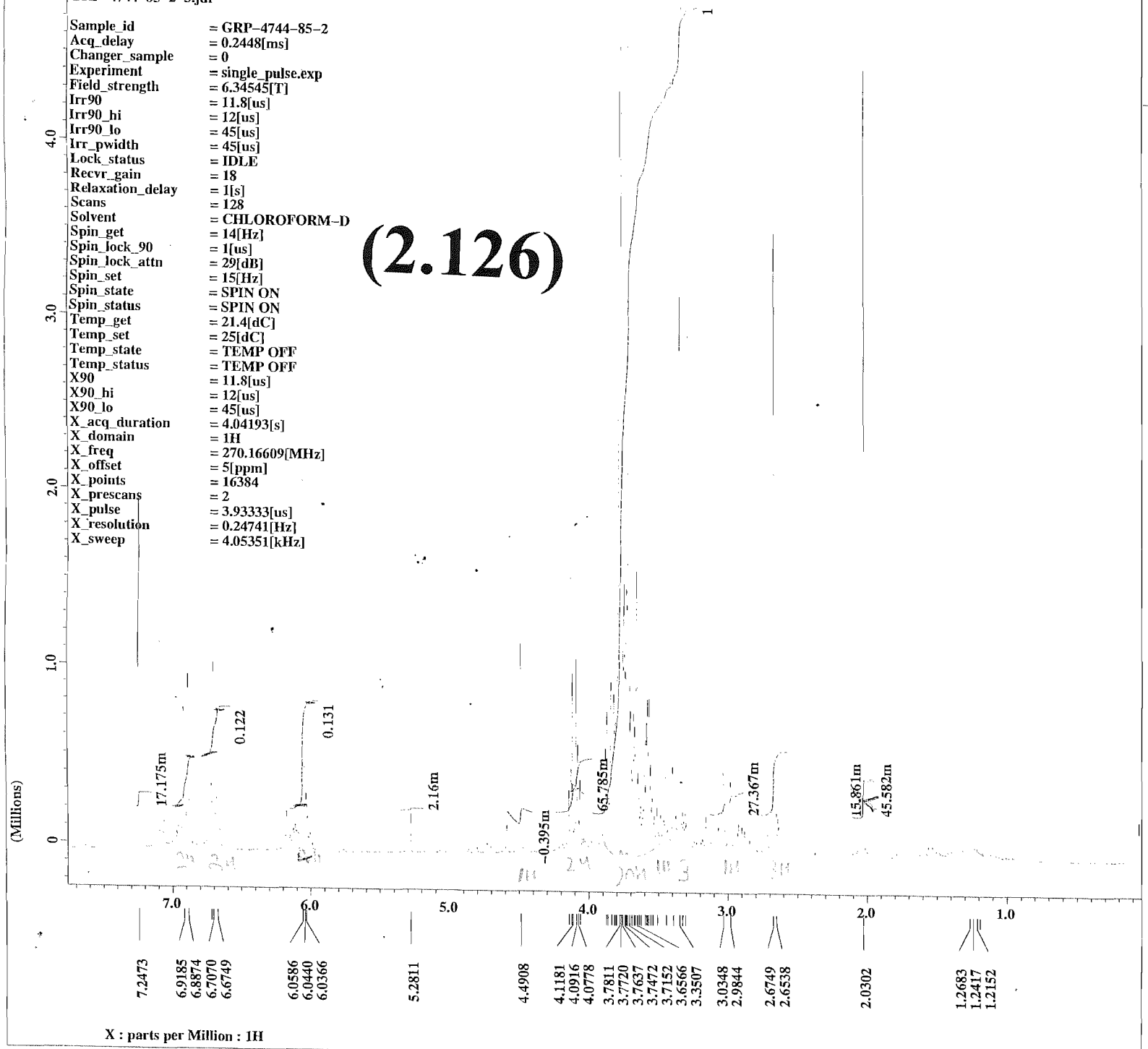
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.345446[T] (270[MHz]
Irr90	= 10[us]
Irr90_hi	= 10[us]
Irr90_lo	= 10[us]
Irr_pwidth	= 1[us]
Lock_status	= IDLE
Recvr_gain	= 16
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 13[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 26.4[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333333[us]
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]



GRP-4744-85-2-3.jdf

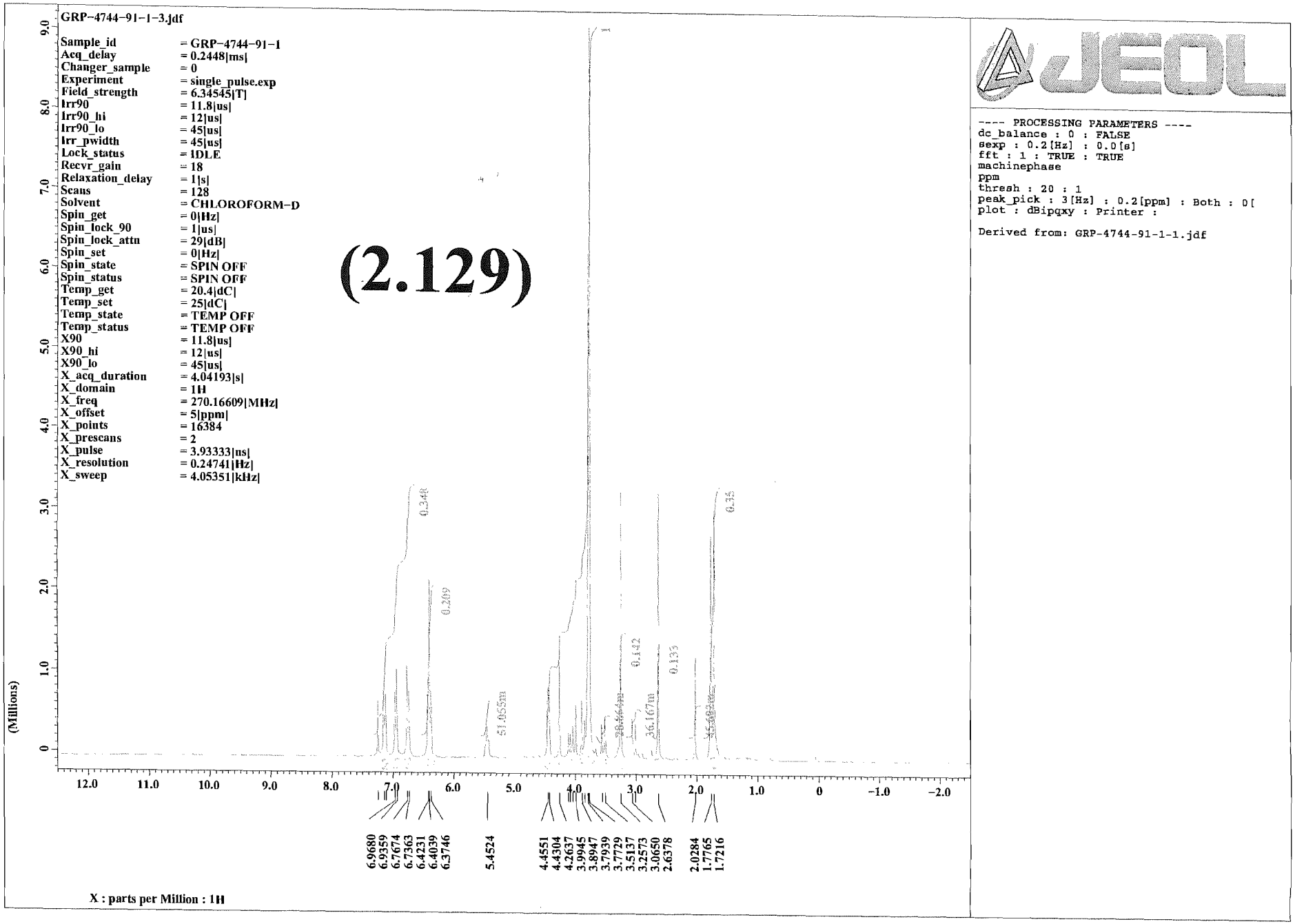
Sample_id	= GRP-4744-85-2
Acq_delay	= 0.2448[ms]
Changer_sample	= 0
Experiment	= single_pulse.exp
Field_strength	= 6.34545[T]
Irr90	= 11.8[us]
Irr90_hi	= 12[us]
Irr90_lo	= 45[us]
Irr_pwidth	= 45[us]
Lock_status	= IDLE
Recvr_gain	= 18
Relaxation_delay	= 1[s]
Scans	= 128
Solvent	= CHLOROFORM-D
Spin_get	= 14[Hz]
Spin_lock_90	= 1[us]
Spin_lock_attn	= 29[dB]
Spin_set	= 15[Hz]
Spin_state	= SPIN ON
Spin_status	= SPIN ON
Temp_get	= 21.4[dC]
Temp_set	= 25[dC]
Temp_state	= TEMP OFF
Temp_status	= TEMP OFF
X90	= 11.8[us]
X90_hi	= 12[us]
X90_lo	= 45[us]
X_acq_duration	= 4.04193[s]
X_domain	= 1H
X_freq	= 270.16609[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_pulse	= 3.93333[us]
X_resolution	= 0.24741[Hz]
X_sweep	= 4.05351[kHz]

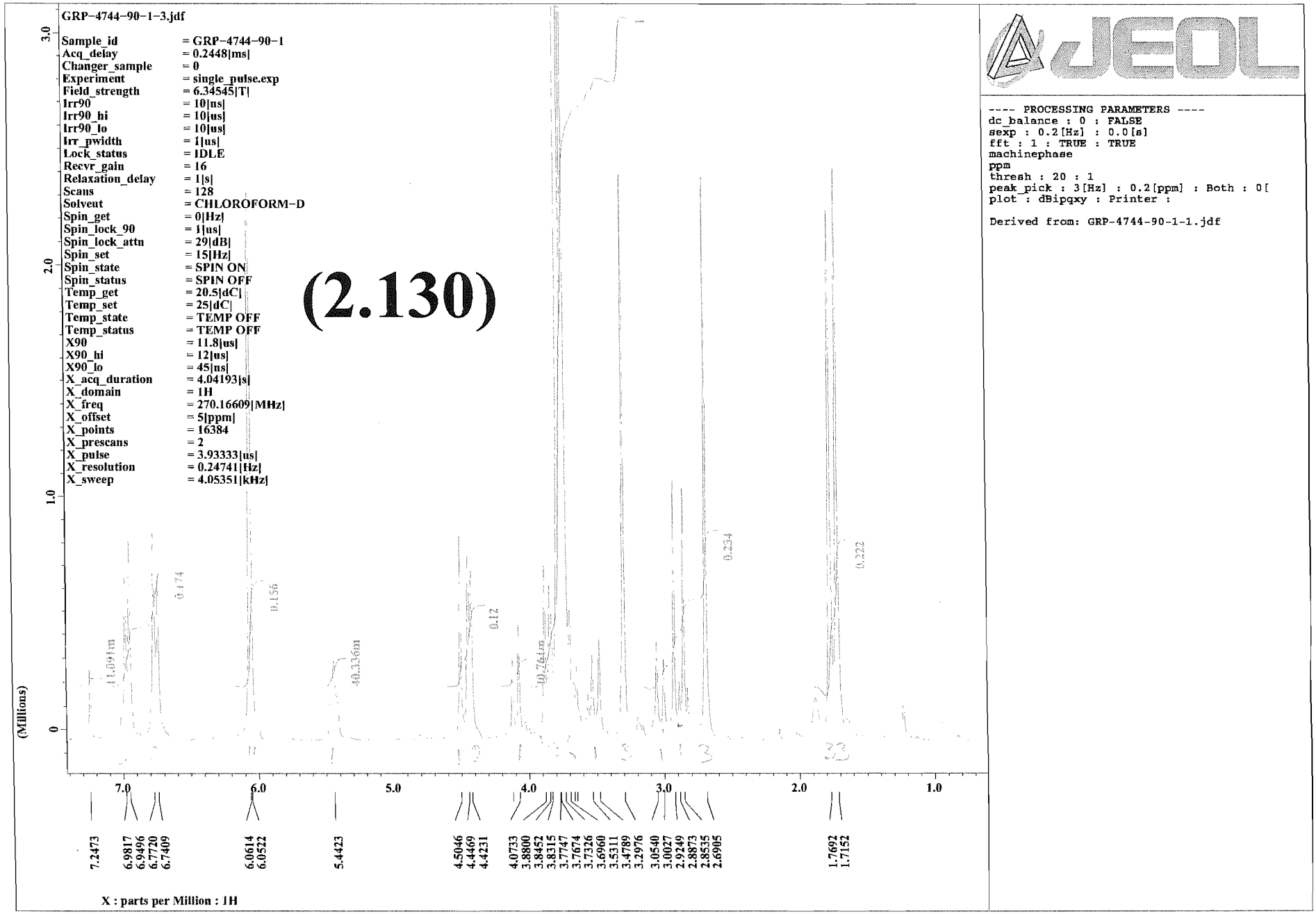
(2.126)

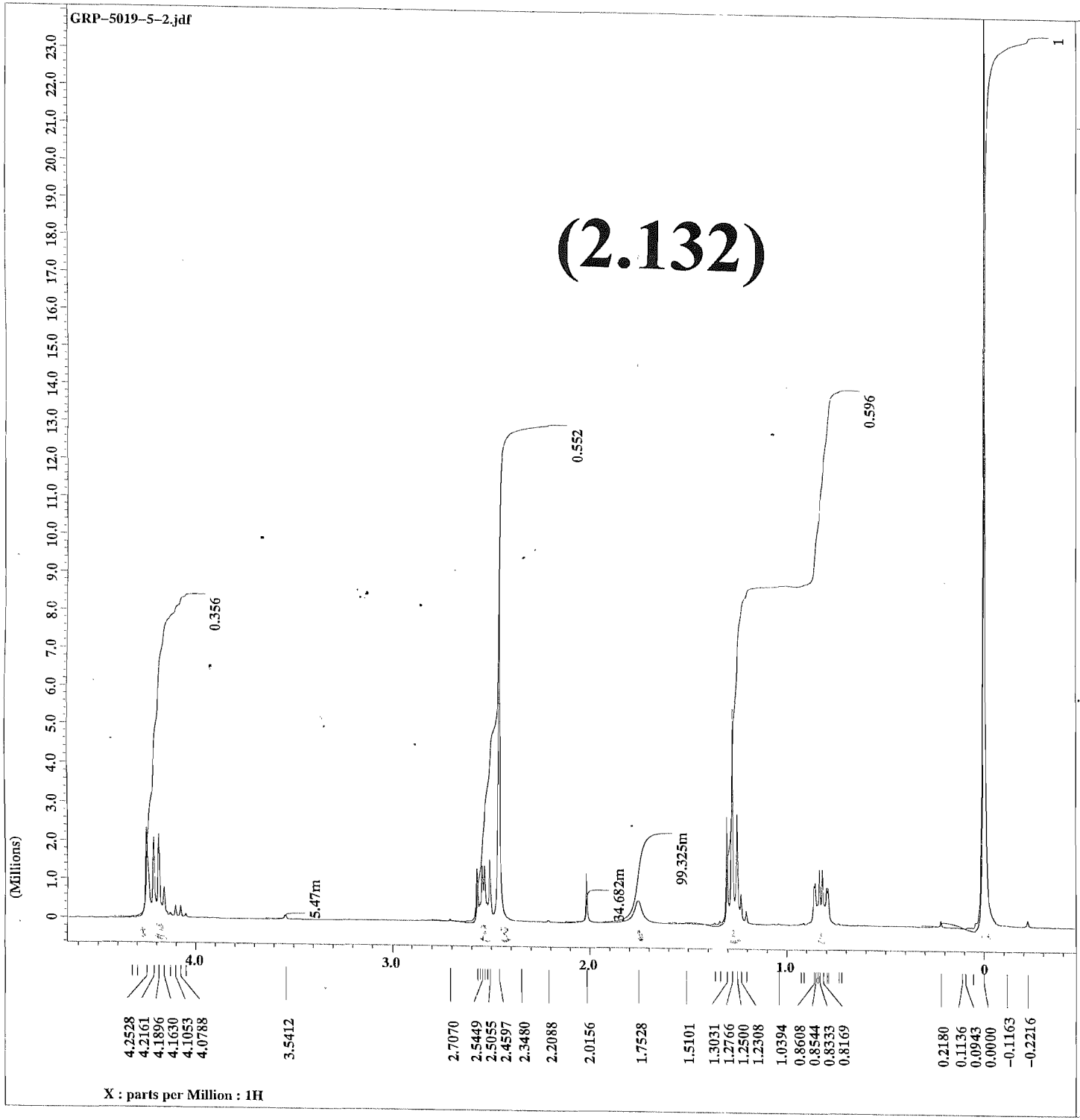


---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :

Derived from: GRP-4744-85-2-1.jdf

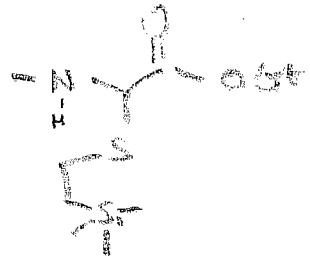

GRP-4744-91-1-3.jdf
Sample_id = GRP-4744-91-1
Acq_delay = 0.2448|ms|
Changer_sample = 0
Experiment = single_pulse.exp
Field_strength = 6.34545|T|
Irr90 = 11.8|us|
Irr90_hi = 12|us|
Irr90_lo = 45|us|
Irr_pwidth = 45|us|
Lock_status = IDLE
Recvr_gain = 18
Relaxation_delay = 1|s|
Scans = 128
Solvent = CHLOROFORM-D
Spin_get = 0|Hz|
Spin_lock_90 = 1|us|
Spin_lock_attn = 29|dB|
Spin_set = 0|Hz|
Spin_state = SPIN OFF
Spin_status = SPIN OFF
Temp_get = 20.4|dC|
Temp_set = 25|dC|
Temp_state = TEMP OFF
Temp_status = TEMP OFF
X90 = 11.8|us|
X90_hi = 12|us|
X90_lo = 45|us|
X_acq_duration = 4.04193|s|
X_domain = 1H
X_freq = 270.16609|MHz|
X_offset = 5|ppm|
X_points = 16384
X_prescans = 2
X_pulse = 3.93333|us|
X_resolution = 0.24741|Hz|
X_sweep = 4.05351|kHz|
(2.129)
(Millions)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
0.348
0.269
51.055m
0.142
0.133
36.167m
0.348
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
6.9680
6.9359
6.7674
6.7363
6.4231
6.4039
6.3746
5.4524
4.4551
4.4304
4.2637
3.9945
3.8947
3.7939
3.7729
3.5137
3.2573
3.0650
2.6378
2.0284
1.7765
1.7216
X : parts per Million : 1H
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :
Derived from: GRP-4744-91-1-1.jdf


GRP-4744-90-1-3.jdf
Sample_id = GRP-4744-90-1
Acq_delay = 0.2448|ms|
Changer_sample = 0
Experiment = single_pulse.exp
Field_strength = 6.34545|T|
Irr90 = 10|us|
Irr90_hi = 10|us|
Irr90_lo = 10|us|
Irr_pwidth = 1|us|
Lock_status = IDLE
Recvr_gain = 16
Relaxation_delay = 1|s|
Scans = 128
Solvent = CHLOROFORM-D
Spin_get = 0|Hz|
Spin_lock_90 = 1|us|
Spin_lock_attn = 29|dB|
Spin_set = 15|Hz|
Spin_state = SPIN ON
Spin_status = SPIN OFF
Temp_get = 20.5|dC|
Temp_set = 25|dC|
Temp_state = TEMP OFF
Temp_status = TEMP OFF
X90 = 11.8|us|
X90_hi = 12|us|
X90_lo = 45|us|
X_acq_duration = 4.04193|s|
X_domain = 1H
X_freq = 270.16609|MHz|
X_offset = 5|ppm|
X_points = 16384
X_prescans = 2
X_pulse = 3.93333|us|
X_resolution = 0.24741|Hz|
X_sweep = 4.05351|kHz|
(2.130)
(Millions)
3.0
2.0
1.0
0
0.174
0.156
0.12
0.234
0.222
7.0
6.0
5.0
4.0
3.0
2.0
1.0
7.2473
6.9817
6.9496
6.7720
6.7409
6.0614
6.0522
5.4423
4.5046
4.4469
4.4231
4.0733
3.8800
3.8452
3.8315
3.7747
3.7674
3.7326
3.6960
3.5311
3.4789
3.2976
3.0540
3.0027
2.9249
2.8873
2.8535
2.6905
1.7692
1.7152
X : parts per Million : 1H
JEOL
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
fft : 1 : TRUE : TRUE
machinephase
ppm
thresh : 20 : 1
peak_pick : 3[Hz] : 0.2[ppm] : Both : 0[
plot : dBipqxy : Printer :
Derived from: GRP-4744-90-1-1.jdf



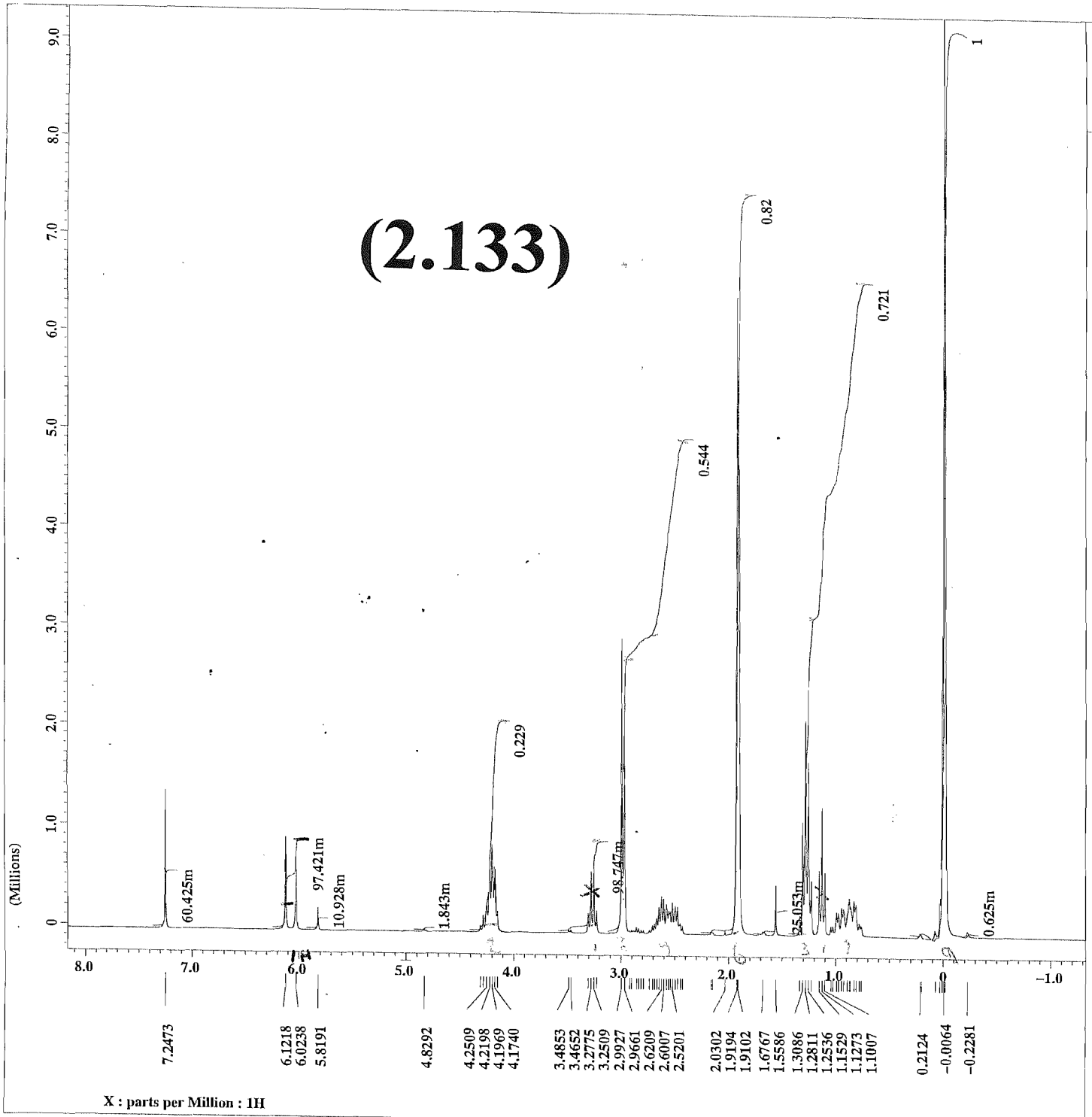
JEOL

Filename	= GRP-5019-5-2.jdf
Author	= nmr
Experiment	= single_pulse.exp
Sample_id	= GRP5019-5
Solvent	= CHLOROFORM-D
Creation_time	= 1-OCT-2007 20:19:33
Revision_time	= 1-OCT-2007 17:43:23
Current_time	= 1-OCT-2007 17:43:56
Content	= Single Pulse Experime
Data_format	= 1D REAL
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= EX-Delta 270
Spectrometer	= DELTA_NMR
Field_strength	= 6.345446[T] (270[MHz]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 128
Total_scans	= 128
X_90_width	= 11.8[us]
X_acq_time	= 4.0419328[s]
X_angle	= 30[deg]
X_pulse	= 3.93333333[us]
Initial_wait	= 1[s]
Phase_preset	= 2[us]
Recvr_gain	= 12
Relaxation_delay	= 1[s]
Temp_get	= 21.3[dC]
Unblank_time	= 2[us]



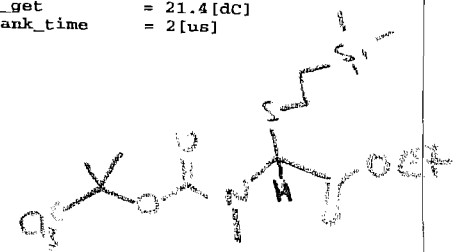
E.37

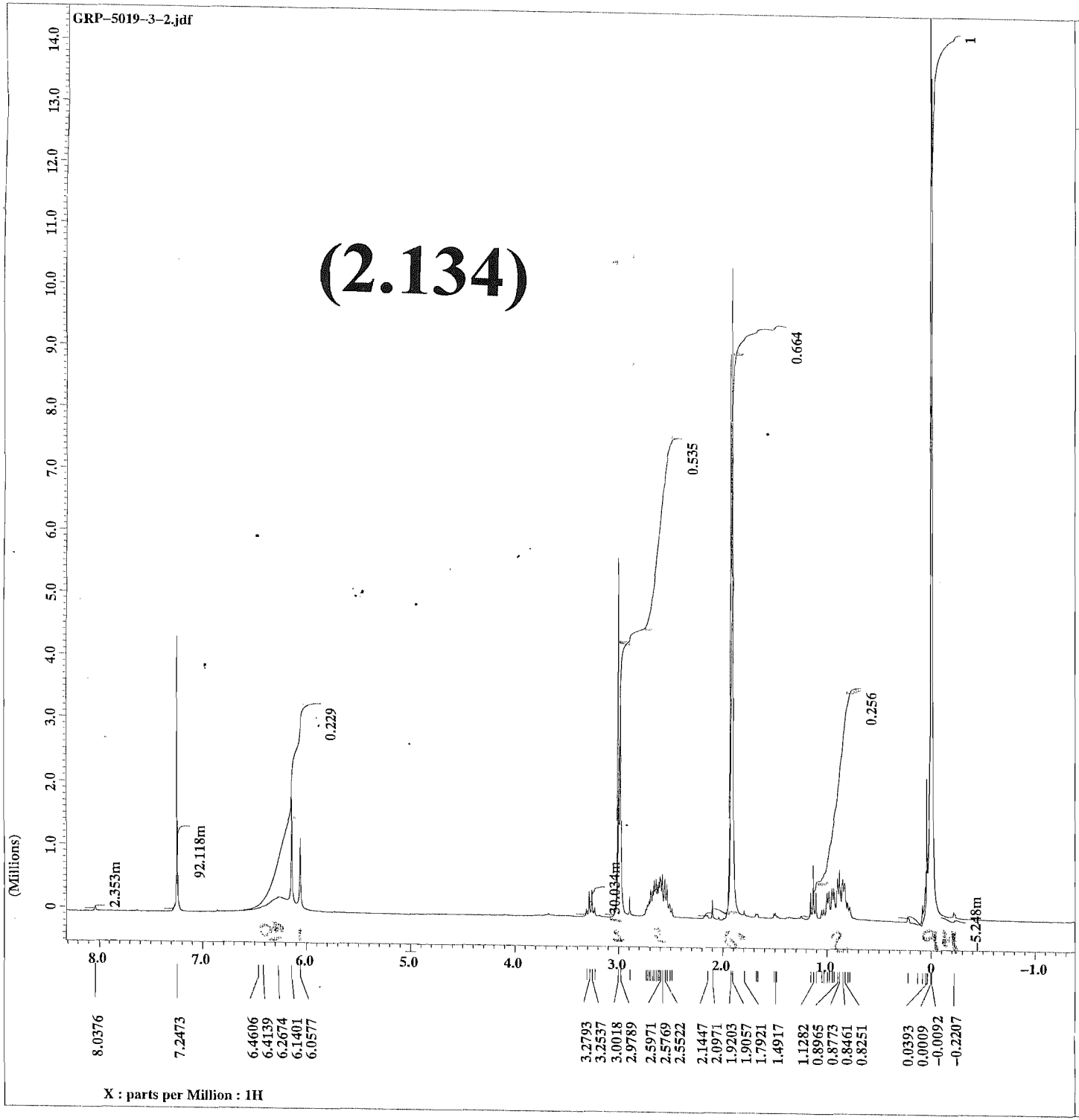
(2.133)



JEOL

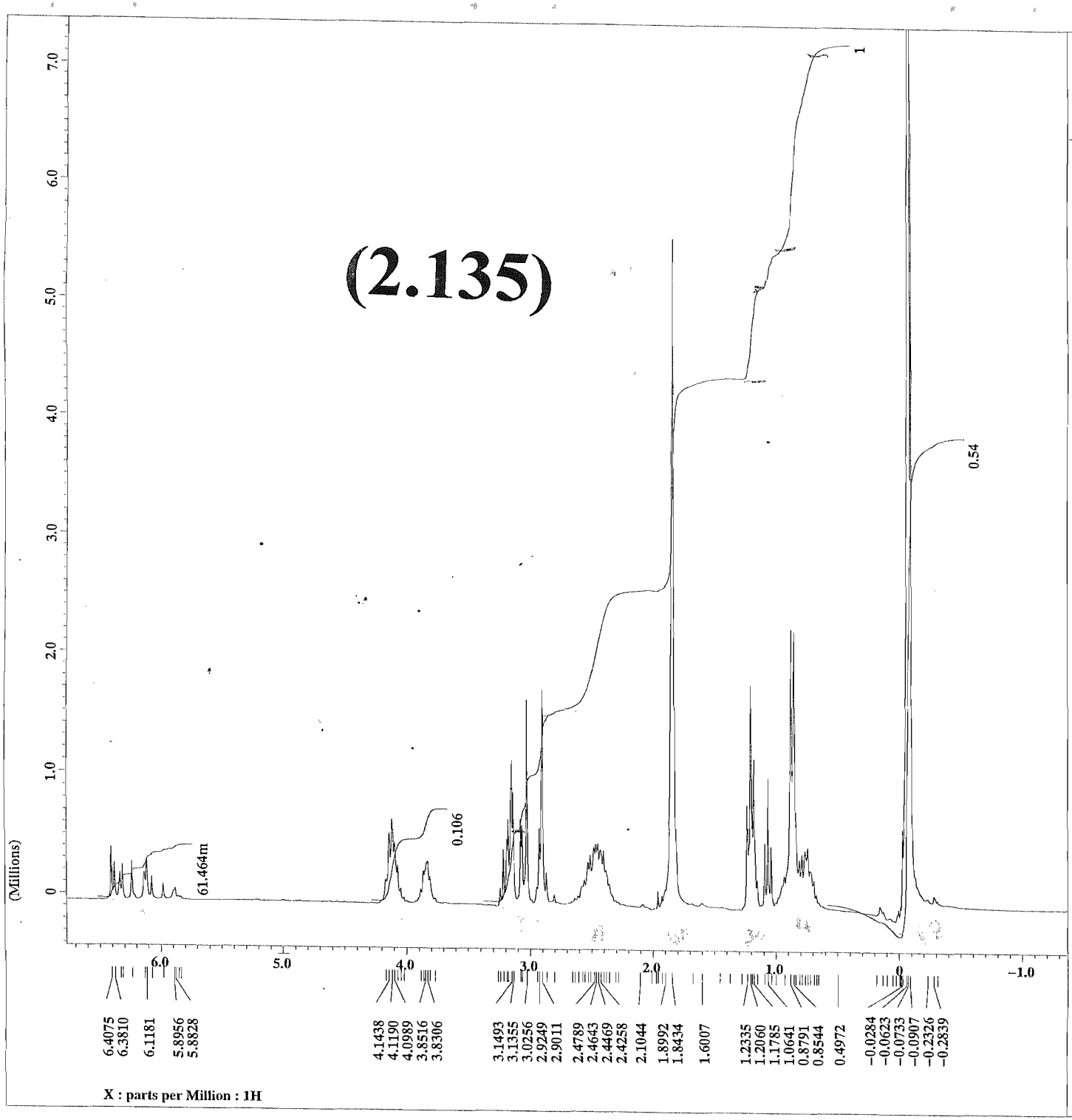
Filename	= GRP-5019-02-2.jdf
Author	= nmr
Experiment	= single_pulse.exp
Sample_id	= GRP-5019-02
Solvent	= CHLOROFORM-D
Creation_time	= 24-SEP-2007 20:08:53
Revision_time	= 24-SEP-2007 17:29:06
Current_time	= 24-SEP-2007 17:29:23
Content	= Single Pulse Experime
Data_format	= 1D COMPLEX
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= EX-Delta 270
Spectrometer	= DELTA_NMR
Field_strength	= 6.345446[T] (270[MHz]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 0
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 64
Total_scans	= 64
X_90_width	= 11.8[us]
X_acq_time	= 4.0419328[s]
X_angle	= 45[deg]
X_pulse	= 5.9[us]
Initial_wait	= 1[s]
Phase_preset	= 3[us]
Recvr_gain	= 19
Relaxation_delay	= 4[s]
Temp_get	= 21.4[dC]
Unblank_time	= 2[us]





JEOL

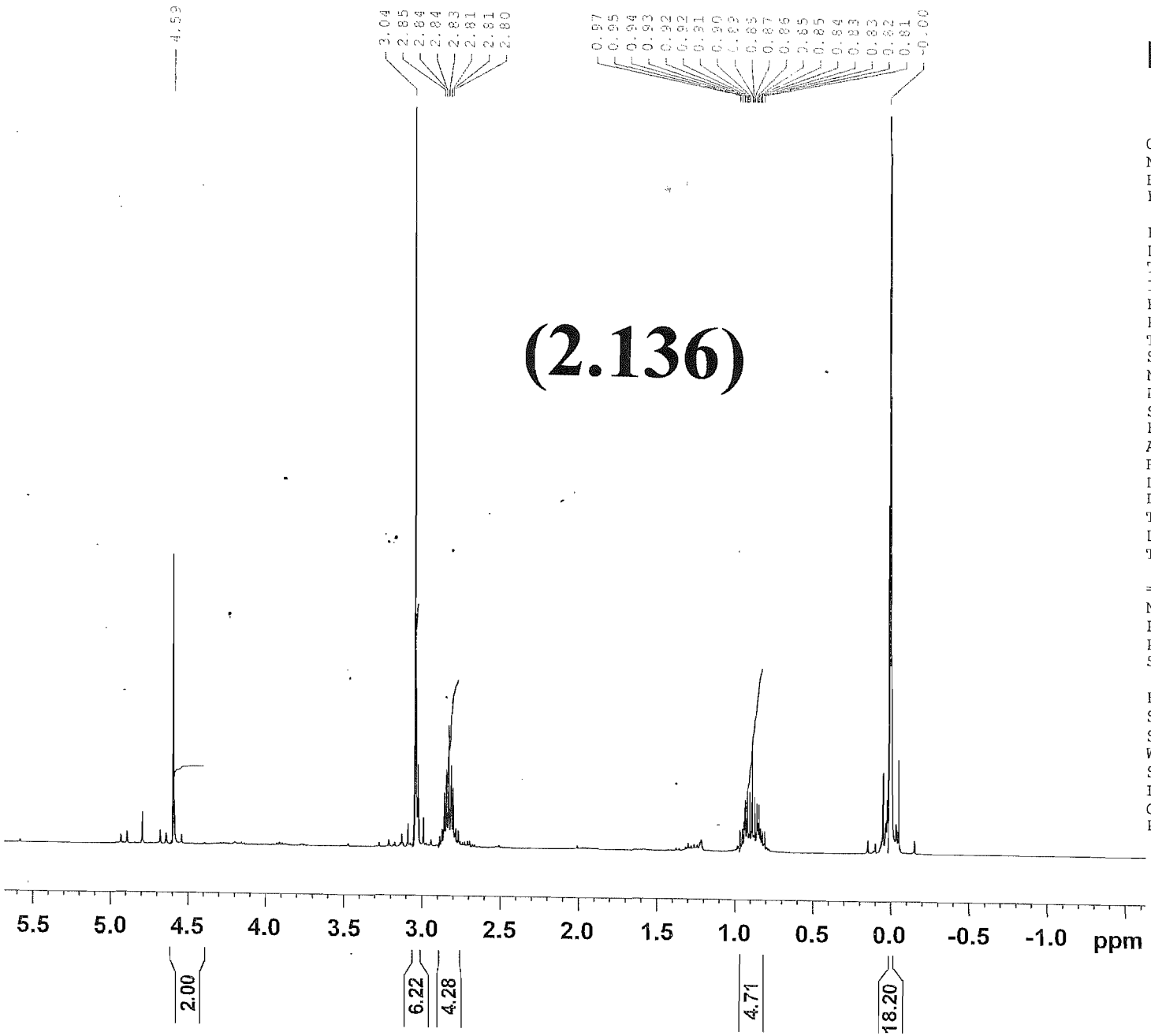
Filename	= GRP-5019-3-2.jdf
Author	= nmr
Experiment	= single_pulse.exp
Sample_id	= GRP-5019-3 acid
Solvent	= CHLOROFORM-D
Creation_time	= 2-OCT-2007 14:38:51
Revision_time	= 2-OCT-2007 12:01:36
Current_time	= 2-OCT-2007 12:01:55
Content	= Single Pulse Experime
Data_format	= 1D COMPLEX
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= EX-Delta 270
Spectrometer	= DELTA_NMR
Field_strength	= 6.345446[T] (270[MHz]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 128
Total_scans	= 128
X_90_width	= 11.8[us]
X_acq_time	= 4.0419328[s]
X_angle	= 30[deg]
X_pulse	= 3.93333333[us]
Initial_wait	= 1[s]
Phase_preset	= 2[us]
Recvr_gain	= 24
Relaxation_delay	= 1[s]
Temp_get	= 20.7[dC]
Unblank_time	= 2[us]



JEOL

Filename	= GRP-5019-6-2.jdf
Author	= nmr
Experiment	= single_pulse.exp
Sample_id	= GRP-5019-6
Solvent	= CHLOROFORM-D
Creation_time	= 5-OCT-2007 15:15:18
Revision_time	= 5-OCT-2007 12:39:10
Current_time	= 5-OCT-2007 12:40:23
Content	= Single Pulse Experime
Data_format	= 1D COMPLEX
Dim_size	= 16384
Dim_title	= 1H
Dim_units	= [ppm]
Dimensions	= X
Site	= EX-Delta 270
Spectrometer	= DELTA_NMR
Field_strength	= 6.345446[T] (270[MHz]
X_acq_duration	= 4.0419328[s]
X_domain	= 1H
X_freq	= 270.16608844[MHz]
X_offset	= 5[ppm]
X_points	= 16384
X_prescans	= 2
X_resolution	= 0.24740639[Hz]
X_sweep	= 4.05350628[kHz]
Clipped	= FALSE
Mod_return	= 1
Scans	= 16
Total_scans	= 16
X_90_width	= 11.8[us]
X_acq_time	= 4.0419328[s]
X_angle	= 30[deg]
X_pulse	= 3.93333333[us]
Initial_wait	= 1[s]
Phase_preset	= 2[us]
Recvr_gain	= 12
Relaxation_delay	= 1[s]
Temp_get	= 20.9[dC]
Unblank_time	= 2[us]

GRP-5019-10



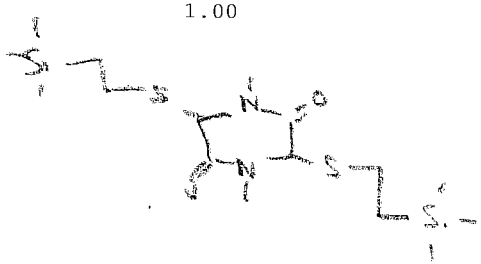
BRUKER

Current Data Parameters
NAME Dec13-2007
EXPNO 70
PROCNO 1

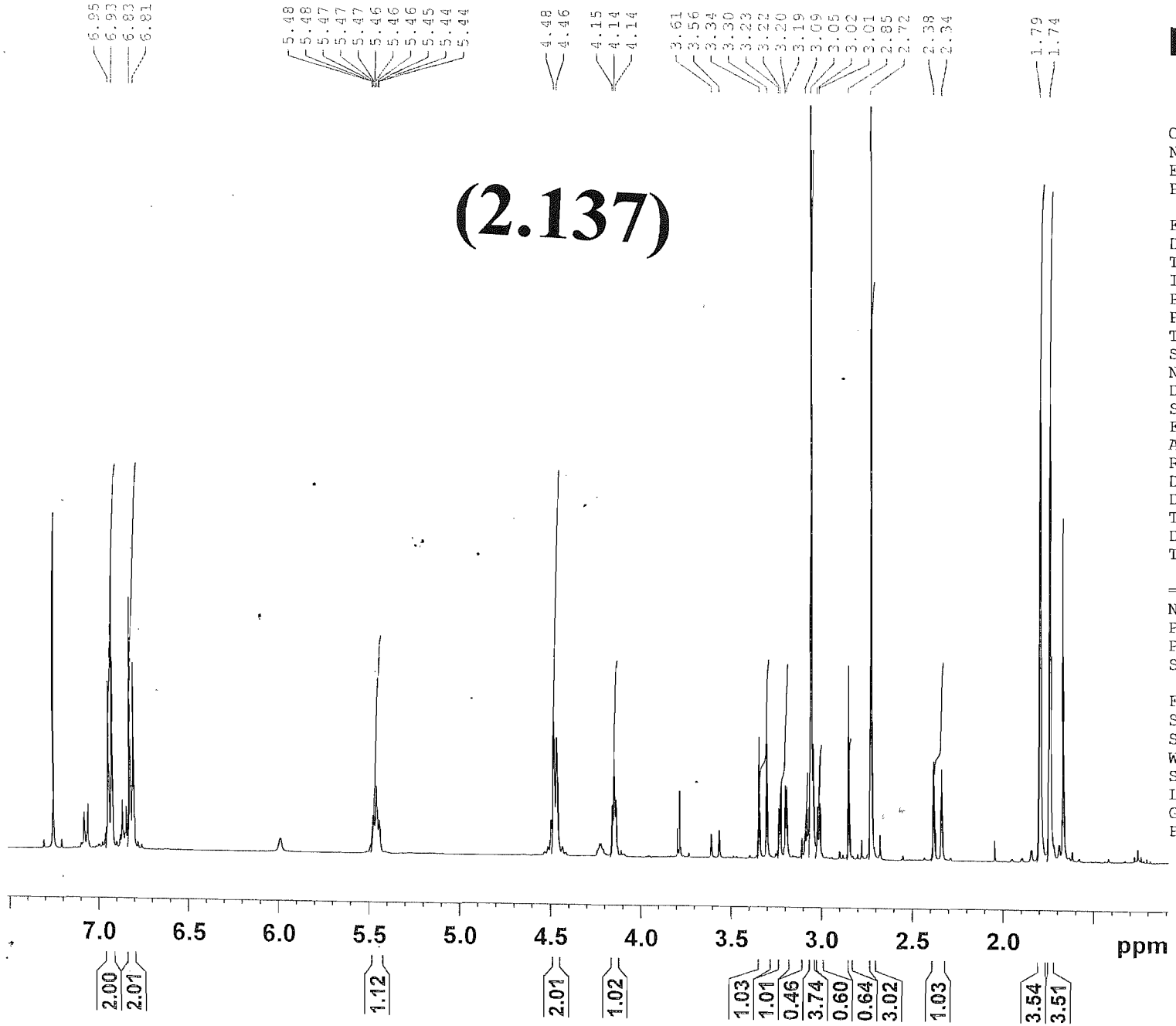
F2 - Acquisition Parameters
Date_ 20071213
Time 17.39
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 101
DW 60.800 usec
DE 6.50 usec
TE 292.4 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300143 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



GRP-5019-21



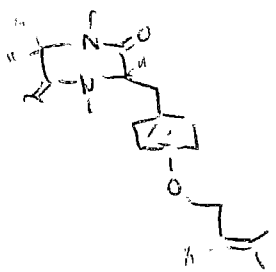
BRUKER

Current Data Parameters
NAME Dec13-2007
EXPNO 100
PROCNO 1

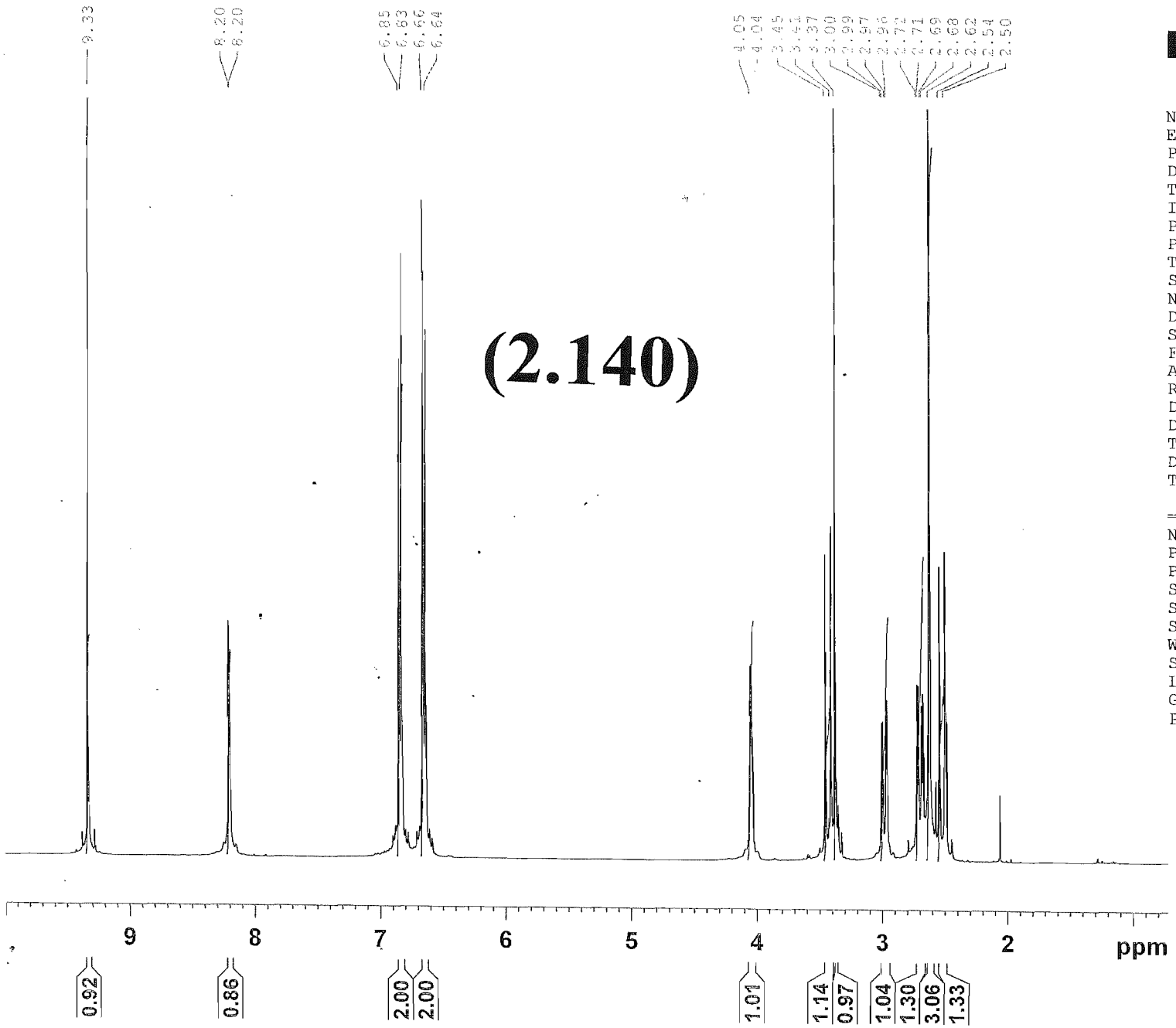
F2 - Acquisition Parameters
Date_ 20071213
Time 22.09
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 512
DW 60.800 usec
DE 6.50 usec
TE 292.5 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300048 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



GRP-5019-18



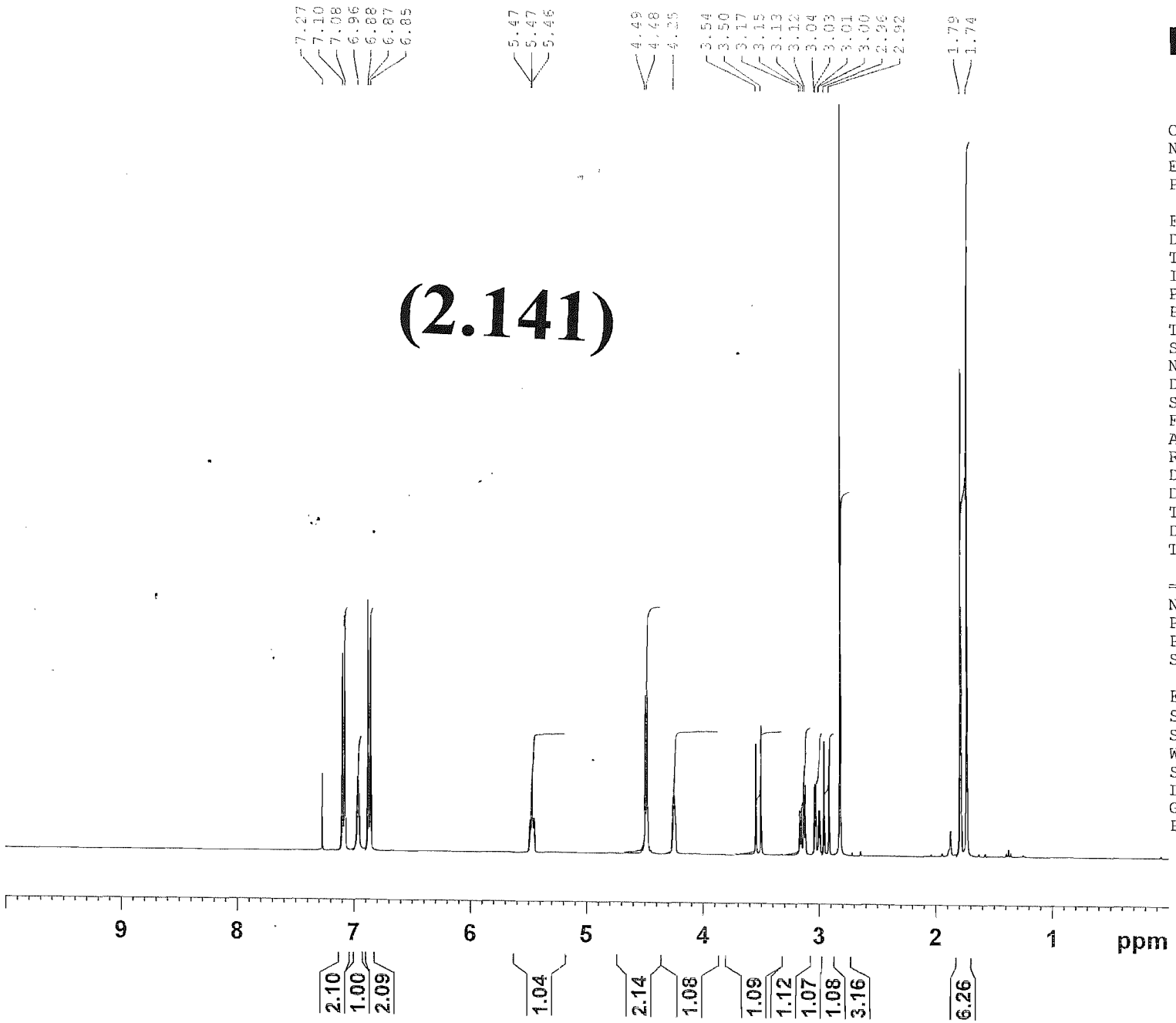
BRUKER

NAME	Dec14-2007
EXPNO	10
PROCNO	1
Date_	20071214
Time	12.20
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	128
DS	0
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	203
DW	60.800 usec
DE	6.50 usec
TE	291.8 K
D1	2.00000000 sec
TD0	1

======== CHANNEL f1 ========

NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
SFO1	400.1324710 MHz
SI	32768
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

GRP-5019-19



BRUKER

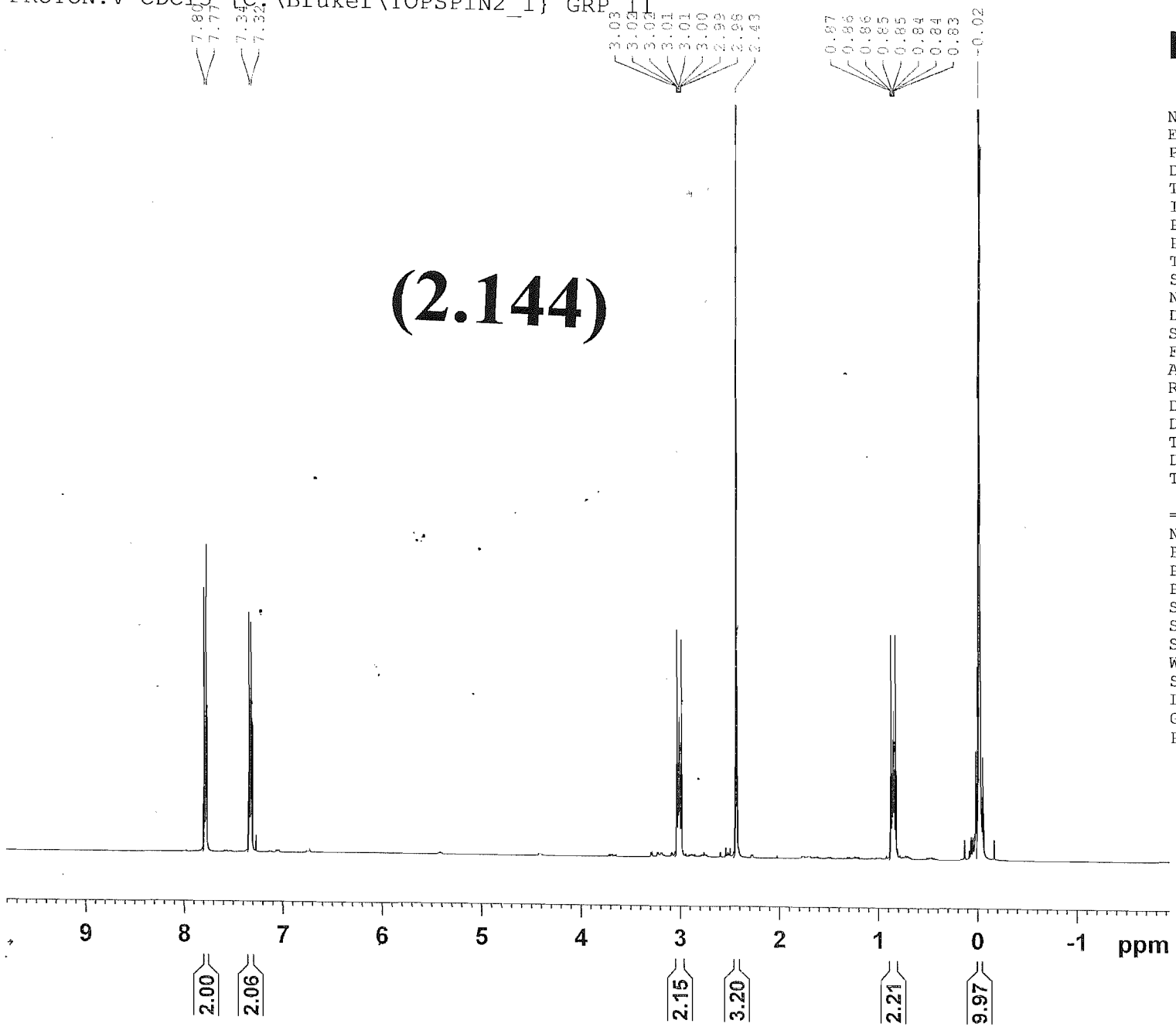
Current Data Parameters
NAME Dec05-2007
EXPNO 20
PROCNO 1

F2 - Acquisition Parameters
Date_ 20071205
Time 11.44
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 128
DW 60.800 usec
DE 6.50 usec
TE 294.8 K
D1 2.00000000 sec
TD0 1

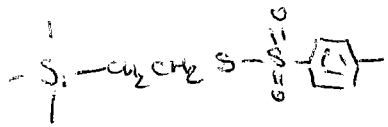
======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300014 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

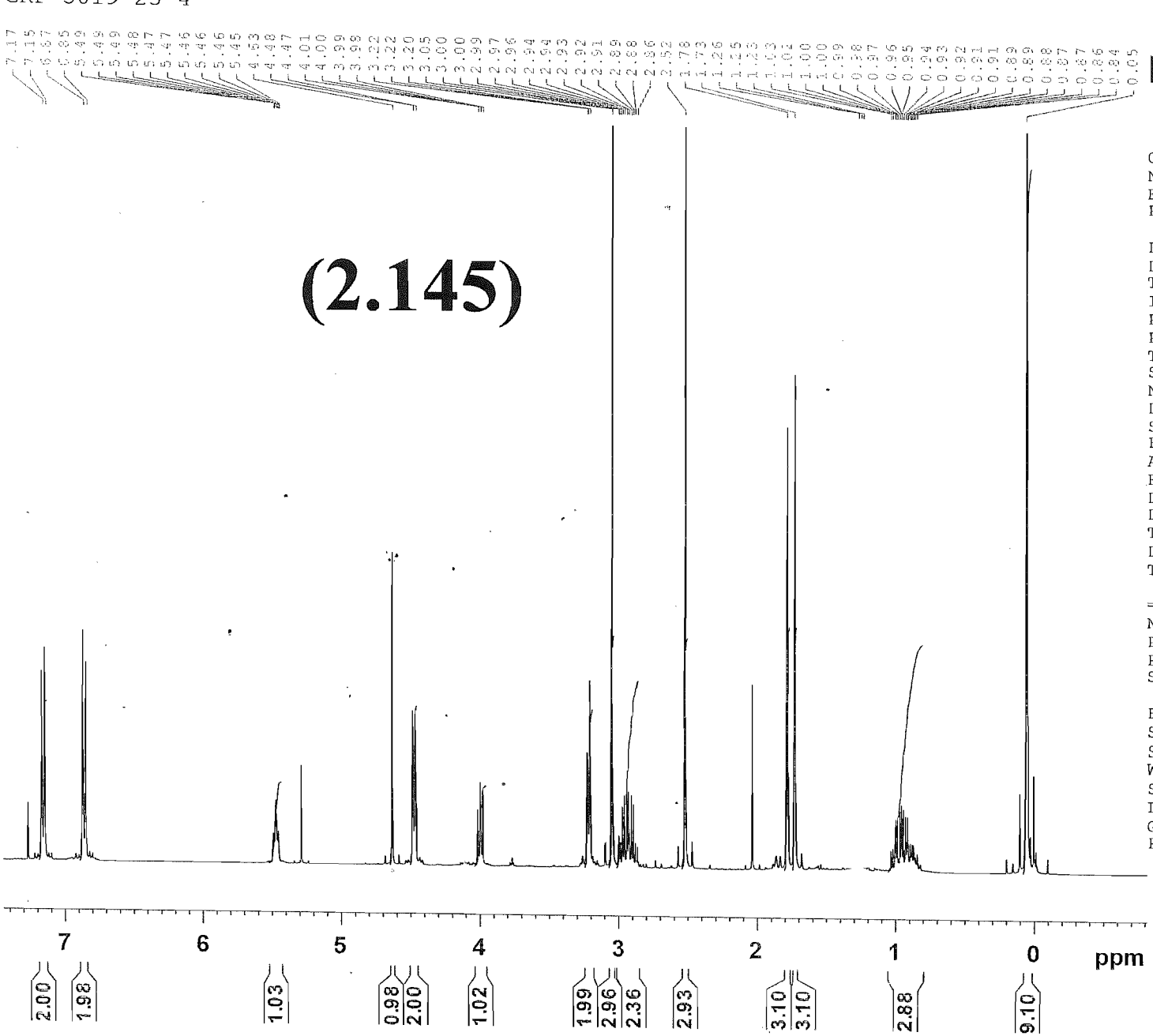
GRP-5019-24
PROTON.v CDCl3 {C:\Bruker\TOPSPIN2_1} GRP_11



NAME	GRP-5019-24
EXPNO	10
PROCNO	1
Date_	20080124
Time	17.43
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDC13
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	32
DW	60.800 usec
DE	16.77 usec
TE	298.2 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300007 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



GRP-5019-23-4



BRUKER

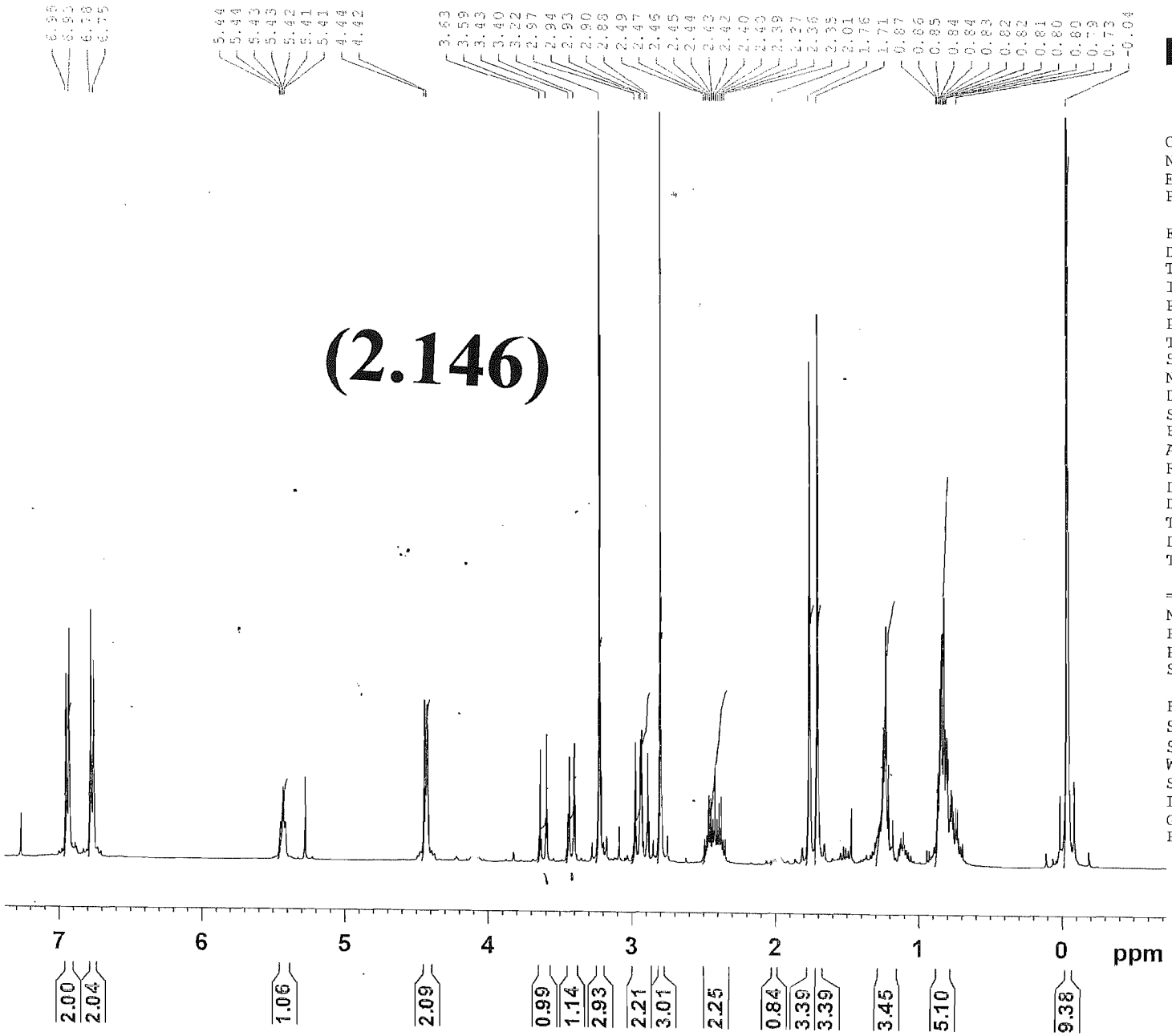
Current Data Parameters
NAME Dec17-2007
EXPNO 40
PROCNO 1

F2 - Acquisition Parameters
Date_ 20071217
Time 15.18
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 64
DW 60.800 usec
DE 6.50 usec
TE 292.0 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300013 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

GRP-5019-23-2



Current Data Parameters

NAME	Dec17-2007
EXPNO	20
PROCNO	1

F2 - Acquisition Parameters

Date_	20071217
Time	12.17
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	128
DS	0
SWH	8223.685 Hz
FIDRES	0.125483 Hz
AQ	3.9846387 sec
RG	45.2
DW	60.800 usec
DE	6.50 usec
TE	291.8 K
D1	2.00000000 sec
TD0	1

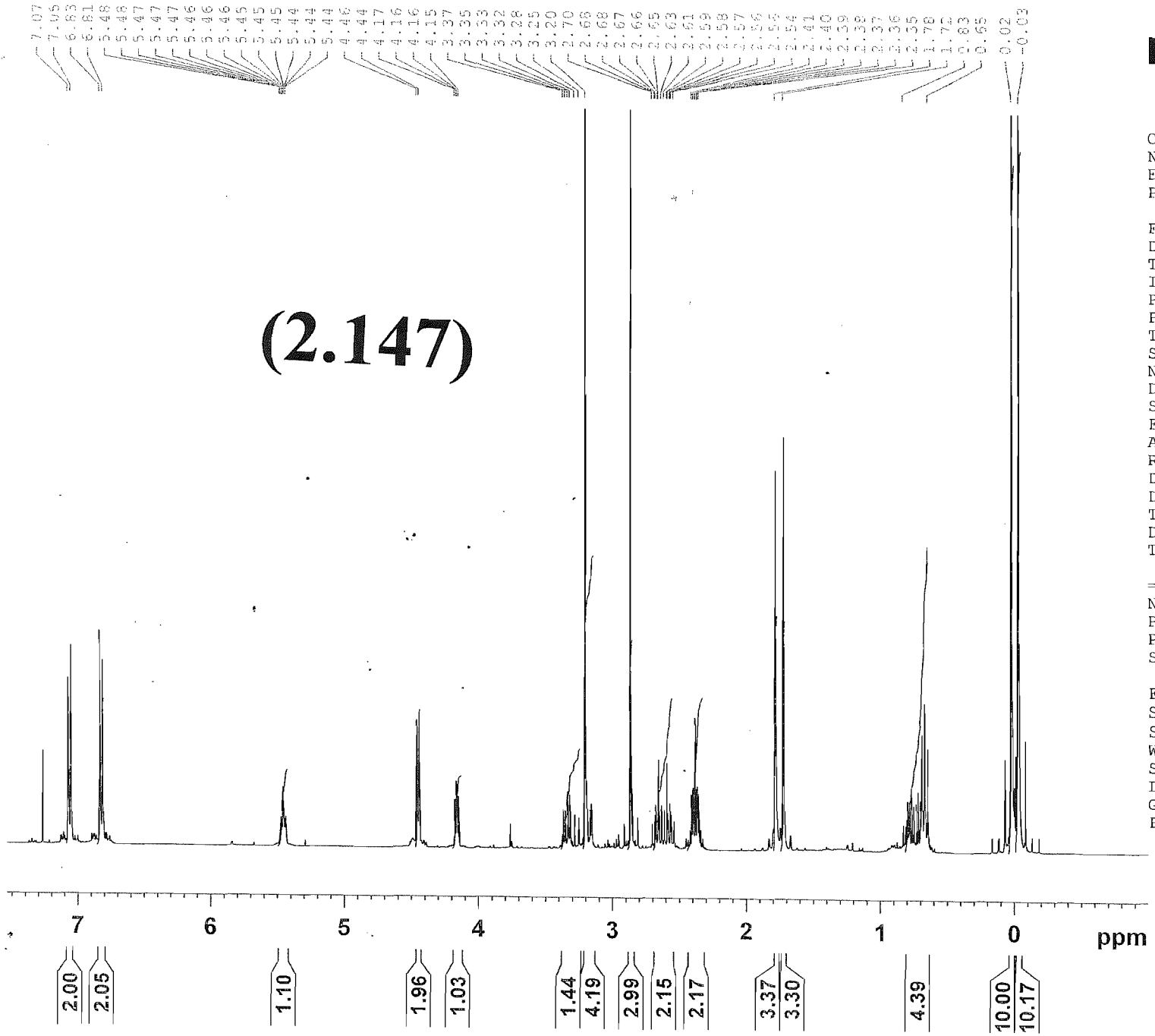
======== CHANNEL f1 ========

NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
SFO1	400.1324710 MHz

F2 - Processing parameters

SI	32768
SF	400.1300025 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

GRP-5019-23-1



BRUKER

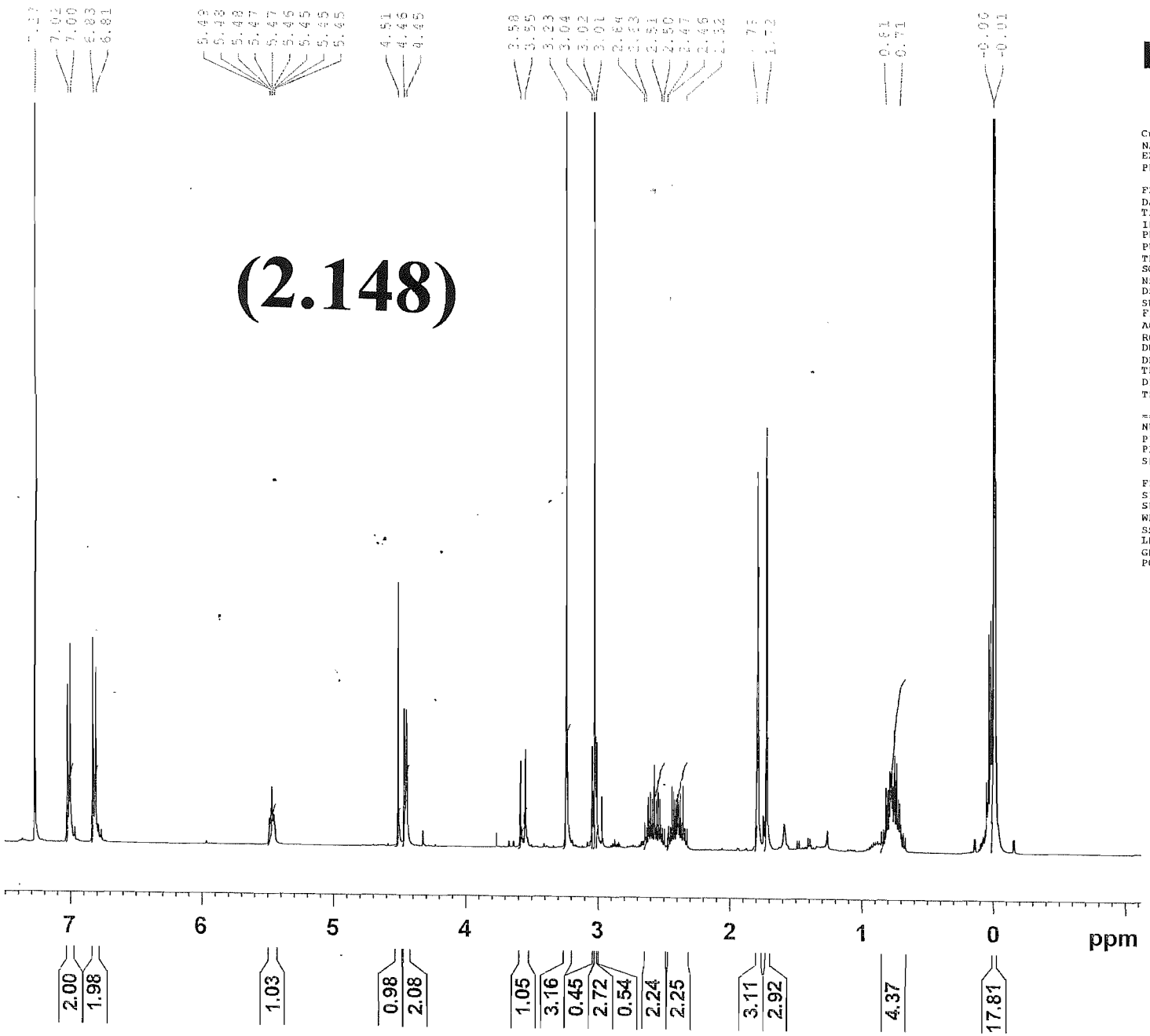
Current Data Parameters
NAME Dec18-2007
EXPNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 20071218
Time 15.54
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 64
DW 60.800 usec
DE 6.50 usec
TE 291.9 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300015 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

GRP-5019-25-1



BRUKER

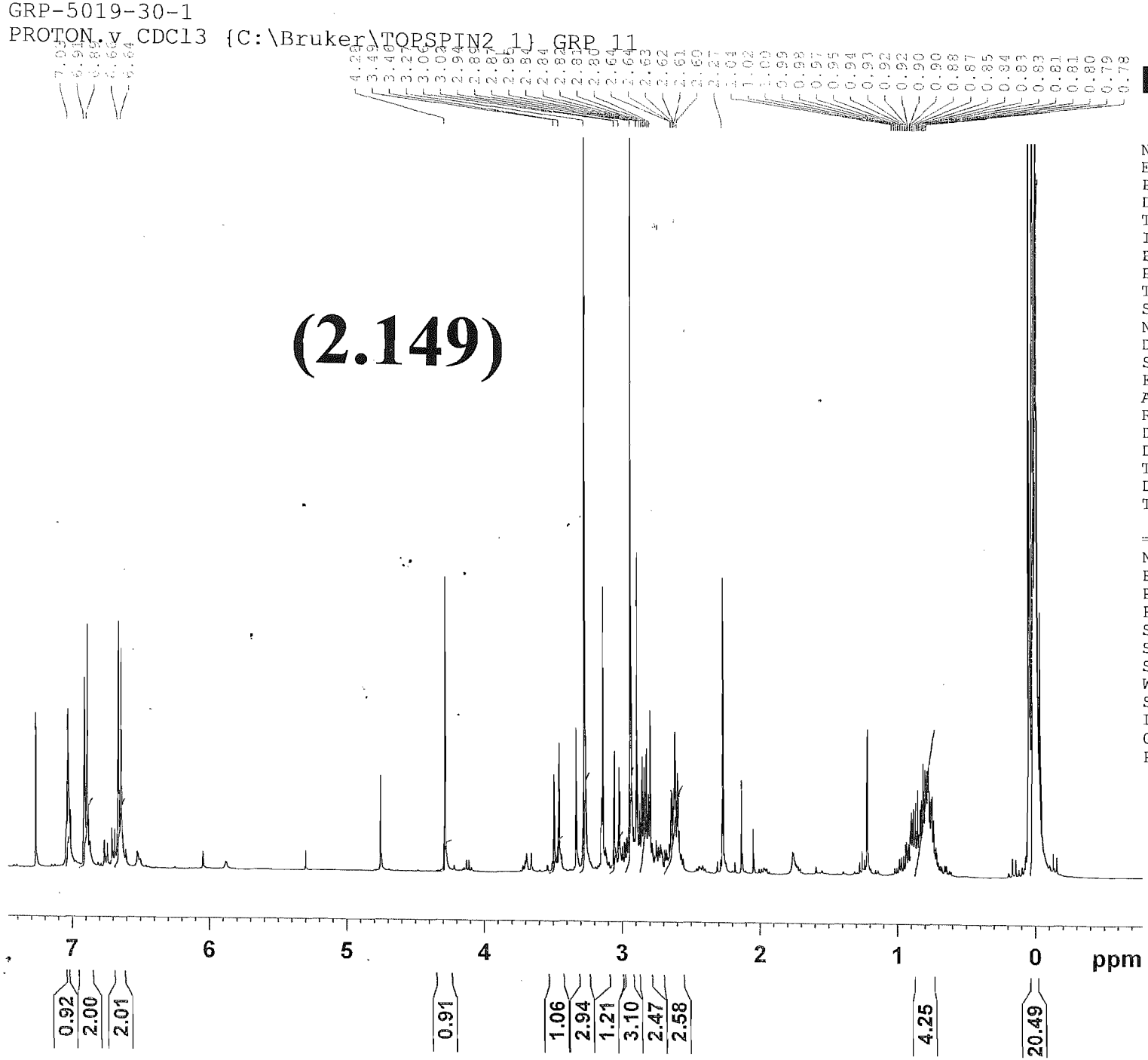
Current Data Parameters
NAME Jan22-2008-FERRINGWORLDAPPL-GB1-LAB
EXPNO 50
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080122
Time 14.13
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 512
DW 60.800 usec
DE 6.50 usec
TE 292.2 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300002 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

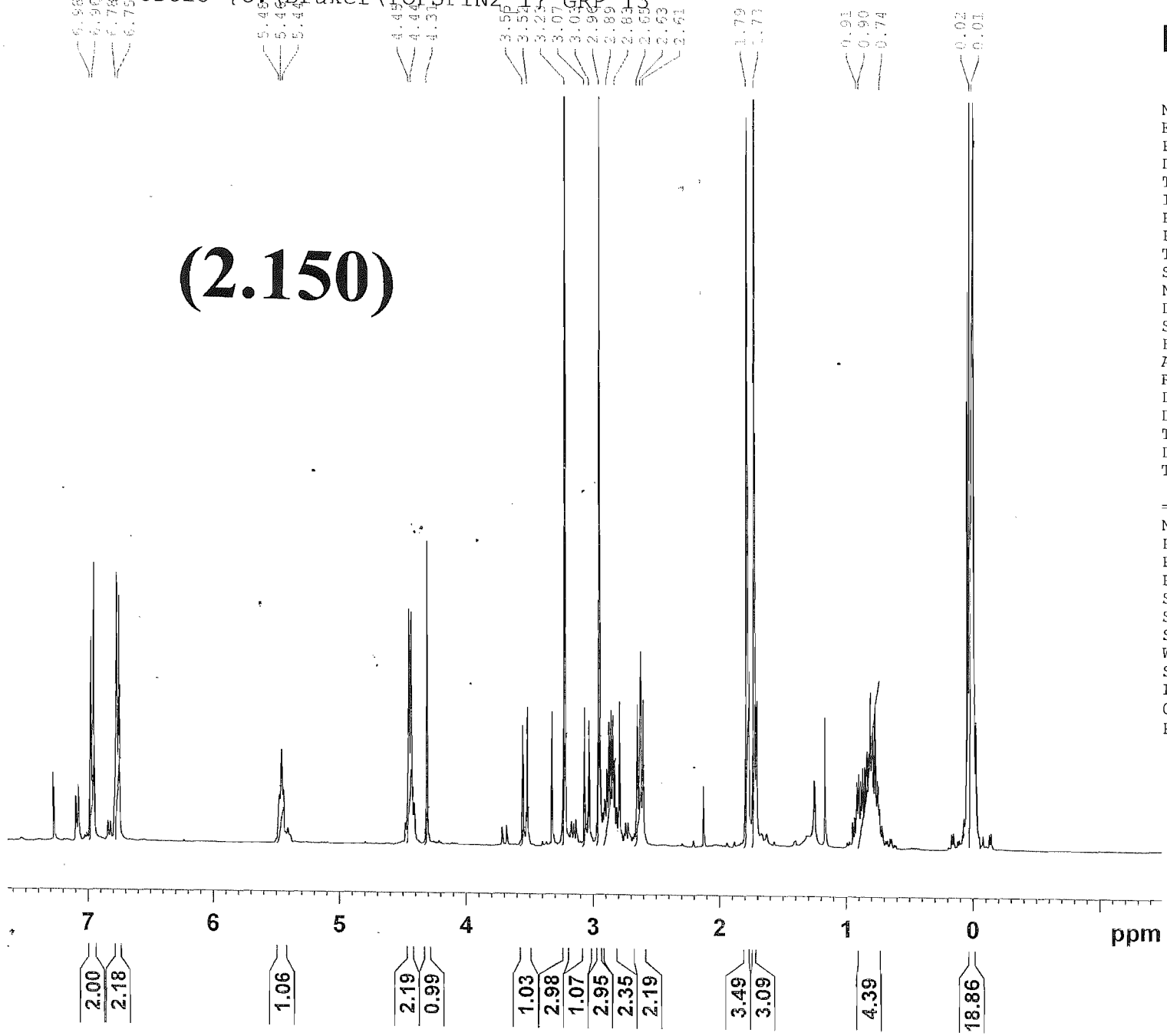
GRP-5019-30-1
PROTON.y CDCl3 {C:\Bruker\TOPSPIN2_1} GRP 11



BRUKER

NAME	GRP-5019-30-2
EXPNO	20
PROCNO	1
Date_	20080219
Time	10.31
INSTRUM	spect
PROBHD	5 mm DUL 13C-1
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	128
DS	2
SWH	8223.685 Hz
FIDRES	0.250967 Hz
AQ	1.9923444 sec
RG	144
DW	60.800 usec
DE	16.77 usec
TE	300.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.50 usec
PL1	-2.00 dB
PL1W	14.49110126 W
SFO1	400.1324710 MHz
SI	16384
SF	400.1300034 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

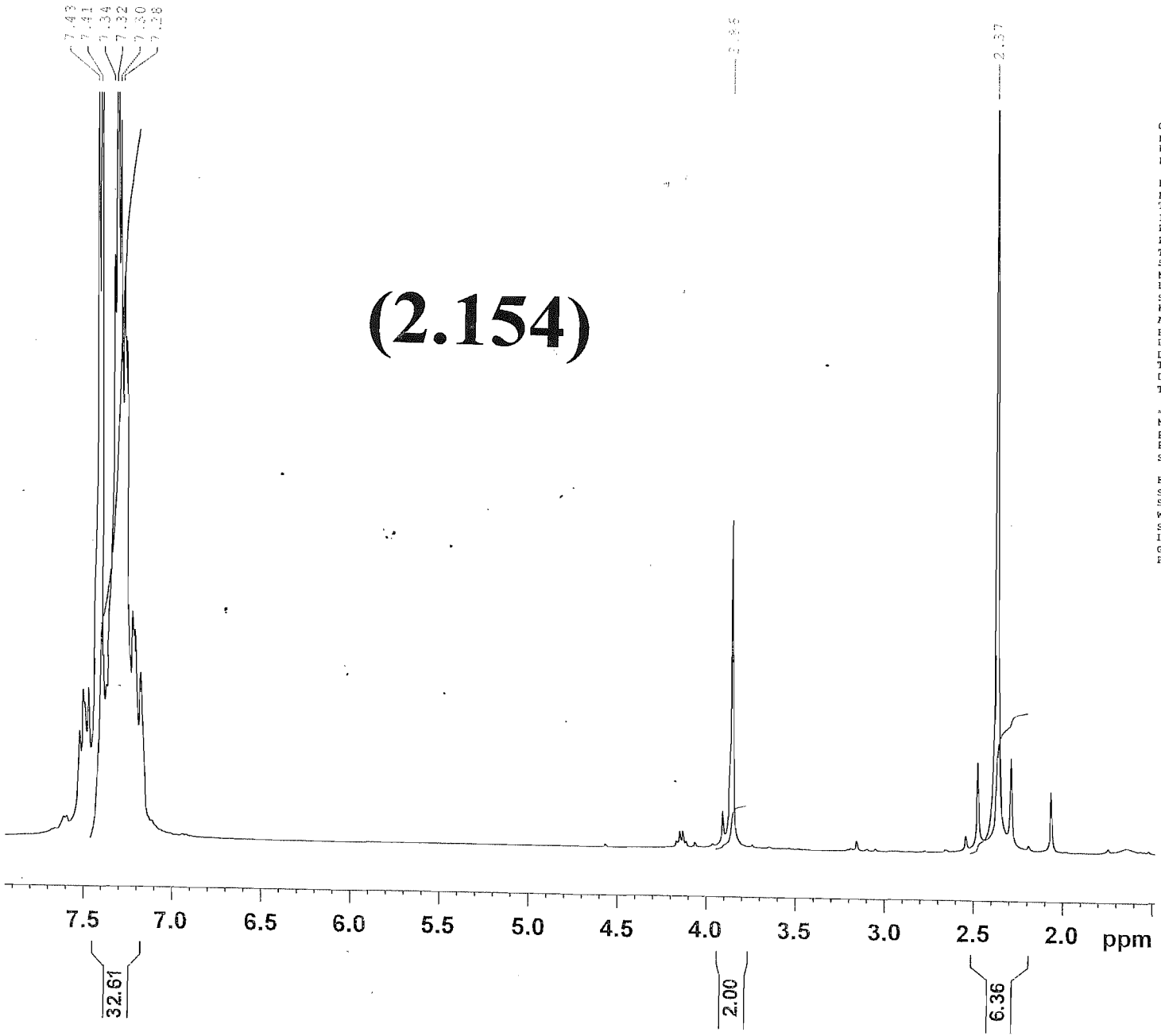
GRP-5019-31
PROTON.y CDCl3 {C:\Bruker\TOPSPIN2_1} GRP_13



BRUKER

NAME	GRP-5019-31	
EXPNO	10	
PROCNO	1	
Date_	20080220	
Time	13.26	
INSTRUM	spect	
PROBHD	5 mm DUL 13C-1	
PULPROG	zg30	
TD	32768	
SOLVENT	CDCl3	
NS	16	
DS	2	
SWH	8223.685	Hz
FIDRES	0.250967	Hz
AQ	1.9923444	sec
RG	57	
DW	60.800	usec
DE	16.77	usec
TE	300.0	K
D1	1.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	10.50	usec
PL1	-2.00	dB
PL1W	14.49110126	W
SFO1	400.1324710	MHz
SI	16384	
SF	400.1300005	MHz
WDW	EM	
SSB	0	
LB	0.30	Hz
GB	0	
PC	1.00	

GRP-5019-11



(2.154)

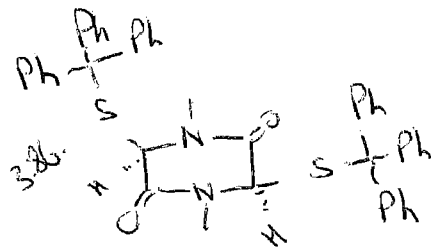
BRUKER

Current Data Parameters
NAME Jan22-2008-FERRINGWORLDAPPL-GB1-LAB
EXPNO 60
PROCNO 1

F2 - Acquisition Parameters
Date_ 20080122
Time 16.03
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 287
DW 60.800 usec
DE 6.50 usec
TE 292.2 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.50 usec
PL1 -2.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



Chapter IV: References

Chapter IV: Reference

1. (a) Murphy, F.; Arthur, M.; Iredale, J. *Expert Opin. Investig. Drugs* **2002**, *11*, 1575-1585. (b) Burt, A. D.; Griffiths, M. R.; Schuppan, D. *Histopathology* **1990**, *16*, 53-58.

2 (a) Ohuchi, E.; Imai, K.; Fujii, Y. *J. Biol. Chem.* **1997**, *272*, 2446-2451. (b) Aimes, R. T.; Quigley, J. P. *J. Biol. Chem.* **1995**, *270*, 5872-5876. (c) Benyon, R. C.; Iredale, J. P.; Goddard, S.; Winwood P. J.; Arthur, M. J. *Gastroenterology* **1996**, *110*, 821-831.

3. (a) Lee, W. M. *N Engl. J. Med.* **1997**, *337*, 1733-1745. (b) Poynard, T.; Bedossa, P, Opolon, P. *Lancet* **1997**, 349, 825-832.

4. (a) Purohit, V.; Brenner, D. A. *Hepatology* **2006**, *43*, 872-878. (b) Kono, H.; Rusyn, I.; Yin, M.; Gäbele, E.; Yamashina, S.; Dikalova, A.; Kadiiska, M. B.; Connor, H.D.; Mason, R. P.; Segal, B. H.; Bradford, B. U.; Holland, S. M.; Thurman, R. G. *J. Clin. Invest.* **2000**, *106*, 867-872.

5. Brewer, G. *J. Curr. Treat. Options Neurol.* **2000**, *2*, 193-203.

6. Loréal, O.; Deugnier, Y.; Moirand, R.; Lauvin, L.; Guyader, D.; Jouanolle, H.; Turlin, B.; Lescoat, G.; Brissot, P. *J. Hepatol.* **1992**, *16*, 122-127.

7. (a) Dufour, J.; DeLellis, R.; Kaplan, M, M. *Dig. Dis. Sci.* **1998**, *43*, 2573-2576. (b) Dufour, J.; DeLellis, R.; Kaplan, M. M. *Ann. Intern. Med.* **1997**, *127*, 981-985. (c) Niederau, C.; Fischer, R.; Sonnenberg. *J. Med.* **1985**, *313*, 1256-1262. (d) Sobesky, R.; Mathurin, P.; Charlotte, F. *Gastroenterology* **1999**, *116*, 378-386.

8. Furui, M.; Takashima, J.; Sudo, K.; Chiba, N.; Mikawa, T. *Japan patent 05239023* **1993**.

9. Furui, M.; Takashima, J.; Mikawa, T.;Yoshikawa, N.; Ogishi, H. *Japan patent 04074163* **1992**.

10. (a) Orr, J. G.; Leel, V.; Cameron, G. A.; Marek, C. J.; Haughton, E. L.; Elrick, L. J.; Trim, J. E.; Hawksworth, G. M.; Halestrap, A. P.; Wright , M. C. *Hepatology* **2004**, *40*, 232-242. (b) Dekel, R.; Zvibel, I.; Brill, S.; Brazovsky, E.; Halpern, Z.; Oren, R. *Digestive Dis. Sci.* **2003**, *48*, 1642-1647. (c) Kweon, Y.; Paik, Y.; Schnabl, B.; Qian, T.; Lemasters, J. J.; Brenner, D. A. *J. Hepatology* **2003**, *39*, 38-46. (d) Wright, M. C.; Issa, R.; Smart, D. E.; Trim, N.; Murray, G. I.; Primrose, J. N.; Arthur, M. J. P.; Iredale, J.; Mann, D. A. *Gastroenterology* **2001**, *121*, 685-698.

11. Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981-2001.

12. Steyn, P. S.; Wessels, P. L. *Tetrahedron Lett.* **1978**, *47*, 4707-4710.

13. Nolte, M. J.; Steyn, P. S.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1057-1065.

14. (a) Hopmann, C.; Kurz, M.; Bronstrup, M.; Wink, J.; Lebeller, D. *Tetrahedron Lett.* **2002**, *43*, 435-438. (b) Yamada, S.; Yaguchi, S.; Matsuda, K. *Tetrahedron Lett.* **2002**, *43*, 647-651. (c) Haskins C. M.; Knight, D. W. *Chem. Commun.* **2005**, 3162-3164. (d) Wolf, D.; Schmitz, I. J.; Qui, F.; Kelly-Borges, M. *J. Nat. Prod.* **1999**, *62*, 170-172. (e) Lang, G.; Cole, A. L. J.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod.* **2006**, *62*, 151-153.

15. (a) Markopoulos, J.; Detsi, A.; Gavrielatos, E.; Athanasellis, G.; Igglessi-Markopoulou, O. *Biomed. Health Res.* **2002**, *55*, 25-32. (b) Mawer, I. M.; Kulagowski, J. J.; Leeson, P. D.; Grimwood, S.; Marshall G. R. *Bioorg. Med. Chem. Lett.* **1995**, *5* , 2643-2648. (c) Karwowski, J. P.; Jackson, M.; Theriault, R. J.; Barlow, G .J.; Coen, L.; Hensey, D. M.; Humphrey, P. E. *Antibiot.* **1992**, *45*, 1125-1132. (d) Radl, S.; *Pharmacol. Therapeut.* **1990**, *48*, 1-17. (e) Tetsuzo, K.; Hitoshi, K.; Akihiro, W.; Toshiki, S.; Mihoko, O.; Hirotaka, S.; Morihiro, K.; Denichi, M. *Yakugaku Zasshi* **1977**, *97*, 676-684.

16. Schobert, R.; Jagusch, C. *Tetrahedron* **2005**, *61*, 2301-2307.

17. Lacey, R. N. *J. Chem. Soc.* **1954**, 850-854.

18. Hart, A. C.; Phillips, A. J. *J. Am. Chem. Soc.* **2006**, *128*, 1094-1095.

19. Ramana, C. V.; Mondal, M. A.; Puranik, V. G.; Gurjar, M. K. *Tetrahedron Lett.* **2006**, *47*, 4061-4064.

20. Schobert, R.; Dietrich, M.; Mullen, G.; Urbina-Gonzalez, JM. *Synthesis* **2006**, *22*, 3902-3914.

21. Jones, R. C. F.; Begley, M. J.; Peterson, G. E.; Sumaria, S. *J. Chem., Soc. Perkin Trans. 1* **1990**, 1959-1968.

22. Jones, R. C. F.; Peterson, G. E. *Tetrahedron Lett.* **1983**, *24*, 4751-4754.

23. Clough, J. M.; Pattenden, G.; Wight, P. G. *Tetrahedron Lett.* **1989,** *30*, 7469-7472.

24. Shimshock, S. J.; Waltermire, R. E.; DeShong, P. *J. Am. Chem. Soc.* **1991**, *113*, 8791-8796.

25. Kulkarni, B. A.; Ganesan, A. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2454-2455.

26. Kulkarni, B. A.; Ganesan, A. *Tetrahedron Lett.* **1998**, *39*, 4369-4372.

27. (a) Kivirikko, K. I.; Myllylä, R. *Methods in Enzymology* **1982**, *82*, 245-304. (b) Prockop, D. P. Kivirikko, K. I.; Tuderman, L.; Guzman, N. A. *N. Engl. J. Med.* **1979**, *301*, 77-85.

28. Bickel, M.; Baader, E.; Brocks, D.; Engelbart, G. K.; Gunzler, V.; Schmidts H. L.; Vogel, G. H. *J. Hepatology.* **1991**, *13*, S26-S34.

29. Matsumura, Y.; Sakaida, I.; Ucida, K.; Kimura, T.; Ishiara, T.; Okita, K. *J. Hepatology* **1997**, *27*, 185-192.

30. Hanauske-Abel, HM. *J. Hepatology* **1991**, *13*, S8-S16.

31. Ratcliffe, P. J.; Schofield, C. P.; *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 343-353.

32. Andrews, M. D.; Brewster, A, G.; Crapnell, K. M.; Ibbett, A. J.; Jones, T.; Moloney, M. G.; Prout, K.; Watkin, D. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2231. (b) Bloomer, J. L.; Kappler, F. E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1485.

33. Weber, L.; Iaiza, P.; Biringer, G.; Barbier, P. *Synlett.* **1998**, 1156-1158.

34. Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**. *43*, 2087-2088.

35. (a) Lensen, N.; Mouelhi, S.; Bellassoued, M. *Synth. Commun.* **2001**, *31*, 1007-1011. (b) Bellassoued, M.; Lensen, N.; Bakasse, M.; Mouelhi, S. *J. Org. Chem.* **1998**, *63*, 8785-8789.

36. Booth, P. M.; Broughton, H. B.; Ford, M. J.; Fox, C. M. J.; Ley, S. V.; Slawin, A. M. Z.; Williams, D. J.; Woodward, P. R. *Tetrahedron* **1989**, *45*, 7565-7580.

37. Ley, S, V.; Smith, S. C.; Woodward, P. R.; *Tetrahedron* **1992,** *48*, 1145-1174.

38. Masamune, S.; Hayase, Y.; Schilling, W.; Chan, W. K.; Bates, G. S. *J. Am. Chem. Soc.* **1977**, *99*, 6756.

39. Mukaiyama, T.; Suzuki, H.; Yamada, T. *Chem. Lett.* **1987**, *2*, 293-296.

40. Vendeville, S.; Goossens, F.; Debreu-Fontaine, M.; Landry, V.; Davioud-Charvet, E.; Grellier, P.; Scharpe, S.; Sergheraert, C. *Bioorg. Med. Chem.* **2002**, *10*, 1719-1729.

41. David, C.; Bischoff, L.; Roques, B. P.; Fournie-Zaluski, M. *Tetrahedron* **2000**, *56*, 209-215.

42. Raillard, S. P.; Chein, W.; Sullivan, E.; Bajjalieh, W.; Bhandari, A.; Baer, T. A. *J. Comb. Chem.* **2002**, *4*, 470-474.

43. Emastenas, H.; Alderin, L.; Almqvist, F. *J. Org. Chem.* **2001**, *66*, 6756-6761.

44. Ley, S. V.; Woodward, P. R. *Tetrahedron Lett.* **1987**, *28*, 345-346.

45. Wadsworth, Jr., W. S. *Org. React.* **1977**, *25*, 73-254.

46. Poncet, J.; Jouin, P.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 611-616.

47. Jones, R. C.F.; Bates, A. D. *Tetrahedron. Lett.* **1987**, *28*, 1565-1568.

48. Neukom, C.; Richardson, D. P.; Myerson, J. H.; Bartlett, P. A. *J. Am. Chem. Soc.* **1986**, *108*, 5559-5567.

49. Cartwright, D.; Lee, V. J.; Rinehart, K. L. *J. Am. Chem. Soc.* **1978,** *100*, 4237-4239.

50. Sorensen, S. U.; Falch, E.; Krogsgaard-Larsen, P. *J. Org. Chem.* **2000**, *65*, 1003-100.

51. Yamamoto, Y.; Watanabe, Y.; Ohnishi, S. *Chem. Pharm. Bull.* **1987**, *35*, 1860-70.

52. Wang, L.; Wang, J.; Wang, BE.; Xiao, PG.; Qiao, YJ.; Tan, XH. *World J. Gastroenterol.* **2004**, *10*, 2831-2835.

53. Gardiner, D. M.; Waring, P.; Howlett, B. J. *Microbiol.* **2005**, *151*, 1021-1032.

54. Hake, S. B.; Xiao, A.; Allis, C. D. *Br. J. Cancer* **2004**, *90*, 761-769.

55. Saveliev, A.; Everett, C.; Sharpe, T.; Webster, Z.; Festenstein, R. *Nature* **2003**, 909-913.

56. Greiner, D.; Bonaldi, T.; Eskeland, R.; Roemer, E.; Imhof, A. *Nature Chem. Bio.* **2005**, *1*, 143-145.

57. Vigushi, D. M.; Mirsaidi, N.; Brooke, G.; Sun, C.; Pace, P.; Inman, L.; Moody, C. J.; Coombes, R. C. *Med. Oncol.* 2004, *21*, 21–30.

58. Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron* **1981**, *37*, 2045-2078.

59. Trown, P. W. *Biochem. Biophys. Res. Commun.* **1968**, *33*, 402-407.

60. Hino, T.; Sato, T. *Tetrahedron Lett.* **1971**, 3127-3129.

61. Öhler, E.; Poisel, H.; Tataruch, F.; Schmidt, U. *Chem. Ber.* **1972**, *105*, 635-641.

62. DeMarinis, R. M.; Filer, C. N.; Waraszkiewicz, S. M.; Berchtold, G. A. *J. Am. Chem. Soc.* **1974**, *96*, 1193-1197.

63. Fukuyama, T.; Kishi, Y. *J. Am. Chem. Soc.* **1976**, *98*, 6723-6724.

64. Hilton, S. T.; Motherwell, W.B.; Selwood, D. L. *Synlett* **2004**, 2609-2611.

65. Aliev, A. E.; Hilton, S. T.; Motherwell, W. B.; Selwood, D. L. *Tetrahedron Lett.* **2006**, *47*, 2387-2390.

66. Kawahara, N.; Nozawa, K.; Nakajima, S.; Kawai, K. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2099-2101.

67. Patel ,S. M.; Currie, J. O.; Olsen, R. K. *J. Org. Chem.* **1973**, *38*, 123-128.

68. Ottenheijm, H. C. J.; Potman, A. D.; van Vroonhoven, T. *J. Royal. Netherlands Chem. Soc.* **1975,** *94*, 135-138.

69. Greene, W. T and Wuts, G. M. P.; *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999; p506.

70. Fukuyama, T.; Nakatsuka, S.; Kishi, Y. *Tetrahedron Lett.* **1976**, *38*, 3393-3396.

71. Malkinson, J. P.; Anim, M. K.; Zloh, M.; Searcey, M. *J. Org. Chem.* **2005,** *70*, 7654-7661.

72. Lorenz, K. B.; Diederichsen, U.; *J. Org. Chem.* **2004**, *69*, 3917-3927.

73. Greene, W. T and Wuts, G. M. P.; *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999; p249.

74. Appel, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 801 - 811.

75. Gillespie, R. J.; Lerpiniere, J.; Gaur, S.; Bamford, S. J.; Stratton, G. C.; Leonardi, S.; Weiss, S. M. PCT int Appl. (**2002**) WO 2002055083.

76. Jobron, L.; Hindsgaul, O. *J. Am. Chem. Soc.* **1999**, *121*, 5835-5836.

77. Collado, D.; Perez-Inestrosa, E.; Suau, R.; Lopez Navarrete, J. L. *Tetrahedron* **2006**, *62*, 2927-2935.

78. Hansen, M. M.; Riggs, J. R. *Tetrahedron Lett.* **1998**, *39*, 2705-2706.

79. Nägele, E.; Schelhaas, M.; Kuder, N.; Waldmann. H. *J. Am. Chem. Soc.* **1998**, *120*, 6889-6902.

80. Carr, J. A.; Bisht, K. S. *Org. Lett.* **2004**, *6*, 3297-3300.

81. Sridhar, M.; Kumar, A.; Narender, R. *Tetrahedron Lett.* **1998**, *39*, 2847-2850.

82. Bell, M. R.; Johnson, J. R.; Wildi, B. S.; Woodward, R. B. *J. Am. Chem. Soc.* **1958**, *80*, 1001.

83. Eleuteri, A.; Reese, C. B.; Song, Q. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2237-2240.

84. Vetter, S. *Synth. Commun.* **1998**, *28*, 3219-3223.

85. Eckert, H.; Listl, M.; Ugi, I. *Angew. Chem., Int. Ed. Engl.* **1978**, *5*, 361-362.

86. Shibata, N.; Baldwin, J. E.; Jacobs, A.; Wood, M. E. *Tetrahedron* **1996**, *39*, 12839-12852.

87. Lin, C.; Richardson, S. K.; Garvey, D. S. *Tetrahedron Lett.* **2002**, *43*, 4531-4533.

88. Munson, M. C.; Garcia-Echeverria, C.; Albericio, F.; Barany, G. *J. Org. Chem.* **1992**, *57*, 3013-3018.

89. Akaji, K.; Tatsumi, T.; Yoshida, M.; Kimura, T.; Fujiwara, Y.; Kisco, Y. *J. Am. Chem. Soc.* **1992**, *114*, 4137-4143.

90. Otaka, A.; Koide, T.; Shide, A.; Fujii, N. *Tetrahedron Lett.* **1991**, *32*, 1223-1226.

91. (a) Albericio, F.; Hammer, R. P.; Garcia-Echeverria, C.; Molins, A. M.; Chang, J. L.; Munson, M. C.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 402-413. (b) Fujii, N.; Otaka, A.; Funakoshi, S.; Bessho, K.; Yajima, H. *J. Chem. Commun.* **1987**, 163-164. (c) Yajima, H.; Fujii, N.; Funakoshi, S.; Watanabe, T.; Murayama, E.; Otaka, A. *Tetrahedron*, **1988**, *44*, 805-819.

92. Koreeda, M.; Yang, W. *J. Am. Chem. Soc.* **1994**, *116*, 10793-10794.

93. Anderson, M. B.; Ranasinghe, M. G.; Palmer, J. T.; Fuchs, P. L. *J. Org. Chem.* **1988**, *53*, 3127-3129.

94. Yonezawa, Y.; Shimizu, K.; Uchiyama, M.; Kagawa, N.; Shin, C. *Heterocycles* **1997**, *45*, 1151-1159.

95. Ranasinghe, P. L, Fuchs, P. L. *Synth. Commun.* **1988**, *18*, 227-232.

96. Nitecki, D. E.; Halpern, B.; Westley, J. W. *J. Org. Chem.* **1967**, *33*, 864-866.

97. Zervas, L.; Photaki, I.; Ghelis, N. *J. Am. Chem. Soc.* **1968**, *85*, 1337.

98. Ramage, R.; Stewart, A. S. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1947-1952.

99. Yuki, H.; Tohira, Y.; Aoki, B.; Kano, T.; Takama, S.; Yamazaki, T. *Chem. Pharm. Bull.* **1967**, *15*, 1107-1111.

100. Bernasconi, C. F.; Ketner, R. J.; Chen, X.; Rappoport, Z. *J. Am. Chem. Soc.* **1998,** *120*, 7461-7468.

101. House, H. O.; Outcalt, R. J.; Cliffton, M. D. *J. Org. Chem.* **1982**, *47*, 2413-2419.

102. Grice, R.; Owen, L. N. *J. Chem. Soc.* **1963**, 1947-1954.