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The Design and Synthesis of Novel Matrix Metalloproteinase Inhibitors

by

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ABSTRACT

FACULTY OF SCIENCE
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Matrix metalloproteinases (MMPs) are a therapeutic target for many pharmaceutical companies as they are involved in numerous disease areas such as tumour metastasis, rheumatoid arthritis, multiple sclerosis and coronary heart disease.

Recently, the ancorinoside natural products were described as micromolar MMP inhibitors. Chapter one describes the synthesis of an array of tetramic acids, the core functional group present in the ancorinosides. The compounds synthesised were then tested in biological assays in order to determine whether any were effective MMP inhibitors. The use of molecular modeling technology in aiding the synthesis of tetramic acids is discussed. In chapter two, keto-pyrrolidinones are introduced as an alternative scaffold to tetramic acids. A series of keto-pyrrolidinones was prepared and tested for MMP activity.

The third chapter of my thesis describes synthetic studies directed towards ageladine A, a recently isolated MMP inhibitor. The synthesis of the structure reported for ageladine A was achieved *via* a Suzuki coupling of the pyrrole and pyridine portions. Analogues of this have been tested for MMP activity.

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ABBREVIATIONS

Ac	acetyl
AIBN	a,a'-azobisisobutyronitrile
anal.	analysis
aq	aqueous
Ar	aryl
Boc	*tert*-butoxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
Bn	benzyl
br	broad (spectral)
Bu	butyl
calcd.	calculated
d	doublet
δ	chemical shift in parts per million
DCC	dicyclohexyl carbodiimide
DIC	diisopropyl carbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DME	ethylene glycol dimethyl ether
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DTNB	dithio bis 2-nitrobenzoic acid
EDC	1-ethyl-3-(dimethylaminopropyl)carbodiimide.HCl
ee	enantiomeric excess
eq	equivalents
ES	electrospray (in mass spectrometry)
Et	ethyl
FT IR	Fourier transform infrared
h	hour
HOBt	*N*-hydroxybenzotriazole
HPLC	high-performance liquid chromatography
Hz	hertz
J	coupling constant

IPA	isopropanol
m	multiplet
M	molar
Me	methyl
min	minute
MMP	matrix metalloproteinase
MOCAc	(7-methoxycoumarin-4-yl)acetyl
mp	melting point
MS	mass spectrometry
NaHMDS	sodium bis(trimethylsilyl)amide
NBS	*N*-bromosuccinimide
NMP	*N*- methyl pyrrolidine
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	parts per million
Pr	propyl
Py	pyridine
PyBrOP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet
R_f	retardation factor
rt	room temperature
s	singlet
SAR	structure activity relationship
t	triplet
TBAF	tetra-*n*-butylammomium fluoride
TBDMS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Ts	*p*-toluenesulfonyl (tosyl)

Chapter One

Tetramic Acids as Potential Matrix Metalloproteinase (MMP) Inhibitors

1.1. Introduction

1.1.1 Matrix metalloproteinases as therapeutic targets

Matrix metalloproteinases (MMPs) are hydrolytic enzymes that cleave peptide bonds. Hydrolysis is mediated by a central zinc atom at the enzyme active site. These enzymes are involved in the remodeling, repair and degradation of the extra-cellular matrix in the body. There are known to be at least 23 enzymes that form part of the MMP subfamily such as collagenases (MMP-1, 8, 13), gelatinases (MMP-2, 9) and stromelysins (MMP-3, 7, 10) to name but a few. Two enzymes, matrylisin (MMP-7) and macrophage metalloelastase (MMP-12) have been identified and do not belong in the three sub-groups mentioned above on the basis of sequence homology. More recently, the MMP family has been extended due to the addition of a new sub-group, the membrane-type MMPs (MT-MMPs). Currently four members of this sub-group have been identified (MMP-14 to MMP-17). MMPs are a therapeutic target for many pharmaceutical companies as their aberrant activity is implicated in numerous disease areas such as tumour metastasis, rheumatoid arthritis, multiple sclerosis and coronary heart disease. [1, 2]

Examination of the catalytic cycle helps us to understand how proteolysis occurs for all MMPs. The figure below shows the accepted mechanism for proteolysis.

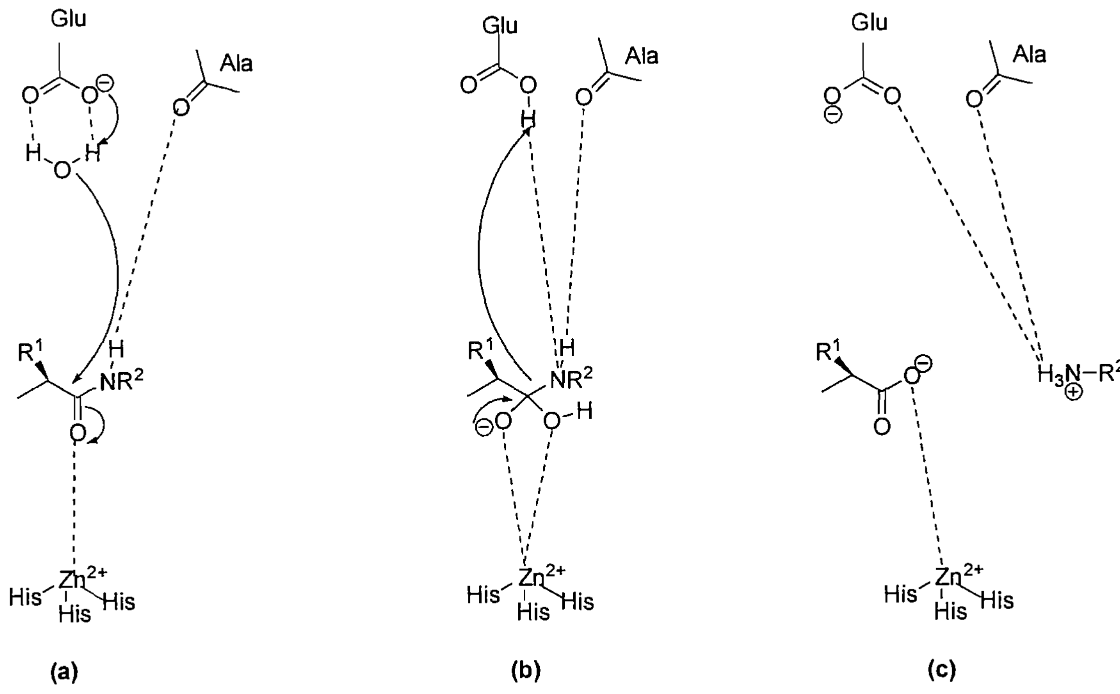


Figure 1.1

The zinc atom is tetrahedrally coordinated to three histidines from the enzyme and a water molecule. The water molecule is also hydrogen bonded to the carboxylate side chain of a glutamic acid residue. The system is then set up for nucleophilic attack **(a)**. Coordination of the carbonyl group of the scissile amide bond to the zinc is followed by donation of a proton from water to the glutamic acid side chain. The transfer of a proton to the nitrogen leads to the tetrahedral intermediate **(b)**. The tetrahedral intermediate then collapses resulting in peptide bond cleavage. The positive charge on the zinc stabilises the negative charge at the carbon of the scissile amide. The conserved alanine residue helps to stabilise the positive charge on the nitrogen **(c)** (Figure 1.1).[3]

Under normal physiological conditions MMPs are inhibited naturally by endogenous inhibitors such as α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs). α2-Macroglobulin is a plasma protein which binds to MMPs to form a complex. The α2-macroglobulin-MMP complex then binds to a scavenger receptor and is irreversibly cleared by endocytosis and subsequently degraded. The endogenous MMP inhibitors, TIMP-1, 2, 3 and 4 irreversibly inhibit MMPs in a 1:1 stoichiometric fashion. The activity of MMPs is therefore controlled by maintaining a

balance between synthesis of the active forms and inhibition by the same tissue inhibitors. The danger occurs when MMP activity becomes excessive as it is linked to the promotion of tumour metastasis, angiogenesis, arthritis and cardiovascular diseases. The imbalance can cause the degradation of fibrillar collagens, type IV collagen and non-helical proteins, proteoglycans, fibronectin and laminin respectively. There has therefore been a great deal of interest in discovering and developing inhibitors with the aim to regulate excessive MMP production. Such medicinal agents could be used to ameliorate arthritis and cardiovascular disease among others.[1]

One difficulty is the design and development of inhibitors possessing high selectivity for a particular enzyme because the MMPs themselves show a high degree of homology. This characteristic has generally resulted in broad spectrum inhibitors, with concern regarding side effects and toxicity. Crystal structures[4-7] of inhibitors bound to various MMPs have given much more structural information regarding the shape of the enzymes. A schematic representation of a hexapeptide substrate bound into an MMP active site (Figure 1.2) shows that there exists a binding groove which is open at S1-S3 and at S3'. The groove narrows at S1' and S2' whereby the S1' pocket penetrates the surface of the enzyme. Therefore any differences in the unprimed sites, S1-S3 are slim. However, the variation between the amino acid residues of MMPs that form the S1' pocket is more noticeable which makes it possible to find selective inhibitors. According to recent structural studies, MMPs can now be classified into two categories dependent on the depth of the S1' pocket. For the majority of the enzymes eg. MMP-2, 9, 3, 8 and 13 the S1' pocket is deep. The shape also varies at the bottom of the pocket and it can be either a channel or closed off. For MMPs-1, 7 and 11 the S1' pocket is practically non-existent due to the increase in size of the amino acid side chain. In general, inhibitors with larger and longer groups at P1' can show selectivity whereas the presence of smaller groups results in broad-spectrum inhibitors. The S2' subsite is solvent exposed and prefers to have hydrophobic moieties present at P2'. The S3' subsite is similar to the unprimed sites in that variation of substituents at P3' doesn't affect inhibitor selectivity greatly.[3] It is important to note that once inhibitors bind to the enzyme they can cause the shape to

alter and so information gained from the crystal structures should be taken as a guide and may not be precise.

Figure 1.2
Schematic representation of MMP active site after Grams et al. Numbering follows that for MMP-8.

There are vast numbers of MMP inhibitors published in the literature. The two main families of inhibitors are based on either hydroxamic acids or carboxamates which have a functional group that can chelate to the zinc atom in the enzyme. Through ionic interaction they become attached to the enzyme active site and irreversibly inhibit catalysis. These early inhibitors were discovered through a substrate-based design approach based on the amino acid sequence, Gly-Ile and Gly-Leu at the collagenase (MMP-1) cleavage sites. Up to this point there were three types of zinc binding group (ZBG) inhibitors classified: i) Those where the zinc binding group (ZBG) is flanked on either side by amino acid residues; ii) Those in which the amino acid residues are on the left-hand side (LHS); iii) Those in which the amino acid residues are on the right-hand side (RHS) of the ZBG (Figure 1.3); Those lying on the right-hand side (P1'-P3') tend to be more potent and compounds of this class have been reported much more frequently.[2]

S3	S2	S1	Zn++	S1'	S2'	S3'	**Enzyme Subsites**
-Pro-	Gln-	Gly-	CONH	-Ile	-Ala	-Gly-	**Natural substrate**
P3-	P2-	P1-	ZBG	-P1'	-P2'	-P3'	**Combined inhib.**
			ZBG	-P1'	-P2'	-P3'	**RHS inhibitor**
P3-	P2-	P1-	ZBG				**LHS inhibitor**

Figure 1.3

Design of MMP inhibitors based upon the sequence of the collagen substrate cleavage site.

1.1.2 Current MMP inhibitors

Research undertaken by British Biotech led to the discovery of batimastat **(1.1)** and marimastat **(1.2)** broad spectrum inhibitors which are examples of hydroxamic acids (Figure 1.4).[2] Both of these have displayed efficacy in animal models of human disease but only marimastat has high oral bioavailability. Marimastat is more soluble due to the introduction of an α-hydroxyl group replacing the thienylthiomethylene substituent in batimastat. The inhibitor has reached phase III clinical trails and is an example of a succinyl hydroxamte. It has low nanomolar activity with an IC_{50} of 5, 6, 20, 2, and 3 nm against MMP-1, 2, 7, 8 and 9 respectively. Variation of the side chains provides analogues such as **(1.3)** which features an α hydroxy group and an α methyl group. In addition the phenyl-propyl group is thought to be the optimal substituent at P1'. The analogue **(1.3)** is a potent inhibitor of both the 'short pocket' enzyme MMP-1 and the 'deep pocket enzymes' MMP-3 and MMP-9 (Figure 1.4).[2]

Batimastat **(1.1)**

Marimastat **(1.2)**

analogue of Marimastat **(1.3)**

Figure 1.4

The hydroxamate inhibitors tend to be the most potent as they contain a bidentate zinc binding group yet they tend to fail at the later stages of clinical trials due to poor bioavailability. Their toxicological problems are thought to be due to the fact that they interact so strongly with zinc. Due to difficulties in the solubility of peptidomimetics the focus has shifted towards non-hydroxamate zinc binding groups (ZBG).

Other inhibitors of MMPs include carboxylates, aminocarboxylates, thiols, and phosphinic acids.[3] Since 1994 the availability of structural information of MMP-inhibitor complexes has enabled rational inhibitor design. The ranking of such inhibitors with respect to zinc binding of MMP-1 has been determined[8] using SAR studies and is as follows: hydroxamate >> formylhydroxylamine > sulfhydryl > phosphinate > aminocarboxylate > carboxylate. The thiol based inhibitors have a monodentate zinc binding group and are consistently less potent than their hydroxamic counterparts. Much of the SAR for the hydroxamates has been applied to the design of thiolate inhibitors. The thiol **(1.4)** is one of the earliest inhibitors with activity against MMP-1 (IC_{50} 2.5 nM). The activity could be due to the side chain S1 interaction or alternatively the ester and the thiol are forming a bidentate interaction

with the zinc atom. Inhibitors based on phosphorus ZBGs have also been developed. The example below is of a phosphinic acid **(1.5)** which shows activity against MMP-1 (IC_{50} 270 nM) (Figure 1.5). It has been observed that the phosphinic acids are more potent than phosphonates and phosphonamidates.[2]

Figure 1.5

There is also interest in the discovery and evaluation of new ZBGs as MMP inhibitors. The 5-substituted-1,3,4-thiadiazole-2-thione, PNU-142371 **(1.6)** (Figure 1.6) has, through screening been identified as an MMP-3 inhibitor (IC_{50} 18 nM). The crystal structure of this compound bound to MMP-3 shows that the thiadiazole sulfur binds with the catalytic zinc. Both the nitrogens in the thiadiazole ring form H-bond interactions with the enzyme.[2]

PNU-142371 (1.6)

Figure 1.6

There has been a great deal of development in this area over the last few decades which has led to a diverse set of inhibitors. A combination of substrate-based design

and the solving of crystal structures[4-7] of the enzymes has aided in the design of potent MMP inhibitors such as the hydroxamates, thiols and carboxylates. It has been stated[1] that the criteria needed for a potent MMP inhibitor is as follows: i) A zinc binding group; ii) At least one functional group for H-bonding and iii) A side chain for van der Waal's interactions.

1.1.3 Tetramic acids as natural products

The tetramic acid (2,4-pyrrolidinedione) heterocycle has been known since the beginning of the 20th century. It was not until the 1960s that the tetramic acid unit was found to be present in many natural products. The majority of those isolated exhibit some biological function such as potent antibiotic, antiviral, antiulcerative, cytotoxic, mycotoxic and inhibition of tumours in animal models. The simplest tetramic acid **(1.7)** (Figure 1.7) was surprisingly only first synthesised[9] in 1972. Previous attempts[10, 11] to synthesise this tetramic acid were later shown to have resulted in formation of the isomer, 2-imino tetronic acid **(1.8)**. Comparisons regarding the structure of the tetramic acid **(1.7)** with the known tetronic acid **(1.9)** were drawn (Figure 1.7). However, the tetramic acid has a pK_a of 6.4 as opposed to 3.7 for the tetronic acid. Consequently, it is not highly enolised and exists in the 2,4-diketo form. IR absorbtions at 3230 (NH stretch), 1782 (C=O), 1696 (lactam C=O) and 1670 (NH bend) confirm this finding. Tetramic acids containing an acyl **(1.10)** or alkoxycarbonyl **(1.11)** substituent are strongly acidic and have pK_a values in the region of 3.0-3.5 and 2.3-2.7 respectively. Proton NMR indicates complete enolisation for the tetramic acids however the spectra can often be complicated due to the existence of tautomeric forms.[12]

(1.7) (1.8) (1.9)

(1.10) (1.11)

Figure 1.7

The 3-acyl tetramic acids of which tenuazonic acid **(1.12)** is an example can exist in four different tautomeric forms due to enolisation (Figure 1.8).[12] 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[13, 14] 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-acyl-tetramic acids.

A fast B

slow slow

C fast D

Figure 1.8

The most commonly found tetramic acid derivatives in nature are those bearing an acyl substituent in the 3-position of which tenuazonic acid **(1.12)** is the simplest. Kohl and co-workers isolated[15] the acyl tetramic acid magnesidin **(1.13)** from *Pseudomonas magnesiorubra* as a 1:1 mixture of the magnesium chelates of the 3-hexanoyl and 3-octanoyl tetramic acid derivative (Figure 1.9). Magnesidin inhibits various Gram-positive bacteria and prevents the decay of foodstuffs caused by spore-generating organisms.[12]

Tenuazonic acid **(1.12)**

Magnesidin **(1.13a)** n = 1, **(1.13b)** n = 2

Figure 1.9

Dienoyl tetramic acids contain a 1-oxopentadienyl substituent at the C-3 position and there have been several natural product tetramic acids containing this subunit. The first, streptolydigin **(1.14)**, obtained from the culture filtrates of the actinomycete *Streptomyces lydicus* is a potent inhibitor of DNA transferase and bacterial RNA polymerase. Studies show that the 3-dienoyl unit is essential for activity and the presence of substituents at the 1- and 5- positions improve potency. Tirandalydigin **(1.15)** was obtained from the fermentation broth of *Streptomyces* and shows an antimicrobial spectrum comparable with that of other members of its series (Figure 1.10).[12]

Streptolydigin (**1.14**) R =

Tirandalydigin (**1.15**) R =

Figure 1.10

The marine sponge *Theonella* sp. contains the orange, chlorine containing cytotoxic pigments aurantosides A and B. Recently, the aurantosides G-I have been isolated[16] from the lithistid sponge *Theonella swinhoei* from Papua New Guinea. The structures of the aurantosides of which aurantoside G (**1.16**) is shown below (Figure 1.11), represent new monochloropentaenoyl tetramic acids with mono-, di- and tri-*N*-saccharide substituents respectively. The aurantosides failed to show any activity against human colon tumour cell lines (HTC116) and were inactive in the anti-HIV assay.[16]

Aurantoside G (**1.16**)

Discodermide (**1.17**)

Figure 1.11

Discodermide **(1.17)** is a tetramic acid containing macrolactam and is obtained[17] from the Caribbean sponge *Discodermia dissolute* (Figure 1.11). This natural product was shown to inhibit *in vitro* proliferation of P388 murine leukaemia cells in addition to the growth of *Candida* fungi.

1.1.4 MT1-MMP inhibitors based on tetramic acid functionality

Ancorinosides B-D have recently been isolated[18] from the marine sponge *Penares sollasi* Thiele found in southern Japan and shown to be inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP). These natural products are of interest as they show activity against MMPs but do not possess the hydroxamate or carboxamate core which have toxicity problems. The structures of ancorinosides B,C and D contain a tetramic acid moiety connected by a long alkyl chain to the final uronic acid unit. The main differences between ancorinoside B-D are that C has an extra methyl group at position 20 and is larger by a CH_2 unit and D has an extra methylene group elongating the chain length by CH_2CH_2 (Figure 1.12).

Ancorinoside B

Ancorinoside C

Ancorinoside D

Figure 1.12

The inhibition constants (IC_{50}) for ancorinoside B,C and D are 500, 370 and 180 μg/mL as opposed to 25 μg/mL for the known inhibitor FN-439 **(1.18)** which is based on a hydroxamate group (Figure 1.13).[18] The measurement of cytotoxicity also indicates that ancorinoside B-D are 10 times less potent than FN-439 **(1.18)**. Further studies were carried out to determine which part of the molecule was actually active in inhibiting MT1-MMP. It was concluded that the two carboxylic acid groups were irrelevant by making comparisons with the aglycon of ancorinoside B and the aglycon methyl ester. The latter two showed a higher degree of potency towards MT1-MMP and MMP-2. It was established that it was the tetramic acid functionality which was active. This was achieved through a comparison with tenuazonic acid **(1.12)**, which contains the tetramic acid moiety but lacks the long alkyl chain and disaccharide. The IC_{50} for tenuazonic acid is much lower at 22 μg/mL. Unlike the ancorinosides, tenuazonic acid is a much more attractive lead for drug discovery, being a small molecule that fits Lipinski's "Rule of Five".

FN-439 **(1.18)**

Tenuazonic acid **(1.12)**

Figure 1.13

1.1.5 Solution and solid phase synthesis of tetramic acids

It was this recent discovery that has sparked our interest in using tetramic acids as potent MMP inhibitors and thus forms the basis of my research. The synthesis of tetramic acids has already been established in solution and on solid phase and general reaction conditions have been optimised.[12, 19-22] 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 moiety. The resulting 1,3-dicarbonyl undergoes a Claisen-type cyclisation with bases such as sodium methoxide to give a

tetramic acid. The last step is often referred to as the Lacey-Dieckmann cyclisation[23] and has been applied to the synthesis of numerous tetramic acid natural products. 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.

Scheme 1.1

The Lacey-Dieckmann method is very flexible and can be used to prepare 3-acyl tetramic 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. Alternative methods[12, 24-29] to the Lacey-Dieckmann route have been reported for the synthesis of tetramic acids and have subsequently been employed in the synthesis of many tetramic acid derived natural products.

Boeckmann first reported[30] the synthesis of a tetramic acid with the acetyl moiety functionalised as a phosphonate group **(1.20)**. Treatement of the isoxazolium salt with aqueous sodium bicarbonate gave the phosphonacetoacetimidate **(1.19)** (Scheme 1.2). Treatment with alkoxide yielded the tetramic acid phosphonate **(1.20)**. The tetramic acid **(1.20)** has the advantage that it can be further functionalised[31] by reacting with a range of aldehydes. It was realised that the phosphonoacetyltetramic acid is not particularly stable and so is better stored as its sodium salt. A problem with this route is that unless the ring nitrogen of the phosphonylacetyltetramic acid is alkylated the proceeding Horner-Wadsworth-Emmons reaction does not work well. The generality of this method is limited as the initial isoxazolium fragmentation gave rise to corresponding *β*-ketoacetimidates for only a narrow range of substituents. (±)-Tirandamycin **(1.21)** was synthesised using this method.

Tirandamycin **(1.21)**

Scheme 1.2

Reagents: i) $NaHCO_3$ (aq); ii) NaOEt.

Schlessinger and colleagues have also prepared[24] the tetramic acid phosphonate by treating bromoacetoacetamido ester **(1.22)** with potassium diethylphosphite to give *N*-alkylated phosphonate **(1.23)** (Scheme 1.3). The phosphonate **(1.23)** behaved as expected upon reaction with the required aldehyde to give the natural product ($\pm$)-tirandamycin **(1.21)**.

Scheme 1.3

Reagents: i) $KPO(OEt)_2$ (2.1 eq).

Direct metalation, in particular lithiation of methyl tetramates **(1.24)** has been investigated (Scheme 1.4). The following reaction is an example of a base-mediated approach, namely the reaction of vinyl lithium derivatives with aldehydes and subsequent conversion to 3-acyl-tetramic acids.[25]

Scheme 1.4

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

Metalation with BuLi, followed by the addition of various aldehydes afforded the aldol adducts. The alcohols were oxidised with manganese dioxide to give the keto-derivatives (**1.25**). They in turn were readily converted to the 3-acyl tetramic acids (**1.26**) by treatment with sodium hydroxide (Scheme 1.4).

Another useful alternative to *β*-ketoacetamides is through a zirconium (IV) catalysed coupling[26] of aldehydes and α-diazoacetamides. The products of the coupling reaction **(1.27)** were readily cyclised to the 3-acyl tetramic acids **(1.28)** (Scheme 1.5).

Scheme 1.5

Reagents: i) Et_3N; ii) RCHO, $ZrCl_4$; iii) *n*-Bu_4NF.

Jones reported[27] a novel route to 3-acyl-tetramic acids involving the ring opening of an α-pyrone system. Treatment of (bromomethyl)pyrone **(1.29)** with sodium *p*-toluenesulfonamide afforded **(1.30)** which, in the presence of alkoxide, led to the tetramic acid **(1.31)**. The 3-acyl tetramic acid **(1.32)** was obtained *via* alkaline hydrolysis followed by decarboxylation (Scheme 1.6). Elaboration of the alkyl substituent can lead to more complex tetramic acids making this a viable route for the synthesis of natural products.

Scheme 1.6

Reagents: i) TsNHNa; ii) NaOMe; iii) NaOH.

Tetramic acids have also been prepared *via* radical cyclisations[28] of propargyl α-bromoamides. Treatment of the α-bromoamide **(1.33)** with tri-n-butyltin hydride in

the presence of AIBN produced the lactam **(1.34)**. Ozone was added to a solution of the lactam to give the corresponding tetramic acid **(1.35)** (Scheme 1.7).

(1.33) i) → **(1.34)** ii) → **(1.35)**

Scheme 1.7

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

Ley and colleagues developed a strategy[29] for the enantioselective synthesis of tetramic acids centred on *tert*-butyl 3-oxobutanethioate **(1.36)** and its 4-diethylphosphono derivative **(1.37)**. Their strategy allows a wide range of *β*-ketoamides to be formed under mild conitions. The Horner-Wadsworth-Emmons reaction of **(1.37)** with aldehydes and ketones gives the (E)-alkene products **(1.38)**. Conversion to the *β*-ketoamides **(1.39)** is accomplished by the reaction with an amine or an α-aminoester in the presence of silver (I) trifluoroacetate. Cyclisation with TBAF or *tert*-butoxide produced the tetramic acids **(1.40)** with retention of configuration (Scheme 1.8). Milder bases and shorter reaction times prevent racemisation occurring.

(1.36) R^1 = H
(1.37) R^1 = $PO(OEt)_2$

i) → **(1.38)** + R^3HN–CH(R^4)–CO_2R^5 ii) → **(1.39)** iii) → **(1.40)**

Scheme 1.8

Reagents: i) R^2CHO, NaH; ii) AgO_2CCF_3; iii) TBAF.

The tetramic acids are an attractive scaffold for combinatorial chemistry. Kulkarni and Ganesan devised[32] an approach suitable for solution-phase parallel synthesis (Scheme 1.9). Amino acid esters are reductively alkylated,[33] and the amine acylated, without purification of these intermediates.

EWG = electron withdrawing group

Scheme 1.9

The Lacey-Dieckmann cyclisation is then effected by a hydroxide ion-exchange resin (Scheme 1.10). The product, being acidic, exchanges with the resin and becomes non-covalently attached. Meanwhile, unreacted starting material and other impurities remain in solution and can be removed by simple filtration. Acidification then releases the tetramic acid 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.

Scheme 1.10

There are several solid phase procedures[19-21] for the combinatorial synthesis of tetramic acids and the following is just one example. Kulkarni and Ganesan adapted their solution-phase approach (Scheme 1.9) to solid-phase conditions. The procedure[19] starts with a protected amino acid attached to Wang resin, followed by acetylation and finally cyclative cleavage[34, 35] from the resin. This relatively quick synthesis afforded the tetramic acid in good yields with simple purification steps such as resin washing after each successive step (Scheme 1.11).

Scheme 1.11

Solid phase synthesis of tetramic acids (Kulkarni and Ganesan)

Reagents: i) a) 20% piperidine in DMF b) $R_3CH_2CO_2H$, DIC, HOBt, CH_2Cl_2; ii) a) $Bu_4N^+OH^-$, THF, b) Amberlyst A-15.

1.2. Results and Discussion

1.2.1 Initial study

An initial study was undertaken in order to gain experience with the chemistry for making the tetramic acids according to the Kulkarni-Ganesan method described previously (page 19).

(1.41) (1.42) (1.43) (1.44)

Scheme 1.12

Reagents and conditions: i) PhCHO (1.2 eq), Et_3N (1.1 eq), $NaBH(OAc)_3$ (1.5 eq), 8 h; ii) cyanoacetic acid (1.2 eq), HOBt (1.2 eq), DCC (1.4 eq), 16 h; iii) Ambersep-900, MeOH then TFA, 30 min.

The initial step (Scheme 1.12) was a reductive alkylation[33] of (*S*)-phenylalanine methyl ester hydrochloride *via* imine formation to give the *N*-alkylated product **(1.42)** in moderate yield (61 %). The next stage was acylation with cyanoacetic acid to give the amide **(1.43)** in moderate yield (48 %). A Lacey-Dieckmann condensation reaction was then performed[32] using a resin-bound base, Ambersep-900 (OH^- form).

This is a cationic resin with a hydroxide counterion functioning as a base. The desired product is captured by the resin and is then detached using acid (Scheme 1.10). The conditions for cyclisation worked well and gave the desired tetramic acid **(1.44)** in moderate yield (60 %).

The synthesis of a solution phase library of tetramic acids was then undertaken with the intent to test the final compounds against MMPs. This library is based on (*S*) and (*R*) isomers of phenylalanine as it is possible that one enantiomer shows inhibition whereas the other may have reduced potency. The four precursors for the library are (*S*) and (*R*)-*N*-benzyl phenylalanine methyl ester **(1.42a,b)** and (*S*) and (*R*)-*N*-methyl phenylalanine methyl ester **(1.45a,b)**. The former was obtained *via* a reductive alkylation[33] with benzaldehyde. *N*-Methyl phenylalanine methyl ester was synthesised from the corresponding acid which is commercially available. Conditions for the esterification[36] using thionyl chloride in methanol were followed. Phenylthioacetic acid and benzylthioglycolic acid were chosen as the acylating agents. They can be further functionalised to the sulfone providing extra oxygens which may aid in chelation to the zinc metal ion in the enzyme. The coupling reagent PyBroP[37] was used for the acylation (Scheme 1.13) to give the corresponding sulfides **(1.46a-h)** in moderate yields (35-45 %).

(1.42a,b)
(1.45a,b)

a $R^1 = CH_2Ph$, (*S*)-enantiomer
b $R^1 = CH_2Ph$, (*R*)-enantiomer
c R^1 = Me, (*S*)-enantiomer
d R^1 = Me, (*R*)-enantiomer

(1.46a-h) a-d R^2 =Ph
e-h $R^2 = CH_2Ph$

(1.48a-h)

(1.47a-h)

(1.49a-h)

Scheme 1.13

Reagents and conditions: i) PyBroP (1.5 eq), $R^2SCH_2CO_2H$ (1.2 eq), diisopropylethylamine (1.2 eq), dichloromethane, 6 h; ii) Oxone® (3 eq), methanol/H_2O (2:1), 16 h; iii) NaHMDS (1M in THF, 1.5 eq), THF, 2 h, 0 °C.

Half of each sulfide **(1.46a-h)** was retained for cyclisation and the remainder oxidised to the sulfone **(1.47a-h)** in good yields (51-98 %) using Oxone®[38] in methanol. This was an efficient method of oxidation and any by-products were removed in the aqueous phase. In the initial study, cyclisation to give the cyano tetramic acid **(1.44)** was effected using the resin-bound base, Ambersep-900. However, initial attempts to cyclise the sulfides **(1.46a-h)** and the sulfones **(1.47a-h)** using the milder resin base, Ambersep 900[32] were unsuccessful. An alternative method of cyclisation was chosen using sodium bis(trimethylsilyl)amide (NaHMDS). Each individual set was cyclised to give the corresponding tetramic acids **(1.48a-h)** and **(1.49a-h)** in good yields (Table 1.1). It was thought that NaHMDS would be a strong enough base for all the cyclisations (Scheme 1.13).

Tetramic acid	R^1	R^2	% Yield of cyclisation	
			(*S*)	(*R*)
1.48a-b	CH_2Ph	Ph	94	90
1.48c-d	CH_2Ph	CH_2Ph	95	90
1.48e-f	Me	Ph	75	69
1.48g-h	Me	CH_2Ph	70	84
1.49a-b	CH_2Ph	Ph	n/a	88
1.49c-d	CH_2Ph	CH_2Ph	n/a	96
1.49e-f	Me	Ph	96	n/a
1.49g-h	Me	CH_2Ph	97	55

Table 1.1: Tetramic acids synthesised from (*S*) and (*R*)-phenylalanine. n/a; cyclisation was not performed.

The main problem faced when making these tetramic acids was finding a suitable method of purification. They are very polar compounds especially when R^1 equals Me and for these column chromatography is not suitable as the Rf is too low. The best methods found were recrystallisation from either methanol or methanol/ether depending on the tetramic acid.

The optical rotations for the following tetramic acids **1.48ab**, **1.48cd**, **1.48ef**, **1.49gh** were taken. The rotations tended to be equal and opposite showing differences of between 5 and 10. It is possible that racemistion may occur if the reaction conditions for cyclisation are harsher e.g. higher temperatures or longer reaction times.

1.2.2 Synthesis of 3-acyl-tetramic acids

A number of tetramic acids were synthesised containing an acyl group in the R^3 position. This group is present in the known MMP inhibitor, tenuazonic acid **(1.12)**. Two routes for the acetoacetylation step were compared. The first using diketene[39] and in the second, using an alternative reagent, 2,2,6-trimethyl-4H-1,3 dioxin-4-one.[40] A solution phase synthesis starting from (*S*)-tryptophan methyl ester hydrochloride **(1.50)** led to the tetramic acid **(1.53)**. The initial step towards the final product was a reductive alkylation[33] to give **(1.51)** in moderate yield (45 %). Acetoacetylation using diketene[20] gave the desired product **(1.52)** (Scheme 1.14). A Lacey-Dieckmann

condensation reaction was then performed using the resin bound base Ambersep 900 and detaching the desired product with TFA. The overall yield after purification by recrystallisation (MeOH/hexanes) was 30 %.

(1.50) (1.51) (1.52) (1.53)

Scheme 1.14

Reagents and conditions: i) PhCHO (1.2 eq), Et_3N (1.1 eq), $NaBH(OAc)_3$ (1.5 eq), 8 h; ii) diketene (1.1 eq), Et_3N (1.1 eq), 16 h; iii) Ambersep 900, MeOH then TFA, 30 min.

The same procedure was employed starting from (*S*) or (*R*)-phenylalanine methyl ester hydrochloride **(1.41a,b)** and (*S*)-isoleucine methyl ester hydrochloride **(1.41c)** (Scheme 1.15). The main difference in the reaction conditions for these three compounds was the cyclisation step where 0.5M sodium methoxide[22] in methanol under reflux for 0.5 hours was used to give **(1.56a) (1.56b)** and **(1.56c)** in good to moderate yields of 90 %, 86 % and 40 % respectively. For the tetramic acids **(1.56a,b)** the reductive alkylation was omitted leaving a hydrogen in the R^2 position. Biological testing of these tetramic acids would enable comparisons to be drawn between compounds containing small or larger groups at the R^2 position.

(1.41a-c) (1.54a-c) (1.55a-c) (1.56a-c)

1.56a R^1 = CH_2Ph, R^2 = H, (*S*)-enantiomer
1.56b R^1 = CH_2Ph, R^2 = H, (*R*)-enantiomer
1.56c R^1 = $CH(CH_3)CH_2CH_3$, R^2 = CH_2Ph, (*S*)-enantiomer

Scheme 1.15

Reagents and conditions: i) PhCHO (1.2 eq), Et_3N (1.1 eq), $NaBH(OAc)_3$ (1.5 eq), 8 h; ii) diketene (1.1 eq), Et_3N (1.1 eq), 16 h; iii) 0.5M NaOMe in MeOH (1 eq), 0.5 h, reflux.

It is possible to use 2,2,6-trimethyl-4H-1,3 dioxin-4-one[40] as a cheaper and stable alternative to diketene in the acetoacetylation. A test reaction was undertaken with (*S*)-tryptophan methyl ester. The mixture was heated in refluxing xylene at 150 °C for 3 hours to give the *β*-keto-amide **(1.57)** in good yield (87 %) (Scheme 1.16). The driving force for this reaction is the evolution of acetone. Although this method was effective, harsher reaction conditions were needed in order to obtain yields matching that with diketene. A disadvantage is that racemisation occurred due to heating.

Scheme 1.16

Reagents and conditions: i) **(1.50)** (free base), dioxinone (3 eq), xylene, 3h, 150 °C.

Two bicyclic tetramic acids were synthesised using slightly stronger cyclisation conditions. (*S*)-proline methyl ester and (*S*)-pipecolinic acid methyl ester are cyclic secondary amines and can be acetoacetylated under the ususal diketene condtions to give **(1.58a)** and **(1.58b)** respectively. These were cyclised using sodium methoxide in methanol under reflux for 3 hours to give **(1.59a)** and **(1.59b)** in 85 % and 75 % respectively (Scheme 1.17). The conditions needed were harsher than for previous tetramic acids and refluxing was necessary in order for the cyclisation to proceed. Unlike previous examples the two products were both oils rather than solids.

(1.58a), HCl salt, n = 1
(1.58b), free base, n = 2

Scheme 1.17

Reagents and conditions: i) diketene (1.1 eq), Et_3N (1.1 eq), 16 h; ii) NaOMe (0.5M in methanol, (1 eq), 3 h.

The bicyclic tetramic acids (**1.59a,b**) were devoid of optical activity and are thought to have racemised under the harsher conditions employed for cyclisation. The next stage was to test the tetramic acids synthesised so far against MMP-2.

1.3. Biological Testing

1.3.1 Biological assay

A biological assay was set up in order to test some of the tetramic acids synthesised against MMP-2, gelatinase. This commercially available assay consisted of the enzyme (catalytic domain), a thiopeptolide substrate, DTNB, NNGH[41] (an inhibitor of MMP-2) and buffer solution.

Enzymatic cleavage

Mimic of peptide substrate for MMP-2

Scheme 1.18

The assay uses a thiopeptolide as a colorimetric substrate and the MMP amide cleavage site is replaced by a thioester. Hydrolysis by the MMP produces a sulfhydryl group which in turn reacts with dithio bis 2-nitrobenzoic acid (DTNB) to give 5-thio-2-nitrobenzoic acid (Scheme 1.18). The production of 5-thio-2-nitrobenzoic acid can be detected at an absorbance of 412 nm. An inhibitor would prevent the hydrolysis of the thioester and no change in absorbance would be detected.

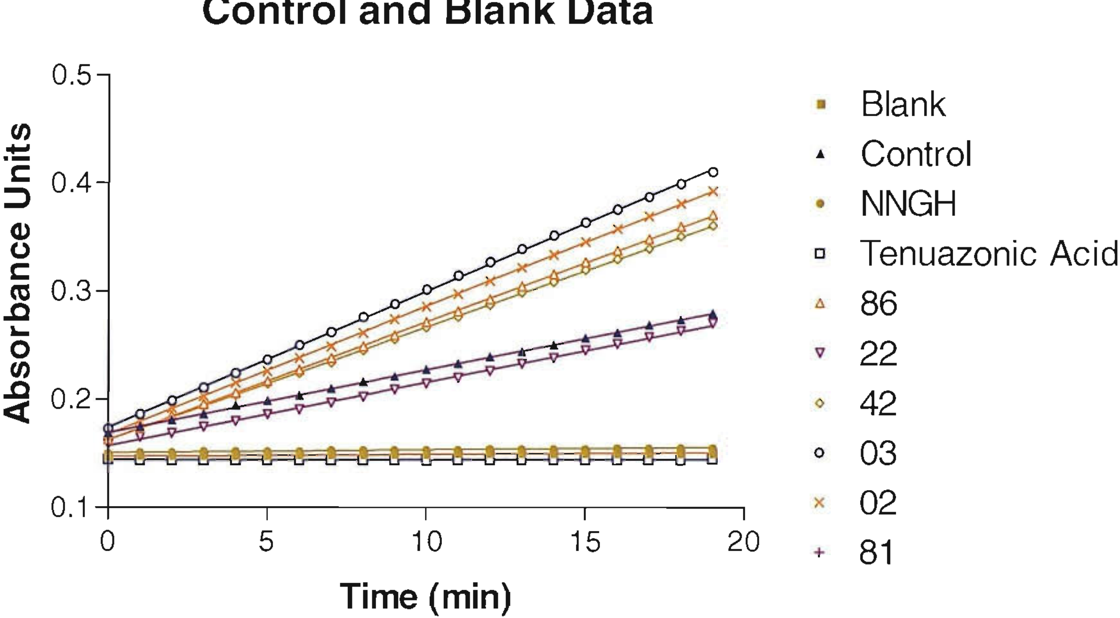
The compounds below (Table 1.2) were selected for the assay as they gave a broad representation of the tetramic acid library synthesised.

Compound	Structure
(1.12) Tenuazonic acid	
(1.56a) HF86	
(1.48a) HF22	
(1.49a) HF42	
(1.59a) HF02	
(1.59b) HF03	
(1.56c) HF81	

Table 1.2: Tetramic acids tested in biological assay against MMP-2.

The graph below shows the results of the assay over a time of 20 minutes. The blank contained the buffer solution and the substrate and as expected the absorbance did not change. The control contained the enzyme, substrate and the buffer and so absorbance increased over time. The known inhibitors tenuazonic acid **(1.12)** and NNGH were also present in the assay and both successfully inhibited the enzyme. The absorbance of these known inhibitors did not change over time and so this was an indication that the assay was working. Finally, there were the tetramic acids synthesised, all of which showed an increase in the rate of absorbance. These

compounds unfortunately showed no inhibition and were not active. Furthermore, the data suggests that the compounds are in fact enhancing the MMP activity.



Graph 1.1: Results from MMP assay.

To test all compounds in this manner is not very cost or time effective. It is therefore not particularly viable to synthesise large numbers of compounds and test them *via* this method. Molecular modeling is an additional tool that can be used to aid in the design of a suitable inhibitor.

1.4. Molecular Modeling

1.4.1 Modeling of tetramic acids

Molecular modeling can be used to aid the process of designing a drug and is an extremely useful tool. Crystal structures of the catalytic domain of many MMPs have now been obtained[4-7] and it is possible to look at them in 3D. Using the computer package Prism, MMP-inhibitor interactions were viewed and analysed accordingly. The information from such interactions helped to ascertain certain facts such as the degree of homology within the MMP family and whether the structure moved much when the inhibitor was attached. Modeling also showed how the inhibitor orientates itself around the zinc atom and gave an indication of other interactions such as hydrogen bonding between the inhibitor and the enzyme.

The diagram below shows the degree of homology between MMP-3, 8, 9, 11 and 13 (chosen because their structures have been determined by crystallography). The basic structure of the MMPs does not vary much which may make it difficult to design a drug specific to one MMP.

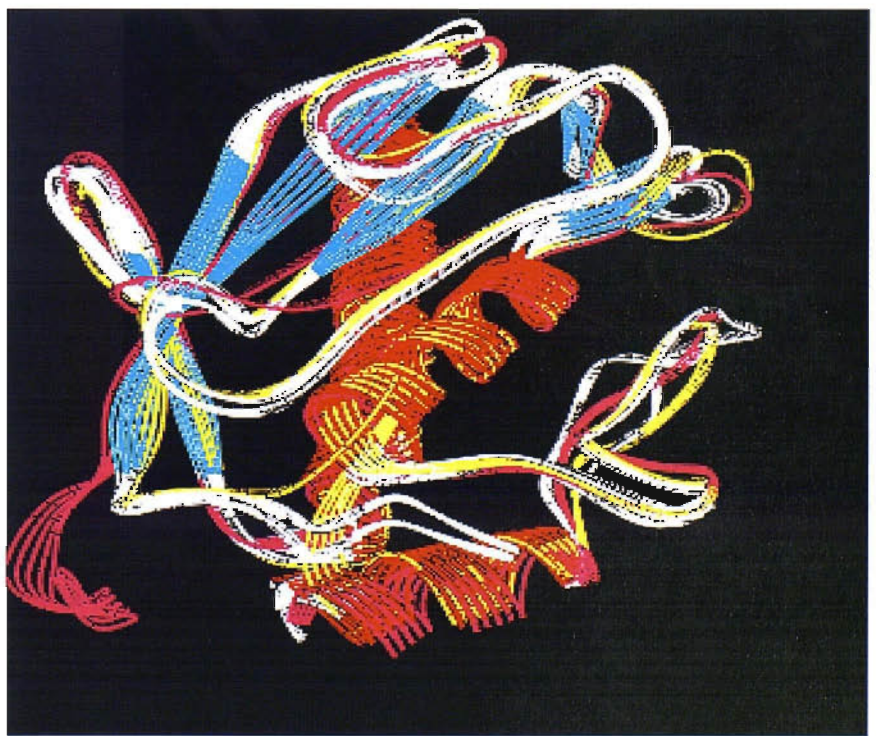


Diagram 1.1: Overlay of MMP-3, 8, 9, 11, 13.

Using the modeling package Prism, it was possible to view interactions between a hydroxamic acid inhibitor and the enzyme, MMP-3 (Diagram 1.2).[42] Coordination to the zinc atom through the two oxygens and also hydrogen bonding between the NH and the glutamic acid side chain can be seen. The diagram also depicts the three histidines around the zinc atom which are conserved in all known MMPs.

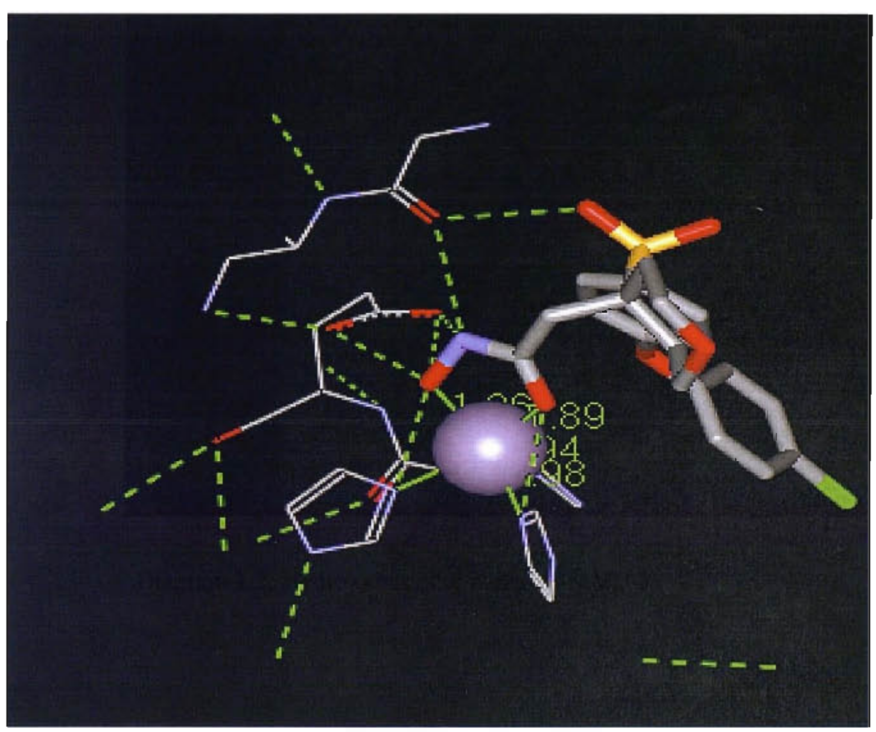


Diagram 1.2: Hydroxamic acid bound to MMP-3.

1.4.2 Docking studies with tenuazonic acid

Tenuazonic acid (**1.12**) was docked into the enzyme to see how it would best orientate itself around the zinc atom. From the results of the docking study we could determine which R groups would fit into the space and design a potential inhibitor accordingly. Tenuazonic acid forms a bidentate complex with zinc and so there are different ways it can fit into the enzyme, as shown for MMP-3 (Diagram 1.3).

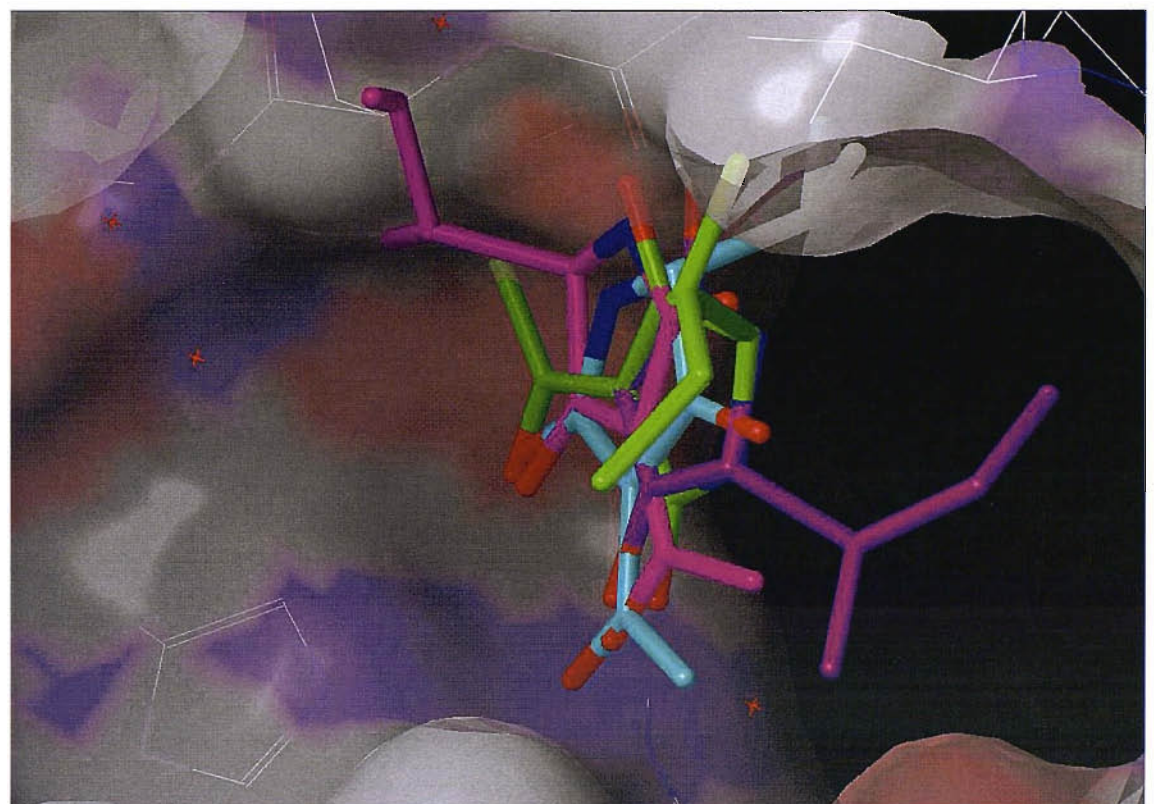


Diagram 1.3: Docking of tenuazonic acid into MMP-3.

Four low energy tautomers of tenuazonic acid were identified, which could coordinate to zinc and fit the active site. These are depicted in the diagram as cyan, green and two purple modes (Diagram 1.3). The cyan mode does not produce bad interactions with critical catalytic residues. Focusing on the position of the isobutyl portion, it is the purple and green orientations that dispose of the hydrophobic moiety in the best manner. Comparing the orientations to those of a bidentate copper complex of tenuazonic acid,[43] it is the cyan and green orientations that make the analogous metal interactions (Figure 1.14). Combining the results of the modeling and the knowledge of the orientation of copper complexes of tenuazonic acid it is the cyan mode which is the preferred orientation. The cyan mode disposes tenuazonic acid to the right hand side (RHS) of the zinc binding group (ZBG). As mentioned previously, inhibitors lying on the right-hand side (RHS) of the (ZBG) tend to be more potent (see page 4).

Cyan and Green Modes

Purple Mode

Figure 1.14

Upon analysis it was expected that a long alkyl or aryl chain which is alpha substituted would be more active. It would also be beneficial to have a free NH group as opposed to *N*-alkylated tetramic acids as this could interact with the glutamic acid side chain. The interaction is important as we know the glutamic acid residue is involved in the mechanism for proteolysis (Page 2). The alpha branched tetramic acid **(1.60)** could be a mimic for this known carboxylate inhibitor[44] **(1.61)** (Figure 1.15). It could coordinate to zinc *via* a bidentate interaction through the oxygens. The hydrophobic moiety could be a long alkyl or aryl chain as a replacement for the biphenyl group in the sulfonamide **(1.61)**. The alpha branched methyl group could act in the same way as the sulfonamide group in the carboxylate inhibitor by fixing the position of the long side chain preventing it from freely moving. Both the tetramic acid **(1.60)** and the sulfonamide **(1.61)** have a free NH group which is available for interaction with the enzyme through hydrogen bonding.

(1.60) **(1.61)**

Figure 1.15

1.5. Second Generation of Tetramic Acids

1.5.1 Synthesis of second generation of tetramic acids

The results from biological testing and also from the docking studies of tenuazonic acid gave a better indication of the type of compound that may fit into the active site. It was decided that threonine would be a suitable precursor to this type of tetramic acid as it has an alpha substituted side chain. Threonine has a free alcohol group which can undergo further functionalisation to give a range of products that fit our model. For this particular series serine has also been used as a precursor. The alpha branching in threonine could help to fix the side chain into a pocket in the enzyme and in turn improve its potency. A direct comparison could be made between the threonine and serine derived tetramic acids to determine whether the steric effect caused by the alpha branching is an important feature.

The synthesis of two tetramic acids starting from either (*S*)-threonine methyl ester or (*S*)-serine methyl ester **(1.62a,b)** *via* protection and deprotection of the alcohol group was undertaken. The initial step was addition of *tert*-butyldimethylsilyl chloride[45] (TBDMSCl) to give the protected alcohols **(1.63a,b)** in good yields (65-85 %) (Scheme 1.19). The next step was acetoacetylation using the usual diketene[39] conditions to give the corresponding *β*-keto amides **(1.64a,b)** in good yields (92-99 %). Initially, the acetoacetylation step was attempted in the presence of the alcohol but crude ^{1}H NMR analysis indicated that acetoacetylation had occurred on both the alcohol and the amine. The TBDMS group was removed with tetrabutylammonium fluoride[46] (TBAF) in THF to give the alcohols **(1.65a,b)** in good yields (84 % and 85 %) respectively followed by cyclisation[22] using NaOMe in methanol to give the corresponding tetramic acids **(1.66ab)**. The acetoacetylation and cyclisation conditions were also used to give the *O*-benzyl substituted threonine and serine derived tetramic acids **(1.65c,d)** in moderate yields (Table 1.3).

Scheme 1.19

Reagents and conditions: i) TBDMSCl (2.1 eq), imidazole (2.1 eq), dichloromethane, 12 h; ii) diketene (1.1 eq), dichloromethane, 16 h; iii) TBAF (1M in THF, 1.1 eq), THF, 1.5 h; iv) NaOMe (1 eq, 0.5M in MeOH), reflux.

Tetramic acid	R^1	% Yield of cyclisation
1.66a	H	58
1.66b	Me	61
1.66c	H	75
1.66d	Me	50

Table 1.3: Tetramic acids synthesised derived from (*S*)-serine and (*R*)-threonine.

The *β*-keto amides **(1.64a,b)** containing a free alcohol group underwent cyclisation very rapidly and in good yields. The conditions needed to cyclise the benzyl substituted precursors **(1.64c,d)** were much harsher and refluxing was necessary in

order to obtain the corresponding tetramic acid **(1.66c,d)**. The low yield (50 %) obtained for the *O*-benzyl-threonine-tetramic acid was thought to be due to the occurrence of a competing elimination reaction. Analysis of ^{1}H NMR data indicated that benzyl alcohol had been produced in the reaction

1.5.2 Synthetic studies towards *O*-biphenyl threonine tetramic acid

Attempts to synthesise *O*-biphenyl substituted threonine based tetramic acid **(1.67)** were undertaken as this compound posseses a biaryl chain similar to the biphenyl chain in the carboxylate inhibitor **(1.61)** (Figure 1.16).

HN O O HO O **(1.67)**

O O S NH R HO O **(1.61)**

Figure 1.16

The initial synthetic route investigated *via* a known literature procedure[47] is outlined in Scheme 1.20. The mono-sodium salt of (*S*)-threonine **(1.68)** was treated with $BF_3.Et_2O$ in THF to give **(1.69)** (Scheme 1.20). The boron complex **(1.69)** was allowed to react with the freshly prepared biphenyl trichloroacetimidate[48] **(1.70)** (replacing benzyl trichloroacetimidate in the literature procedure) in dioxane to give intermediate **(1.71)**. Upon treatment with aqueous NaOH the desired product **(1.72)** was expected.

Scheme 1.20

Reagents and conditions: i) $BF_3.Et_2O$ (5 eq), THF, 6 h (rt), 2 h (45 °C); ii) 2,2,2-trichloroacetimidic acid biphenyl-4-yl methyl ester **(1.70)** (1.2 eq), dioxane; iii) NaOH (aq), 3 h.

It was very difficult to follow the reaction as the reagents and products were in suspension in THF or dioxane. The substituted amino acid **(1.71)** was not isolated. At pH 6 the desired product **(1.72)** would not partition into the organic phase. At this point the aqueous phase was evaporated *in vacuo* to give a white solid which was insoluble in any organic medium or water. Whether the white solid was the desired product or a mixture of products was undetermined.

The crude material was esterified with thionyl chloride[36] in methanol under reflux in order to try and ascertain whether the previous reaction had worked (Scheme 1.21). If there was a mixture of products then it would be easier to separate them as the ester. ^{1}H NMR analysis of the crude material showed that the boron protection reaction had not given the desired product and the only product isolated from the reaction was the starting amino acid. This was later confirmed by ^{13}C NMR data.

(1.72) **(1.73)**

Scheme 1.21

Reagents and conditions: i) $SOCl_2$ (3 eq), methanol, 6 h, reflux.

An alternative route to the desired compound involves a greater number of steps but would protect the amine and acid from deprotonation. The remaining alcohol group could then freely undergo deprotonation and addition of the biphenyl group (Scheme 1.22). Protection of the amine to give the phthaloyl derivative[49] **(1.74)** proceeded in quantitative yield. Treatment of the phthaloyl derivative **(1.74)** with 4-bromomethylbiphenyl in the presence of potassium *tert*-butoxide did not give the desired ether **(1.75)**.

(1.62b) **(1.74)** **(1.75)**

Scheme 1.22

Reagents: i) phthalic anhydride (1.2 eq), dioxane; ii) 4-bromomethylbiphenyl (1.2 eq), KO^tBu (1.1 eq), THF.

The Boc protected threonine **(1.76)**, synthesised from (*S*)-threonine methyl ester hydrochloride in good yield was reacted with an excess of biphenylmethyl bromide in the presence in sodium hydride under refluxing conditions but also failed to give any

desired product. Instead the major product obtained was the biphenylmethyl ester **(1.77)** (Scheme 1.23).

(1.76) i) **(1.77)**

Scheme 1.23

Reagents and conditions: i) biphenylmethyl bromide (20 eq), NaH (2 eq), DMF (2 mL), reflux.

Attempts so far to synthesise *O*-biphenyl threonine have been unsuccessful. The first *via* a boron intermediate[47] was a difficult reaction to monitor and the reaction did not yield the desired product. Alternative routes *via* protection of the acid and the amine have also been unsuccessful. According to a literature precedent[50] the reaction between (*S*)-threonine and benzyl alcohol in toluene under Dean-Stark conditions for 24 hours gives the dibenzyl derivative in a very low yield (23%). The procedure was followed using biphenylmethyl alcohol as a replacement for benzyl alcohol. After 24 hours, all the water had been collected and the reaction gave **(1.78)** in low yield (20%) (Scheme 1.24). One reason for the low yield was that the alcohol reacted with itself to form by-products such as dimers and trimers confirmed by ^{1}H NMR.

Scheme 1.24

Reagents and conditions: i) biphenylmethanol (10 eq), *p*-TsOH (0.12 eq), toluene (30 mL), Dean-Stark, 18 h; ii) diketene (1.1 eq), dichloromethane, 16 h; iii) NaOMe (1 eq, 0.5 M in methanol), reflux.

The product **(1.78)** was treated with diketene[39] to give the *β*-ketoamide **(1.79)** but upon cyclisation[22] did not give the corresponding tetramic acid **(1.80)**. Upon cyclisation a mixture of inseparable products was obtained. It is possible that elimination had occurred. Crude ^1H NMR indicated the presence of biphenyl methanol and also of the eliminated tetramic acid **(1.81)**.

1.5.3 Synthetic route towards ester functionalised tetramic acid

A coupling reaction between the alcohol on (*S*)-threonine and a long alkyl chain carboxylic acid could potentially give an interesting alternative tetramic acid. The esterification[51] of dodecanoic acid (lauric acid) with the alcohol on threonine using DCC and a catalytic amount of DMAP gave the desired product **(1.82)** in good yield (77%) This functionalisation gave a long alkyl chain in the desired position according to molecular modeling results. Deprotection of the Boc group with TFA proceeded

smoothly in quantitative yield to give the free amine **(1.83)**. The product was reacted under the usual diketene conditions to give the *β*-ketoamide **(1.84)** (Scheme 1.25). However, upon cyclisation with sodium methoxide the major product isolated was dodecanoic acid. None of the desired product was formed. An alternative route would be to synthesise the ester after the cyclisation step.

Scheme 1.25

Reagents and conditions: i) dodecanoic acid (1 eq), DMAP (0.05 eq), DCC (1 eq), dichloromethane (5 mL), 3 h; ii) TFA (10 eq), dichloromethane (2 mL), 2 h; iii) diketene (1.1 eq), dichloromethane, 18 h; iv) NaOMe (1 eq, 0.5 M in methanol), reflux.

1.5.4 Zinc binding studies of serine derived tetramic acid

Attempts to bind zinc to a tetramic acid have been undertaken. This would confirm that the compounds synthesised thus far will actually bind to zinc which is an important feature in the inhibition of all MMPs. As tetramic acids can exist in tautomeric forms it would be useful to determine which orientation is favoured around zinc. The sodium salt of the tetramic acid **(1.66c)** was reacted with zinc chloride to give the zinc-bound product **(1.86)** (Scheme 1.26).[52] The product obtained can be in either orientation B or C and it was possible to ascertain which was more favoured.

(1.66c)

A

1) NaOH 0.2 M

2) $ZnCl_2$

(1.86)

B

OR

C

Scheme 1.26

The ^{13}C NMR data of the starting material and the product were compared (Table 1.4). There are significant shifts in the NMR data for C6 and C7. Comparing C4 with C2, there is a greater chemical shift for C2 indicating that the major zinc bound product binds through the amide carbonyl. These results are consistent with similar findings in the literature.[52]

Tetramic acid	^{13}C					
	C2	C3	C4	C5	C6	C7
A	175.7	102.1	193.6	62.8	184.4	19.6
B	177.6	100.2	193.1	60.5	191.7	26.3
Difference (ppm)	+1.9	-1.9	-0.5	-2.3	+7.3	+6.7

Table 1.4: ^{13}C NMR data of zinc bound and non-zinc bound tetramic acid.

1.5.5 Results of MMP inhibition assays

Dr Yoichi Nakao at the University of Tokyo tested the compounds shown in Table 1.5 in two assays, against MMP-2 and MT1-MMP. The assay used is based upon fluorescence. MMPs cleave substrate 1 and produce substrate 2 which is fluorescent. An inhibitor prevents the cleavage of substrate 1 by the MMP, and so the fluorescence associated with substrate 2 is reduced (Scheme 1.27). The lower the fluorescence the more active the compound.

MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2 —(MMPs / incubation)→ MOCAc-Pro-Leu-Gly

substrate 1 **substrate 2** (fluoresence)

Scheme 1.27

The majority of these compounds did not show any inhibition against the enzymes. Entries **2**, **8** and **9** all showed very weak inhibition at greater than 50 μg/mL. This lack of inhibition indicates that the substituents around the core tetramic acid unit are more sensitive to the enzyme active site than was first thought. Variation of these groups have provided compounds with less activity than tenuazonic acid or the ancorinosides.

Entry	Tetramic acid	Entry	Tetramic acid
1		6	
2		7	
3		8	
4		9	
5		10	

Table 1.5: Tetramic acids tested against MMP-2 and MT1-MMP.

1.6 Conclusions and Future Work

An initial library of tetramic acids has been synthesised successfully and a selection of these were tested for biological activity against MMP-2, gelatinase. The results showed that none of the tetramic acids synthesised were active. Molecular modeling aided in designing a second set of tetramic acids based upon the binding mode of tenuazonic acid. The second series of tetramic acids synthesised from threonine and serine along with the remainder of the first set were tested against MMP-2 and MT1-MMP. None of the tetramic acids synthesised showed significant inhibition. It would appear that the substituents around the tetramic acid unit are particularly sensistive to alterations.

The tetramic acid library did not give any hits in the assays against MMP-2 and MT1-MMP. However, the scope of the reaction can be explored further. There are other amino acids which can be used as the starting materials to give enantiomerically enriched products. Tyrosine would be a good alternative as the alcohol could be further functionalised. Further exploration into substituents for R^3 could be undertaken. One approach would be to vary the acyl group whilst retaining the remaining groups associated with tenuazonic acid. This could be achieved using the enantioselective synthesis developed by Ley and colleagues mentioned previously (page 18). A library of compounds could be synthesised and tested for biological activity.

Chapter Two

Keto-pyrrolidinones as Potential MMP Inhibitors

2.1 Introduction

2.1.1 Synthesis and biological activity of keto-pyrrolidinones

Keto-pyrrolidinones have similar functionalities to tetramic acids whereby there is a possibility of chelation to zinc through the carbonyl oxygens. Like the tetramic acids they therefore have the possibility of binding to zinc in the enzyme and thus inhibit MMP acvtivity. The keto group is now in the 3-position of the heterocycle compared to the previous examples of tetramic acids where it is in the 4-position (Figure 2.1).

Tetramic acid

Keto-pyrrolidinone

Figure 2.1

This class of compounds is known to possess biological properties in particular as CCR2 antagonists.[53] Chemokine receptors are members of the large receptor family known as G-protein coupled receptors (GPCRs). MCP-1 is a chemokine produced by a number of cell types and it binds to CCR2. CCR2 is an appropriate target for inhibiting the excessive inflammatory responses that contribute to disease. It has been shown that specific keto-pyrrolidinones block the biological effects of MCP-1 and inhibit the inflammatory process mediated by the chemokine. Results of a binding and chemotaxis assay show that many compounds inhibit MCP-1 with IC_{50} values of less than 5 μM of which the following compounds **(2.1a-c)** are examples (Figure 2.2).

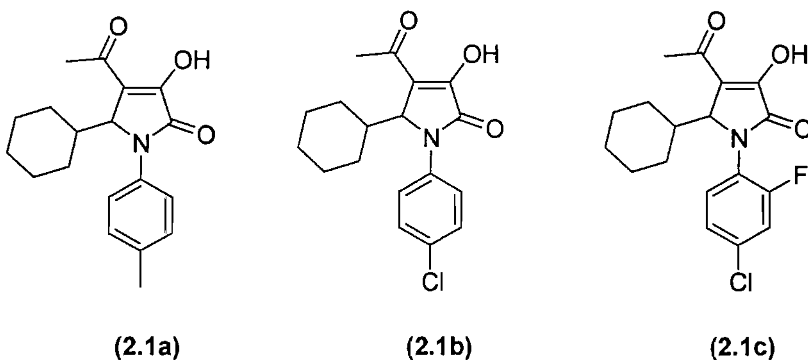


Figure 2.2

A small array consisting of ten 1,5-diaryltetrahydropyrrole-2,3-diones were synthesised and shown to have antiviral activity with respect to the following viruses: herpes simplex type 1 (VHS), smallpox vaccine (SPV), classical foul plaque (VCFP), vesicularstomatitis (VVS), respiratory syncytial (RS) and Venezuela equine encephalomyelitis (VEE).[54] Pronounced activity was observed for compounds such as **(2.2a-c)** bearing aryl substituents in the 1 and 5 positions and an ethoxycarbonyl group in the 4 position (Figure 2.3). Introduction of an acetyl group or a cyano group in the 4 position resulted in a decrease of antiviral activity.

EtO O OH N O Br

(2.2a) **(2.2b)** **(2.2c)**

Figure 2.3

The synthesis of keto-pyrrolidinones by a one-pot condensation is known.[54-58] The method can incorporate a broad range of groups for the R^1, R^2 and R^3 positions (Scheme 2.1). In the literature these compounds are often synthesised using acid catalysis.[53] However, it also known for the reaction to proceed without any acid present.[54]

Scheme 2.1

Reagents: i) AcOH (20 %), THF.

An interesting alternative to the standard synthesis is the conversion of dioxopyrrolines to the corresponding keto-pyrrolidinone. The reaction of 4-benzoyl-1-benzyl-5-phenyldioxopyrroline **(2.3)** with formamide causes reduction[59] to give 1,5-dihydro-3-hydroxy-2H-pyrrol-2-one **(2.4)** in good yield (Scheme 2.2).

Scheme 2.2

Reagents: i) $HCONH_2$.

The conversion of dioxopyrrolines **(2.5)** into keto-pyrrolidinones **(2.6)** *via* a 1,4-reduction using a large excess of sodium hydrogensulfite has also been reported.[60] The reaction is versatile and can tolerate a range of substituents. Methylation of the products with diazomethane gave the methyl ethers **(2.7)** in good yield. The pyrrolidinone **(2.7)** can be reacted further and treatment of these products with triethyloxonium tetrafluoroborate (Meerwein reagent) gave 2-ethoxy-3-methoxy-1H-pyrrole **(2.9)** (Scheme 2.3).

R = H, Me, Ph, CH_2Ph

(2.5) (2.6) (2.7) (2.8) (2.9)

Scheme 2.3

Reagents: i) $Na_2S_2O_4$ (20 eq), dioxane/H_2O; ii) CH_2N_2 (excess), dichloromethane; iii) Et_3OBF_4 (excess), K_2CO_3, dichloromethane.

The synthesis of 1-substituted 5-(4-dimethylaminophenyl)-4-aroyl-3-hydroxy-2,5-dihydro-2-pyrrolones **(2.13a-f)** has been reported.[61] These compounds are formed as a result of the reaction between furandiones **(2.10)** and *N*-(4-dimethylaminobenzylidene)amines **(2.11)**. For cases where the azomethine contains a dimethylamino group in the para position the reaction can be explained as ring opening of the furandiones. Attack of the lactone carbonyl by the nitrogen atom causes the ring to open. The zwitterionic intermediate then undergoes cyclisation to the substituted tetrahydro-2,3-pyrroledione **(2.12a-f)** which then isomerises to the keto-pyrrolidinones **(2.13a-f)** (Scheme 2.4).

(2.10) **(2.11)** **(2.12a-f)** **(2.13a-f)**

2.13a R^1 = H, R^2 = H
b R^1 = CH_3, R^2 = H
c R^1 = Cl, R^2 = H
d R^1 = H, R^2 = CHO
e R^1 = CH_3 R^2 = CHO
f R^1 = Cl, R^2 = CHO

Scheme 2.4

2.2 Results and Discussion

2.2.1 One-pot synthesis of pyrrolidinediones

An initial study was performed to evaluate the known one-pot methods for keto-pyrrolidinone synthesis. A 1:1:1 mixture of acetaldehyde **(2.14)**, benzylamine **(2.15)** and ethyl-2,4-dioxovalerate **(2.16)** were added together in THF and 20 % acetic acid.[53] A white solid precipitate was recrystallised from methanol to give **(2.17a)** in a good yield (83 %) (Scheme 2.5). The one-pot synthesis is efficient and can tolerate a broad range of aliphatic and aromatic groups for R^1, R^2 and R^3. For R^3 there are a limited number of commercially available compounds (methyl, *tert*-butyl, and thienyl). However, the diketo-esters can be prepared[62] from any methyl ketone and diethyl oxalate.

(2.14) + **(2.15)** + **(2.16)** —i)→ **(2.17a)**

Scheme 2.5

Reagents and conditions: i) 20 % acetic acid, THF, 6 h.

An initial set of keto-pyrrolidinones were synthesised using the above method from ethyl-2,4-dioxovalerate (Scheme 2.6).

R^1CHO + R^2-NH_2 + ethyl-2,4-dioxovalerate —i)→ **(2.17b-s)**

Scheme 2.6

Reagents and conditions: i) AcOH (20 %), THF, 3 h.

Various keto-pyrrolidinones were synthesised from ethyl-2,4-dioxovalerate and their corresponding yields are shown below (Table 2.1). Further experimentation showed that acid catalysis was not needed in the one-pot condensation. Simply mixing the three components was sufficient, and this method was used for the majority of the compounds in Table 2.1.

Entry	R^1	R^2	Yield (%)
2.17a[a]	Me	CH_2Ph	83
2.17b	CH_2Ph	CH_2Ph	66
2.17c[a]	CH_2CH_2Ph	CH_2Ph	60
2.17d	2-Thienyl	CH_2Ph	90
2.17e[a]	2-(5-Chloro-Thienyl)	CH_2Ph	72
2.17f	2-Furyl	CH_2Ph	70
2.17g	4-Pyridyl	CH_2Ph	65
2.17h	$CH_2CH(CH_3)_2$	CH_2Ph	68
2.17i	Ph	CH_2-*p*-Cl-C_6H_4	83
2.17j	Ph	CH_2Ph	88
2.17k[a]	Ph	Ph	83
2.17l[a]	$CH_2CH(CH_3)_2$	Ph	43
2.17m	Ph	Me	66
2.17n	*p*-OMe-C_6H_4	Me	48
2.17o	*p*-Cl-C_6H_4	Me	37
2.17p	Ph	$CH_2CH(CH_3)_2$	70
2.17q	*p*-OMe-C_6H_4	$CH_2CH(CH_3)_2$	63
2.17r	Me	$CH_2CH(CH_3)_2$	86
2.17s[a]	Ph	H	70

Table 2.1: Keto-pyrrolidinones synthesised from ethyl-2,4-dioxovalerate. [a] Keto-pyrrolidinones synthesised in the presence of acetic acid.

The scope of this one-pot condensation was explored and a small array was synthesised employing various aldehydes and amines. The synthesis of thienyl keto-pyrrolidinones **(2.18a-h)** has also been achieved *via* the same method (Scheme 2.7).

Scheme 2.7

Entry	R^1	R^2	Yield (%)
2.18a	Ph	CH_2Ph	56
2.18b	*p*-OMe-C_6H_4	CH_2Ph	60
2.18c	2-Thienyl	CH_2Ph	73
2.18d	$CH_2CH(CH_3)_2$	CH_2Ph	85
2.18e	Ph	CH_2-*p*-Cl-C_6H_4	57
2.18f[a]	Ph	Ph	70
2.18g	Ph	Me	71
2.18h	Ph	H	40

Table 2.2: Keto-pyrrolidinones synthesised from thienyl valerate. [a] This compound was synthesised using acetic acid.

A third set of keto-pyrrolidinones were synthesised from the bulkier *tert*-butyl valerate (Scheme 2.8).

Scheme 2.8

Entry	R^1	R^2	Yield (%)
2.19a	Ph	CH_2Ph	57
2.19b[a]	*p*-OMe-C_6H_4	CH_2Ph	55
2.19c	*p*-Cl-C_6H_4	CH_2Ph	47
2.19d[a]	Ph	Ph	31
2.19e	Ph	Me	34
2.19f[a]	Ph	H	44
2.19g	Ph	CH_2-*p*-*m*-Cl_2- C_6H_4	46

Table 2.3: Keto-pyrrolidinones synthesised from *t*-butyl valerate. [a] Keto-pyrrolidinone synthesised using acetic acid.

The one-pot condensation proceeds *via* imine formation and so is faster when R^1 is an electron withdrawing group and R^2 is an electron donating group. The best yields occur when imine formation is favoured.

2.2.2 Zinc binding studies of keto-pyrrolidinones

Zinc binding is an important feature in the inhibition of all MMPs. An attempt to bind zinc to the keto-pyrrolidinone **(2.17i)** was undertaken. As keto-pyrrolidinones and tetramic acids both exist in tautomeric forms it would be useful to determine which orientation is favoured around zinc and whether the keto-pyrrolidinones behave similarly to the tetramic acids. ^{13}C NMR analysis can aid in this determination. The sodium salt of the keto-pyrrolidinone **(A, 2.17i)** was reacted with zinc chloride[63] to give the product **(B or C)** (Scheme 2.9). The product obtained can be either **(B, 2.20i)** or **(C)** and it was possible to ascertain which was the more favoured.

A (2.17i) i) B (2.20i) OR C

Scheme 2.9

Reagents: i) a) NaOH (1 eq, 0.2 M), b) $ZnCl_2$ (1 eq, 0.1 M).

The ^{13}C NMR data of the starting material **(A, 2.17i)** and the product **(B, 2.20i)** were compared (Table 2.4). Significant shifts in the NMR data for C2, C3 and C4 of 11.5, 4.7 and –6.9 ppm respectively were observed. Interestingly, the change in chemical shift for C6 was only slight and this small difference suggests that keto-pyrrolidinones bind to zinc through the amide carbonyl **(B, 2.20i)** (Scheme 2.9). These results are consistent with binding studies of tetramic acids.

Keto-pyrrolidinone	**^{13}C (ppm)**				
	C2	**C3**	**C4**	**C5**	**C6**
A (2.17i)	154.6	165.6	120.5	60.4	191.6
B (2.20i)	166.1	170.3	113.6	60.7	190.9
Difference	11.5	4.7	-6.9	0.3	-0.7

Table 2.4: Comparison between non-bound and zinc bound keto-pyrrolidinone.

2.2.3 Asymmetric synthesis of keto-pyrrolidinones

So far all the compounds shown have been racemic. According to Zou[53] and co-workers it is possible to resolve certain keto-pyrrolidinones using a chiral HPLC column (Chiral Cel OD-H), (15 % iPrOH/hexane and 0.1 % TFA with a flow rate of 0.5 mL/min). In order to determine which enantiomer, if any, is active in the biological assay it would be beneficial to try to induce chirality into the final product. It may be possible to induce chirality in these reactions with an organic catalyst such as proline. Proline has been shown to be an effective organocatalyst[64-66] for many asymmetric transformations such as the aldol, Mannich, and Michael reactions.
Asymmetric enamine catalysis was first realised with the discovery of the proline-catalysed asymmetric intramolecular aldol reaction (also known as the Hajos-Parrish-Eder-Sauer-Wiechert reaction) in the 1970s. It was reported[67] that the reaction of triketones **(2.21)** in the presence of proline gave the aldol products **(2.22)** in good yields and high enantiomeric excess (Scheme 2.10). This reaction has been applied in the synthesis of many natural products such as the steroid, cortisone. [68, 69]

(2.21) i) ii) → **(2.22)** → → Cortisone

Scheme 2.10

Reagents: i) (*S*)-proline (3 mol%), DMF; ii) *p*-TsOH, benzene.

Proline catalysis has been extended to the 3-component Mannich reaction of ketones, aldehydes and amines to give *β*-amino ketones **(2.23)** in good yields and high ee's (Scheme 2.11). [65, 66] The scope of this asymmetric multi-component reaction has been explored. Various ketones can be employed and the most useful amine component has been shown to be *p*-anisidine. The reaction is tolerant of a broad and diverse range of aldehydes. Both aliphatic and aromatic aldehydes can be employed.

Scheme 2.11

Reagents: i) (*S*)-proline (25-30 mol%), DMSO.

The proposed mechanism[64] for the Mannich reaction is described as a proline-enamine reacting with an imine in the C-C bond forming and enantioselectivity-determining step. Both the imine and the enamine are formed *in situ* from the aldehyde and ketone in two separate pre-equilibria (Scheme 2.12).

Scheme 2.12

Our proposed mechanism shows how it may be possible to preform the enamine and react this with the imine to give an enantiomerically enriched compound (Scheme 2.13).

Scheme 2.13

To evaluate the success of this asymmetric synthesis, a method was needed for determination of the enantiomeric excess. Initially, a europium chiral shift reagent[70] was added to the keto-pyrrolidinone **(2.19g)** to determine whether we could use NMR to evaluate the enantiomeric excess. The results from this were disappointing as only a broadening of peaks was seen and not a separation. Separation of the racemate **(2.19g)** by chiral HPLC (Diacel, 250 mm x 4.6 mm, AD column) using the conditions outlined by Zou (page 57) was also unsuccessful.

The final method investigated to determine enantiomeric excess was to synthesise the Mosher ester. The Mosher ester was prepared[71] by deprotonation of keto-pyrrolidinone **(2.19g)** with sodium hydride followed by addition of R (-)-α-methoxy-trifluoromethyl-phenylacetyl chloride to give **(2.24g)** in low yield (28 %) (Scheme 2.14). Success was confirmed by a doubling of all peaks in the proton NMR spectrum. Synthesis of the Mosher ester is potentially a good method to determine whether the proline-catalysed reactions are providing enantioselectivity. A drawback may be that one enantiomer of the racemate may react at a different rate to the other and therefore would fail to give accurate results. Furthermore, under these basic conditions the chiral center may undergo racemisation. The ^{1}H NMR of the racemic

Mosher ester showed two sets of peaks although these were not of exact equal intensity, but in a ratio of 58:42.

(2.19g)

i)

(2.24g)

Scheme 2.14

Reagents: i) a) NaH (1 eq), dichloromethane, b) R (-)-α-methoxy-trifluoromethyl-phenylacetyl chloride (1.1 eq).

The one-pot condensation was undertaken using exactly the same starting materials as before, this time with the addition of (*S*)-proline (20 mol %) (Scheme 2.15). The enamine portion was preformed from the pyruvate and proline. The imine portion, also pre-mixed, was then added to the enamine in THF at room temperature. The Mosher ester of the product was then prepared and the ^{1}H NMR analysed. A ratio of 62:38 was observed which did not look very promising when compared with the racemic version.

Scheme 2.15

Reagents: i) dichloromethane ii) a) NaH (1 eq), dichloromethane; b) R (-)-α-methoxy-trifluoromethyl-phenylacetyl chloride (1.1 eq).

At this point several attempts had been made to try and induce chirality into keto-pyrrolidinones using proline as a catalyst under standard conditions.[65] A recent literature precendent[66] showed that proline catalysis induces chirality into Mannich products using NMP as the solvent at –20 °C. It was established that the reaction temperature was very important for this transformation. Following the report, several reactions were set up at this low temperature and the conditions were altered slightly. For the first reaction the imine and the enamine portion were preformed and the two portions added together at –20 °C. In the second reaction the imine was preformed with the proline present and the pyruvate was added later at –20 °C.

The Mosher ester[71] of both products were synthesised and the ^1H NMR analysed accordingly. The ratio of each reaction was 62:38 and 60:40 respectively and again did not look very promising when comparing with the racemic version. The attempts to induce chirality using proline do not appear to have worked. It is possible that the

background reaction is too fast even at low temperatures and the favoured pathway is not *via* a proline intermediate. Alternatively, derivatisation to the Mosher ester may not be a suitable method for determining the enantioselectivity of these reactions.

2.2.4 Reults of MMP inhibition assays

Dr Nakao tested the keto-pyrrolidinones synthesised in two assays, against MMP-2 and MT1-MMP. An original attempt indicated that the majority of the keto-pyrrolidinones were in fact inhibiting both of the enzymes. However, a subsequent assay was set up taking into account these compounds ability to quench fluorescence. As previously mentioned, this assay detects the amount of fluorescence produced, which in turn indicates the level of inhibition. After control experiments, two hits remained with low micromolar activity. Against MMP-2, the best results were for the keto-pyrrolidinones **(2.18a)** and **(2.18h)** which gave an IC_{50} of 35 and 50 μg/mL respectively (Figure 2.4).

(2.18a) (2.18h)

Figure 2.4

The same keto-pyrrolidinones **(2.18a)** and **(2.18h)** showed inhibition against MT1-MMP with an IC_{50} of 22 and 30 μg/mL respectively (Table 2.6). Care must be taken when analysing assay results. These compounds are fluorescenct and so initially gave false readings of enhanced activity. A control for each compound was set up to counteract these readings and give accurate results.

2.3 Conclusions and Future Work

A small array of keto-pyrrolidinones was synthesised using a broad and diverse range of aldehydes, amines and valerates. Attempts to determine the zinc binding mode of these compounds were made. It was concluded that the preferred mode is through the amide carbonyl which is consistent with data for the tetramic acids. Attempts to induce chirality into the keto-pyrrolidinone synthesis using proline-catalysed asymmetric reaction conditions proved unsuccessful but could be further explored. Repetition of the proline catalysed reactions using chiral HPLC as a detector could be investigated.

The array of keto-pyrrolidinones were tested for inhibition against MMP-2 and MT1-MMP. An initial assay gave hits for the majority of keto-pyrrolidinones. However, a second assay was performed taking into account the fluorescence quenching of the compounds which gave two hits. The keto-pyrrolidinones **(2.18a)** and **(2.18h)** showed inhibition against both MMP-2 and MT1-MMP. These results can be used to design a second-generation array of keto-pyrrolidinones to further improve activity.

The one-pot keto-pyrrolidinone synthesis has great scope as it can tolerate a wide variety of functional groups. For the imine component there is the possibility of using sulfonylimines or cyanoimines where the nitrogen atom has a cyano or sulfonyl group adding extra diversity. Focused libraries could be synthesised taking into account certain 'drug-like' properties such as whether they fit Lipinski's 'Rule of Five'. These compounds could also be tested against targets other than MMPs especially where chelation to a metal is involved.

Chapter Three

Synthetic Studies Towards the Structure Reported for Ageladine A

3.1 Introduction

3.1.1 Ageladine A as an MMP inhibitor

Recently, the hydrophilic extract of the marine sponge, *Agelas nakamuri*, found in southern Japan, has been shown to have significant inhibition[72] against MMP-2. MMP-2 (gelatinase A) is known to form a complex with MT1-MMP and TIMP-2 in the process of tumor metastasis and degrade type IV collagen, the main component of the extra-cellular matrix (ECM). MMP-2 is not only implicated in tumor metastasis but also in angiogenesis making it an attractive target for drug discovery. A fluorescent alkaloid, ageladine A (**3.1**) was isolated[72] from the extract and shown to be an inhibitor of MMP-2, 1, 8, 9, 12 and 13 with IC_{50} values of 2.0, 1.2, 0.39, 0.79, 0.33, and 0.47 μg/mL respectively.

Figure 3.1

A comparison between ageladine A and its *N*-methylated derivatives showed that while the former inhibited MMP-2 the derivatives **(3.2)**, **(3.3)** and **(3.4)** did not. Ageladine A **(3.1)** also inhibits cell migration of bovine endothelial cells at 5 μg/mL. Furthermore, it significantly inhibits vascular formation in mouse cells indicating it has an antiangiogenic effect. Many potent inhibitors of MMPs contain a zinc binding group. Thus, the chelating ability of ageladine A to Zn^{2+} was examined by way of a zinc chloride titration experiment. The results indicated that ageladine A does not exhibit chemical shift changes with up to 200 mol % of zinc(II)chloride. Kinetic analysis was performed using six substrate concentrations and seven ageladine concentrations. The lines were intersected on the X-axis in a Lineweaver-Burk plot indicating that ageladine A is a non-competitive inhibitor. It is presumed that the inhibition mechanism of ageladine A differs from those of other MMP inhibitors that chelate to the active site zinc.

The structure of ageladine A was elucidated using the following methods. LR-FABMS showed an $(M + H)^+$ peak at 356/358/360 in a ratio of 1:2:1, indicating that there are 2 bromines present. The molecular formula was established as $C_{10}H_7N_5Br_2$ on the basis of HR-FABMS and ^{13}C NMR data and indicates nine degrees of unsaturation. From ^1H, ^{13}C and 2D NMR correlation experiments the following signals have been assigned: δ_c 160.8 (C13), 147.1 (C10), 136.7 (C11), 133.0 (C8), 128.5 (C6), 125.7 (C2), 115.1 (C4), 107.7 (C5), 105.4 (C9), 102.3 (C3) and δ_H 8.04 (H8), 7.41 (H9), 7.17 (H4). The guanidine moiety was presumed as there were three nitrogen atoms remaining with exchangeable protons and a characteristic chemical shift at δ_c 160.8. The exchangeable protons were not observed in the ^1H NMR spectrum using various solvents such as CD_3OD, DMSO, $CDCl_3$, C_5H_5N with or without 1 % of TFA, even at low temperatures such as -20 °C.

So far, in these structural studies there were no long-range 2D-NMR correlations obtained between the pyrrole and pyridine fragments of the alkaloid. The *N*-methyl derivatives **(3.2)**, **(3.3)** and **(3.4)** were prepared in order to undertake further correlation studies and confirm the structure. For the tri-methyl derivative **(3.2)** correlations were observed between Me14 and Me15 with C13 and a further correlation between Me14 and C10 indicating the presence of the 2-aminoimidazole

ring. The tetra-methyl derivative **(3.3)** showed a correlation between H4 and C6 indicating a connection between C5 and C6. For the penta-methyl derivative **(3.4)** there was a correlation observed between Me4 and C3. These critical correlations, among others secured the structure of ageladine A.

3.1.2 Bromopyrroles from the marine sponges of the genus *Agelas*

In addition to ageladine A, there are over 60 bioactive bromopyrroles reported and isolated from the marine sponges of the genus *Agelas*. The majority are C11 or C22 oroidin derivatives[73] of which the polycyclic monomers, dibromocantharelline, dibromophakellin and odiline are examples (Scheme 3.1).

Scheme 3.1

It is proposed[73] that the biogenetic synthesis of this class of pyrrole-imidazole alkaloids begins from two simple starting blocks, 3-amino-1-(2-aminoimidazolyl)-prop-1-ene **(3.5)** and pyrrole-2-carboxylic acid or its bromo derivatives **(3.6)** (Scheme 3.1). The ambivalent reactivity of the aminoimidazole helps to explain how these compounds can be derived from just two building blocks. The electrophilic and nucleophilic nature depends on which tautomeric isomer of the aminoimidazole is involved (Scheme 3.2). A similar reactivity is exhibited by the vinylogous building block, 3-amino-1-(2-aminoimidazolyl)-prop-1-ene **(3.5)**. It is proposed that each tautomer engaged in the process may act as an initiator of controlled chain reactions

leading to a complex set of natural products. Due to the various modes of intramolecular cyclisation shown by the linear monomers such as oroidin it is possible to see how such polycyclic monomers, mentioned previously have been derived.[73]

nucleophilic position electrophilic position

Scheme 3.2

4,5-Dibromopyrrole-2-carbonitrile **(3.7)** and Latonduine A **(3.8)** which has a C10 skeleton are the two known exceptions to this class of amino-imidazole pyrroles. Latonduine A is a new alkaloid of this family with an unprecendented heterocyclic skeleton isolated[74] from the Indonesian marine sponge, *Stylissa carteri*. The structure was elucidated using spectroscopic methods. However, an alternate structure **(3.9)** was also proposed which could account for all the data (Figure 3.2). The alternate structure **(3.9)** was dismissed by comparison with the oroidin biogenetic building block **(3.10)**. The oroidin building block **(3.10)** has a minimum four carbon linear chain separating the amide nitrogen and the first point of attachment of a guanidine nitrogen (Figure 3.2). Verification of this biogenetic proposal was confirmed *via* a comparison between the synthetic and natural product.

Latonduine A **(3.8)** **(3.9)** Oroidin building block **(3.10)**

(3.7)

Figure 3.2

Latonduine A **(3.8)** does appear to be biogenetically related to the oroidin family of alkaloids. However, their skeletons are not derived from the same building blocks. This alkaloid has a six-membered aminopyrimidine substructure in place of the five membered aminoimidazole substructure of the oroidin family. Similar to latonduine A, ageladine A **(3.1)** appears to be related to the oroidin family, however it is the first of this family to contain a 2-aminoimidazolopyridine skeleton.

3.1.3 Proposed retrosynthesis of ageladine A and the Suzuki-Miyaura reaction

For our retrosynthetic analysis of ageladine A, the disconnection 'a' would give two fragments, a pyridine portion, **(3.11)** and a pyrrole portion, **(3.14)** (Scheme 3.3). This C-C bond could be formed by a palladium catalysed coupling of the two fragments. The imidazolopyridine **(3.11)** can be synthesised from the pyridinediamine **(3.12)**, which in turn can be synthesised from the commercially available starting material **(3.13)** (Scheme 3.3). The pyrrole boronic acid needed for cross coupling **(3.15)** is commercially available. Incorporation of the two bromines could be achieved after the cross coupling of the two fragments in order to avoid any self cross coupling of the pyrrole.

Scheme 3.3

The most obvious disconnection leaves us with an organometallic cross coupling reaction between the two fragments. The palladium catalysed, Suzuki-Miyaura reaction[75, 76] is one of the most powerful C-C bond forming reactions and uses Pd(0) as the catalyst. The general mechanism[77] of the cross coupling of organometallics with halides begins with oxidative addition of the halide to Pd(0) to generate a Pd(II) intermediate (Scheme 3.4). Oxidative addition is often the rate-determining step in the catalytic cycle. The relative reactivity decreases in the order of I > OTf > Br >> Cl. Less reactive halides can be activated by the proximity of electron withdrawing groups and vice versa. Transmetallation with the organometallic followed by reductive elimination expels the product and regenerates the Pd(0) catalyst (Scheme 3.4). For the Suzuki-Miyaura reaction, the transmetallation step requires that there is a base present as this accelerates the step, leading to a borate via a more nucleophilic 'ate' complex. An essential feature of the reaction is that R^1M and R^2X are electronically different so that R^2X combines with Pd(0) and R^1M combines with Pd(II). The halide partner must not have any β-hydrogens as elimination would decompose the intermediate whereas the metallic partner can be almost anything.

$$R^1M + R^2X \xrightarrow{Pd(0)} R^1\text{-}R^2 + M\text{-}X$$

Scheme 3.4

The most commonly used palladium(0) catalyst for the Suzuki-Miyaura reaction is tetrakis(triphenylphosphine)palladium(0), $Pd(PPh_3)_4$. However, this air sensitive catalyst will decompose readily to the inactive Pd(II). It can be readily prepared[77] from Pd(II) complexes such as $PdCl_2$ or $Pd(OAc)_2$ with an excess of PPh_3 and is best used *in situ*. $Pd(dba)_2$, another Pd(0) complex is prepared from the reaction of $PdCl_2$ with dibenzylideneacetone in methanol and is more air stable than $Pd(PPh_3)_4$. $Pd(dba)_2$ can be recrystallised[78] from chloroform to give the $Pd_2(dba)_3.CHCl_3$ complex which is equally as active as $Pd(dba)_2$. Both complexes ($Pd(dba)_2$ and $Pd_2(dba)_3.CHCl_3$) can be easily handled and stored. Upon treatment with phosphines they produce yellow solutions of catalytically active PdL_n species.[76]

The palladium-mediated cross coupling of aryl halides has become widely used for the formation of carbon-carbon bonds. However, most methods do not extend to aryl chlorides. In particular much less has been reported on the cross coupling of electron rich aryl chloride substrates with organoboron reagents. Literature precedents[79, 80] by Buchwald show that it is possible to use a mixture of palladium acetate with various ligands such as the biphenyl examples, *o*-(dicyclohexylphosphino)biphenyl **(3.16)** or *o*-(di-tert-butylphosphino)biphenyl) **(3.17)** to catalyse room temperature Suzuki couplings of aryl chlorides (Figure 3.3). The biphenyl-based phosphine ligand **(3.18)** has been used to promote the cross coupling of aryl sulfonates.[80] Moreover, these ligands are air stable and require no special handling unlike simpler phosphine ligands used in the Suzuki reacton.

Figure 3.3

By varying the conditions, in particular the ligand, Buchwald successfully performed a cross coupling reaction[81] of 3-chloropyridine with 3-acetylphenylboronic acid to give the product **(3.19)** in good yield (92 %) (Scheme 3.5). A temperature of 50 °C was necessary in order for this reaction to proceed as opposed to room temperature for the bromide substrates.

Cl, B(OH)$_2$, i), N, O, **(3.19)**

Scheme 3.5

Reagents and conditions: i) Pd(OAc)$_2$ (1 mol %), Ligand **(3.17)** (2 mol %), KF (3 eq), THF, 50 °C.

Fu reported a general method[82] for the Suzuki cross coupling reaction of aryl chlorides and boronic acids in the presence of a Pd$_2$(dba)$_3$/P(*t*-Bu)$_3$ catalyst system with KF as the base in dioxane. In addition, the reactions were performed at room temperature using very low catalyst loadings making this an attractive system to use. 2-Chloro-substitued pyridines, which have the potential to bind to palladium through the nitrogen atom are suitable substrates for room temperature Suzuki reactions. Under these conditions 2-chloropyridine reacted with 2-methylphenylboronic acid to give the coupled product **(3.20)** in excellent yield (97 %) (Scheme 3.6).

N, Cl, B(OH)$_2$, i), **(3.19)**

Scheme 3.6

Reagents and conditions: i) Pd$_2$(dba)$_3$ (0.5 mol %), P(*t*-Bu)$_3$ (1 mol %), KF (3.3 eq), THF, r.t.

Electron rich aryl chlorides need much harsher reaction conditions, including higher temperatures and higher ligand to catalyst ratio in order to proceed. An example is 4-chloroaniline whereby there is an electron donating amine group present.[82] The reaction with phenylboronic acid proceeded at 90 °C with a ratio of 1.5:1 of P(t-Bu)$_3$:Pd$_2$(dba)$_3$ to give the biaryl (**3.21**) in good yield (80 %) (Scheme 3.7).

H$_2$N–C$_6$H$_4$–Cl + C$_6$H$_5$–B(OH)$_2$ —i)→ H$_2$N–C$_6$H$_4$–C$_6$H$_5$ **(3.21)**

Scheme 3.7

Reagents and conditions: i) Pd$_2$(dba)$_3$ (1.5 mol %), P(*t*-Bu)$_3$ (4.5 mol %), KF (3.3 eq), THF, 90 °C.

Furthermore, a cross coupling reaction of a Boc-pyrrole boronic acid **(3.22)**, similar to our Boc-pyrrole boronic acid **(3.15)** was reported.[83] The pyrrole boronic acid **(3.22)** was successfully coupled to the triflate **(3.23)** to give the biaryl **(3.24)** in moderate yield (57 %) (Scheme 3.8). The particularly low yield was ascribed to the inherent lability of the boronic acid **(3.22)**. Deborylation could not be avoided even under non-aqueous conditions.

(3.22) + **(3.23)** —i)→ **(3.24)**

Scheme 3.8

Reagents and conditions: i) Pd(PPh$_3$)$_4$ (0.05 mol %), LiCl (3 eq), DME, 85 °C.

3.1.4 Conclusion

The alkaloid, ageladine A has been isolated and shown to be an MMP inhibitor. It appears to be biogenetically related to the oroidin family of alkaloids. However, the skeleton is not derived from the same building blocks. Ageladine A is the first of this family to contain a 2-aminoimidazolopyridine skeleton. Retrosynthetic analysis of ageladine A has led to a cross coupling reaction between the pyrrole and pyridine fragments.

3.2 Results and Discussion

3.2.1 Synthesis of fragments of ageladine A

Initial attempts to synthesise the two fragments, **(3.11)** and **(3.15)** highlighted in the retrosynthetic analysis were undertaken. It is possible to synthesise the halide fragment for the Suzuki reaction from the cheap and commercially available starting material, 4-aminopyridine. 4-Aminopyridine was nitrated *via* a known literature procedure[84] to give 4-nitraminopyridine **(3.25)** which isomerised to give the product **(3.26)** in good yield (78 %). 3-Nitro-4-aminopyridine **(3.26)** was reductively chlorinated[85] with stannous chloride in hot concentrated HCl to give the desired fragment, 2-chloro-3,4-diaminopyridine **(3.27)** in good yield (90 %) (Scheme 3.9). This chosen route to the halide fragment is very efficient as it is high yielding (71 %) and only three steps.

NH_2 —i)→ $NHNO_2$ **(3.25)** —ii)→ NH_2, NO_2 **(3.26)** —iii)→ NH_2, NH_2, Cl **(3.27)**

Scheme 3.9

Reagents and conditions: i) HNO_3, H_2SO_4 5 h; ii) H_2SO_4, 30 min, 90 °C; iii) $SnCl_2$(10 eq), HCl, 2 h, 90 °C.

The next step in the synthesis of fragment 1 was preparation of the imidazolopyridine skeleton. Cyclisation of the diamine **(3.27)** to give the guanidine moiety **(3.28)** was achieved[86] by addition of cyanogen bromide in methanol (Scheme 3.10). The yield for this reaction was particularly low (23 %) and would need optimising for this synthesis to be viable. Protection of the primary amine as a Boc group[87] to give **(3.29)** proceeded in moderate yield (59 %). This protection facilitated handling of the guanidine moiety as it was less polar. A second benefit is that protection makes the amine less electron donating. The aryl chloride bond would then be potentially more reactive to the palladium cross coupling reaction with the Boc group present rather than the free guanidine.

Scheme 3.10

Reagents and conditions: i) CNBr (5 eq), MeOH, 24 h, reflux; ii) Boc_2O (1.5 eq), Et_3N (1.2 eq), MeOH, 30 min (0 °C), 18 h (r.t.).

For the synthesis of the second fragment, the boronic acid **(3.15)** was initially purchased from Aldrich. However, due to the cost it was later prepared from pyrrole in two steps following known literature procedures. Pyrrole was protected[88] using di-*tert*-butyl-dicarbonate to give the protected derivative **(3.30)** in good yield (80 %). This reaction is suitable for scale-up and large quantities of Boc-pyrrole can be synthesised and stored. The desired boronic acid **(3.15)** was synthesised from Boc-pyrrole using a known literature procedure[89] in good yield (61 %). The boronic acid **(3.15)** is best stored below 0 °C as it is unstable and decomposes back to Boc-pyrrole.

Scheme 3.11

Reagents and conditons: i) Boc_2O (1.5 eq), Et_3N (1.2 eq), MeCN; ii) Li(TMP) (1.24 eq), $B(OMe)_3$ (5 eq), THF, 2 h (-78 °C), 18 h (r.t.).

The synthesis of the two fragments **(3.27)** and **(3.15)** was completed in good overall yields (71 %) and (48 %) respectively. The next stage in the synthesis was to couple the two fragments together using palladium chemistry.

There are many different conditions[79-82, 90] published in the literature for the Suzuki reaction. However, the standard conditions[75] employ tetrakis(triphenylphosphine)palladium(0) (1-5 mol %) with a base such as sodium carbonate and the reaction performed under argon atmosphere. A model study of the Suzuki cross coupling reaction using these standard conditions was attempted for some simple systems. The halide partners chosen for the trial reactions were bromobenzene, 2-bromopyridine and 2-chloropyridine. Each halide was reacted with Boc-pyrrole-boronic acid under the standard Suzuki conditions. The desired products **(3.31)** and **(3.32)** from the bromo-precursors were obtained in reasonable yields (59 %) and (29 %) respectively. Chloropyridine is much less reactive in the Suzuki reaction than its bromopyridine counterpart and was confirmed by isolation of the product **(3.32)** in low yield (18 %).

Ar—X + (3.15) [N-Boc-pyrrole-2-B(OH)$_2$] —i)→ [N-Boc-2-Ar-pyrrole]

(3.31) Ar = Ph, X = Br
(3.32) Ar = 2-Pyridyl, X = Br or Cl

Scheme 3.12

Reagents and conditions: i) $Pd(PPh_3)_4$ (3 mol %), Na_2CO_3 (2 eq), DME/EtOH/H_2O, 16 h, 80 °C.

Further trial reactions were undertaken with 2-bromopyridine, varying the Suzuki conditions to obtain the optimum yield (Scheme 3.13). The complex $Pd_2(dba)_3.CHCl_3$ **(3.34)** was easily prepared[76] from the reaction of dibenzylidene acetone **(3.33)** with palladium(II)chloride and can be used to catalyse the Suzuki reaction. The best conditions[82] found so far for the cross coupling reaction were with $Pd_2(dba)_3.CHCl_3$ as the catalyst, triphenylphosphine as the ligand and sodium carbonate as the base at 80 °C to give **(3.32)** in moderate yield (43 %) (Table 3.1, Entry 2). High temperatures and prolonged reaction times caused the Boc group to fall off and often two products were obtained from these reactions, the protected **(3.32)** and unprotected **(3.35)** pyrrole. A trial Suzuki reaction employing EnCat™, an encapsulated palladium source[91] was performed (Table 3.1, Entry 3). The best yield obtained for these

conditions (26 %) was considerably lower than for the standard conditions (Table 3.1, Entry 3).

Scheme 3.13

Entry	Catalyst/Ligand	Solvent	Base	Temp	Yield (3.32) vs (3.35)	
1	$Pd_2(dba)_3.CHCl_3$/ PPh_3 3 mol/ 6 mol %	tol/EtOH/ H_2O	Na_2CO_3	rt	10 %	
2	$Pd_2(dba)_3.CHCl_3$/ PPh_3 5 mol/ 15 mol %	tol/EtOH/ H_2O	Na_2CO_3	80 °C	43 %	35 %
3	EnCat™	IPA	Na_2CO_3	80 °C	26 %	
4	$Pd(PPh_3)_4$ 3 mol%	DME/EtOH/ H_2O	Na_2CO_3	reflux	28 %	44 %

Table 3.1: Conditions for the Suzuki cross coupling of 2-bromopyridine and Boc-pyrrole boronic acid.

The model study also allowed us to test our idea of late stage halogenation for the ageladine A total synthesis. In a trial reaction, bromination was effected[92] by addition of NBS to a solution of the free pyrrole **(3.35)** at -78 °C (Scheme 3.14). The reaction was difficult to monitor by TLC as the change in Rf from starting material to brominated products was negligible. Four equivalents of NBS were added in this reaction as it appeared initially that no reaction had occurred. A mixture of tri- and di-brominated products **(3.36)** and **(3.37)** were obtained and were inseparable by column chromatography.

(3.35) (3.36) (3.37)

80% combined

Scheme 3.14

Reagents and conditions: i) NBS (4 eq), THF, 30 min (-78 °C), 2 h (-20 °C).

3.2.2 Synthesis of chloropyridine precursors for the Suzuki cross coupling reaction

Initial attempts using standard Suzuki conditions to couple our chloropyridines **(3.27)**, **(3.28)** and **(3.29)** and boronic acid **(3.15)** together proved unsuccessful. It is more difficult for palladium to insert into a carbon-chloride bond especially in our system where there are neighbouring electron-donating groups which deactivate the C-Cl bond and may poison the catalyst by coordination. A recent paper by Caron reported[90] that the Suzuki coupling of 2-chloro-3-aminopyridine **(3.38)** with phenylboronic acid proved unsuccessful. However, upon protection of the aminopyridine as the acetamide **(3.39)**, the palladium mediated coupling proceeded to give **(3.40)** in good yield (80 %) (Scheme 3.15).

(3.38) (3.39) (3.40)

Scheme 3.15

Reagents and conditions: i) AcCl (1.1 eq), Et_3N (1.1 eq), dichloromethane; ii) phenylboronic acid (1.3 eq), $Pd(PPh_3)_4$ (0.3 mol %), Na_2CO_3 (1.6 eq), EtOH/toluene/H_2O, 80 °C.

Based upon the information above, we thought that in order for the Suzuki cross coupling reaction to have any chance of success then at least one of the amines

needed to be protected. The synthesis of the mono- and di-Boc protected amines **(3.41)** and **(3.42)** was undertaken[93] using Boc-anhydride to give the products in low yields (56 %) and (6 %) respectively (Scheme 3.16). The diamine **(3.27)** was also protected as the diacetamide[90] **(3.43)** using acetyl chloride in the presence of base to give the product in low yield (12 %). The isolated yields of these protection reactions were not very promising but there was enough material to react on further in the Suzuki reaction.

NH2 NH2 N Cl **(3.27)** i) → NHBoc NH2 N Cl **(3.41)** + NHBoc NHBoc N Cl **(3.42)**

ii) ↓

NHAc NHAc N Cl **(3.43)**

Scheme 3.16

Reagents and conditions: i) Boc_2O (2.2 eq), Et_3N (2.2 eq), MeOH, 16 h, reflux; ii) AcCl (2.2 eq), Et_3N (2.2 eq), dichloromethane, 18 h.

Various conditions were employed for the cross coupling reaction between the protected amino chlorides **(3.41)**, **(3.42)**, and **(3.43)** and the boronic acid **(3.15)** (Table 3.2). A variety of catalysts and ligands were used, such as replacing triphenylphosphine with the more labile *tert*-tributylphosphine. Fu reported[82] that the *tert*-tributylphosphine ligand gives improved results for the cross coupling reaction of less reactive chlorides especially when used in conjunction with the $Pd_2(dba)_3.CHCl_3$ catalyst. All attempts to use this system with a variety of bases and solvents proved unsuccessful. The X-phos, Buchwald ligand[80] **(3.18)** was used as a replacement to the *tert*-tributylphosphine ligand with no success (Table 3.2, Entry 4). The various conditions attempted (Table 3.2) did not give any of the desired products.

Furthermore, prolonged reaction times and high temperatures caused deborylation and the boronic acid decomposed back to the Boc-pyrrole.

R–Cl + (N-Boc-pyrrol-2-yl)$B(OH)_2$ —Suzuki→ (N-Boc-pyrrol-2-yl)R

Scheme 3.17

	Substrate R	Catalyst/Ligand	Solvent	Base	Temp
1	NHBoc, NH_2, N, Cl	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3	50 °C
2	NHBoc, NH_2, N, Cl	$Pd_2(dba)_3.CHCl_3$/ $P(tBu)_3$ 1.5 mol/4.5 mol %	Dioxane	KF	80 °C
3	NHBoc, NH_2, N, Cl	$Pd_2(dba)_3.CHCl_3$/ $P(tBu)_3$ 3 mol/9 mol %	DME/EtOH/ H_2O	Na_2CO_3	80 °C
4	NHBoc, NH_2, N, Cl	$Pd_2(dba)_3.CHCl_3$/ X-Phos 3 mol/9 mol %	THF	KF	80 °C
5	NHBoc, NHBoc, N, Cl	$Pd_2(dba)_3.CHCl_3$/ $P(tBu)_3$ 1.5 mol/4.5 mol %	Dioxane	KF	80 °C
6	NHAc, NHAc, N, Cl	$Pd_2(dba)_3.CHCl_3$/ PPh_3 5 mol/15 mol %	DME/EtOH/ H_2O	Na_2CO_3	80 °C

Table 3.2: Suzuki reactions attempted with a variety of protected diamines and boronic acid **(3.15)**.

The synthesis of a second series of precursors was undertaken from the diamino-chloropyridine **(3.27)** (Scheme 3.18). The urea **(3.44)** was synthesised by condensation[94] with trichloromethyl chloroformate in moderate yield (56 %). The thiourea ring was generated[95] by treatment of the diamine with carbon disulfide to give the desired product **(3.46)** in good yield (79%). Both of these compounds could be converted to the desired guanidine moiety by known methods.[96] The thiourea was further functionalised using methyl iodide[97] to give the S-methyl derivative **(3.47)** in good yield (71 %). Another idea was to perform a Finkelstein reaction[98] and generate

the iodide from the chloride, which would be more susceptible to palladium insertion. Treatment of diamine **(3.27)** with anhydrous sodium iodide in acetone gave the iodopyridine **(3.45)** in low yield (30%) (Scheme 3.18). Further halogen exchange reactions on alternative precursors all failed. These precursors have the potential to be more susceptible than the diamino-chloropyridine **(3.27)**, to palladium insertion in the Suzuki reaction. Furthermore, if the cross coupling reactions proved to be successful, then the products formed are potential analogues of ageladine A and can be tested for activity against MMPs.

NH_2 NH_2 N Cl **(3.27)**

i) ii) iii)

O HN NH N Cl **(3.44)**

NH_2 NH_2 N I **(3.45)**

S HN NH N Cl **(3.46)**

iv)

SMe N NH N Cl **(3.47)**

Scheme 3.18

Reagents and conditions: i) $ClCOOCCl_3$ (1.2 eq), MeOH, 16 h; ii) NaI (5 eq), acetone, 24 h, reflux; iii) CS_2 (2 eq), Et_3N (2 eq), EtOH, 48 h, 45 °C; iv) MeI (1.05 eq), Et_3N (1.1 eq), DMF, 24 h, 100 °C.

Attempts at palladium mediated cross-couplings, with these new synthetic precursors, were undertaken. Initially, the conditions[90] as described by Caron, (Page 80) were employed. The following chlorides, urea **(3.44)**, thiourea **(3.46)**, the *S*-methyl derivative **(3.47)**, the *N*-Boc guanidine **(3.29)** and also the 2-iodo-3,4-diaminopyridine **(3.45)** were treated with $Pd(PPh_3)_4$ and Na_2CO_3 with various boronic acids under argon in order to achieve cross-coupling (Table 3.3, Entry 1, 2, 4, 5 and 10). These initial conditions did not give any of the desired products. A combination of boronic acids, bases, catalysts, ligands and solvents have further been employed. The conditions[80] set by Buchwald were followed for our substrates using the biphenyl-based phosphine ligand **(3.18)** (2 mol %) and $Pd(OAc)_2$ (1 mol %) as the source of palladium (II) (Table 3.3, Entries 3,6). The reaction did not proceed at room temperature or under refluxing conditions and the starting material was recovered. The conditions[82] set by Fu were also followed and $P(t\text{-}Bu)_3$ was used in conjuntion with $Pd_2(dba)_3$ but did not give any desired product (Table 3.3, Entries 13, 9). The conditions shown in Table 3.3 did not yield any of the desired products.

	Substrate	Boronic acid	Catalyst/Ligand	Solvent	Base
1	NH_2 NH_2 N I	$PhB(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3
2	S HN NH N Cl	N Boc $B(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3
3	S HN NH N Cl	N Boc $B(OH)_2$	$Pd(OAc)_2$/**(3.18)** 1 mol/2 mol %	THF	KF
4	O HN NH N Cl	$PhB(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3
5	NHBoc N NH N Cl	$B(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3
6	NHBoc N NH N Cl	$B(OH)_2$	$Pd(OAc)_2$/**(3.18)** 1 mol/2 mol %	THF	KF
7	NHBoc N NH N Cl	$B(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	THF	KF
8	NHBoc N NH N Cl	N Boc $B(OH)_2$	$Pd(OAc)_2/PPh_3$ 5 mol/15 mol %	DMF	Na_2CO_3
9	NHBoc N NH N Cl	N Boc $B(OH)_2$	$Pd_2(dba)_3.CHCl_3$/ $P(tBu)_3$ 1.5 mol/4.5 mol %	Dioxane	KF
10	SMe N NH N Cl	$B(OH)_2$	$Pd(PPh_3)_4$ 3 mol %	tol/EtOH/H_2O	Na_2CO_3

11	SMe, N=, NH, N, Cl	B(OH)2	$Pd(PPh_3)_4$ 3 mol %	DME/EtOH/ H_2O	Na_2CO_3
12	SMe, N=, NH, N, Cl	N, Boc, B(OH)2	$Pd(OAc)_2/PPh_3$ 5 mol/15 mol %	DMF	Na_2CO_3
13	SMe, N=, NH, N, Cl	N, Boc, B(OH)2	$Pd_2(dba)_3.CHCl_3/$ $P(tBu)_3$ 1.5 mol/4.5 mol %	Dioxane	KF

Table 3.3: Suzuki conditions employed for the coupling of chloro precursors to various boronic acids.

Alternative metal cross-couplings were investigated. The reaction of bromopyridine with pyrrole in the presence of silver triflate did not give the desired product. Similarly, attempts at Ulmann chemistry using copper or zinc chloride[99] with either the free pyrrole or its corresponding magnesium salt[100] and coupling with bromopyridine all proved unsuccessful (Scheme 3.19).

No cross-coupling ← , AgOTf (N-H pyrrole) — 2-bromopyridine (N, Br) — (N-H pyrrole) or (N-MgCl pyrrole), CuCl or Zn → No cross-coupling

Scheme 3.19

So far, all of the Suzuki reactions attempted with our desired precursors have been unsuccessful. Even with protection of both of the amines thus reducing their electron donating ability, it appears that the chloride precursors are too unreactive to palladium insertion. The scope of the Suzuki-Miyaura reaction is vast and there are many more conditions in the literature for the cross coupling of chlorides using a wide variety of catalysts, ligands and bases. However, examples were not found in the literature with two electron donating groups present in the halide partner. Attempts at cross coupling using other metals such as copper and zinc did not give any desired products. In the initial trials, 2-bromopyridine was coupled with Boc-pyrrole-boronic acid **(3.15)** employing $Pd(dba)_2.CHCl_3$ as the palladium(II) source in moderate yield (43 %) (Table 3.1, Entry 2). The success of the reaction suggested that we needed a bromo-

rather than a chloropyridine as our precursor for the Suzuki reaction. The next stage was to synthesise a bromo partner for the cross coupling.

3.2.3 Synthesis of bromopyridine alternative for the Suzuki reaction

The attractiveness of the reductive chlorination[85] step to give the diamino chloride **(3.27)** led us to investigate the reaction with tin bromide to give the corresponding diamino bromide **(3.48)**. Nitro-aminopyridine **(3.26)** was treated with either tin bromide in HBr or tin chloride in HBr and heated to 90 °C. These conditions did not yield the desired bromide **(3.48)** and the only product isolated from the reaction was the reduced 3,4-diaminopyridine **(3.49)** confirmed by crude ^{1}H NMR analysis (Scheme 3.20).

NH2 NO2 N **(3.26)** i) i) NH2 NH2 N Br **(3.48)** NH2 NH2 N **(3.49)**

Scheme 3.20

Reagents and conditions: i)a) $SnBr_2$ (10 eq), HBr, 1 h, 90 °C; b) $SnCl_2$ (10 eq), HBr, 1 h, 90 °C.

An alternative route to our desired bromopyridine was *via* an initial nitration[101] of dihydroxypyridine to give the desired product **(3.51)** in good yield (75 %). Conversion of the dihydroxy-nitropyridine **(3.51)** to the corresponding dichloropyridine is known in the literature.[101] The procedure was adapted using $POBr_3$ instead of $POCl_3$ and upon heating for 3 days gave a mixture of compounds from which the dibromopyridine **(3.52)** was isolated in low yield (22 %) (Scheme 3.21). Attempts to improve upon the yield of this step using NBS in conjunction with triphenylphosphine and also resin bound triphenylphosphine were both

unsuccessful.[102] Selective displacement[103] of the bromine in the 4-position is known and efforts to displace this bromine using methanolic ammonia gave the desired product **(3.53)** but in a very low yield (8 %). This route gave the desired 4-amino-3-nitro-bromopyridine **(3.53)** in an overall yield of 13 %. The yields of the last two steps were very poor as a result of mixtures of products being obtained. The selective displacement of the bromine at the 4-position was confirmed *via* a comparison between the ^{1}H NMR of the product against the ^{1}H NMR of the same compound synthesised by a different route.

OH, OH, N (3.50) —i)→ OH, NO2, OH, N (3.51) —ii)→ Br, NO2, Br, N (3.52) —iii)→ NH2, NO2, Br, N (3.53)

Scheme 3.21

Reagents and conditions: i) HNO_3, H_2SO_4, 30 min; ii) $POBr_3$ (20 eq), 150 °C; iii) NH_3/MeOH (excess).

A longer yet more efficient route to the same amino-nitro-bromopyridine **(3.53)** was achieved on a gram scale based upon several literature precedents.[104, 105] Nitration of 2-bromopyridine-*N*-oxide **(3.54)** gave the 4-nitro compound[104] **(3.55)** in good yield (74 %) (Scheme 3.22). A dual reduction[104] of the nitro group and the *N*-oxide in the presence of iron in acetic acid gave 4-amino-2-bromopyridine **(3.56)** in a good yield (80 %). Nitration[105] of the amine initially gave the 4-nitramino-2-bromopyridine **(3.57)** in excellent yield (99 %). Conversion to the isomer **(3.53)** by treatment of **(3.57)** with conc H_2SO_4 and heating to 90 °C proceeded in a moderate yield (55 %).[106] The isomerisation also produced the alternative isomer **(3.58)** in a low yield (7 %) (Scheme 3.22).

Scheme 3.22

Reagents and conditions: i) HNO_3, H_2SO_4, 2 h, 90 °C; ii) Fe (5 eq), AcOH, 1 h, 100 °C; iii) HNO_3, H_2SO_4, 5 h; iv) H_2SO_4, 0.5 h, 90 °C.

3.2.4 Suzuki cross coupling of bromopyridine and subsequent bromination reactions

To complete the synthesis of ageladine A the two fragments needed to be coupled together, two bromines incorporated, the diamine reduced and finally the guanidine formed. A Suzuki cross coupling of the two fragments **(3.53)** and **(3.15)** using standard conditions[90] of $Pd(OAc)_2$ as the palladium source in conjunction with triphenylphosphine as the ligand and sodium carbonate as the base worked in moderate yield (55 %) to give the desired product **(3.59)** (Scheme 3.23). The reaction needed to be heated to 80 °C in order to go to completion as indicated by the disappearance of the boronic acid. However, a competing reaction with the Suzuki-Miyaura was deborylation and at high temperatures this occured quite readily to give Boc-pyrrole which would no longer react. Also, under these conditions the Boc group was removed and the free pyrrole **(3.60)** was obtained in a low yield (10 %). It was thought that bromination at this stage would favour addition to the pyrrole rather than the pyridine as it is more electron rich and therefore more susceptible to electrophilic substitution.

(3.53) (3.14) (3.59) (3.60)

55 % 10 %

Scheme 3.23

Reagents and conditions: i) $Pd(OAc)_2$ (5 mol %), PPh_3 (15 mol %), Na_2CO_3 (2 eq), DME/EtOH/H_2O, 5 h, 80 °C.

Bromination of the Boc protected compound **(3.59)** at –50 °C using NBS[92] gave the mono-brominated pyrrole **(3.61)** as the major product in moderate yield (48 %) (Scheme 3.24). The dibrominated compound **(3.62)**, whereby the second substitution occurred on the pyridine and not on the pyrrole was obtained in moderate yield (42 %). The presence of the Boc group deactivates the pyrrole and makes it less electron rich. This effect, in conjuntion with the electron donating amine group on the pyridine, making the 5-position more electron rich, means that the second bromination prefers to occur on the pyridine.

(3.59) (3.61) (3.62)

48% 42%

Scheme 3.24

Reagents and conditions: i) NBS (2 eq), THF, 10 min (-50 °C) then 20 min (r.t.).

To avoid the problem of bromination occurring on the pyridine, the Boc group was removed[107] in quantitative yield using TFA to give the free pyrrole **(3.60)** (Scheme 3.25). As mentioned previously, the bromination reactions were difficult to monitor

by TLC analysis (Page 79). The Rf of the product did not vary considerably from the starting material and so it was difficult to estimate completion of the reaction. Initally, an overall amount of four equivalents of NBS were added to the free pyrrole **(3.60)** to give tetra-brominated **(3.63)** and tri-brominated **(3.64)** compounds in good yields (49 %) and (43 %) respectively (Scheme 3.25). Bromination of the pyridine fragment occurred when an excess of NBS was employed. Treatment of the pyrrole **(3.60)** with just two equivalents of NBS for 2 h at –78 °C gave the tri-brominated and **(3.65)** di-brominated **(3.66)** compounds in low to moderate yields (17 %) and (56 %) respectively (Scheme 3.25). The major product from this reaction was the desired di-brominated pyrrole **(3.66)** which is a precursor to ageladine A. A reduction of the nitro group, followed by formation of the guanidine would give ageladine A in only 5 steps overall from the synthetic precursors **(3.53)** and **(3.15)**.

NH_2 NO_2 Boc **(3.59)** i) → NH_2 NO_2 HN **(3.60)** ii) → Br NH_2 NO_2 Br Br HN Br **(3.63)** 49 % + NH_2 NO_2 Br Br HN Br **(3.64)** 43 %

(3.60) iii) ↓ NH_2 NO_2 Br Br HN Br **(3.65)** 17 % + NH_2 NO_2 Br HN Br **(3.66)** 56 %

Scheme 3.25

Reagents and conditions: i) TFA (3 eq), dichloromethane, 0.5 h; ii) NBS (4 eq), THF, 10 min (-50 °C) then 16 h (r.t.); iii) NBS (2 eq), THF, 2 h, -78 °C.

Reduction of the nitro group using standard metal reduction conditions,[84] in our case tin(II)chloride/HCl at 90 °C for 2 h worked well. However, the two bromines were

sensitive to these reaction conditions and the pyrrole was debrominated to give the diamine **(3.67)** in quantitative yield (99 %) (Scheme 3.26). Complete reduction did not occur if the reaction was performed for less than 2 h. After 1 h, using reduction conditions[108] of zinc(II)chloride in HCl, an unidentified intermediate product was obtained which was neither the nitro compound **(3.60)** nor the final amine **(3.67)**. Under these conditions the two bromines were again removed.

NH_2 NO_2 N HN Br Br **(3.66)** i) NH_2 NH_2 N HN **(3.67)**

Scheme 3.26

Reagents and conditions: i) a) $SnCl_2$ (5 eq), HCl, 2 h, 90 °C; b) $ZnCl_2$ (5 eq), HCl, 1 h, 90 °C.

Alternative conditions for this transformation were investigated. Reduction using hydrogenation[109] conditions, H_2 and Pd/C did not remove the bromines nor reduce the nitro group. An alternative neutral reduction[110] with Fe/NH_4Cl was also unsuccessful (Scheme 3.27).

NH_2 NO_2 N HN Br Br **(3.66)** i) NH_2 NH_2 N HN Br Br

Scheme 3.27

Reagents: i)a) H_2, Pd/C; b) Fe/NH_4Cl.

The next stage in the synthesis was to find an alternative order of events in order to overcome the problems with over-bromination. Reduction of the nitro group and removal of the Boc group was effected using tin(II)chloride in HCl to give the

diamine **(3.67)** in good yield (84 %) (Scheme 3.28). To complete the synthesis of ageladine A the guanidine moiety needed to be formed followed by a bromination. Bromination as the penultimate step would could occur preferentially on the 2 and 3 positions of the pyrrole as the 4 position is more hindered.

Scheme 3.28

Reagents and conditions: i) $SnCl_2$ (5 eq), HCl, 3 h, 90 °C.

Preparation of the guanidine moiety was not as straightforward as was anticipated. Treatment of the diamine **(3.67)** with cyanogen bromide[86] using various conditions did not give any of the desired guanidine **(3.68)**. This reaction worked previously (Page 77) for the diamino-chloropyridine **(3.27)** in low yield but would not work on this precursor. An alternative guanidine forming reaction known in the literature[111] reacts a diamine with guanidine and heats to very high temperatures. These conditions were also tried on our system but were again unsuccessful. Di(imidazole-1-yl)methanimine[112] **(3.69)** can be prepared from the reaction between imidazole and cyanogen bromide. Another attempt at guanylation using di(imidazole-1-yl)methanimine **(3.69)** did not give any isolable product. The diamine was then treated with an excess of cyanogen bromide and heated for 3 days in a sealed vessel. These harsh conditions did not give the desired product. Instead the tri-brominated diamine **(3.70)** and traces of the tri-brominated guanidine **(3.71)** were obtained.

Major Product   Trace

Scheme 3.29

Reagents and conditions: i) a) BrCN (5 eq), EtOH, 48 h, 60 °C; b) guanidine (10 eq), 250 °C; c) di(imidazole-1-yl)methanimine **(3.69)** (5 eq), MeOH; iii) BrCN (10 eq), EtOH, sealed tube, 3 days, 100 °C.

Attempts to convert the thiourea **(3.72)** to the desired guanidine **(3.68)** were undertaken. The thiourea **(3.72)** was synthesised using a standard literature procedure[95] whereby the diamine **(3.67)** was heated to 50 °C and treated with carbon disulfide in the presence of base, Et_3N, to give the thiourea **(3.72)** in moderate yield (49 %) (Scheme 3.30). There are numerous methods[4, 113-115] for converting thioureas to the corresponding guanidines and they all generally use an excess of ammonia in a sealed vessel. Conversion of the thiourea **(3.72)** to the guanidine **(3.68)** in the presence of ammonia in a sealed vessel, using activators such as DCC and the water soluble EDC (Table 3.4) were attempted[4, 113, 114] but did not work for our system. The use of lead oxide in the presence of ammonia to convert thioureas to guanidines is also a known method.[115] The lead coordinates to the sulfur making it a better leaving

group so the ammonia can displace it. These conditions were used for our substrate **(3.42)** but no desired product was isolated from the reaction. The conditions employed were predominantly on the chloro-substrate **(3.42)** (Table 3.4).

(3.67) —i)→ **(3.72)** ---NH_3, sealed vessel---> **(3.68)**

Scheme 3.30

Reagents and conditions: i) CS_2 (17 eq), Et_3N (3 eq), EtOH, 48 h, 50 °C.

Substrate	Conditions	Solvent	Temperature °C
(3.68)	DCC	DMF	100
(3.42)	DCC	DMF	100
(3.42)	EDC/DIPEA	^{i}prOH	65
(3.42)	EDC/DIPEA/DMAP	DMF	100
(3.42)	PbO	MeOH	110

Table 3.4: Conditions for the conversion of thiourea to guanidine using NH_3 in a sealed vessel.

The Katritzky reagent,[116] di(benzotriazole-1-yl)methanimine **(3.73)** was synthesised from the reaction between cyanogen bromide and benzotriazole. This reagent is a good alternative guanylating agent compared to cyanogen bromide and also to di(imidazole-1-yl)methanimine **(3.69)**. It is robust so longer reaction times and higher temperatures can be tolerated. The diamine **(3.67)** was heated at reflux for 48 h in the presence of the guanylating reagent[116] **(3.73)** to give the guanidine **(3.68)** in moderate yield (61 %) (Scheme 3.31). The benzotriazole by-product **(3.74)** formed in the reaction was easily removed by column chromatography.

Scheme 3.31

Reagents and conditions: i) THF, 48 h, reflux.

3.2.5 Biomimetic late-stage bromination

The remaining challenge in the synthesis of ageladine A was to selectively brominate at the 2 and 3 positions on the pyrrole. Initial bromination of the pyrrole **(3.68)** with NBS at low temperature gave a mixture of products. The major product was the tri-brominated compound **(3.75)** whereby the third bromine has gone on the 5 position of the pyridine. There were smaller quantities of the mono **(3.76)** and the di-bromo **(3.1)** compounds of which the latter was thought to be the desired natural product, ageladine A. The mono-bromo **(3.76)** and di-bromo **(3.1)** compounds were characterised by MS and ^{1}H NMR of the crude mixture. These two products were inseparable by column chromatography and so were separated by HPLC (MeCN/MeOH, 0.05 % TFA). However, the ^{1}H NMR of what was thought to be ageladine A did not correlate to the ^{1}H NMR of the isolated natural product. Both ^{1}H NMRs were of the TFA salt of the guanidine due to the HPLC solvent conditions (MeCN/MeOH) containing 0.05 % of TFA. In order to ascertain whether our product had brominated in the correct positions or alternatively whether the structure proposed is incorrect we needed to obtain more material. It may be possible to obtain a crystal structure of our 'ageladine A' if we could synthesise and purify more of the di-bromo compound.

After HPLC obtained 1-2 mg of each.

Conditions : $MeCN/H_2O/0.05$ % TFA.

Scheme 3.32

Reagents and conditions: i) NBS (2 eq), MeOH, 2 h, -78 °C.

Attempts to brominate the diamino compound **(3.67)** in order to avoid over bromination were undertaken. Bromination using the same conditions gave predominately the tri-bromo product **(3.70)** with three bromines residing on the pyrrole and a small quantity of the di-bromo compound **(3.77)**. Switching to the diamine and brominating has eliminated any products whereby the bromines reside on the pyridine portion. However the problem of over-bromination of the pyrrole portion has not been resolved.

Scheme 3.33

Reagents and conditions: i) NBS (2 eq), MeOH, 2 h, -78 °C.

As an alternative, a radical bromination reaction[117] was attempted and the guanidine **(3.68)** was treated with 0.5 equivalents of dibromo-hydantoin in the presence of a catalytic amount of AIBN to give only the mono-brominated compound **(3.76)** in moderate yield (44 %). The mono-brominated guanidine **(3.76)** was reacted further with another 0.5 equivalents of dibromo-hydantoin to give predominately the tri-

brominated compound (3.75) confirmed by analysis of crude ^{1}H NMR (Scheme 3.34). It was possible to control the addition of the first bromination as the 2-position on the pyrrole is the most reactive. However, this method has given no advantage over the NBS method of bromination as there was still no control over the addition of the second bromine. The enhanced reactivity of the pyridine moiety means that bromination on position 5 in the pyridine was competing with bromination on the pyrrole moiety.

(3.68) → (3.76) 44 % → (3.75)

Scheme 3.34

Reagents and conditions: i) *N,N*-dibromo-dimethyldihydantoin (0.5 eq), AIBN cat., THF, 2 h, -78 °C.

A known alternative method[118] for bromination is using bromine in acetic acid. In our system the presence of acid would deactivate the guanidine moiety and reduce the reactivity of the pyridine fragment. A solution of bromine in acetic acid was added to the guanidine (3.68) at room temperature. After 4 h the reaction gave a 1:1 mixture of mono-bromo (3.76) and di-bromo guanidine (3.1) as determined by ^{1}H NMR of the crude product. The reaction mixture was then brominated again for a further 4 h to give a ratio of 85:15 in favour of the dibromo guanidine (3.1) as determined by ^{1}H NMR. The difference in these reaction conditions than for previous examples were longer reaction times, performed at room temperature and with acid present.

15:85

Scheme 3.35

Reagents and conditions: i) Br_2 (2 eq), AcOH, 8 h, r.t.

At present, our structure synthesised does not correspond to the reported structure for ageladine A. The MS for both compounds shows $(M + H)^+$ as 356/358/360 in a ratio of 1:2:1, indicating the presence of two bromines. A comparison of the ^1H NMR data between the natural and synthetic product indicates differences in chemical shifts significant enough for there to be considerable doubt that the two compounds are the same (Figure 3.4). Dr Yoichi Nakao at the University of Tokyo, whose group isolated ageladine A, has compared the natural and synthetic material by HPLC analysis and also for inhibition of MMP-2 and MT1-MMP. This confirms our suspicion that the two compounds are not the same. Furthermore, our compound is not biologically active. One possible explanation for the differences is that the two bromines have added unusually in the 2 and 4 positions on the pyrrole. Another possibility is that the natural product is an isomer of the structure we synthesised. It is unusual that no exchangeable protons are exhibited in the ^1H NMR spectrum of ageladine A with the following solvents; DMSO, $CDCl_3$, C_5H_5N, MeOH even at low temperatures. The ^1H NMR spectrum, in DMSO, of our debromoagealdine **(3.68)** does show the guanidine NH_2 and the pyrrole NH exchangeable protons. Furthermore, not all of the two and three bond HMBC correlations are detected in the natural product and the guanidine moiety was inferred to fit the molecular formula.

ageladine A (Fusetani) ^{1}H NMR (MeOH, TFA salt)

'ageladine A' (Helen) ^{1}H NMR (MeOH, TFA salt)

Figure 3.4

3.2.6 Results from biological assay

Dr Nakao has tested our synthetic intermediates for inhibition against MMP-2 and MT1-MMP using the same fluorescent assay metioned previously (Page 47). The analogues **(3.68)**, **(3.35)**, **(3.72)** and **(3.67)** showed inhibition against MMP-2 with IC_{50} values of 5, 24, 25 and 42 μg/mL respectively. The inhibition of ageladine A against MMP-2 is given as 1.7 μg/mL.

(3.35) **(3.68)** **(3.72)** **(3.67)**

Figure 3.5

The results for inhibition against MT1-MMP are shown below in decreasing order of activity (Table 3.5). The analogues **(3.66)**, **(3.68)**, **(3.62)** and **(3.61)** all showed significant inhibition against MT1-MMP with IC_{50} values of 4.6, 8.8, 10, and, 13 µg/mL (Table 3.5, Entry 1-4). The most active analogue against both enzymes was the guanidine **(3.68)** (Table 3.5, Entry 2).

Entry	**Structure**	**IC_{50} µg/mL**
1 (3.66)		4.6
2 (3.68)		**8.8**
3 (3.62)		10
4 (3.61)		13
5 (3.72)		13
6 (3.63)		15
7 (3.53)		16

8 (3.67)	NH2, NH2, N, HN	28
9 (3.59)	NH2, NO2, N, BocN	33
10 (3.35)	N, HN	36
11 (3.65)	NH2, NO2, Br, Br, Br, N, HN	36
12 (3.70)	NH2, NH2, Br, Br, Br, N, HN	46

Table 3.5: Results from biological assay against MT1-MMP

3.3. Conclusions and Future Work

The structure reported for ageladine A has been successfully synthesised. The key step in the synthesis was a Suzuki-Miyaura cross coupling of the pyridine and pyrrole fragments. The final bromination step gave a mixture of products. The ratio of this step in favour of the dibromo-compound over the mono-bromo compound was improved using bromine in acetic acid as opposed to NBS. However, there are discrepancies in the ^{1}H NMR data between our synthetic ageladine A and the isolated natual product, and our compound is also not biologically active. It is possible that the bromination step has yielded an alternative brominated pyrrole. Alternatively the natural product could be an isomer of the structure reported. Conclusive proof has been difficult to achieve due to the small quantity of ageladine synthesised (1-2 mg). A crystal structure of either the natural product or the synthetic product would fully confirm which is the correct structure. Analogues of ageladine A have been tested against MMP-2 and MT1-MMP. Many of these showed some level of inhibition with the guanidine analogue giving the best results for both enzymes.

Future work would involve obtaining a crystal structure of the synthesised ageladine A in order to fully confirm the structure. Also synthesis of the isomer would be beneficial as this could potentially be the real structure of the natural product. A proposed synthesis would be to start from 4-bromo-pyridine and oxidise this to the N-oxide using hydrogen peroxide (Scheme 3.37). Nitration would give the corresponding nitro product **(3.79)**. A dual reduction would give the 2-amino-4-bromo pyridine **(3.80)** which could then undergo a further nitration to give the desired precursor **(3.81)**. The final product **(3.82)** could be obtained following the synthesis for ageladine A.

Scheme 3.36

Reagents: i) H_2O_2; ii) HNO_3, H_2SO_4; iii) Fe, AcOH; iv) HNO_3, H_2SO_4.

Many of the analogues, including the guanidine **(3.68)** showed inhibition against the enzymes. This area could be further explored by synthesising analogues whereby the guanidine fragment remains constant and the boronic acid is varied. There were also hits whereby the guanidine moiety was not present therefore another option would be to retain the pyrrole fragment and vary the halide partner. There are many different routes that could be taken to produce compounds with potentially greater inhibition than ageladine itself.

Chapter Four

Experimental Section

4.1 General

Chemicals and general reagents were purchased from commercial suppliers, and unless otherwise stated were used without further purification. Anhydrous dichloromethane was freshly distilled from calcium hydride and tetrahydrofuran from sodium wire with benzophenone as indicator. All other anhydrous solvents were purchased as Aldrich sure-seal® bottles.

Analytical TLC was carried out on pre-coated plastic plates, normal phase Merck 60 F_{254} silica plates. Visualisation was carried out either with shortnwave UV, or staining with aqueous potassium permanganate or phosphomolybdic acid. Column chromatography was performed using silica (Apollo, 70-230 mesh). Melting points were taken on a hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM spectrometer at 300 or 400 and 75 or 100 MHz respectively. Chemical shifts are given in ppm. Characteristic splitting patterns due to spin spin coupling are expressed as follows: s = singlet, br = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. All coupling constants are measured in Hertz. Low resolution mass spectra were obtained with a Micromass® platform single quadrupole mass spectrometer using acetonitrile as delivery eluent. Infrared spectra were obtained using a Perkin Elmer spectrometer with Golden-Gate© attachment. The term *in vacuo* refers to the removal of solvents by means of evaporation at reduced pressure, using a Buchi rotary evaporator.

4.2 Experimental section for chapter one

(*S*)-*N*-Benzyl phenylalanine methyl ester (1.42a)

1.42 was prepared following a general reductive amination procedure.[33]

A mixture of (*S*)-phenylalanine methyl ester hydrochloride (2.4 g, 11.0 mmol), benzaldehyde (1.3 mL, 13.2 mmol), triethylamine (1.86 mL, 13.2 mmol) and sodium triacetoxyborohydride (3.5 g, 16.5 mmol) in dichloromethane (50 mL) were stirred at room temperature for 8 h. The organic phase was washed with brine (3 x 20 mL), and dried over $MgSO_4$. The solution was concentrated, and the crude product was purified by column chromatography (0-30 % EtOAc/hexane) to give a colourless oil (1.8 g, 61 %).

^{1}H NMR, FT IR and ms data are consistent with literature reference.[87]

FT IR ν 1739 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.99 (d appt, *J* = 7.5, 2H), 3.55 (t appt, *J* = 7.5, 1H), 3.70 (s, 3H), 3.71 (d, *J* = 13.0, 1H), 3.85 (d, *J* = 13.0, 1H), 7.19 – 7.39 (m, 10H);

MS $(M + H)^+$ 270.

(*R*)-*N*-benzyl phenylalanine methyl ester (1.42b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.42a.

(*S*)-2-[Benzyl-(2-cyano-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.43)

1.43 was prepared following a known literature procedure[32].

To a solution of (*S*)-*N*-benzylphenylalanine methyl ester **(1.42a)** (468 mg, 1.7 mmol) in CH_2Cl_2 (10 mL) was added cyanoacetic acid (177 mg, 2.1 mmol) and 1-hydroxy 1 *H*-benzotriazole hydrate (HOBt, 280 mg, 2.1 mmol). The solution was cooled to 0 °C, followed by addition of dicyclohexyl carbodiimide (DCC, 501 mg, 2.4 mmol). The reaction mixture was warmed to room temperature, stirred for 6 h, filtered, washed with water (5 mL), saturated $NaHCO_3$ (5 mL) and brine (5 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography (0-40 % EtOAc/hexane) yielded the amide which was recrystallised (EtOAc/hexane) to give a white solid (280 mg, 48 %). Mp 91 - 92 °C. Lit mp[32] 90 – 92 °C.

^{1}H NMR and ms data are consistent with literature reference.[32]

FT IR ν 1736, 1647 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.25 – 3.41 (m, 4H), 3.70 (s, 3H), 3.84 (d, *J* = 17.0, 1H), 4.32 (d, *J* = 17.0, 1H), 4.43 (dd, *J* = 6.0, 9.5, 1H), 7.01 – 7.27 (m, 5H), 7.29 – 7.36 (m, 5H);

MS $(M + H)^+$ 337.

(*S*)-1,5-Dibenzyl-4-hydroxy-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile (1.44)

1.44 was prepared following a known literature procedure.[32]

To a stirred solution of **(1.43)** (230 mg, 0.7 mmol) in MeOH (5 mL) was added Ambersep-900 resin (2.3 g, 3.4 mmol, OH^- form). After 16 h the resin was filtered and washed three times with MeOH (10 mL). The resin was then stirred for 30 min with MeOH (5 mL) and trifluoroacetic acid (TFA, 0.3 mL, 3.4 mmol), filtered and washed three times with MeOH (10 mL). Concentration of the filtrate afforded the 2,4 pyrrolidinedione hydrate (175 mg), which was recrystallised (MeOH/hexane) to give a white solid (125 mg, 60 % yield). Mp 234-235 °C. Lit mp[32] 234 – 237 °C.

^{1}H NMR, FT IR and ms data are consistent with literature reference.[32]

FT IR ν 1695 (br), 1591 (br) cm^{-1};

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94 (dd, *J* = 4.5, 13.8, 1H), 3.16 (dd, *J* = 4.5, 13.8, 1H), 3.97 (t appt, *J* = 4.4, 1H), 4.04 (d, *J* = 15.4, 1H), 4.86 (d, *J* = 15.4, 1H), 7.09 – 7.22 (m, 5H), 7.25 – 7.31 (m, 5H);

MS (M+H) 305.

(*S*)-*N*-methyl phenylalanine methyl ester (1.45a)

1.45a was prepared following a standard esterification procedure.[36]

Thionyl chloride (2.0 mL, 27.0 mmol) was added dropwise to a solution of (*S*)-*N*-methylphenylalanine (1.0 g, 5.6 mmol) in methanol (30 mL) at 0 °C and the reaction was stirred for 4 h. The organic phase was washed with brine (3 x 30 mL) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude material was purified by column chromatography (10 % MeOH/dichloromethane) to give a pale

yellow oil that solidifies on standing (750 mg, 69 %). Mp 77-78 °C. Lit mp[87] 75-76 °C.

^{1}H NMR, FT IR and ms are consistent with literature reference.[87]

FT IR ν 1743 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (s, 3H), 2.96 (d appt, *J* = 7.0, 2H), 3.47 (t appt, *J* = 7.0, 1H,), 3.66 (s, 3H), 7.16 - 7.3 (m, 5H);

MS $(M + H)^+$ 194.

(*R*)-*N*-methyl phenylalanine methyl ester (1.45b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.45c.

(*S*)-2-[Benzyl-(2-phenylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46a)

A mixture of (*S*)-*N*-benzylphenylalanine (600 mg, 2.2 mmol), phenylthioacetic acid (453 mg, 2.7 mmol), PyBrOP (1.57 g, 3.4 mmol) and diisopropylethylamine (0.47 mL, 2.7 mmol) in dichloromethane (40 mL) was stirred at room temperature for 6 h. The organic phase was washed with brine (3 x 15 mL) dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (20-50 % EtOAc/hexane) to give a colourless oil (405 mg, 44 %).

FT IR ν 1743, 1644, 1441 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.22 (dd, J = 9.5, 14.5, 1H), 3.34 (dd, J = 5.5, 14.5, 1H,), 3.61 (s, 3H), 3.72 (s, 2H), 3.86 (d, J = 16.5, 1H), 4.28 (dd, J = 5.5, 9.5, 1H), 4.52 (d, J = 16.5, 1H), 7.07 – 7.27 (m, 15H);

^{13}C NMR (100 MHz) δ 35.6, 37.4, 52.6, 53.4, 61.9, 127.1, 127.2, 127.8, 128.6, 128.9, 129.1, 129.4, 129.8, 130.2, 135.6, 136.1, 138.2, 169.4, 170.8;

MS $(M + H)^+$ 420, $(M + Na)^+$ 442.

(*R*)-2-[Benzyl-(2-phenylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.46a.

(*S*)-2-[Benzyl-(2-benzylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46c)

Procedure as for 1.46a with benzylthioglycolic acid replacing phenylthioacetic acid gave a colourless oil (359 mg, 40 %).

FT IR ν 1710, 1649, 1431 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.11 (s, 2H), 3.22, (dd, J = 9.0, 14.0, 1H), 3.40 (dd, J = 5.5, 14.0, 1H), 3.66 (s, 3H), 3.81 (s, 2H), 3.89 (d, J = 16.5, 1H), 4.33 (dd, J = 5.5, 9.0, 1H), 4.47 (d, J = 16.5, 1H) 7.09 – 7.23 (m 15H);

^{13}C NMR (100 MHz) δ 32.7, 35.7, 36.3, 52.6, 53.1, 61.5, 127.1, 127.5, 127.7, 128.1, 128.9, 128.99, 129.0, 129.7, 129.8, 135.9, 137.9, 138.0, 170.1, 171.0;

MS $(M + H)^+$ 434, $(M + Na)^+$ 456.

(*R*)-2-[Benzyl-(2-benzylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46d)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.46c.

(*S*)-2-[Methyl-(2-phenylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46e)

Procedure as for 1.46a with (*S*)-*N*-methyl phenylalanine methyl ester replacing (*S*)-*N*-benzyl phenylalanine methyl ester gave a colourless oil (124 mg, 34 %).

FT IR ν 1739, 1639, 1431 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 2.89 (s, 3H), 3.07 (dd, J = 11.0, 14.0, 1H), 3.39 (dd, J = 5.5, 14.0, 1H), 3.64 (s, 3H), 3.71 (s, 2H), 5.22 (dd, J = 5.5, 11.0, 1H), 7.16 – 7.20 (m, 10H);

^{13}**C NMR** (100 MHz) δ 34.2, 35.7, 36.9, 52.7, 59.4, 127.2, 127.3, 127.7, 129.5, 130.4, 130.41, 136.6, 136.9, 168.9, 171.1;

MS $(M + H)^+$ 344, $(M + Na)^+$, 366.

(*R*)-2-[(2-phenylsulfanyl-acetyl)-methyl-amino]-3-phenyl-propionic acid methyl ester (1.46f)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.46e.

(*S*)-2-[(2-benzylsulfanyl-acetyl)-methyl-amino]-3-phenyl-propionic acid methyl ester (1.46g)

Procedure as for 1.46e with benzylthioglycolic acid replacing phenylthioacetic acid gave a colourless oil (126 mg, 37 %).

FT IR ν 1743, 1654, 1431 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 2.86 (s, 3H), 3.07 (s, 2H), 3.08 (dd, J = 11.0, 14.3, 1H), 3.4 (dd, J = 5.0, 14.3, 1H), 3.57 (s, 2H), 3.75 (s, 3H), 5.34 (dd, J = 5.0, 11.0, 1H), 7.14 – 7.26 (m, 10H);

^{13}**C NMR** (100 MHz) δ 32.2, 33.8, 35.5, 36.2, 52.7, 58.9, 126.8, 127.0, 128.4, 128.6, 129.0, 129.6, 136.9, 137.6, 169.0, 171.1;

MS $(M + H)^+$ 358, $(M + Na)^+$ 380.

(*R*)-2-[Methyl-(2-benzylsulfanyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.46h)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.46g.

(*S*)-2-[Benzyl-(2-benzenesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47a)

1.46a was prepared follwing a general oxone oxidation procedure.[38]

The sulfide **(1.46a)** (63 mg, 0.15 mmol) was added to a stirred solution of Oxone® (275 mg, 0.45 mmol) in a 2:1 mixture of methanol/water (10 mL) under nitrogen for 16 h. The solvent was concentrated and the aqueous phase extracted with dichloromethane (3 x 5 mL). The combined filtrates were washed with brine (2 mL), dried over $MgSO_4$, filtered and and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography (20-50 % EtOAc/hexane) to give a colourless oil (60 mg, 89 %).

FT IR ν 1743, 1649, 1568 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.15 (dd, J = 9.0, 14.0, 1H), 3.41 (dd, J = 6.0, 14.0, 1H), 3.62 (s, 3H), 4.14 (d, J = 17.0, 1H), 4.14 - 4.18 (m, 2H), 4.42 (dd, J = 6.0, 9.0, 1H), 4.79 (d, J = 17.0, 1H), 7.11 – 7.28 (m, 15H);

^{13}C NMR (75MHz) δ 35.7, 52.6, 53.2, 60.5, 62.1, 127.6, 128.3, 128.9, 129.1, 129.3, 129.5, 129.8, 129.9, 134.6, 135.6, 137.9, 139.4, 162.7, 170.3;

MS $(M + H)^+$ 452, $(M + Na)^+$ 474.

(*R*)-2-[Benzyl-(2-benzenesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.47a.

(S)-2-[Benzyl-(2-phenylmethanesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47c)

Procedure as for 1.47a gave a colourless oil (68 mg, 97 %).

FT IR ν 1743, 1649, 1427 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26 (dd, J = 9.0, 14.0, 1H), 3.47 (dd, J = 6.0, 14.0, 1H), 3.69 (s, 3H), 3.78 (s, 2H), 4.07 (d, J = 16.5, 1H), 4.48 (dd, J = 6.0, 9.0, 1H), 4.51 – 4.56 (m, 2H), 4.60 (d, J = 16.5, 1H), 7.07 – 7.25 (m, 15H);

^{13}C NMR (75 MHz) δ 36.9, 53.9, 54.3, 55.3, 61.1, 63.7, 127.3, 127.34, 128.4, 129.2, 129.3, 129.4, 129.5, 129.8, 131.7, 132.8, 136.4, 138.9, 165.2, 171.5;

MS $(M + H)^+$ 466, $(M + Na)^+$ 488.

(R)-2-[Benzyl-(2-phenylmethanesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47d)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.47c.

(*S*)-2-[(2-benzenesulfonyl-acetyl)-methyl-amino]-3-phenyl-propionic acid methyl ester (1.47e)

Procedure as for 1.47a gave a colourless oil (14 mg, 98 %).

FT IR ν 1743, 1649, 1445 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (m, 1H), 3.07 (s, 3H), 3.43 (dd, *J* = 5.1, 14.7, 1H), 3.75 (s, 3H), 4.13 (d, *J* = 14.0, 1H), 4.21 (d, *J* = 14.0, 1H), 5.28 (dd, *J* = 5.1, 10.2, 1H), 7.20 – 7.35 (m, 10H);

^{13}C NMR (100 MHz): δ 34.5, 34.9, 59.4, 60.3, 63.6, 127.8, 128.9, 129.0, 129.3, 129.4, 129.5, 134.5, 138.9, 162.5, 170.9;

MS $(M + H)^+$ 376, $(M + Na)^+$ 398.

(*R*)-2-[(2-benzenesulfonyl-acetyl)-methyl-amino]-3-phenyl-propionic acid methyl ester (1.47f)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.47e.

(*S*)-2-[Methyl-(2-phenylmethanesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47g)

Procedure as for 1.47a gave a colourless oil (10 mg, 51 %).

FT IR ν 1734, 1649, 1441 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.95 (s, 3H), 3.10 (dd, *J* = 5.0, 14.5, 1H), 3.46 (dd, *J* = 5.5, 14.5, 1H), 3.78 (s, 3H), 3.80 (s, 2H), 4.35 (s, 2H), 5.30 (dd, *J* = 5.5, 10.5, 1H), 7.10 – 7.36 (m, 10H);

^{13}C NMR (75 MHz) δ 34.7, 35.1, 52.9, 54.1, 59.5, 63.2, 129.4, 129.8, 130.7, 130.9, 131.0, 133.2, 138.0, 138.6, 165.2, 172.4;

MS $(M + NH_4)^+$ 407, $(M + Na)^+$ 412.

(*R*)-2-[Methyl-(2-phenylmethanesulfonyl-acetyl)-amino]-3-phenyl-propionic acid methyl ester (1.47h)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.47g.

(*S*)-1,5-Dibenzyl-4-hydroxy-3-phenylsulfanyl-1,5-dihydro-pyrrol-2-one (1.48a)

Sodium hexamethyldisilazane (NaHMDS, 1M in THF, 0.16 mL) was added to a solution of the sulfide **(1.46a)** (45 mg, 0.1 mmol) in THF (5 mL) at 0 °C and the reaction mixture was stirred for 2 h. The solvent was evaporated *in vacuo* and water (2 mL) was added to the residue. The aqueous phase was acidified with 4 M H_2SO_4 to pH 2 and the precipitated free acid was filtered and dried. The crude material was recrystallised from MeOH/ether to give a white solid (40 mg, 94 %). Mp 182-183 °C. $[\alpha]_D$ = - 89.4 (*c* 0.5, MeOH);

FT IR ν 1578, 1389 cm^{-1};

^{1}**H NMR** (400 MHz, MeOD) δ 3.13 (dd, *J* = 4.0, 14.5, 1H), 3.30 (dd, *J* = 8.0, 14.5, 1H), 4.17 (t appt, *J* = 4.0, 8.0, 1H), 4.31 (d, *J* = 15.5, 1H), 5.22 (d, *J*= 15.5, 1H), 6.97 – 7.02 (m, 15H);

^{13}**C NMR** (100 MHz) δ 35.2, 45.7, 61.6, 96.4, 125.9, 126.9, 128.2, 128.8, 129.1, 129.5, 129.8, 129.2, 131.1, 136.1, 137.6, 138.6, 173.8, 180.2;

MS $(M + H)^+$ 388, $(M + Na + MeCN)^+$ 451.

Anal. Calcd for $C_{24}H_{21}NO_2S$: C, 74.39; H, 5.46; N, 3.61. Found C, 74.03; H, 5.68; N, 3.20.

(*R*)-1,5-Dibenzyl-4-hydroxy-3-phenylsulfanyl-1,5-dihydro-pyrrol-2-one (1.48b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.49a. $[\alpha]_D$ = + 99.6 (*c* 0.5, MeOH).

(*R*)-1,5-Dibenzyl-3-benzylsulfanyl-4-hydroxy-1,5-dihydro-pyrrol-2-one (1.48c)

Procedure as for 1.48a gave a white solid (31 mg, 95 %). Mp 179-181 ºC.

$[\alpha]_D$ = + 75.6 (*c* 0.5, MeOH);

FT IR ν 1568, 1408 cm^{-1};

^{1}H NMR (400 MHz, MeOD) δ 2.87 (dd, *J* = 5.0, 14.5, 1H), 3.17 (dd, *J* = 4.5, 14.5, 1H), 3.58 (d, *J* = 12.0, 1H), 3.69 (d, *J* = 12.0, 1H), 3.90 (t appt, *J* = 4.5, 1H), 4.05 (d, *J* = 15.0, 1H), 5.09 (d, *J* = 15.0, 1H), 7.04 – 7.21 (m, 15H);

^{13}C NMR (75MHz) δ 36.5, 39.2, 45.5, 61.1, 98.6, 128.3, 128.5, 128.9, 129.4, 129.6, 129.63, 130.2, 130.6, 131.1, 137.1, 138.9, 139.7, 174.4, 178.4;

MS $(M + H)^+$ 402, $(M + Na)^+$ 424

Anal. Calcd for $C_{25}H_{23}NO_2S$: C, 74.78; H, 5.77; N, 3.49. Found C, 74.37; H, 5.37; N, 3.33.

(*S*)-1,5-Dibenzyl-3-benzylsulfanyl-4-hyddroxy-1,5-dihydro-pyrrol-2-one (1.48d)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.48c. $[\alpha]_D$ = - 70.9 (*c* 0.4, MeOH).

(*S*)-5-Benzyl-4-hydroxy-1-methyl-3-phenylsulfanyl-1,5-dihydro-pyrrol-2-one (1.48e)

Procedure as for 1.48a gave a white solid (28 mg, 75 %). Mp 202-204 °C.

$[\alpha]_D$ = - 130.5 (*c* 0.3, MeOH);

FT IR ν 1564, 1398 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.98 (s, 3H), 3.14 (dd, *J* = 4.0, 14.3, 1H), 3.26 (dd, *J* = 4.5, 14.3, 1H), 4.38 (t appt, *J* = 4.0, 1H), 6.97 – 7.32 (m, 10H);

^{13}C NMR (100 MHz) δ 27.9, 33.8, 62.2, 94.0, 124.8, 125.2, 127.1, 128.5, 129.1, 130.2, 135.5, 137.3, 169.9, 178.1;

MS $(M + H)^+$ 312, $(M + Na + MeCN)^+$ 375.

(*R*)-5-Benzyl-4-hyrdoxy-1-methyl-3-phenylsulfanyl-1,5-dihydro-pyrrol-2-one (1.48f)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.48e. **$[\alpha]_D$** = + 123.5 (*c* 0.5, MeOH).

(*S*)-5-Benzyl-3-benzylsulfanyl-4-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one (1.48g)

Procedure as for 1.49a gave a white solid (25 mg, 70 %). Mp 195-197 °C.

FT IR ν 1568, 1375 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.92 (s, 3H), 2.98 (dd, J = 4.5, 14.0, 1H), 3.19 (dd, J = 4.5, 14.0, 1H), 3.49 (d, J = 12.0, 1H), 3.57 (d, J = 12.0, 1H), 4.14 (t appt, J = 4.5, 1H), 7.11 – 7.14 (m, 10H);

^{13}C NMR (100 MHz) δ 28.6, 36.3, 39.6, 64.0, 99.3, 128.3, 128.4, 129.6, 129.7, 130.5, 131.0, 136.9, 139.7, 174.4, 178.0;

MS (M+H)$^+$ 326, (M + Na)$^+$ 348.

(*R*)-5-Benzyl-3-benzylsulfanyl-4-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one (1.48h)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.48g.

(*R*)-3-Benzenesulfonyl-1,5-dibenzyl-4-hydroxy-1,5-dihydro-pyrrol-2-one (1.49b)

Procedure as for 1.48a gave a white solid (37 mg, 88 %). Mp 197-199 °C.

$[\alpha]_D$ = + 89.7 (*c* 0.5, MeOH);

FT IR ν 1579, 1406 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.97 (dd, *J* = 5.0, 14.5, 1H), 2.86 (dd, *J* = 5.3, 14.5, 1H), 3.38 (br m, 1H), 3.73 (d, *J* = 15.2, 1H), 4.83 (d, *J* = 15.2, 1H), 7.0 – 7.45 (m, 15H);

MS $(M + H)^+$ 420, $(M + MeCN)^+$ 460.

(*R*)-1,5-Dibenzyl-4-hydroxy-3-phenylmethanesulfonyl-1,5-dihydro-pyrrol-2-one (1.49d)

Procedure as for 1.48a gave a white solid (25 mg, 96 %). Mp 210-212 °C.

$[\alpha]_D$ = + 82.1 (*c* 0.5, MeOH);

FT IR ν 1743, 1564, 1408 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.82 (dd, *J* = 6.0, 14.0, 1H), 2.86 (dd, *J* = 5.5, 14.0, 1H), 3.49 (t appt, *J* = 5.0, 1H), 3.82 (d, *J* = 15.3, 1H), 4.30 (s, 2H), 4.88 (d, *J* = 15.3, 1H), 7.1 – 7.18 (m, 15H);

MS $(M + H)^+$ 434.

(*S*)-3-Benzenesulfonyl-5-benzyl-4-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one (1.49e)

Procedure as for 1.48a gave a white solid (42 mg, 96 %). Mp 196-198 °C.

FT IR ν 1594, 1389 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.65 (s, 3H), 3.03 (dd, J = 4.0, 14.0, 1H), 3.12 (dd, J = 4.0, 14.5, 1H), 4.29 (t, J = 4.0, 1H), 6.89 – 7.51 (m, 10H);

^{13}C NMR (100 MHz) δ 27.2, 34.1, 62.1, 105.1, 126.9, 127.1, 128.5, 129.4, 129.6, 133.1, 134.9, 142.6, 165.2, 178.7;

MS $(M - H)^-$ 342;

Anal. Calcd for $C_{18}H_{17}NO_4S$: C, 62.96; H, 4.99; N, 4.08. Found C, 62.61; H, 4.59; N, 3.70.

(*S*)-5-Benzyl-4-hydroxy-1-methyl-3-phenylmethanesulfonyl-1,5-dihydro-pyrrol-2-one (1.49g)

Procedure as for 1.48a gave a white solid (9 mg, 97 %). Mp 200-202 °C.

$[\alpha]_D$ = -118.5 (*c* 0.5, MeOH);

FT IR ν 1594, 1392 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.57 (s, 3H), 2.79 (dd, *J* = 4.0, 14.0, 1H), 3.30 (dd, *J* = 4.5, 14.0, 1H), 3.52 (t, appt, *J* = 5.0, 1H), 4.09 (s, 2H), 7.24 – 7.28 (m, 10H);

^{13}C NMR (100 MHz) δ 28.3, 36.6, 59.8, 66.1, 91.7, 126.4, 126.9,128.9, 129.3, 129.9, 131.3, 138.7, 144.8, 162.8, 171.4;

MS $(M + H)^+$ 358.

(*R*)-5-Benzyl-4-hydroxy-1-methyl-3-phenylmethanesulfonyl-1,5-dihydro-pyrrol-2-one (1.49h)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.49g. $[\alpha]_D$ = +110.9 (*c* 0.5, MeOH).

(*S*)-Methyl-2-(benzylamino)-3-(1H-indol-3-yl)propanoate (1.51)

Procedure as for 1.42a replacing (*S*)-tryptophan methyl ester hydrochloride for (*S*)-phenylalanine methyl ester hydrochloride. Purified by column chromatography (50 % EtOAc/hexane) to give a white gum (281 mg, 45 %).

^{1}H and MS data are consistent with literature reference.[119]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (br s, 1H, N*H*), 3.17-3.21 (m, 2H), 3.64 (s, 3H), 3.66-3.86 (m, 3H, C*H*N, NCH_2), 7.02-7.60 (m, 10H), 8.07 (br s, 1H, N*H*);

MS $(M+H)^+$ 309.

(*S*)-2-Benzylamino-3-methyl-pentanoic acid methyl ester (1.54c)

Procedure as for 1.42a replacing (*S*)-isoleucine methyl ester hydrochloride with (*S*)-phenylalanine methyl ester hydrochloride. Purified by column chromatography (5-30 % EtOAc/hexane) to give an off-white solid (405 mg, 78 %); Mp 92-93 °C. Lit mp[120] 90-93 °C.

Melting point and optical rotation are consistent with literature reference.[120]

$[\alpha]_D$ = -1.8 (*c* 0.5, EtOH), $[\alpha]_D^{lit}$ = -2.0 (*c* 1.5, EtOH).

FT IR ν 1720, 1445 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 3.0, 3H), 0.89 (br dd, 3H), 1.19 (m, 1H), 1.61 (m, 1H), 1.66 (m, 1H), 1.81, (br s, 1H, N*H*), 3.11 (d, *J* = 6.5, 1H), 3.59 (d, *J* = 13.0, 1H), 3.71 (s, 3H), 3.82 (d, *J* = 13.0, 1H), 7.21 – 7.25 (m, 5H);

^{13}C NMR (100 MHz) δ 9.9, 14.1, 24.0, 36.9, 49.6, 51.1, 63.9, 125.5, 126.75, 126.8, 138.6, 174.2;

MS $(M+H)^+$ 236.

(*S*)-1-(3-Oxo-butyryl)-pyrrolidine-2-carboxylic acid methyl ester (1.58a)

1.58a was prepared following a known acetoacetylation procedure.[39]

A solution of (*S*)-proline methyl ester hydrochloride (630 mg, 3.8 mmol) in dichloromethane (10 mL) was cooled and a solution of 4 M diketene in dichloromethane (0.95 mL, 3.8 mmol) and triethylamine (0.53 mL, 3.8 mmol) were added dropwise. After 16 h the mixture was quenched with water (3 x 10 mL), washed with brine (3 x 10 mL), and dried over $MgSO_4$ and concentrated. Purification by column chromatography (50-70 % EtOAc/hexane) gave a yellow oil (700 mg, 87 %).

^{1}H NMR and ms are consistent with literature reference.[14]

FT IR ν 1748, 1644 1446 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$, major form) δ 1.97 (m, 4H), 2.18 (m, 1H), 2.29 (s, 3H), 3.52 (s, 2H), 3.61 (m, 1H), 3.73 (s, 3H), 4.53 (dd, J = 7.5, 9.0, 1H);

^{13}C NMR (100 MHz) δ 25.1, 29.6, 30.6, 47.9, 52.6, 51.5, 59.2, 165.7, 172.7, 202.6;

MS $(M + H)^+$ 214, $(M + Na)^+$ 236.

(*S*)-1-(3-Oxo-butyryl)-piperidine-2-carboxylic acid methyl ester (1.58b)

Procedure as for 1.58a with (*S*)-pipecolinic acid methyl ester replacing (*S*)-proline methyl ester hydrochloride gave a yellow oil (70 mg, 42 %).

FT IR ν br 1753, 1644, 1450 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$, major form) δ 1.47 (m, 2H), 1.63 (m, 4H), 2.23 (s, 3H), 2.24 (m, 1H), 3.23 (ddd, J = 4.0, 17.5, 1H), 3.54 (s, 2H), 3.69 (s, 3H), 5.33 (d, appt, J = 7.5, 1H);

^{13}C NMR (100 MHz) δ 20.9, 25.2, 26.6, 30.1, 50.7, 51.4, 52.2, 57.0, 166.8, 171.5, 202.4;

MS $(M + H)^+$ 228, $(M + Na)^+$ 250.

(*S*)-2-(3-Oxo-butyrylamino)-3-phenyl-propionic acid methyl ester (1.55a)

Procedure as for 1.58a with (*S*)-phenylalanine methyl ester hydrochloride replacing (*S*)-proline methyl ester hydrochloride. Purification by column chromatography (70-90 % EtOAC/hexane) gave a yellow oil (204 mg, 78 %).

^{1}H NMR and ms are consistent with literature reference.[22]

FT IR ν 1734, 1658 cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$, major form) δ 2.20 (s, 3H), 3.09 (dd, *J* = 7.0, 14.0, 1H), 3.19 (dd, *J* = 5.5, 14.0, 1H), 3.37 (s, 2H), 3.71 (s, 3H), 4.93 (dd appt, *J* = 5.5, 7.0, 1H), 7.15 (d, *J* = 7.5, 1H, N*H*), 7.22 – 7.32 (m, 5H);

^{13}C NMR (75 MHz) δ 30.8, 37.8, 49.6, 52.4, 53.4, 127.5, 129.0, 129.6, 136.1, 165.4, 171.7, 203.7;

MS (M+Na) 286, (M+K) 302.

(*R*)-2-(3-Oxo-butyrylamino)-3-phenyl-propionic acid methyl ester (1.55b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.55a.

(rac)-3-(1*H*-indol-3-yl)-2-(3-oxo-butyrylamino)-propionic acid methyl ester (1.57)

1.57 was prepared following a known acetoacetylation procedure.[40]

To a solution of (*S*)-tryptophan methyl ester (50 mg, 0.2 mmol) in xylene (5 mL) was added dioxinone (0.07 mL, 50.0 mmol). The reaction vessel was placed in an oil bath that had been preheated to 150 °C and the solution was vigorously stirred. The evolution of acetone became apparent within several minutes and heating was continued for 3 h. The reaction mixture was cooled and the solvent was removed *in vacuo*. The crude material was purified by column chromatography (50-80 %, EtOAc/hexane) to give a colourless oil (61 mg, 87 %).

$[\alpha]_D$ = 0.0 (*c* 0.5, MeOH); Lit $[\alpha]_D$[119] = +9.7° (opposite enantiomer).

FT IR ν br 1743, 1654 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.1 (s, 3H), 3.28 (s, 2H), 3.33 (d appt, *J* = 7.8, 2H), 3.69 (s, 3H), 4.95 (dd, *J* = 7.8, 9.8, 1H), 7.02-7.37 (m, 5H), 8.51 (br s, 1H, N*H*);

^{13}C **NMR** (100 MHz) δ 27.5, 30.7, 49.5, 52.5, 52.9, 109.6, 111.4, 118.4, 119.5, 122.1, 123.2, 127.4, 136.1, 165.7, 172.3, 204.0;
MS $(M+H)^+$ 303.

(*S*)-1-Benzyl-3-(1-hydroxy-ethylidiene)-5-(1H-indol-3-ylmethyl)-pyrrolidine-2,4-dione (1.53c)

Cyclisation procedure as for 1.44 apart from the filtrate being recrystallised (MeOH/hexane) to give a white solid (11 mg, 30 %). Mp 146-148 °C.
^{1}H NMR and ms data are consistent with literature reference.[22]
^{1}**H NMR** (300 MHz DMSO-d_6) δ 2.37 (s, 3H) 3.32 (d appt, *J* = 6.0, 2H), 3.98 (t appt, *J* = 6.0, 1H), 3.99 (d, *J* = 14.0, 1H), 5.23 (d, *J* =14.0, 1H), 7.04-7.28 (m, 5H), 7.29 (m, 5H), 7.55 (br d, 1H,), 8.1(br d, 1H, N*H*);
MS $(M + 113)^-$ 473.

(rac)-2-(1-Hydroxy-ethylidene)-tetrahydro-pyrrolizine-1,3-dione (1.59a)

1.59a was prepared following a known procedure.[22]
To a refluxing solution of diketone **(1.58a)** (250 mg, 1.17 mmol) in anhydrous methanol (10 mL) under argon was added over 10 minutes a 0.5 M solution of sodium methoxide in methanol (2.4 mL, 1.2 mmol). The reflux was continued for 3 h, the solvent was removed and the resulting mixture dried *in vacuo*. The sodium salt was dissolved in water (3 mL) and 4 M H_2SO_4 was added. The free acid was extracted with dichloromethane (3 x 5 mL), dried over $MgSO_4$, filtered and the solvent

evaporated in *vacuo*. The crude material was purified by column chromatography (2-5 % MeOH/EtOAc) to give a yellow oil (179 mg, 85 %).

^{1}H NMR and ms are consistent with literature reference.[14]

FT IR ν 1706, br 1616 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 2.15 (m, 4H), 2.44 (s, 3H), 3.29 (ddd, J = 6.5, 7.5, 9.0, 1H), 3.78 (ddd, J = 7.5, 8.0, 9.0, 1H), 3.98 (dd, J = 6.5, 7.0, 1H);

^{13}**C NMR** (100 MHz) δ 19.1, 26.4, 26.9, 42.6, 68.6, 103.2, 176.4, 184.4, 194.8;

MS (M-H)$^-$ 180.

(rac)-2-(1-Hydroxy-ethylidene)-tetrahydro-indolizine-1,3-dione (1.59b)

Procedure as for 1.58a. Purified by column chromatography (5 % MeOH/EtOAc) to give a yellow oil (45 mg, 75 %).

FT IR ν 1716, 1624, 1475 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 1.28 (ddd, J = 3.5, 13.0, 1H), 1.44 (m, 2H), 1.79 (m, 1H), 1.99 (m, 1H), 2.18 (m, 1H), 2.43 (s, 3H), 2.84 (ddd, J = 3.5, 13.0, 1H), 3.60 (dd, J = 4.0, 12.0, 1H), 4.31 (dd, J = 4.5, 5.0, 1H);

^{13}**C NMR (100 MHz)** δ 19.8, 23.7, 25.4, 28.0, 38.9, 63.8, 102.0, 171.0, 184.2, 195.1;

MS (M-H)$^-$ 194.

(*S*)-1-Benzyl-5-sec-butyl-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.56c)

Procedure as for 1.58a. Purified by column chromatography (2-5 % MeOH/dichloromethane) to give a yellow oil (11 mg, 40 %);

Data is consistent with literature reference.[121]

FT IR ν 1739, 1654 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 0.83 (d, J = 3.0, 3H), 0.86 (br dd, 3H), 1.38-1.57 (m, 2H), 1.95 (m, 1H), 3.01 (s, 3H), 3.69 (d, J = 3.0, 1H), 3.97 (d, J = 15.0, 1H), 5.36 (d, J = 15.0, 1H), 7.26 – 7.37 (m, 5H);
MS $(M+Na)^+$ 310.

(*S*)-5-Benzyl-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.56a)

1.56a was prepared following an adapted procedure.[22]

To a refluxing solution of diketone **(1.55a)** (200 mg, 0.8 mmol) in anhydrous methanol (10 mL) under argon was added over 10 minutes a 0.5 M solution of sodium methoxide in methanol (1.52 mL, 0.8 mmol). The reflux was continued for 0.5 h. After cooling the mixture was concentrated to 2 mL and the sodium salt was precipitated out with diethyl ether (15 mL). The salt was filtered, dried and dissolved in water (5 mL). The free acid was precipitated out with 4 M H_2SO_4, filtered and dried. The product was recrystallised (MeOH) to give white needles (158 mg, 90 %). Mp 86-87 °C; Lit mp[22] 85-87 °C.
^{1}H NMR and ms data are consistent with literature reference.[22]

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 2.98 (dd, J = 6.0, 14.0, 1H), 2.99 (dd, J = 4.5, 14.0, 1H), 4.16 (dd appt, J = 5.5, 10.6, 1H), 8.70 (br s, N*H*), 7.14 – 7.27 (m, 5H);

MS $(M + H)^+$ 232.

(*S*)-5-Benzyl-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.56b)

This compound was synthesised in a manner analogous to that used for its enantiomer. Data as for 1.56a .

(*S*)-2-Amino-3-(*tert*-butyl-dimethyl-silanyloxy)-propionic acid methyl ester (1.63a)

O
NH2
O
TBDMSO

1.63 was prepared following a standard procedure.[45]

Imidazole (1.2 g, 17.3 mmol) was added to a solution of (*S*)-serine methyl ester (1.0 g, 8.3 mmol) in dichloromethane (20 mL). ^{t}Butyldimethylsilyl chloride (2.6 g, 17.3 mmol) was added and the reaction was stirred for 12 h at room temperature. The organic phase was extracted with slightly acidic aqueous HCl (pH 5) (3 x 20 mL), dried, filtered and the solvent evaporated *in vacuo*. Purification by column chromatography (50-80 % EtOAc/hexane) gave the product as a clear oil (1.3 g, 65 %).

^{1}H NMR and ms data are consistent with literature reference.[122]

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.84 (s, 9H), 1.69 (br s, 2H, NH_2) 3.48 (t appt, J = 4.0, 1H), 3.68 (s, 3H), 3.78 (dd, J = 4.5, 9.5, 1H), 3.88 (dd, J = 4.0, 9.5, 1H);

MS $(M + H)^+$ 234.

(*S*)-2-Amino-3-(*tert*-butyl-dimethyl-silanyloxy)-butyric acid methyl ester (1.63b)

O
NH2
O
TBDMSO

Procedure as for 1.63a with (*S*)-threonine methyl ester replacing (*S*)-serine methyl ester gave a clear oil (3.12 g, 85 %).

^{1}H NMR and ms data are consistent with literature reference.[123]

^{1}H NMR (400 MHz, $CDCl_3$) δ -0.05 (s, 3H), 0.00 (s, 3H), 0.80 (s, 9H), 1.21 (d, J = 6.0, 3H), 1.54 (br s, 2H, NH_2), 3.24 (d appt, J = 3.0, 1H), 3.66 (s, 3H), 4.25 (dq, J = 3.0, 6.0, 1H);

MS $(M + H)^+$ 248, $(M + MeCN)^+$ 288.

(*S*)-3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(3-oxo-butyrylamino)-propionic acid methyl ester (1.64a)

1.64a was prepared following an acetoacetylation procedure.[39]

To a cooled solution of **(1.63a)** (1.0 g, 4.3 mmol) in dichloromethane (10 mL) was added dropwise diketene (0.36 mL, 4.7 mmol). The mixture was stirred at room temperature for 16 h. The solvent was evaporated *in vacuo* and the residue redissolved in ethyl acetate (30 mL). The organic phase was washed with 1M HCl (15 mL) and saturated Na_2CO_3 (15 mL), dried over $MgSO_4$, filtered and the solvent evaporated in *vacuo*. Purification by column chromatography (50-80 % EtOAc/hexane) gave the product as a yellow oil (1.3 g, 92 %).

FT IR ν 1744, 1717, 1656 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.02 (s, 3H), 0.85 (s, 9H), 2.25 (s, 3H), 3.43 (s, 2H), 3.72 (s, 3H), 3.82 (dd, *J* = 3.5, 10.0, 1H), 4.10 (dd, *J* = 3.0, 10.0, 1H), 4.64 (dt appt, *J* = 3.0, 6.0, 1H), 7.45 (d, *J* = 6.0, 1H, N*H*);

^{13}C NMR (100 MHz) δ 18.2, 25.8, 30.9, 49.9, 52.5, 54.5, 63.4, 91.0, 165.7, 170.6, 203.5;

MS $(M + H)^+$ 318, $(M + Na)^+$ 340.

(*S*)-3-(*tert*-Butyl-dimethyl-silanyloxy)-2-(3-oxo-butyrylamino)-butyric acid methyl ester (1.64b)

Procedure as for 1.64a gave a yellow oil (3.3 g, 99 %).

FT IR ν 1749, 1716, 1657 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.06 (s, 3H), 0.88 (s, 9H), 1.19 (d, J = 6.5, 3H), 2.29 (s, 3H), 3.49 (s, 2H), 3.71 (s, 3H), 4.47 (dq, J = 1.5, 6.5, 1H), 4.56 (dd, J = 1.5, 9.0, 1H), 7.41 (d, J = 9.0, 1H, N*H*);

^{13}**C NMR** (100 MHz) δ 17.9, 21.1, 25.7, 30.9, 49.9, 52.8, 58.2, 68.7, 91.1, 168.2, 170.8, 203.5;

MS $(M+H)^+$ 332, $(M+Na)^+$ 354, $(M+Na+MeCN)^+$ 395.

(*S*)-3-Benzyloxy-2-(3-oxo-butyrylamino)-propionic acid methyl ester (1.64c)

1.64c was prepared following an acetoacetylation procedure.[39]

To a cooled suspension of *O*-benzyl-(*S*)-serine methyl ester (1.1 g, 5.1 mmol) in dichloromethane (10 mL) were successively added dropwise triethylamine (0.8 mL, 5.6 mmol) and diketene (1.4 mL, 5.6 mmol). The reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated *in vacuo* and the residue redissolved in ethyl acetate (10 mL). The organic phase was washed with 1 M HCl (10 mL) and saturated Na_2CO_3 (10 mL), dried, filtered and the solvent evaporated in *vacuo*. Purification by column chromatography (50-70 % EtOAC/hexane) gave the product as a clear oil (1.3 g, 90 %).

FT IR ν 1746, 1719, 1651 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 2.20 (s, 3H), 3.36 (s, 2H), 3.63 (dd, J = 3.5, 9.5, 1H), 3.65 (s, 3H), 3.84 (dd, J = 3.5, 9.5, 1H), 4.44 (d, J = 12.0, 1H), 4.50 (d, J = 12.0, 1H), 4.70 (dd, J = 3.5, 7.0 1H), 7.19 – 7.28 (m, 5H), 7.39 (d, J = 7.0, 1H, N*H*);

^{13}**C NMR** (100 MHz) δ 30.9, 50.0, 52.7, 53.2, 69.5, 73.4, 127.0, 127.8, 128.6, 137.6, 165.7, 170.5, 203.4;

MS $(M+H)^+$ 294.

(*S*)-3-Benzyloxy-2-(3-oxo-butyrylamino)-butyric acid methyl ester (1.64d)

Procedure as for 1.64c with *O*-benzyl-(*S*)-threonine-methyl ester replacing *O*-benzyl-(*S*)-serine methyl ester gave a clear oil (1.89g, 69%).

$[\alpha]_D$ = -0.15 (c = 0.66, CH_2Cl_2);

FT IR ν 1747, 1721, 1651 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (d, *J* = 6.5, 3H), 2.33 (s, 3H), 3.52 (s, 2H), 3.71 (s, 3H), 4.22 (dq, *J* = 2.0, 6.5, 1H), 4.45 (d, *J* = 11.7, 1H), 4.66 (d, *J* = 11.7, 1H), 4.71 (dd, *J* = 2.0, 9.0, 1H), 7.28 - 7.37 (m, 5H), 7.46 (d, *J* = 9.0, 1H, N*H*);

^{13}C NMR (100 MHz) δ 16.4, 30.9, 50.3, 52.5, 56.9, 70.8, 73.9, 127.9, 127.94, 128.5, 137.9, 166.3, 170.8, 203.3;

MS $(M+H)^+$ 308 $(M+Na)^+$ 330.

(*S*)-3-Hydroxy-2-(3-oxo-butyrylamino)-propionic acid methyl ester (1.65a)

1.65a was prepared following a deprotection procedure.[46]

To a solution of silyl ether (**1.64a**) (560 mg, 1.7 mmol) in THF (5 mL) was added 1 M TBAF (1.7 mL) at 0 °C over 10 minutes. The mixture was stirred for 1.5 h at room temperature. The solvent was evaporated *in vacuo* and purification of the crude material by column chromatography (90 % EtOAC/hexane) gave a pale yellow oil (300 mg, 84 %).

^{1}H NMR and ms data are consistent with literature reference.[22]

^{1}H NMR (400 MHz, MeOD-d_3) δ 2.23 (s, 2H), 3.75 (s, 3H), 3.82 (dd, *J* = 4.0, 11.0, 1H), 3.92 (dd, *J* = 4.0, 11.0, 1H), 4.55 (t appt, *J* = 4.0, 1H), 4.79 (s, 3H);

MS $(M + Na)^+$ 226.

(*S*)-3-Hydroxy-2-(3-oxo-butyrylamino)-butyric acid methyl ester (1.65b)

Procedure as for 1.65a gave a yellow oil (110 mg, 85 %).

^{1}H NMR and ms data are consistent with literature reference.[22]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.5, 3H), 2.22 (s, 3H), 3.20 (br s, 1H, O*H*), 3.46 (s, 2H), 3.69 (s, 3H), 4.29 (dq, *J* = 2.5, 6.5, 1H), 4.51 (dd, *J* = 2.5, 8.5, 1H), 7.48 (d, *J* = 8.5, N*H*);

MS $(M + H)^+$ 218.

(*S*)-3-(1-Hydroxy-ethylidene)-5-hydroxymethyl-pyrrolidine-2,4-dione (1.66a)

1.66a was prepared following an adapted procedure.[22]

To a refluxing solution of diketone **(1.65a)** (200 mg, 1.0 mmol) in anhydrous methanol (10 mL) under argon was added over 10 minutes a 0.5 M solution of sodium methoxide in methanol (1.95 mL, 1.0 mmol). The reflux was continued for 30 mins. After cooling, the mixture was concentrated to 2 mL and the sodium salt was precipitated out with diethyl ether (5 mL). The salt was filtered, dried and dissolved in water (5 mL). The free acid was precipitated out with 4 M H_2SO_4, filtered and dried to give a white solid which was recrystallised from methanol to give white needles (98 mg, 58 %). Mp 237-238 °C. Lit mp 237-239 °C

^{1}H NMR and mp data are consistent with literature reference.[124]

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (s, 3H), 3.61 (d, *J* = 3.5, 1H), 3.62 (br s, 1H, OH), 3.65 (d, *J* = 4.0, 1H), 3.85 (t, appt *J* = 3.5, 1H), 8.7 (br s, N*H*);

MS $(M +113)^-$ 284.

(*S*)-5-(1-Hydroxy-ethyl)-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.66b)

Procedure as for 1.66a gave a white solid (161 mg, 61 %). Mp 148-150 °C. Lit mp[22] 148-149 °C.

^{1}H NMR and ms data are consistent with literature reference.[22]

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 1.17 (d, *J* = 6.5, 3H), 2.34 (s, 3H), 3.68 (d, *J* = 3.0, 1H), 3.97 (dq, *J* = 3.0, 6.5, 1H), 8.78 (br s, N*H*);

MS $(M + H)^-$ 298.

(*S*)-5-Benzyloxymethyl-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.66c)

Procedure as for 1.66a gave a white solid (266 mg, 75 %). Mp 104-105 °C.

$[\alpha]_D$ = -0.1 (c = 0.12, CH_2Cl_2);

FT IR ν 1657, 1620 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (s, 3H), 3.48 (dd, *J* = 3.0, 9.5, 1H), 3.75 (dd, *J* = 3.5, 9.5, 1H), 3.98 (dd, *J* = 3.0, 3.5, 1H), 4.45 (s, 2H), 6.63 (br s, N*H*), 7.19 – 7.28 (m, 5H), 10.51 (br s, O*H*);

^{13}C NMR (100 MHz) δ 19.8, 62.7, 70.1, 73.7, 101.9, 127.9, 128.1, 128.6, 137.4, 175.4, 185.6, 192.6;

MS $(M\text{-}H)^-$ 260, $(M+113)^-$ 374;

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found C, 63.96; H, 5.78; N, 5.23.

(*S*)-5-(1-Benzyloxy-ethyl)-3-(1-hydroxy-ethylidene)-pyrrolidine-2,4-dione (1.66d)

1.66a was prepared following an adapted procedure.[22]

To a refluxing solution of diketone **(1.65d)** (500 mg, 1.6 mmol) in anhydrous methanol (10 mL) under argon was added over 10 mins a 0.5 M solution of sodium methoxide in methanol (3.25 mL, 1.6 mmol). The reflux was continued for 3 h and the solvent evaporated *in vacuo*. The sodium salt was dissolved in water (10 mL) and 4 M H_2SO_4 was added. The free acid was extracted with dichloromethane (3 x 10 mL), dried and filtered. The crude material was purified by column chromatography (50-80 % EtOAc/hexane) to give a yellow oil (223 mg, 50 %).

$[\alpha]_D$ = -1.2 (c = 0.55, CH_2Cl_2);

FT IR ν 1653, 1612 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (d, *J* = 5.5, 3H), 2.36 (s, 3H), 3.65 (d, *J* = 5.5, 1H), 3.69 (br dq *J* = 5.5, 1H), 4.33 (d, *J* = 11.0, 1H), 4.53 (d, *J* = 11.0, 1H), 6.76 (br s, N*H*), 7.17 – 7.24 (m, 5H), 10.31 (br s, O*H*);

^{13}C NMR (100 MHz) δ 16.8, 19.7, 66.9, 71.3, 74.7, 102.3, 127.8, 127.9, 128.5, 137.9, 175.3, 185.0, 193.1;

MS $(M-H)^-$ 274, $(M+113)^-$ 388.

2,2,2-Trichloro-acetimidic acid biphenyl-4-ylmethyl ester (1.70)

1.70 was prepared following a known procedure.[125]

To a cooled solution of 4-biphenylmethanol (2.2 g, 12.0 mmol) in dry THF (20 mL) was added sodium hydride (200 mg, 0.1 mmol). The reaction mixture was left to stir for 10 min. Trichloroacetonitrile (1.26 mL, 12.6 mmol) was added and the reaction mixture was left to stir for 8 h. The solvent was evaporated *in vacuo*, the residue redissolved in hexane (5 mL) and filtered to remove any unreacted alcohol. Evaporation of the solvent in *vacuo* gave a white solid (1.0 g, 26 %). Lit mp not given.

^{1}H NMR and FTIR data are consistent with literature reference. [125]

FT IR ν 3333, 1664 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 5.38 (s, 2H), 7.23 – 7.79 (m, 9H), 8.41 (br s, N*H*);

(*S*)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-hydroxy-butyric acid methyl ester (1.74)

1.74 was prepared following a known procedure.[126]

To a solution of phthalic anhydride (3.7 g, 25.2 mmol) in dry dioxane (25 mL) was added (*S*)-threonine methyl ester (2.8 g, 21.0 mmol). The mixture was heated at 105 °C for 5 h. The solvent was evaporated *in vacuo* and the product purified by column chromatography (50-80 % EtOAc/hexane) to give a white solid (5.5 g, 99 %). Mp 94-96 °C. Lit mp[126] 95 °C.

^{1}H NMR and mp data are consistent with literature reference.[126]

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 1.38 (d, J = 6.5, 3H), 3.87 (s, 3H), 4.04 (d, J = 9.5, 1H, O*H*), 4.40 (dq, J = 3.0, 3.5, 6.5, 1H), 4.83 (d, J = 3.5, 1H), 7.47 – 7.58 (m, 4H).

(*S*)-2-tert-Butoxycarbonylamino-3-hydroxy-butyric acid methyl ester (1.76)

1.76 was prepared following a known procedure.[127]

Triethylamine (4.25 mL, 30.0 mmol) was added to a solution of (*S*)-threonine methyl ester hydrochloride (2.0 g, 11.7 mmol) in a 1:1 mixture of dioxane:water (25 mL). The reaction flask was cooled to 0 °C and di-*tert*-butyl dicarbonate (2.8 g, 12.9 mmol) was added. After 30 min the cold bath was removed and the reaction mixture was stirred at ambient temperature for 18 h. The reaction mixture was concentrated and the residue diluted with water (15 mL) and washed with ethyl acetate (3 x 20 mL). The aqueous layer was acidified to pH 1 with 1 N HCl and back extracted with ethyl acetate (3 x 20 mL). The organic extracts were washed with brine (20 mL), dried over $MgSO_4$ and the solvent evaporated *in vacuo* to give the protected amino acid as a clear oil (2.6 g, 96 %).

FT IR and ^{1}H NMR data are consistent with literature reference.[127]

FT IR ν 3410, 1712, 1692 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (d, *J* = 7.0, 3H), 1.46 (s, 9H), 2.26 (br s, O*H*), 3.80 (s, 3H), 4.30 (br m, 1H), 5.33 (br m, 1H);

(*S*)-2-*tert*-Butoxycarbonylamino-3-hydroxy-butyric acid biphenyl-4-ylmethyl-ester (1.77)

To a solution of protected amine (**1.76**) (112 mg, 0.5 mmol) in DMF (5 mL) was added sodium hydride (38 mg, 0.8 mmol) at 0 °C. After the evolution of hydrogen, biphenyl methyl bromide (132 mg, 96.0 mmol) was added and the reaction mixture was stirred at ambient temperature for 5 h. The solvent was removed, the residue redissolved in H_2O (2 mL) and extracted with ether (3 x 5 mL). The aqueous layer was acidified with 3 N HCl and extracted with ethyl acetate (3 x 5 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Purification by column chromatography (10-50 % EtOAc/hexane) gave a white solid (91 mg, 49 %). Mp 70-71 °C.

FT IR ν 3417, 1712, 1692 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 1.25 (d, *J* = 7.0, 3H), 1.44 (s, 9H), 2.57 (br d, 1H, O*H*), 4.33 (br m, 1H), 5.21 (d, *J* = 12.3, 1H), 5.26 (d, *J* = 12.3, IH), 5.48 (d appt, *J* = 9.5, 1H), 7.34 (d, *J* = 9.5, 1H, N*H*), 7.35-7.57 (m, 9H);

^{13}**C NMR** (100 MHz) δ 18.9, 27.1, 57.9, 65.9, 67.0, 79.0, 126.0, 126.3, 126.4, 127.7, 129.2, 133.2, 139.5, 140.3, 155.1, 170.4;

MS $(M+H)^+$ 386, $(M+Na)^+$ 408.

(*S*)-2-Amino-3-(biphenyl-4-ylmethoxy)-butyric acid biphenyl-4-ylmethylester (1.78)

1.78 was prepared following an adapted procedure.[50]

A mixture of 4-biphenylmethanol (1.55 g, 8.4 mmol), (*S*)-threonine (100 mg, 0.84 mmol), and *p*-toluene sulfonic acid monohydrate (207 mg, 1.1 mmol) in toluene (30 mL) was refluxed with a Dean-Stark trap until no more water was collected (18 h). The mixture was chilled, diluted with ethyl acetate (10 mL) and shaken with cold 0.5 M Na_2CO_3 (10 mL). The organic phase was separated and washed with water (3 x 10 mL). The aqueous phase was back extracted with ethyl acetate (3 x 10 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Purification by column chromatography (50-90 % EtOAc/hexane) gave a clear oil (75 mg, 20 %).

FT IR ν 2972, 1737 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 1.27 (d, *J* = 6.0, 3H), 1.99 (br s, 2H, NH_2), 3.43 (br m, 1H), 3.99 (dq, *J* = 4.0, 6.0, 1H), 4.31 (d, *J* = 12.0, 1H), 4.52 (d, *J* = 12.0, 1H), 5.11 (s, 2H), 7.17-7.31 (m, 18H);

^{13}**C NMR** (100 MHz) δ 16.5, 59.7, 66.8, 70.6, 75.6, 127.17, 127.2, 127.38, 127.4, 127.6, 128.0, 128.2, 128.9, 128.92, 129.0, 134.7, 137.4, 140.6, 140.64, 140.9, 141.43, 174.2;

MS $(M+H)^+$ 452, $(M+Na)^+$ 474.

(*S*)-3-(Biphenyl-4-ylmethoxy)-2-(3-oxo-butyrylamino)-butyric acid biphenyl-4-ylmethyl ester (1.79)

1.79 was prepared following an acetoacetylation procedure.[39]

Diketene (74 µL, 0.1 mmol) was added dropwise to a cooled solution of amine **(1.78)** (50 mg, 0.1 mmol), in dichloromethane (2 mL) and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* and the residue redissolved in ethyl acetate (5 mL). The organic phase was washed with 1 M HCl (3 x 5 mL) and saturated Na_2CO_3 solution (3 x 5mL), dried over $MgSO_4$, filtered and the solvent evaporated. Purification by column chromatography (80 % EtOAc/hexane) gave a yellow oil (51 mg, 87 %).

FT IR ν 1732, 1655, 1547 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (d, J = 7.0, 3H), 2.19 (s, 3H), 3.41 (s, 2H), 4.15 (dq, J = 3.0, 7.0, 1H), 4.28 (d, J = 12.0, 1H), 4.51 (d, J = 12.0, 1H), 4.69 (dd, J = 3.0, 9.0, 1H), 5.10 (s, 2H), 7.16 (br d, 1H, N*H*), 7.18-7.31 (m, 18H);

^{13}C NMR (100 MHz) δ 16.5, 30.8, 50.4, 57.1, 67.1, 70.6, 74.3, 127.1, 127.2, 127.4, 128.0, 128.2, 128.6, 128.8, 128.88, 128.9, 129.0, 134.3, 136.9, 140.6, 140.8, 140.82, 141.5, 166.4, 170.3, 203.2;

MS $(M+H)^+$ 536, $(M+Na)^+$ 558.

(*S*)-Dodecanoic acid 2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-1-methyl-ethyl ester (1.82)

1.82 was prepared following a general esterification procedure.[51]

Dimethylamino-pyridine (DMAP, 6 mg, 0.05 mmol) and protected threonine (**1.76**) (230 mg, 1.1 mmol) were added to a solution of dodecanoic acid (100 mg, 0.5 mmol) in anhydrous dichloromethane (10 mL). The solution was cooled to 0 °C and DCC (103 mg, 0.5 mmol) was added over 5 min. The reaction mixture was stirred for a further 5 min at 0 °C and then for 3 h at ambient temperature. The precipitate was filtered and the filtrate evaporated to give an oily residue. The residue was dissolved in dichloromethane (10 mL) and washed with 0.5 M HCl (3 x 5 mL) and saturated $NaHCO_3$ (3 x 5 mL), dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Purification by column chromatography (50-90 % EtOAc/hexane) gave a clear oil (351 mg, 77 %).

FT IR ν 2924, 1741, 1720 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (t, *J* = 7.0, 3H), 1.16 (br m, 16H), 1.21 (d, *J* = 7.0, 3H), 1.37 (s, 9H), 1.47 (m, 2H), 2.15 (t, *J* = 8.0, 2H), 3.62 (s, 3H), 4.33 (dd, *J* = 2.0, 10.0, 1H), 5.10 (d, *J* = 10.0, 1H), 5.31 (dq, *J* = 2.0, 7.0, 1H);

^{13}C NMR (100 MHz) δ 14.2, 17.1, 22.7, 25.0, 28.3, 29.1, 29.3, 29.4, 29.5, 29.8, 29.7, 31.9, 34.3, 52.6, 57.2, 70.3, 80.3, 155.9, 170.8, 172.6;

MS $(M+H)^+$ 416, $(M+Na)^+$ 438.

(*S*)-Dodecanoic acid 2-amino-2-methoxycarbonyl-1-methyl-ethyl ester (1.83)

O
NH2
O
O
O

TFA (0.24 mL, 2.40 mmol) was added to a solution of Boc threonine (**1.82**) (100 mg, 0.24 mmol) in dichloromethane (5 mL) and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated *in vacuo* and the residue was purified by column chromatography (60-90 % EtOAc/hexane) to give a white solid (75 mg, 99 %). Mp 79-80 °C.

FT IR ν 2920, 1711, 1643 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0, 3H), 1.22 (d, *J* = 7.0, 3H), 1.26 (br m, 16H), 1.65 (m, 2H), 2.29 (t, *J* = 8.0, 2H), 3.76 (s, 3H), 4.34 (dq, *J* = 2.0, 7.0, 1H), 4.62 (dd, *J* = 2.0, 9.0, 1H), 6.41 (d, *J* = 9.0, 2H);

^{13}**C NMR** (100 MHz) δ 14.2, 20.0, 22.8, 25.1, 26.1, 25.8, 29.4, 29.5, 29.6, 29.7, 32.0, 36.7, 52.7, 57.3, 68.1, 171.8, 174.3;

MS $(M+H)^+$ 316, $(M+Na)^+$ 339.

(*S*)-Dodecanoic acid 2-methoxycarbonyl-1-methyl-2-(3-oxo-butyrylamino)-ethyl ester (1.84)

1.84 was prepared following an acetoacetylation procedure.[39]

Diketene (0.6 mL, 0.8 mmol) was added dropwise to a cooled solution of amine **(1.86)** (270 mg, 0.8 mmol), in dichloromethane (5 mL) and the reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* and the residue redissolved in ethyl acetate (5 mL). The organic phase was washed with 1 M HCl (10 mL) and saturated Na_2CO_3 (10 mL), dried over $MgSO_4$, filtered and the solvent evaporated. Purification by column chromatography (30-50 % EtOAc/hexane) gave a yellow oil (205 mg, 60 %).

FT IR ν 2924, br 1742, br 1657 cm^{-1};

^{1}**H NMR** (400 MHz, $CDCl_3$) δ 0.67 (t, *J* = 7.0, 3H), 1.05 (br m, 16H), 1.07 (d, *J* = 7.0, 3H), 1.38 (m, 2H), 2.07 (t, *J* = 8.0, 2H), 2.08 (s, 3H), 3.3 (s, 2H), 3.5 (s, 3H), 4.58 (dd, *J* = 2.0, 9.0, 1H), 5.24 (dq, *J* = 2.0, 7.0, 1H), 7.29 (d, *J* = 9.0, 1H, N*H*);

^{13}**C NMR** (100 MHz) δ 14.2, 17.2, 22.7, 24.9, 29.1, 29.3, 29.4, 29.5, 29.7, 29.73, 30.9, 31.9, 34.3, 49.6, 52.7, 55.7, 69.9, 166.2, 169.9, 172.6, 203.7;

MS $(M+H)^+$ 400, $(M+Na)^+$ 422.

Zinc coordinated tetramic acid (1.86)

1.85 was prepared following an adapted procedure.[52]

Aqueous NaOH (0.1 M) solution was added dropwise to a solution of the ligand **(1.66c)** (80 mg, 0.3 mmol) in dichloromethane (1 mL) until the pH had reached 10.5. The resulting solution was then concentrated to a small volume by evaporation of dichloromethane. The obtained mixture was dissolved in water (1 mL) and 10 % aqueous solution of zinc chloride was added (3 mL, 0.3 mmol). The resulting precipitate was filtered, washed with water and dried *in vacuo* over phosphorous pentoxide to give a pale yellow solid (40 mg). Mp > 300 °C.

FT IR ν 3278, 1594, 1474 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.30 (s, 3H), 3.48 (br dd, *J* = 6.5, 9.5, 1H), 3.65 (br dd, *J* = 3.0, 9.5, 1H), 3.74 (br m, 1H), 4.48 (d, *J* = 13.0, 1H), 4.52 (d, *J* =13.0, 1H), 7.25-7.36 (m, 5H), 8.09 (br s, N*H*);

^{13}C NMR (100 MHz) δ 26.3, 60.5, 70.4, 72.2, 100.2, 127.4, 127.5, 128.2, 138.2, 177.6, 191.7, 193.1.

4.3 Experimental section for chapter two

General procedure for keto-pyrrolidinone synthesis with acid[53] (a)

Aldehyde (1.0 mmol), amine (1.0 mmol) and valerate (1.0 mmol) were mixed together in THF (3 mL). After 30 min, 20 % acetic acid (0.2 mL) was added and the reaction mixture was stirred for 6 h. Ether (5 mL) was added to precipitate out the solid which was filtered and dried. Recrystallisation from methanol gave the product.

General procedure for keto-pyrrolidinone synthesis without acid[53] (b)

Aldheyde (1.0 mmol), amine (1.0 mmol) and valerate (1.0 mmol) were mixed together in THF. The reaction mixture was left to stir for 6 h. Ether was added to precipitate out the solid which was filtered and dried. Recrystallisation from methanol gave the product.

4-Acetyl-1-benzyl-3-hydroxy-5-methyl-1,5-dihydro-pyrrol-2-one (2.17a)

O
OH
N
O

2.17a was prepared following procedure (a) to give a white solid (204 mg, 83 %). Mp 191-192 °C.

FT IR ν 1702, 1648 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (d, J = 6.5, 3H), 2.36 (s, 3H), 3.96 (q, J = 6.5, 1H), 4.36 (d, J = 15.0, 1H), 4.91 (d, J = 15.0, 1H), 7.24 – 7.36 (m, 5H), 12.12 (br s, O*H*);

^{13}C NMR (100 MHz) δ 17.7, 30.5, 43.7, 52.5, 121.2, 127.9, 128.2, 129.1, 137.5, 154.1, 165.2, 193.0;

MS $(M - H)^-$ 244, $(M + TFA)^-$ 358.

4-Acetyl-1,5-dibenzyl-3-hydroxy-1,5-dihydro-pyrrol-2-one (2.17b)

2.17b was prepared following procedure (b) to give a white solid (205 mg, 66 %). Mp 196-198 °C.

FT IR ν 3085, 1685, 1634 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H), 3.13 (dd, *J* = 3.5, 14.1, 1H), 3.31 (dd, *J* = 3.5, 14.1, 1H), 4.21 (t, *J* = 3.5, 1H), 4.47 (d, *J* = 15.1, 1H), 5.05 (d, *J* = 15.1, 1H), 7.15 – 7.37 (m, 10H), 11.96 (br s, O*H*);

^{13}C NMR (100 MHz) δ 28.1, 31.6, 41.5, 53.5, 116.1, 125.8, 125.9, 126.2, 126.6, 126.7, 127.4, 133.2, 134.8, 152.2, 163.2, 191.1;

MS $(M - H)^-$ 320, $(M + TFA)^-$ 434.

4-Acetyl-1-benzyl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.17c)

2.17c was prepared following procedure (a) to give a white solid (198 mg, 60%). Mp 197-198 °C.

FT IR ν 3131, 1681, 1630, 1413 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (m, 3H), 2.25 (m, 1H), 2.34 (s, 3H), 4.21 (br s, 1H), 4.45 (d, *J* = 15.2, 1H), 4.88 (d, *J* = 15.2, 1H), 6.91 – 7.41 (m, 10H), 12.25 (br s, O*H*);

^{13}**C NMR** (100 MHz) δ 28.4, 30.0, 30.5, 44.5, 56.6, 118.7, 126.4, 128.2, 128.6, 128.8, 129.1, 129.4, 137.8, 141.9, 155.1, 166.3, 193.3;

MS $(M - H)^-$ 334.

4-Acetyl-1-benzyl-3-hydroxy-5-thiophene-2-yl-1,5-dihydro-pyrrol-2-one (2.17d)

2.17d was prepared following procedure (b) to give a white solid (281 mg, 90 %). Mp 219-220 °C.

FT IR ν 3142, 1684, 1650 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.32 (s, 3H), 3.81 (d, J = 15.2, 1H), 4.88 (d, J = 15.2, 1H), 5.15 (s, 1H), 6.81 (d, J = 3.6, 1H), 7.14 (m, 2H), 7.31 (m, 3H), 7.48 (m, 2H);

^{13}**C NMR** (100 MHz) δ 30.1, 44.0, 55.7, 119.9, 125.3, 126.1, 127.1, 127.8, 128.2, 128.9, 136.9, 137.5, 154.7, 165.3, 191.9;

MS $(M - H)^-$ 312;

Anal. Calcd for $C_{17}H_{15}NO_3S$: C, 65.16; H, 4.82; N, 4.47. Found C, 64.98; H, 4.81; N, 4.39.

4-Acetyl-1-benzyl-5-(5-chloro-thiophene-2-yl)-3-hydroxy-1,5-dihydro-pyrrol-2-one (2.17e)

2.17e was prepared following procedure (a) to give a white solid (239 mg, 72 %). Mp 206-207 °C.

FT IR ν 3153, 1684, 1670, 1638 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.36 (s, 3H), 4.04 (d, J = 15.4, 1H), 4.87 (d, J = 15.4, 1H), 5.30 (s, 1H), 6.99 (d, J = 4.0, 1H), 7.05 (d, J = 4.0, 1H), 7.16-7.34 (m, 5H);

^{13}**C NMR** (100 MHz) δ 29.2, 43.4, 55.2, 118.6, 126.3, 127.1, 127.4, 127.6, 127.9, 128.2, 135.9, 139.3, 154.5, 164.2, 190.8;

MS $(M - H)^-$ 346;

Anal. Calcd for $C_{17}H_{15}NO_3SCl$: C, 58.78; H, 4.04; N, 4.04; S, 9.22. Found C, 58.40; H, 4.09; N, 3.98; S, 9.47.

4-Acetyl-1-benzyl-5-furan-2-yl-3-hydroxy-1,5-dihydro-pyrrol-2-one (2.17f)

2.17f was prepared following procedure (b) to give a white solid (209 mg, 70 %). Mp 174-175 °C. Lit mp[53] 174 °C.

Mp data is consistent with literature reference. [53]

FT IR ν 3125, 1681, 1644 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.34 (s, 3H), 3.92 (d, J = 15.2, 1H), 4.89 (d, J = 15.2, 1H), 5.03 (s, 1H), 6.23 (s, 1H), 7.17-7.37 (m, 5H), 7.60 (d, J = 2.0, 1H), 7.77 (d, J = 2.0, 1H);

^{13}**C NMR** (100 MHz) δ 32.1, 45.9, 53.9, 110.8, 121.4, 123.4, 129.8, 130.1, 130.8, 139.0, 144.8, 144.3, 156.3, 167.3, 193.8;

MS $(M - H)^-$ 296.

4-Acetyl-1-benzyl-3-hydroxy-5-pyridin-4-yl-1,5-dihydro-pyrrol-2-one (2.17g)

2.17g was prepared following procedure (b) to give a white solid (201 mg, 65 %). Mp 239-240 °C.

FT IR ν 1698, 1647 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32 (s, 3H), 3.81 (d, J = 15.4, 1H), 4.87 (d, J = 15.4, 1H), 5.01 (s, 1H), 7.09-7.32 (m, 7H), 8.53 (d, J = 5.2, 2H);

^{13}C NMR (100 MHz) δ 29.8, 44.5, 59.4, 119.5, 123.4, 127.9, 128.1, 128.9, 136.5, 146.7, 149.8, 155.9, 166.0, 191.4;

MS $(M - H)^-$ 307;

Anal. Calcd for $C_{18}H_{16}NO_3$: C, 70.12; H, 5.23; N, 9.08. Found C, 69.86; H, 5.22; N, 9.01.

4-Acetyl-1-benzyl-3-hydroxy-5-isobutyl-1,5-dihydro-pyrrol-2-one (2.17h)

2.17h was prepared following procedure (b) to give a white solid (196 mg, 68 %). Mp 157-159 °C.

Literature reference does not give any characterisation.[53]

FT IR ν 3107, 1685, 1638 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 0.67 (d, J = 6.5, 3H), 0.69 (d, J = 6.5, 3H), 1.25 (m, 1H), 1.72 (m, 2H), 2.36 (s, 3H), 4.04 (t, J = 4.5, 1H), 4.31 (d, J = 15.3, 1H), 4.93 (d, J = 15.3, 1H), 7.23-7.37 (m, 5H), 12.01 (br s, O*H*);

^{13}C NMR (100 MHz) δ 23.4, 23.5, 24.3, 30.6, 36.9, 44.1, 55.7, 119.8, 127.9, 128.2, 129.1, 137.3, 153.9, 169.0, 193.2;

MS (M + TFA)$^-$ 400.

4-Acetyl-1-(4-chloro-benzyl)-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one(2.17i)

O
OH
N
O
Cl

2.17i was prepared following procedure (b) to give a white solid (284 mg, 83 %). Mp 218-219 °C.

FT IR ν 3167, 1685, 1644 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.31 (s, 3H), 3.74 (d, J = 15.2, 1H), 4.82 (d, J = 15.2, 1H), 5.02 (s, 1H), 7.10-7.38 (m, 9H);

^{13}C NMR (100 MHz) δ 29.9, 43.4, 60.4, 120.5, 127.9, 128.5, 128.8, 128.9, 129.9, 132.3, 135.8, 136.6, 154.6, 165.6, 191.6;

MS (M - H)$^-$ 340.

4-Acetyl-1-benzyl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.17j)

O
OH
N
O

2.17j was prepared following procedure (b) to give a white solid (111 mg, 88 %). Mp 188-189 °C. Lit mp[53] 188 °C.

Mp data is consistent with literature reference.[53]

FT IR ν 3149, 1683, 1649, cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.27 (s, 3H), 3.62 (d, *J* = 15.6, 1H), 4.86 (d, *J* = 15.6, 1H), 4.94, (s, 1H), 7.06-7.35 (m, 10H);

MS $(M – H)^-$ 306, $(M + TFA)^-$ 420.

4-Acetyl-3-hydroxy-1,5-diphenyl-1,5-dihydro-pyrrol-2-one (2.17k)

2.17k was prepared following procedure (a) to give a white solid (245 mg, 83 %). Mp 232-233 °C;

Data is consistent with literature reference.[57]

FT IR ν 3227, 1673, 1641 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.37 (s, 3H), 6.09 (s, 1H), 7.11 – 7.63 (m, 10H), 12.33 (br s, O*H*);

^{13}**C NMR** (100 MHz) δ 30.5, 60.8, 120.8, 125.8, 128.0, 128.1, 128.5, 128.8, 129.1, 136.6, 137.4, 153.6, 165.0, 192.1;

MS $(M - H)^-$ 292.

4-Acetyl-3-hydroxy-5-isobutyl-1-phenyl-1,5-dihydro-pyrrol-2-one (2.17l)

2.17l was prepared following procedure (a) to give a white solid (116 mg, 43 %). Mp 186-187 °C.

FT IR ν 3136, 1675, 1647 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.43 (d, J = 6.4, 3H), 0.44 (d, J = 6.4, 3H) 0.96 (m, 1H), 1.31 (m, 1H), 1.59 (m, 1H), 2.20 (s, 3H), 4.92 (t, J = 4.0, 1H), 7.04 – 7.37 (m, 5H), 12.03 (br s, O*H*);

^{13}C NMR (100 MHz) δ 23.0, 23.5, 24.3, 30.6, 37.6, 56.6, 119.4, 123.5, 126.2, 129.4, 136.5, 153.6, 164.6, 193.2;

MS $(M - H)^-$ 272.

4-Acetyl-3-hydroxy-1-methyl-5-phenyl-1,5-dihydro-pyrrol-2-one (2.17m)

O
OH
N
O

2.17m was prepared following procedure (b) to give a white solid (151 mg, 66 %). Mp 214-215 °C.

Data is consistent with literature reference.[128]

FT IR ν 1682, 1647 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38 (s, 3H), 2.74 (s, 3H), 5.23 (s, 1H), 7.27-7.46 (m, 5H), 12.03 (br s, O*H*);

^{13}C NMR (100 MHz) δ 27.6, 29.9, 61.5, 120.1, 127.8, 128.5, 128.8, 137.2, 155.5, 165.4, 191.4;

MS $(M - H)^-$ 230.

4-Acetyl-3-hydroxy-5-(4-methoxy-phenyl)-1-methyl-1,5-dihydro-pyrrol-2-one (2.17n)

2.17n was prepared following procedure (b) to give a white solid (125 mg, 48 %). Mp 224-225 °C.

FT IR ν 3148, 1684, 1636 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.30 (s, 3H), 2.66 (s, 3H), 3.77 (s, 3H), 5.11 (s, 1H), 6.92 (d, *J* = 8.4, 2H), 7.12 (d, *J* = 8.4, 2H);

^{13}C NMR (100 MHz) δ 27.5, 30.1, 55.4, 61.5, 114.3, 120.3, 128.8, 128.9, 154.8, 159.3, 165.2, 191.9;

MS $(M - H)^-$ 260;

Anal. Calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found C, 64.07; H, 5.82; N, 5.23.

4-Acetyl-5-(4-chloro-phenyl)-3-hydroxy-1-methyl-1,5-dihydro-pyrrol-2-one (2.17o)

2.17o was prepared following procedure (b) to give a white solid (77 mg, 37 %). Mp 239-240 °C.

Data is consistent with literature reference.[128]

FT IR ν 3108, 1687, 1636 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.67 (s, 3H), 5.18 (s, 1H), 7.25 (d, *J* = 8.6, 2H), 7.43 (d, *J* = 8.6, 2H);

^{13}**C NMR** (100 MHz) δ 26.9, 29.4, 60.7, 119.4, 128.3, 129.2, 132.2, 135.7, 154.7, 164.8, 190.9;

MS $(M - H)^-$ 264.

4-Acetyl-3-hydroxy-1-isobutyl-5-phenyl-1,5-dihydro-pyrrol-2-one (2.17p)

2.17p was prepared following procedure (b) to give a white solid (192 mg, 70 %). Mp 195-197 °C.

FT IR ν 3131, 1685, 1643 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 0.84 (d, *J* = 6.5, 3H), 0.87 (d, *J* = 6.5, 3H), 1.89 (m, 1H), 2.39 (s, 3H), 2.49 (dd, *J* = 6.0, 13.0, 1H), 3.42 (dd, *J* = 9.0, 13.0, 1H), 5.28 (s, 1H), 7.27 -7.45 (m, 5H);

^{13}**C NMR** (100 MHz) δ 21.1, 21.4, 28.1, 31.2, 48.7, 61.7, 121.4, 128.9, 129.4, 129.9, 138.2, 155.2, 166.8, 192.8;

MS $(M - H)^-$ 272, $(M + TFA)^-$ 386.

4-Acetyl-1-benzyl-3-hydroxy-5-(4-methoxy-phenyl)-1,5-dihydro-pyrrol-2-one (2.17q)

2.17q was prepared following procedure (b) to give a white solid (191 mg, 63 %). Mp 185-187 °C.

FT IR ν 3148, 1683, 1645 cm^{-1};

^{1}H NMR (400 MHz, MeOD-d_3) δ 0.83 (d, *J* = 6.8, 3H), 0.86 (d, *J* = 6.8, 3H), 1.86 (m, 1H), 2.31 (s, 3H), 2.54 (dd, *J* = 5.5, 13.5, 1H), 3.40 (dd, *J* = 9.0, 13.5, 1H), 4.92 (s, 3H), 5.22 (s, 1H), 6.92 (d, *J* = 8.0, 2H), 7.13 (d, *J* = 8.0, 2H);

^{13}C NMR (100 MHz) δ 20.7, 20.9, 28.9, 29.9, 49.2, 60.8, 62.5, 115.5, 115.7, 121.9, 130.2, 130.4, 161.7, 167.5, 195.4;

MS $(M + TFA)^-$ 416.

4-Acetyl-3-hydroxy-1-isobutyl-5-methyl-1,5-dihydro-pyrrol-2-one (2.17r)

2.17r was prepared following procedure (b) to give a white solid (192 mg, 86 %).

FT IR ν 1307, 1684, 1635 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, *J* = 6.5, 3H), 0.98 (d, *J* = 6.5, 3H), 1.38 (d, *J* = 6.5, 3H), 1.97 (m, 1H), 2.5 (s, 3H), 3.0 (dd, *J* = 5.5, 14.0, 1H), 3.68 (dd, *J* = 10, 14.0, 1H), 4.29 (q, *J* = 6.5, 1H);

^{13}C NMR (100 MHz) δ 17.5, 20.2, 20.7, 27.9, 30.5, 47.9, 53.8, 121.9, 153.8, 166.1, 194.3;

MS $(M - H)^-$ 210, $(M + TFA)^-$ 324.

4-Acetyl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.17s)

2.17s was prepared following procedure (a) to give a white solid (152 mg, 70 %). Mp 187-188 °C.

FT IR ν 3080, 1698, 1650 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H), 5.20 (s, 1H), 7.23-7.35 (m, 5H), 9.31 (s, N*H*);

^{13}C NMR (100 MHz) δ 29.8, 55.8, 120.6, 126.8, 127.2, 127.8, 138.5, 154.3, 166.5, 191.7;

MS $(M - H)^-$ 216.

1-Benzyl-3-hydroxy-5-phenyl-4-(thiophene-2-carbonyl)-1,5-dihydro-pyrrol-2-one (2.18a)

2.18a was prepared following procedure (b) to give a white solid (195 mg, 56 %). Mp 244-245 °C.

FT IR ν 3142, 1687, 1655, 1604 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (d, J = 15.0, 1H), 4.94 (d, J = 15.0, 1H), 5.24 (s, 1H), 7.12 – 7.97 (m, 11H), 8.12 (s, 1H), 8.13 (s, 1H), 12.22 (br s, O*H*);

^{13}C NMR (100 MHz) δ 44.3, 61.1, 119.8, 127.8, 128.1, 128.2, 128.8, 128.82, 129.04, 129.1, 134.7, 135.3, 136.1, 136.9, 144.3, 150.6, 165.7, 180.3;

MS $(M - H)^-$ 374;

Anal. Calcd for $C_{22}H_{17}NO_3S$: C, 70.38; H, 4.56; N, 3.73. Found C, 69.99; H, 4.53; N, 3.74.

1-Benzyl-3-hydroxy-5-(4-methoxy-phenyl-4-(thiophene-2-carbonyl)1,5-dihydro-pyrrol-2-one (2.18b)

2.18b was prepared following procedure (b) to give a white solid (244 mg, 60 %). Mp 240-241 °C.

FT IR ν 3102, 1681, 1661, 1607 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.96 (s, 1H), 3.97 (s, 3H), 5.16 (d, J = 15.2, 1H), 5.47 (d, J = 15.2, 1H), 7.13 – 7.61 (m, 10H), 8.21 (d, J = 3.2, 1H), 8.34 (d, J = 3.2, 1H), 12.38 (br s, O*H*);

^{13}C NMR (100 MHz) δ 41.6, 52.9, 58.0, 112.0, 125.1, 125.4, 125.6, 125.7, 126.3, 126.6, 126.9, 132.2, 132.8, 134.4, 141.8, 148.0, 157.1, 163.3, 177.9;

MS $(M - H)^-$ 404;

Anal. Calcd for $C_{23}H_{19}NO_4S$: C, 68.13; H, 4.72; N, 3.45; S, 7.91 Found C, 68.41; H, 4.66; N, 3.48; S, 8.24.

1-Benzyl-3-hydroxy-4-(thiophene-2-carbonyl)-5-thiophene-2-yl-1,5-dihydro-pyrrol-2-one (2.18c)

2.18c was prepared following procedure (b) to give a white solid (279 mg, 73 %). Mp 229-230 °C.

FT IR ν 3119, 1684, 1658, 1607 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (d, *J* = 15.2, 1H), 4.90 (d, *J* = 15.2, 1H), 5.42 (s, 1H), 6.94-7.57 (m, 9H), 7.99 (s, 1H), 8.12 (s, 1H), 12.01 (br s, O*H*);

^{13}C NMR (100 MHz) δ 44.4, 56.6, 119.3, 125.8, 126.2, 127.5, 127.8, 128.1, 128.5, 129.0, 134.9, 135.3, 136.9, 137.1, 144.2, 150.4, 165.3, 180.6;

MS $(M - H)^-$ 380.

1-Benzyl-3-hydroxy-5-isobutyl-4-(thiophene-2-carbonyl)-1,5-dihydro-pyrrol-2-one (2.18d)

2.18d was prepared following procedure (b) to give a white solid (289 mg, 85 %). Mp 161-162 °C.

FT IR ν 3074, 1684, 1655, 1610 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 (d, *J* = 6.8, 3H), 0.72 (d, *J* = 6.8, 3H), 1.35 (m, 1H), 1.65 (m, 2H), 4.33 (t, *J* = 4.5, 1H), 4.37 (d, *J* = 15.5, 1H), 4.99 (d, *J* = 15.6, 1H), 7.24 – 7.99 (m, 6H), 8.0 (d, *J* = 1.0, 1H), 8.04 (d, *J* = 1.0, 1H), 11.83 (br s, O*H*);
^{13}C NMR (100 MHz) δ 23.5, 23.7, 37.6, 44.3, 56.3, 61.1, 119.7, 127.8, 128.1, 128.8, 129.1, 134.6, 135.3, 137.4, 144.3, 150.7, 165.7, 181.2;

MS $(M - H)^-$ 354;

Anal. Calcd for $C_{20}H_{21}NO_3S$: C, 67.58; H, 5.95; N, 3.94. Found C, 67.46; H, 6.01; N, 3.82.

1-(4-Chloro-benxyl)-3-hydroxy-5-phenyl-4-(thiophene-2-carbonyl)1,5-dihydro-pyrrol-2-one (2.18e)

2.18e was prepared following procedure (b) to give a yellow solid (236 mg, 57 %). Mp 245-246 °C.

FT IR ν 3126, 1685, 1662, 1609 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (d, J = 15.7, 1H), 4.86 (d, J = 15.7, 1H), 5.23 (s, 1H), 7.13-7.39 (m, 10H), 7.97 (d, J = 4.8, 1H), 8.13 (t, J = 3.0, 1H);

^{13}C NMR (100 MHz) δ 43.8, 61.3, 119.5, 128.7, 128.8, 128.9, 129.5, 129.6, 129.9, 129.96, 132.4, 134.5, 134.9, 135.0, 144.5, 164.3, 180.1, 193.6;

MS $(M-H)^-$ 403;

Anal. Calcd for $C_{22}H_{17}NO_3SCl$: C, 64.50; H, 4.16; N, 3.42. Found C, 64.20; H, 3.99; N, 3.34.

3-Hydroxy-1,5-diphenyl-4-(thiophene-2-carbonyl)-1,5-dihydro-pyrrol-2-one (2.18f)

2.18f was prepared following procedure (a) to give a yellow solid (251 mg, 70 %). Mp 234-235 °C.

FT IR ν 3144, 1679, 1671, 1598 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 6.35 (s, 1H), 7.13-7.38 (m, 10H), 7.67 (d, J = 7.6, 1H), 7.99 (d, J = 4.4, 1H), 8.10 (d, J = 3.0, 1H), 12.18 (br s, OH);

^{13}C NMR (100 MHz) δ 62.3, 121.2, 123.7, 126.4, 128.8, 128.9, 129.4, 129.5, 129.7, 135.6, 136.1, 137.3, 137.33, 144.9, 149.9, 165.6, 181.3;

MS $(M - H)^-$ 360.

3-Hydroxy-1-methyl-5-phenyl-4(thiophene-2-carbonyl)-1,5-dihydro-pyrrol-2-one (2.18g)

2.18g was prepared following procedure (b) to give a yellow solid (215 mg, 71 %). Mp 191-192 °C.

FT IR ν 3115, 1685, 1653, 1601 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.73 (s, 3H), 5.45 (s, 1H), 7.22-7.38 (m, 6H), 7.97 (d, J = 4.8, 1H), 8.09 (t, J = 2.5, 1H);

^{13}C NMR (100 MHz) δ 26.3, 61.4, 118.1, 126.5, 127.2, 127.3, 127.6, 133.1, 133.7, 135.1, 142.3, 149.4, 163.8, 178.9;

MS $(M - H)^-$ 298;

Anal. Calcd for $C_{16}H_{13}NO_3S$: C, 64.20; H, 4.38; N, 4.68. Found C, 63.81; H, 4.32; N, 4.60.

3-Hydroxy-5-phenyl-4-(thiophene-2-carbonyl)-1,5-dihydro-pyrrol-2-one (2.18h)

2.18h was prepared following procedure (b) to give a yellow solid (113 mg, 40 %). Mp 216-217 °C.

FT IR ν 3394, 3160, 1705, 1662 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 5.49 (s, 1H), 7.22-7.33 (m, 6H), 7.98 (d, *J* = 4.8, 1H), 8.07 (d, *J* = 4.0, 1H), 9.41 (s, N*H*);

^{13}**C NMR** (100 MHz) δ 56.7, 119.8, 126.8, 127.5, 128.0, 128.4, 133.8, 134.4, 137.9, 143.7, 150.4, 166.5, 180.1;

MS $(M - H)^-$ 284.

1-Benzyl-4-(2,2-dimethyl-propionyl)3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.19a)

2.19a was prepared following procedure (b) to give a white solid (199 mg, 57 %). Mp 175-176 °C.

FT IR ν 3176, 1681, 1636 cm^{-1};

^{1}**H NMR** (400 MHz, DMSO-d_6) δ 1.01 (s, 9H), 3.62 (d, *J* = 15.0, 1H), 4.89 (d, *J* = 15.0, 1H), 5.01 (s, 1H), 7.10 – 7.36 (m, 10H);

^{13}**C NMR** (100 MHz) δ 25.1, 42.8, 61.4, 90.6, 119.2, 127.8, 127.9, 128.4, 128.9, 129.0, 129.3, 136.9, 137.4, 149.5, 166.3, 200.5;

MS $(M - H)^-$ 348.

1-Benzyl-4-(2,2-dimethyl-propionyl)-3-hydroxy-5-(4-methoxy-phenyl)-1,5-dihydro-pyrrol-2-one (2.19b)

2.19b was prepared following procedure (a) to give a white solid (210 mg, 55 %). Mp 182-183 °C.

Data is consistent with literature values.[129]

FT IR ν 3176, 1681, 1641 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (s, 9H), 3.63 (d, *J* = 15.0, 1H), 3.77 (s, 3H), 4.88 (d, *J* = 15.0, 1H), 4.97 (s, 1H), 6.89- 7.38 (m, 9H), 12.25 (br s, O*H*);

^{13}C NMR (100 MHz) δ 25.0, 43.9, 44.1, 55.4, 60.8, 114.3, 119.8, 127.8, 128.1, 128.5, 128.9, 136.9, 137.0, 148.0, 159.3, 162.8, 200.9;

MS $(M - H)^-$ 378.

1-Benzyl-5-(4-chloro-phenyl)-4-(2,2-dimethyl-propionyl)-3-hydroxy-1,5-dihydro-pyrrol-2-one (2.19c)

2.19c was prepared following procedure (b) to give a white solid (180 mg, 47 %). Mp 179-180 °C.

FT IR ν 3159, 1687, 1638 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (s, 9H), 3.72 (d, *J* = 15.2, 1H), 4.86 (d, *J* = 15.2, 1H), 5.03 (s, 1H), 7.01 – 7.39 (m, 9H);

^{13}C NMR (100 MHz) δ 25.0, 42.9, 44.3, 60.8, 119.3, 127.8, 128.1, 129.0, 129.05, 129.8, 132.8, 136.4, 136.9, 148.8, 160.0, 200.5;

MS $(M - H)^-$ 382.

4-(2,2-Dimethyl-propionyl)-3-hydroxy-1,5-diphenyl-1,5-dihydro-pyrrol-2-one (2.19d)

2.19d was prepared following procedure (a) to give a white solid (105 mg, 31 %). Mp 233-234 °C.

Data is consistent with literature reference.[129]

FT IR ν 3208, 1682, 1644 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (s, 9H), 6.12 (s, 1H), 7.11-7.61 (m, 10H), 12.15 (br s, O*H*);

^{13}C NMR (100 MHz) δ 25.3, 43.3, 62.3, 120.8, 123.0, 125.8, 127.9, 128.2, 128.6, 129.2, 136.8, 137.6, 146.9, 165.3, 201.7;

MS $(M - H)^-$ 334.

4-(2,2-Dimethyl-propionyl)-3-hydroxy-1-methyl-5-phenyl-1,5-dihydro-pyrrol-2-one (2.19e)

2.19e was prepared following procedure (b) to give a white solid (92 mg, 34 %). Mp 150-151 °C.

FT IR ν 3074, 1688, 1630 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (s, 9H), 2.66 (s, 3H), 5.21 (s, 1H), 7.16-7.38 (m, 5H);

^{13}C NMR (100 MHz) δ 25.0, 27.7, 42.9, 63.4, 119.5, 127.3, 128.3, 128.8, 137.6, 148.4, 165.6, 200.8;

MS $(M - H)^-$ 272.

4-(2,2-Dimethyl-propionyl)-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.19f)

O OH N H O

2.19f was prepared following procedure (a) to give a white solid (114 mg, 44 %). Mp 191-192 °C.

FT IR ν 3336, 3101, 1709, 1631 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (s, 9H), 5.23 (s, 1H), 7.19-7.33 (m, 5H), 9.29 (s, N*H*), 11.87 (br s, O*H*);

^{13}C NMR (100 MHz) δ 24.4, 42.2, 57.2, 119.9, 126.6, 127.2, 127.8, 138.9, 147.7, 166.8, 200.6;

MS $(M - H)^-$ 258.

1-(3,4-Dichloro-benzyl)-4-(2,2-dimethyl-propionyl)-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one (2.19g)

2.19g was prepared following procedure (b) to give a white solid (380 mg, 46 %). Mp 269-271 °C.

FT IR ν 3173, 1681, 1642 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (s, 9H), 3.64 (d, J = 15.0, 1H), 4.89 (d, J = 15.0, 1H), 5.15 (s, 1H), 6.93 – 7.37 (m, 8H);

^{13}C NMR (100 MHz) δ 24.4, 42.6, 43.1, 61.9, 120.2, 127.1, 127.8, 128.3, 129.8, 130.2, 131.6, 132.3, 134.3, 135.3, 147.9, 165.7, 176.4, 201.6;

MS $(M - H)^-$ 416.

Zinc coordinated keto pyrrolidinone (2.20i, B)

Aqueous NaOH (0.1M) solution was added dropwise to a solution of the ligand (155 mg, 0.45 mmol) **(2.21i)** in dichloromethane until the pH had reached 10.5. The resulting solution was then concentrated to a small volume by evaporation of the solvent. The obtained mixture was dissolved in water and 10 % aqueous solution of zinc chloride was added (4.5 mL, 0.45 mmol). The resulting precipitate was filtered,

washed with water and dried *in vacuo* over phosphorous pentoxide to give a white solid (110 mg). Mp 269-271 °C.

FT IR ν 3359, 1630, 1669, 1492, cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 3H), 3.82 (d, *J* = 15.5, 1H), 4.92 (d, *J* = 15.5, 1H), 5.08 (s, 1H), 7.16 – 7.36 (m, 9H);

^{13}C NMR (100 MHz) δ 28.2, 43.8, 60.7, 113.6, 128.1, 128.2, 128.8, 128.9, 129.8, 132.4, 135.7, 138.6, 166.1, 170.3, 190.9.

2-Methoxy-2-phenyl-propionic acid 1-(3,4-dichloro-benzyl)-4-(2,2-dimethyl propionyl)-2-oxo-5-phenyl-2,5-dihydro-1H-pyrrol-3-yl ester (2.24g)

Sodium hydride was added to a solution of the keto-pyrrolidinone **(2.19g)** (115 mg, 0.28 mmol) in dichloromethane (5 mL) at 0 °C. The solution was allowed to reach room temperature and R (-)-α-methoxy-trifluoromethyl-phenylacetyl chloride (54 mL, 0.29 mmol) was added. After 16 h the mixture was filtered and the solvent was evaporated in *vacuo*. The crude material was purified by column chromatography (50 % EtOAc/hexane) to give a colourless oil (49 mg, 28 %, two diasteroisomers). ^{1}H NMR and ^{13}C NMR data is given for one diasteroisomer.

FT IR ν 1780, 1715, 1681, 1172 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 (s, 9H), 3.71 (d, *J* = 6.5, 1H), 3.85 (s, 3H), 5.03 (d, *J* = 6.5, 1H), 5.21 (s, 1H), 6.95 – 7.77 (m, 13H);

^{13}**C NMR** (100 MHz) δ 25.6, 43.9, 44.2, 56.8, 63.8, 85.0, 124.8, 127.9, 128.1, 128.9, 129.0, 129.7, 129.8, 130.0, 130.1, 130.5, 130.8, 131.3, 136.5, 140.4, 163.2, 164.0, 171.5, 204.4.

4.4 Experimental section for chapter three

3-Nitropyridin-4-amine (3.26)

3.26 was prepared following a known procedure.[84]

Fuming HNO_3 (5 mL) was added dropwise to a solution of 4-aminopyridine (10.0 g, 106 mmol) in concentrated H_2SO_4 (23 mL). The mixture was stirred for 5 h, poured onto crushed ice and neutralised with aqueous ammonium hydroxide. The precipitate was filtered and recrystallisation from water gave the intermediate, 4-nitraminopyridine **(3.25)** as a yellow solid (7.0 g, 48 %).

4-nitraminopyridine **(3.25)** (7.0 g, 50 mmol) was gradually added to concentrated H_2SO_4 (25 mL) and the mixture heated to 150 °C for 30 min. The mixture was poured onto crushed ice, neutralised with aqueous ammonium hydroxide and the precipitate was filtered. Recrystallisation from water gave a yellow solid (5.4 g, 78 %). Mp 200-202 °C. Lit mp[84] 202-205 °C.

Mp and NMR data are consistent with literature reference.[84]

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.89 (d, J = 6.0, 1H), 7.91 (br s , 2H, NH_2), 8.14 (d, J = 6.0, 1H), 8.97 (s, 1H);

^{13}C NMR (100 MHz) δ 112.6, 128.7, 148.0, 149.4, 151.7;

2-Chloropyridine-3,4-diamine (3.27)

3.27 was prepared following a known procedure.[85]

Finely ground tin (II) chloride (17.0 g, 90 mmol) was added to a mixture of 4-amino-3-nitro pyridine **(3.26)** (2.5 g, 18 mmol) and hot concentrated HCl (40 mL). The mixture was heated to 90 °C for 1 h, cooled and further stirred for 1 h. The white precipitate was filtered through celite, dissolved in water and desalted on Dowex 50X 8(H^+ form) (100 mL). After elution with water the Dowex was transferred to a

beaker, neutralized with 10 % aqueous ammonia, filtered and washed with 10 % aqueous ammonia (100 mL). The solvent was evaporated *in vacuo* and the residue purified by column chromatography (5-10 % MeOH/dichloromethane) to give a white solid (2.3 g, 90 %). Mp 155-156 °C. Lit mp[85] 154-155 °C.

Mp and ^{1}H NMR data are consistent with literature reference.[85]

FT IR ν 3181, 3223, 1580 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 4.64 (br s, 2H, NH_2), 5.75 (br s, 2H, NH_2), 6.44 (d, J = 5.0, 1H), 7.30 (d, J = 5.0, 1H);

^{13}C NMR (100 MHz) δ 108.2, 135.1, 137.5, 138.4, 142.9;

MS (M+H)$^+$ 145, (M+MeCN)$^+$ 185.

4-Chloro-3*H*-imidazo[4,5-c]pyridin-2-amine (3.28)

To a solution of the diamine **(3.27)** (529 mg, 3.7 mmol) in methanol (5 mL) was added a solution of cyanogen bromide (1.9 g, 1.8 mmol) in methanol (5 mL). The mixture was heated under reflux for 24 h. The precipitate formed was filtered and the solvent evaporated *in vacuo*. Neutralisation on an SCX-2 column (elution with methanolic ammonia) gave a crude mixture. The crude material was purified by column chromatography (5-15 % MeOH/dichloromethane) to give a white solid (144 mg, 23 %). Mp 215-216 °C.

FT IR ν 2788, 1657 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 6.75 (br s, 2H, NH_2), 7.12 (d, J = 5.0, 1H), 7.78 (d, J = 5.0, 1H), 11.37 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 106.1, 138.5, 147.7, 155.3, 156.7, 163.1;

MS (M+H)$^+$ 169, (M+MeCN)$^+$ 210.

tert-Butyl 4-chloro-3*H*-imidazo[4,5-c]pyridin-2-ylcarbamate (3.29)

3.29 was prepared following a general procedure.[87]

To a solution of guanidine **(3.28)** (121 mg, 0.7 mmol) in methanol (5 mL) was added triethylamine (0.2 mL, 1.6 mmol). The reaction flask was cooled to 0 °C and di-*tert*-butyl dicarbonate (343 mg, 1.6 mmol) was added. After 30 min at 0 °C the reaction mixture was stirred at room temp for 18 h. The solvent was evaporated *in vacuo*. Purification by column chromatography (dichloromethane/MeOH) gave a white solid (115 mg, 59 %). Mp 256-257 °C.

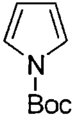
FT IR ν 1743, 1659 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.72 (s, 9H), 7.54 (d, J = 5.0, 1H), 7.70 (br s, 2H, 2 x N*H*), 8.03 (d, J = 5.0, 1H);

^{13}C NMR (100 MHz) δ 28.7, 88.0, 110.1, 137.6, 138.4, 140.9, 150.1, 155.9, 181.7;

MS $(M+H)^+$ 269, $(M+MeCN)^+$ 310.

tert-Butyl 1*H*-pyrrole-1-carboxylate (3.30)



3.30 was prepared following a known procedure.[88]

4-(*N*,*N*-Dimethylamino)pyridine (2.4 g, 19 mmol), and di-*tert*-butyl-dicarbonate (50.1 g, 230 mmol), were added to a solution of pyrrole (15.5 mL, 230 mmol) in dry acetonitrile (25 mL) and stirred for 24 h at rt. The mixture was concentrated *in vacuo* and the residue purified by column chromatography (hexane) to give a colourless liquid (30.9 g, 80 %).

^{1}H and ^{13}C NMR data are consistent with literature reference.[88]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H), 6.12 (t, J = 2.0, 2H), 7.15 (t, J = 2.0, 2H);

^{13}C NMR (100 MHz) δ 28.0, 83.6, 111.9, 120.1, 149.0.

1-(*tert*-Butoxycarbonyl)-1*H*-pyrrol-2-yl-2-boronic acid (3.15)

3.15 was prepared following a known procedure.[89]

n-BuLi (2.3 M in hexane, 6.4 mL, 14.9 mmol), was slowly added to a solution of 2,2,6,6-tetramethyl-piperidine (2.5 mL, 14.9 mmol) in THF (60 mL) at -78 °C under argon. After stirring for 15 min at that temperature, the mixture was allowed to warm to 0 °C over 30 min. After cooling again to -78 °C a solution of Boc-pyrrole **(3.30)** (2.0 g, 12.0 mmol), in THF (10 mL), was added at such a rate the temperature remained below -65 °C. The reaction mixture was stirred for 2 h at -78 °C prior to the addition of trimethyl borate (10.2 mL, 60.0 mmol). The solution was allowed to warm to ambient temperature overnight. Aqueous HCl (0.25 M, 80 mL, 19.0 mmol), was added, the solvent evaporated, the residue extracted with ether (3 x 20 mL) and the combined organic extracts washed with water and dried over Na_2SO_4. The solution was slowly concentrated until a precipitate formed. The mixture was cooled to 0 °C and the precipitated product filtered. Trituration with cold ether (20 mL) and drying of the precipitate *in vacuo* afforded the boronic acid as a white solid (1.5 g, 61 %). Mp 101- 102 °C. Lit mp[89] 101.0-101.5 °C.

Mp and ^{1}H NMR data are consistent with literature reference.[89]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9H), 6.19 (t, *J* = 4.0, 1H), 6.77 (s, 2H, 2 x O*H*), 7.02 (dd, *J* = 2.0, 4.0, 1H), 7.38 (dd, *J* = 2.0, 4.0, 1H).

(1*E*, 4*E*)-1,5-Diphenylpenta-1,4-dien-3-one (3.33)

3.33 was prepared following a known procedure.[130]

An aqueous solution of NaOH (3 M, 15 mL) in ethanol (10 mL) was cooled to 0 °C. A solution of benzaldehyde (1.6 mL, 16.0 mmol) in acetone (0.6 mL, 8.0 mmol) was added dropwise to this solution whilst maintaining the temperature below 25 °C.

After 1 h the precipitate was collected and washed with water (20 mL) until the washings were neutralised. Recrystallisation from ethanol gave yellow crystals (1.4 g, 71 %). Mp 105-107 °C. Lit mp[130] 104-107 °C.

Mp and NMR data are consistent with literature values.[130]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 16.0, 2H), 7.42 (m, 6H), 7.62 (m, 4H), 7.76 (d, J = 16.0, 2H);

^{13}C NMR (100 MHz) δ 125.6, 128.5, 129.1, 130.6, 134.9, 143.4, 189.0.

Tris(dibenzylideneacetone)dipalladium(0)chloroform (3.34)

3.34 was prepared following a known procedure.[77]

Palladium dichloride (225 mg, 1.3 mmol) was added to hot (*ca* 50 °C) methanol (35 mL) containing dibenzylidene acetone (990 mg, 4.2 mmol) and sodium acetate (833 mg, 10.2 mmol). The mixture was stirred for 4 h at 40 °C to give a reddish purple precipitate and then allowed to cool. The precipitate was removed by filtration, washed (H_2O/acetone, 10 mL) and dried *in vacuo.* The product, (dibenzylideneacetone)palladium(0) (847 mg) was dissolved in hot chloroform (30 mL) and filtered to give a deep violet solution. Diethyl ether (20 mL) was added slowly and deep purple needles precipitated which were filtered and dried *in vacuo* to give $(Pd_2dba)_3(CHCl_3)$[77] (850 mg, 25 %).

Mp 133-135 °C, Lit mp (Strem) 131-135 °C

tert-Butyl 2-phenyl-1*H*-pyrrole-1-carboxylate (3.31)

3.31 was prepared following a known procedure.[131]

To a solution of bromobenzene (42 μL, 0.4 mmol) and $Pd(PPh_3)_4$ (14 mg, 0.014 mmol), in DME (2 mL) under argon was added a degassed solution of *N*-Boc-pyrrole-boronic acid **(3.15)** (100 mg, 0.5 mmol) in ethanol (0.5 mL), followed by a degassed solution of sodium carbonate (83 mg, 0.8 mmol) in H_2O (0.5 mL). The mixture was heated to 80 °C for 16 h. The reaction mixture was cooled and *tert*-butyl methyl ether

(5 mL) was added. The organic layer was separated, washed with brine (3 x 5 mL), dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Purification by column chromatography (20-50 % EtOAc/hexane) gave a yellow oil (56 mg, 59 %).

^{1}H NMR and ^{13}C NMR data are consistent with literature reference.[131]

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (s, 9H), 6.15 – 6.09 (m, 2H), 7.26 – 7.15 (m, 6H);
^{13}C NMR (100 MHz) δ 26.6, 82.5, 109.5, 113.3, 121.5, 126.1, 126.5, 128.2, 133.5, 133.9, 148.4.

***tert*-Butyl 2-(pyridin-2-yl)-1*H*-pyrrole-1-carboxylate (3.32) and 2-(1*H*-Pyrrol-2-yl)pyridine (3.35)**

N Boc N (3.32) N HN (3.35)

3.32 and **3.35** were prepared following a general procedure.[82]
Triphenylphosphine (80 mg, 0.3 mmol) was added to a solution of $Pd_2(dba)_3.CHCl_3$ (20 mg, 0.02 mmol) in toluene (2 mL) under argon. The reaction mixture was stirred for 5 min. To this solution was added bromopyridine (36 μL, 0.4 mmol) and a degassed solution of *N*-Boc-pyrrole-boronic acid **(3.15)** (96 mg, 0.5 mmol) in ethanol (0.5 mL), followed by a degassed solution of sodium carbonate (81 mg, 0.8 mmol) in H_2O (0.5 mL). The mixture was heated to 80 °C for 16 h. The reaction mixture was cooled and *tert*-butyl methyl ether (5 mL) was added. The organic layer was separated, washed with brine (3 x 5 mL), dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Separation of these products was achieved by column chromatography (10-50 % EtOAc/hexane).

(3.32): yellow oil (40 mg, 43 %).
FT IR ν 1737, 1312 cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (s, 9H), 6.17 (t, *J* = 4.0, 1H), 6.33 (dd, *J* = 2.0, 4.0, 1H), 7.11 (ddd, *J* = 1.0, 6.0, 1H), 7.29 (m, 2H), 7.59 (ddd, *J* = 2.0, 10.0, 1H), 8.53 (ddd, *J* = 2.0, 6.0, 1H);

^{13}C **NMR** (100 MHz) δ 27.7, 83.7, 110.6, 115.7, 121.8, 123.6, 134.2, 135.8, 136.9, 148.9, 149.7, 153.1;
MS $(M+H)^+$ 245.

(3.35): as a yellow solid (19 mg, 35 %).
Mp 89 – 90 °C, Lit mp[132] 88 – 90 °C.
Mp and ^1H NMR data are consistent with literature reference.[132]
^1H **NMR** (400 MHz, $CDCl_3$) δ 6.22 (1H, m), 6.65 (1H, m), 6.81 (1H, m), 6.96 (m, 1H), 7.51 (2H, m), 8.34 (1H, m), 10.20 (br s, 1H, N*H*).

2-(3,4,5-tribromo-1H-pyrrol-2-yl)pyridine (3.36) and 2-(4,5-Dibromo-1H-pyrrol-2-yl)pyridine (3.37)

Br N Br HN Br **(3.36)** N Br HN Br **(3.37)**

3.36 and **3.37** were prepared following a general procedure.[92]
The pyrrole **(3.35)** (70 mg, 0.50 mmol), was dissolved in THF (3 mL) and cooled in a dry ice/2-propanol bath. NBS (173 mg, 1.0 mmol) was added and the mixture swirled for a short time, after removal from the dry ice bath, until all the NBS was in solution. The solution was allowed to stand in the freezer for 2 h. Sodium sulfite was added to the solution and the solvent was removed *in vacuo*. Purification by column chromatography (50-80 % EtOAc/hexane) gave an inseparable mixture of tri and di-brominated products, **(3.36)** and **(3.37)** respectively.

Data for **(3.36)**:
^1H **NMR** (400 MHz, $CDCl_3$) δ 7.22 (m, 1H), 7.69 (ddd, J = 2.0, 8.0, 1H), 7.80 (m, 1H), 8.49 (dd, J = 1.0, 4.0, 1H);
MS $(M+H)^+$ 382.

Data for **(3.37)**:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (m, 1H), 7.41 (ddd, *J* = 1.0, 4.0, 1H), 7.80 (m, 1H), 8.12 (dd, *J* = 1.0, 8.0, 1H), 8.72 (m, 1H);

MS $(2M+H)^+$605.

tert-Butyl-3-amino-2-chloropyridin-4-ylcarbamate (3.41) and Di-_tert_-butyl-2-chloropyridin-3,4-yl-bis-carbamate (3.42)

(3.41) **(3.42)**

3.41 and **3.42** were prepared following an adapted procedure.[93]

Di-*tert*-butyl dicarbonate (1.2 g, 5.5 mmol), 2-chloro-3,4-diaminopyridine (360 mg, 2.5 mmol) and chloroform (10 mL) were stirred and heated at reflux for 16 h. The solvent was evaporated *in vacuo*. Analysis of the crude mixture indicated that there were two products present. Separation of these products was achieved by column chromatography (60-80 % EtOAc/hexane).

3.41: white solid (341 mg, 56 %). Mp 182-183 °C.

FT IR ν 1694, 1366 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9H), 6.12 (br s, 2H, N*H_2*), 6.59 (d, *J* = 5.5, 1H), 7.69 (d, *J* = 5.5, 1H), 8.19 (br s, 1H, N*H*)

^{13}C NMR (100 MHz) δ 28.5, 78.7, 109.2, 115.8, 146.2, 151.8, 153.6, 180.5

MS $(M+H)^+$ 244, $(M+H+MeCN)^+$ 285.

3.42: white solid (54 mg, 6 %). Mp 163-164 °C.

FT IR ν 3430, 3217, 1790, 1743 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 18H), 4.51 (br s, 2H, 2 x N*H*), 6.55 (d, *J* = 5.5, 1H), 7.90 (d, *J* = 5.5, 1H);

^{13}C NMR (100 MHz) δ 27.9, 28.3, 82.1, 83.7, 109.7, 119.0, 140.5, 148.0, 150.1, 150.6, 151.3;

MS $(M+H)^+$ 344, $(M+Na+MeCN)^+$ 407;

Anal. Calcd for $C_{15}H_{22}NO_4Cl$: C, 52.40; H, 6.45; N, 12.22. Found C, 52.11; H, 6.13; N, 11.86.

***N,N'*-(2-Chloro-pyridin-3,4-yl)bis-acetamide (3.43)**

3.43 was prepared following an adapted procedure.[90]

To a solution of diamine **(3.27)** (200 mg. 1.4 mmol) in dichloromethane (5 mL) at 0 °C was added Et_3N (0.4 mL, 3.1 mmol) followed by AcCl (0.2 mL, 3.1 mmol). The reaction was allowed to warm to room temperature and was stirred for 18 h. The reaction mixture was poured onto water (5 mL) and the layers separated. The organic layer was washed with brine (3 x 5 mL) dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Purification by column chromatography (5 % MeOH/dichloromethane) gave a white solid (38 mg, 12 %).

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 2.09 (s, 3H), 2.17 (s, 3H), 8.15 (d, J = 5.5, 1H), 8.18 (d, J = 5.5, 1H), 9.45 (s, 1H, N*H*), 9.53 (s, 1H, N*H*);

^{13}C NMR (100 MHz) δ; 23.1, 24.2, 114.5, 120.9, 144.9, 147.3, 149.8, 169.2, 169.6;

MS $(M+H)^+$ 228, $(M+H+MeCN)^+$ 270.

4-Chloro-1*H*-imidazo[4,5-c]pyridin-2(3*H*)-one (3.44)

3.44 was prepared following a known procedure.[133]

To a 0 °C suspension of diamine **(3.27)** (426 mg, 2.9 mmol) and *N*-methyl-morpholine (1.4 mL, 11.0 mmol) in dichloromethane (10 mL) was added trichloromethyl chloroformate (0.4 mL, 3.5 mmol) dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. After evaporating, the residue was refluxed in 15 % H_2O/dioxane for 1.5 h. Ethyl acetate (5 mL) was

added and filtration gave a white powder which was recrystallised from methanol to give a white solid (285 mg, 56 %). Mp 339-340 °C. Lit mp[133] 342 °C.
Mp data is consistent with literature reference.[133]

FT IR ν 2771, 2723, 1728 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.01 (d, *J* = 5.0, 1H), 7.92 (d, *J* = 5.0, 1H), 11.39 (br s, 2H, 2 x N*H*);

^{13}C NMR (100 MHz) δ 104.2, 124.4, 128.9, 137.6, 141.4, 154.3;

MS $(M+H)^+$ 170, $(M+MeCN)^+$ 211.

4-Chloro-1*H*-imidazo[4,5-c]pyridine-2(3*H*)-thione (3.46)

S
HN
NH
N
Cl

3.46 was prepared following a general procedure.[95]

To a solution of diamine **(3.27)** (118 mg, 0.8 mmol) in ethanol (5 mL) were added carbon disulfide (1 mL, 1.6 mmol) and triethylamine (0.35 mL, 0.3 mmol). The reaction mixture was heated at 45 °C for 48 h. The solvent was evaporated *in vacuo* and the residue resuspended in methanol (5 mL). Filtration followed by recrystallisation from methanol gave a yellow solid (117 mg, 79 %). Mp 345-346 °C.

FT IR ν 2845, 2775, 1613 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.17 (d, *J* = 5.0, 1H), 8.05 (d, *J* = 5.0, 1H), 13.14 (br s, 1H, N*H*), 13.34 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 104.8, 127.4, 129.9, 139.7, 142.4, 171.1;

MS $(M+H)^+$ 186, $(M+MeCN)^+$ 227.

2-Iodo-3,4-diaminopyridine (3.45)

NH_2
NH_2
N
I

3.45 was prepared following a general procedure.[98]

To a stirred solution of (**3.27**) (400 mg, 2.8 mmol), in acetone (20 mL) was added sodium iodide (2.1 g, 13.9 mmol). The resulting mixture was heated under reflux for 24 h in the dark. The solid precipitate was filtered and washed with acetone. The solvent was evaporated *in vacuo* and the resulting crude material was purified by column chromatography (5 % dichloromethane/methanol) to give a yellow oil (180 mg, 30 %).

FT IR ν 1657, 1024 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.16 (br s, 2H, NH_2), 6.23 (d, *J* = 5.0, 1H), 7.24 (br s, 2H, NH_2), 7.40 (d, *J* = 5.0, 1H);

^{13}C NMR (100 MHz) δ 105.6, 136.1, 139.0, 140.6, 153.8.

4-Chloro-2-(methylthio)-1*H*-imidazo[4,5-c]pyridine (3.47)

HN SMe N N Cl

3.47 was prepared following a general procedure.[97]

To a solution of thiourea (**3.46**) (111 mg, 0.6 mmol) in DMF (2 mL) was added methyl iodide (0.04 mL, 0.6 mmol). The reaction mixture was heated to 100 °C for 24 h. The reaction mixture was cooled and ether (5 mL) was added. The solid precipitate was collected by filtration and the product purified by column chromatography (5-10 % dichloromethane/methanol) to give a white solid (85 mg, 71 %). Mp 224-225 °C.

FT IR ν 2922, 1610 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ (s, 3H) 7.46 (d, *J* = 7.0, 1H), 8.04 (d, *J* = 7.0, 1H), 13.32 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 13.8, 104.8, 106.3, 130.1, 139.7, 140.3, 142.4;

MS $(M+H)^+$ 200, $(M+MeCN)^+$ 241.

3-Nitropyridine-2,4-diol (3.51)

3.51 was prepared following a known procedure.[101]

Fuming HNO_3 (1 mL) was added dropwise to a solution of 2,4 dihydroxypyridine **(3.50)** (200 mg, 1.8 mmol) in H_2SO_4 (1 mL) at 0 °C. The reaction mixture was stirred for 30 min, poured onto crushed ice and chilled for 2 h. The resulting precipitate was filtered, washed with cold water and dried *in vacuo* to give a yellow solid (210 mg, 75 %).

NMR data is consistent with literature reference.[101]

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.05 (d, J = 7.3, 1H), 7.46 (d, J = 7.3, 1H), 11.87 (br s, O*H*), 12.39 (br s, O*H*);

^{13}C NMR (100 MHz) δ 98.0, 127.5, 138.0, 156.2, 160.6;

4-Nitro-2-bromopyridine-N-oxide (3.55)

3.55 was prepared following a known procedure.[104]

2-Bromopyridine-*N*-oxide hydrochloride (15.0 g, 72.0 mmol) was dissolved in H_2SO_4 (125 mL). Fuming HNO_3 (28 mL) was added dropwise and the reaction mixture was heated to 90 °C for 2 h. The liquid was cooled, poured onto ice, neutralised with ammonium hydroxide and the precipitate collected by filtration. Recrystallisation from methanol gave a yellow solid (11.5 g, 74 %). Mp 146-147 °C. Lit mp[104] 145-146 °C.

FT IR ν 1398, 1515 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 6.9, 1H), 8.63 (d, J = 6.9, 1H), 8.75 (d, J = 2.0, 1H);

^{13}C NMR (100 MHz) δ 119.7, 125.3, 133.0, 140.8, 141.5;

2-Bromopyridin-4-amine (3.56)

3.56 was prepared following a general procedure.[104]

Iron powder (10.9 g, 196.0 mmol) was added to a solution of nitropyridine **(3.55)** (8.6 g, 39.2 mmol) in acetic acid (140 mL). The reaction was heated to 100 °C for 1 h. The mixture was basified with ammonium hydroxide and extracted with ether. The organic phase was dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Recrystallisation from methanol/ether gave a white solid (5.5 g, 80 %). Mp 95-96 °C. Lit mp[106] 95-97 °C.

FT IR ν 891, 1337 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.38 (br s, 2H, N*H*), 6.48 (dd, *J* = 2.0, 5.6, 1H), 6.62 (d, *J* = 2.0, 1H), 7.74 (d, *J* = 5.6, 1H);

^{13}C NMR (100 MHz) δ 108.8, 110.6, 142.0, 149.4, 156.5;

MS $C_5H_5N_2Br^{79}$ $(M+H)^+$ 173, $C_5H_5N_2Br^{81}$ $(M+H)^+$ 175.

2-Bromo-3-nitropyridin-4-amine (3.53) and 2-Bromo-5-nitropyridin-4-amine (3.58)

(3.53) **(3.58)**

3.53 and **3.58** were prepared following a general procedure.[84]

Fuming HNO_3 (16 mL) was added dropwise to a solution of 4-amino-2-bromopyridine (8.0 g, 46.1 mmol) in concentrated H_2SO_4 (40 mL). The mixture was stirred for 5 h, poured onto crushed ice and neutralised with aqueous ammonium hydroxide. The precipitate was filtered and recrystallisation from water gave the intermediate, 4-nitramino-2-bromopyridine **(3.57)** (10.2 g, 99 %).

4-nitramino-2-bromopyridine **(3.57)** (10.2 g, 46.1 mmol) was gradually added to concentrated H_2SO_4 (80 mL) and the was mixture heated to 90 °C for 30 min. The mixture was poured onto crushed ice, neutralised with aqueous ammonium hydroxide

and the precipitate was filtered. Recrystallisation from water gave a mixture of two isomers (6.2 g, 62 %). Separation of these isomers was achieved by column chromatography (2-5 % MeOH/dichloromethane).

Literature reference[106] does not give experimental data.

(3.53): yellow solid (5.5 g, 55 %). Mp 195-196 °C.

FT IR ν 3423, 1228, 1365 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (d, *J* = 6.0, 1H), 7.25 (br s, 2H, N*H*$_2$), 7.87 (d, *J* = 6.0, 1H);

^{13}C NMR (100 MHz) δ; 112.0, 132.0, 133.3, 148.4, 149.2;

MS $C_5H_4N_3O_2Br^{79}$ $(M+H)^+$ 218, $C_5H_4N_3O_2Br^{81}$ $(M+H)^+$ 220.

(3.58): yellow solid (700 mg, 7 %). Mp 164-166 °C.

FT IR ν 3355, 1598, 1351 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.11 (d, *J* = 3.5, 1H), 8.03 (br s, 2H, N*H*$_2$), 8.76 (d, *J* = 3.5, 1H);

^{13}C NMR (100 MHz) δ 114.6, 129.0, 144.7, 148.6, 150.5;

MS $C_5H_4N_3O_2Br^{79}$ $(M+H)^+$ 218, $C_5H_4N_3O_2Br^{81}$ $(M+H)^+$ 220.

2-Bromopyridine-3,4-diamine (3.48)

NH_2
NH_2
N Br

3.48 was prepared following a general procedure.[104]

Iron powder (256 mg, 4.6 mmol) was added to a solution of nitropyridine **(3.53)** (200 mg, 0.9 mmol) in acetic acid (4 mL) and the reaction mixture was heated to 100 °C for 2 h. The mixture was allowed to cool, neutralised with aqueous ammonium hydroxide solution and extracted with ether (3 x 20 mL). The aqueous layer was further extracted with dichloromethane (3 x 20 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Recrystallisation from methanol gave a white solid (104 mg, 62 %). Mp 151-152 °C. Lit mp is not given.

^{1}H NMR is consistent with literature values.[134]

FT IR ν 820 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 4.65 (br s, 2H NH_2), 5.76 (br s, 2H, NH_2), 6.46 (d, J = 6.8, 1H), 7.31 (d, J = 6.8, 1H);

^{13}C NMR (100 MHz) δ 108.2, 126.1, 135.1, 137.5, 142.9;

MS $C_5H_6N_3Br^{79}$$(M+H)^+$ 188, $C_5H_6N_3Br^{81}$ $(M+H)^+$ 190.

***tert*-Butyl 2-(4-amino-3-nitropyridin-2-yl)-1*H*-pyrrole-1-carboxylate (3.59)**

NH_2 NO_2 N BocN

3.59 was prepared following an adapted procedure.[82]

To a degassed solution of the nitroamino-bromopyridine **(3.53)** (2.1 g, 9.9 mmol) in EtOH (10 mL) were added degassed solutions of boronic acid **(3.14)** (2.6 g, 11.9 mmol) in EtOH (5 mL) and Na_2CO_3 (2.1 g, 20.0 mmol) in H_2O (10 mL). This mixture was added to a freshly prepared, degassed suspension mixture of palladium(II)acetate (111 mg, 0.5 mmol) and triphenylphosphine (391 mg, 1.5 mmol) in DME (15 mL). The reaction was heated to 80 °C for 5 h. The reaction mixture was cooled and *tert*-butyl methyl ether (20 mL) was added. The organic layer was separated, washed with brine (3 x 20 mL), dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Purification by column chromatography (50-80 % EtOAc/hexane) gave a yellow solid (1.95 g, 55 %). Mp 155-157 °C.

FT IR ν 2980 1738, 1314 cm^{-1};

^{1}H NMR (400 MHz, MeOD-d_3) δ 1.23 (s, 9H), 6.12 (dd, J = 2.0, 3.5, 1H), 6.14 (dd, J = 3.0, 3.5, 1H), 6.80 (d, J = 6.0, 1H), 7.26 (dd, J = 2.0, 3.0, 1H), 7.92 (d, J = 6.0, 1H);

^{13}C NMR (100 MHz) δ 27.8, 85.2, 111.9, 113.4, 115.5, 123.2, 131.0, 133.5, 149.5, 150.0, 151.0, 151.2;

MS $(M+H)^+$ 305, $(M+Na)^+$ 327.

3-Nitro-2-(1*H*-pyrrol-2-yl)pyridin-4-amine (3.60)

The protected pyrrole **(3.59)** (100 mg, 0.3 mmol) was added to a 10 % solution of TFA in dichloromethane (3.3 mL, 0.3 mmol). The reaction mixture was left to stir for 30 min and the solvent was evaporated in *vacuo*. Purification by column chromatography gave a yellow solid (67 mg, 99 %). Mp 144-145 °C.

FT IR ν 3425, 1601,1452, cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.30 (br s, 2H, N*H*$_2$), 6.57 (d, *J* = 5.5, 1H), 6.68 (dd, *J* = 3.0, 3.5, 1H), 6.94 (dd, *J* = 1.5, 3.5, 1H), 7.62 (dd, *J* = 1.5, 3.0, 1H), 7.96 (d, *J* = 5.5, 1H), 10.75 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 104.5, 105.9, 112.7, 114.8, 129.7, 131.5, 140.7, 143.5, 144.3;

MS $(M+H)^+$ 205.

Anal. Calcd for $C_9H_8N_4O_2$: C, 52.94; H, 3.95; N, 27.43. Found C, 52.58; H, 3.87; N, 27.01.

2-(1*H*-Pyrrol-2-yl)pyridine-3,4-diamine (3.67)

3.67 was prepared following a general procedure.[85]

Finely ground tin (II) chloride (3.7 g, 20mmol) was added to solution of the nitro derivative **(3.59)** (1.2 g, 4 mmol) in hot concentrated hydrochloric acid (10 mL). The mixture was heated to 90 °C for 2 h, cooled and further stirred for 1 h. The white precipitate was filtered through celite, dissolved in water and desalted on Dowex 50X8 (H^+ form) (100 mL). After elution with water the Dowex was transferred to a beaker, neutralized with aqueous ammonia, filtered and washed with aqueous ammonia (100 mL). The solvent was evaporated *in vacuo* and the residue purified by column chromatography (10-15 % MeOH/dichloromethane) to give a brown solid. Recrystallisation from methanol gave a white solid (584 mg, 84 %). Mp 189-191°C.

FT IR ν 3454, 3394, 3167 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.20 (br s, 2H, NH_2), 6.30 (dd, J = 2.5, 5.0, 1H), 6.71 (m, 1H), 6.74 (d, J = 6.5, 1H), 7.10 (dd, J = 1.5, 2.5, 1H), 7.51 (br s, 2H, NH_2), 7.68 (d, J = 6.5, 1H), 11.66 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 105.4, 109.4, 111.3, 121.1, 121.7, 126.8, 127.4, 131.2, 148.6;

MS $(M+H)^+$ 175.

Di(1*H*-imidazol-1-yl)methanimine (3.69)

3.69 was prepared following a known procedure.[112]

To a solution of imidazole (2.2 g, 30.5 mmol) in dichloromethane (160 mL) was added cyanogen bromide (1.24 g, 11.7 mmol). The mixture was heated at reflux for 30 min and then cooled to room temperature. The white precipitate was removed by filtration. The filtrate was concentrated to 20 mL and cooled to 0 °C for 24 h. The crystallised solid was collected by filtration, washed with ether (10 mL) and dried in *vacuo* to give a white solid (1.44 g, 76 %).

^{1}H and ^{13}C NMR data are consistent with literature reference.[112]

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (s, 1H), 7.56 (br s, 1H), 8.07 (br s, 1H), 10.20 (br s, 1H);

^{13}C NMR (75 MHz) δ 118.9, 129.6, 137.4, 140.9

Di(benzotriazole-1-yl)methanimine (3.73) and 1*H*-Benzotriazole-1-yl-(2*H*-benzotriazol-2-yl)methanimine (3.73*), Mixture of isomers

The mixture of isomers **3.73** and **3.73*** was prepared following a known procedure.[116]

To a solution of benzotriazole (1.0 g, 8.4 mmol) in ethanol (20 mL) at 0 °C was added a solution of cyanogen bromide (445 mg, 4.2 mmol) in acetone (2 mL) followed by a

10 % solution of NaOH (42 mL, 4.2 mmol). The reaction was left to stir for 30 min and the white precipitate formed was filtered and washed with ice cold ethanol (3 x 10 mL). Recrystallisation from benzene gave white microneedles (890 mg, 80 %). Mp 162-163 °C. Lit mp[116] 162-163 °C.

Mp and NMR data are consistent with literature reference.[116]

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49-7.68 (m, 6H), 7.86-7.89 (m, 6H), 8.29 (d, J = 8, 1H), 8.36-8.38 (m, 8H), 11.74 (s, 2H);

4-(1*H*-Pyrrol-2-yl)-3*H*-imidazo[4,5-c]pyridin-2-amine (3.68)

3.68 was prepared following an adapted procedure.[116]

To a solution of di(benzotriazole-1-yl)methanimine **(3.73)** (1.0 g, 3.8 mmol) in THF (10 mL), under argon was added amine **(3.67)** (223 mg, 1.3 mmol) and the reaction mixture was heated under reflux for 48 h. The mixture was concentrated *in vacuo* and redissolved in dichloromethane (2 mL). Purification by column chromatography (10-15 % MeOH/dichloromethane) gave a brown solid which was recrystallised from methanol to give a white solid (156 mg, 61 %). Mp >300 °C.

FT IR ν 2932, 2671, 2488 cm^{-1};

^{1}H NMR (300 MHz, MeOD-d_3) δ 6.80 (d, J = 6.7, 1H), 6.99 (dd, J = 3.0, 3.5, 1H), 7.28 (dd, J = 1.1, 3.5, 1H), 7.82 (dd, J = 1.1, 3.0, 1H), 7.92 (d, J = 6.7, 1H);

^{13}C NMR (100 MHz) δ 105.2, 106.2, 115.9, 116.4, 123.8, 126.9, 129.0, 138.8, 145.7, 154.4;

MS $(M+H)^+$ 200.

4-(1*H*-Pyrrol-2-yl)-1*H*-imidazo[4,5-c]pyridine-2(3*H*)-thione (3.72)

3.72 was prepared following a general procedure.[95]

To a solution of diamine **(3.67)** (100 mg, 0.6 mmol) in ethanol (2 mL) were added CS_2 (0.6 mL, 9.7 mmol) and Et_3N (0.2 mL, 1.8 mmol). The reaction mixture was heated to 50 °C for 48 h. The reaction vessel was cooled and the solvent evaporated *in vacuo*. Purification by column chromatography (5-10 % MeOH/dichloromethane) gave a brown solid which was recrystallised from methanol to give a white solid (60 mg, 49 %). Mp >300 °C.

FT IR ν 2923, 1612, 1194 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.21 (br s, 1H), 6.92 (br s, 1H), 6.98 (dd, J = 1.5, 5.5, 1H), 7.28 (br s, 1H), 8.21 (dd, J = 1.0, 5.5, 1H), 11.45 (br s, 1H, N*H*), 12.56 (br s, 1H, N*H*), 12.94 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 101.9, 109.5, 110.2, 120.9, 123.9, 127.6, 133.6, 138.9, 141.9, 170.4;

MS $(M+H)^+$ 217,

***tert*-Butyl 2-(4-amino-3-nitropyridin-2-yl)-5-bromo-1*H*-pyrrole-1-carboxylate (3.61) and *tert*-Butyl 2-(4-amino-5-bromo-3-nitropyridin-2-yl)-5-bromo-1*H*-pyrrole-1-carboxylate (3.62)**

NH2 NO2 N BocN Br **(3.61)**

NH2 Br NO2 N BocN Br **(3.62)**

3.61 and **3.62** were prepared following an adapted procedure.[92]

To a solution of pyrrole **(3.59)** (100 mg, 0.3 mmol) in THF (2 mL) at -50 °C was added NBS (117 mg, 0.6 mmol). The reaction mixture was stirred for 10 min at this temperature and then for 20 min at room temperature. The reaction mixture was stirred at room temperature for 20 min. The solvent was evaporated *in vacuo* and the residue re-dissolved in $CDCl_3$. Analysis of the crude material indicated there were two products. Separation of these products was achieved by column chromatography (50 –80 % EtOAc/hexane).

(3.61): yellow solid (61 mg, 48 %). Mp 118-120 °C.

FT IR ν 1753, 1617, 1294 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 9H), 6.0 (br s, 2H, N*H_2*), 6.20 (d, *J* = 3.5, 1H), 6.25 (d, *J* = 3.5, 1H), 6.66 (d, *J* = 6.0, 1H), 8.12 (d, *J* = 6.0, 1H);

^{13}C NMR (100 MHz) δ 27.9, 86.0, 104.8, 105.6, 112.4, 114.5, 115.8, 116.3, 147.1, 148.8, 149.7, 177.9;

MS $C_{14}H_{15}N_4O_4Br^{79}(M+H)^+$ 383, $C_{14}H_{15}N_4O_4Br^{81}$ $(M+H)^+$ 385.

(3.62): yellow solid (75 mg, 42 %). Mp 127-128 °C.

FT IR ν 1752, 1600, 1293 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9H), 6.17 (d, *J* = 3.5, 1H), 6.20 (br s, 2H, N*H_2*), 6.24 (d, *J* = 3.5, 1H), 8.36 (s, 1H);

^{13}C NMR (100 MHz) δ 27.8, 85.7, 104.0, 105.8, 107.9, 114.7, 116.3, 131.6, 145.5, 147.9, 151.1, 151.3;

MS $C_{14}H_{14}N_4O_4Br^{79}Br^{79}$ $(M+H)^+$ 461, $C_{14}H_{14}N_4O_4Br^{79}Br^{81}$ $(M+H)^+$ .463, $C_{14}H_{14}N_4O_4Br^{81}Br^{81}$ $(M+H)^+$ .465.

Anal. Calcd for $C_{14}H_{14}N_4O_4Br_2$: C, 36.39; H, 3.05; N, 12.12. Found C, 35.94; H, 3.03; N, 11.65.

5-Bromo-2-(3,4,5-tribromo-1*H*-pyrrol-2-yl)-3-nitropyridin-4-amine (3.63)

3.63 was prepared following an adapted procedure.[92]

To a solution of **(3.60)** (33 mg, 0.16 mmol) in methanol (2 mL) at –50 C was added NBS (114 mg, 0.64 mmol). The reaction mixture was stirred for 10 min at this temperature and then at room temperature for 16 h. The solvent was evaporated *in vacuo*. Analysis of the crude material indicated that there were tetra and tri-brominated products present. Purification by column chromatography (50-70 % EtOAc/hexane) gave a yellow solid (41 mg, 49 %). Mp 248-250 °C

FT IR ν 3368, 1606, 1514, 1263 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (br s, 2H, N*H_2*), 8.55 (s, 1H), 12.99 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 98.6, 100.8, 102.4, 107.4, 127.9, 131.9, 143.4, 145.4, 151.1;

MS $C_9H_4N_4O_2Br^{79}Br^{79}Br^{81}Br^{81}$ $(M+H)^+$ 521.

Anal. Calcd for $C_9H_4N_4O_2Br_4$: C, 20.80; H, 0.78; N, 10.77. Found C, 20.88; H, 0.90; N, 10.45.

2-(4,5-Dibromo-1*H*-pyrrol-2-yl)-3-nitropyridin-4-amine (3.66)

3.66 was prepared following an adapted procedure.[92]

To a solution of pyrrole **(3.60)** (51 mg, 0.25 mmol) in methanol (2 mL) at -78 °C was added NBS (89 mg, 0.50 mmol). The reaction mixture was stirred for 2 h maintaining a temperature below -50 °C. The solvent was evaporated *in vacuo*. Analysis of the crude material indicated di- and tri-brominated products were present. Purification by column chromatography (50-80 % EtOAc/hexane) gave a yellow solid (51 mg, 56 %).

Mp 185-186 °C

FT IR ν 1738, 1366, 1216 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.25 (s, 1H), 6.72 (d, *J* = 6.0, 1H), 6.90 (br s, 2H, N*H_2*), 8.09 (d, *J* = 6.0, 1H), 12.58 (br s, 1H, N*H*);

^{13}C NMR (100 MHz) δ 98.4, 104.1, 109.9, 111.2, 128.4, 130.7, 141.6, 147.1, 149.0;

MS $C_9H_6N_4O_2Br^{79}Br^{79}$ $(M+H)^+$ 361, $C_9H_6N_4O_2Br^{79}Br^{81}$ $(M+H)^+$ 363. $C_9H_6N_4O_2Br^{81}Br^{81}$ $(M+H)^+$ 365.

Anal. Calcd for $C_9H_6N_4O_2Br_2$: C, 29.86; H, 1.67; N, 15.47. Found C, 29.52; H, 1.64; N, 15.27.

4-(4,5-Dibromo-1*H*-pyrrol-2-yl)-3*H*-imidazo[4,5-c]pyridin-2-amine (3.1) and 4-(5-Bromo-1*H*-pyrrol-2-yl)-3*H*-imidazo[4,5-c]pyridin-2-amine (3.76) and 7-Bromo-4-(4,5-dibromo-1*H*-pyrrol-2-yl)-1*H*-imidazo[4,5-c]pyridin-2-amine (3.75)

(3.1) (3.76) (3.75)

3.1, **3.75** and **3.76** were prepared following an adapted procedure.[92]

To a solution of guanidine **(3.68)** (65 mg, 0.33 mmol) in methanol (1 mL) at -78 °C was added NBS (116 mg, 0.65 mmol) in portions. The reaction mixture was left to stir for 2 h maintaining the temperature below -50 °C. The solvent was evaporated *in vacuo* and analysis of the crude material indicated that mono, di and tri-brominated products were present. The tri-brominated compound **(3.75)** was separated by column chromatography (5-10 % MeOH/dichloromethane). The mono and di-brominated compounds **(3.76)** and **(3.1)** were inseparable by column chromatography. Purification by HPLC (MeCN/H_2O, 0.05 % TFA) gave the mono-brominated **(3.76)** and the di-brominated product **(3.1)** as the TFA salt (~1-2 mg).

(3.1):

^{1}H NMR (400 MHz, MeOD-d_3) δ 6.77 (d, *J* = 7.0, 1H), 7.10 (s, 1H), 7.90 (d, *J* = 7.0, 1H);

MS $C_{10}H_7N_5Br^{79}Br^{79}$ $(M+H)^+$ 356, $C_{10}H_7N_5Br^{81}Br^{79}$ $(M+H)^+$ 358, $C_{10}H_7N_5Br^{81}Br^{81}$ $(M+H)^+$ 360.

(3.76):

^{1}H NMR (400 MHz, MeOD-d_3) δ 6.78 (d, *J* = 7.0; 1H), 7.04 (d, *J* = 4.0, 1H), 7.27 (d, *J* = 4.0, 1H), 7.90 (d, *J* = 7.0, 1H);

MS $C_{10}H_8N_5Br^{79}$ $(M+H)^+$ 278, $C_{10}H_8N_5Br^{81}$ $(M+H)^+$280.

(3.75):

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.91 (s, 1H), 8.16 (s, 1H);

MS $C_{10}H_6N_5Br^{79}Br^{79}Br^{81}$ $(M+H)^+$ 438.

The synthesis of the mono-brominated product **(3.76)** was also achieved by an alternative route.[117]

A solution of guanidine **(3.68)** (34 mg, 0.17 mmol) in methanol (0.2 mL) was added to a solution of AIBN (1 mg, 5 mol %) in THF (1 mL) at -78 °C. The reaction mixture was left to stir for 5 min. 1,3-Dibromo-5,5-dimethyl hydantoin (24 mg, 0.09 mmol) was added to the solution over 5 min. The reaction mixture was stirred for a further 10 min and then allowed to stand for 2 h maintaining the temperature below -50 °C. The solvent was evaporated *in vacuo* and the residue redissolved in dichloromethane. Purification by column chromatography (5-10 % MeOH/dichloromethane) gave a yellow solid **(3.76)** (21 mg, 44 %). Mp-254-256 °C

^{1}H NMR (400 MHz, MeOD-d_3) δ 6.58 (d, *J* = 5.5; 1H), 6.81 (d, *J* = 4.0, 1H), 7.12 (d, *J* = 4.0, 1H), 7.88 (d, *J* = 5.5, 1H);

^{13}C NMR (100 MHz) δ 93.9, 105.2, 106.3, 120.7, 124.0, 134.9, 135.1, 144.5, 145.3, 150.7;

MS $C_{10}H_8N_5Br^{79}$ $(M+H)^+$ 278, $C_{10}H_8N_5Br^{81}$ $(M+H)^+$280.

2-(3,4,5-Tribromo-1H-pyrrol-2-yl)pyridine-3,4-diamine (3.70)

NH2
NH2
Br
N
Br
HN
Br

3.70 was prepared following an adapted procedure.[92]

To a solution of **(3.67)** (30 mg, 0.17 mmol) in methanol (1 mL) at -78 C was added NBS (61.3 mg, 0.34 mmol). The reaction was left to stir for 2 h and warmed to room temperature over 30 min. The solvent was evaporated *in vacuo*. Purification by column chromatography gave the tri-brominated compound as the major product (15 mg, 21 %). Mp 229-231 °C

^{1}**H NMR** (400 MHz, MeOD-d_3) δ 6.71 (d, *J* = 6.5; 1H), 7.59 (d, *J* = 6.5, 1H).

^{13}**C NMR** (100 MHz) δ 105.2, 106.1, 107.0, 108.3, 112.0, 115.9, 116.3, 120.9, 139.0;

MS $C_9H_7N_5Br^{79}Br^{81}Br^{81}$ $(M+H)^+$ 413.

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Appendix

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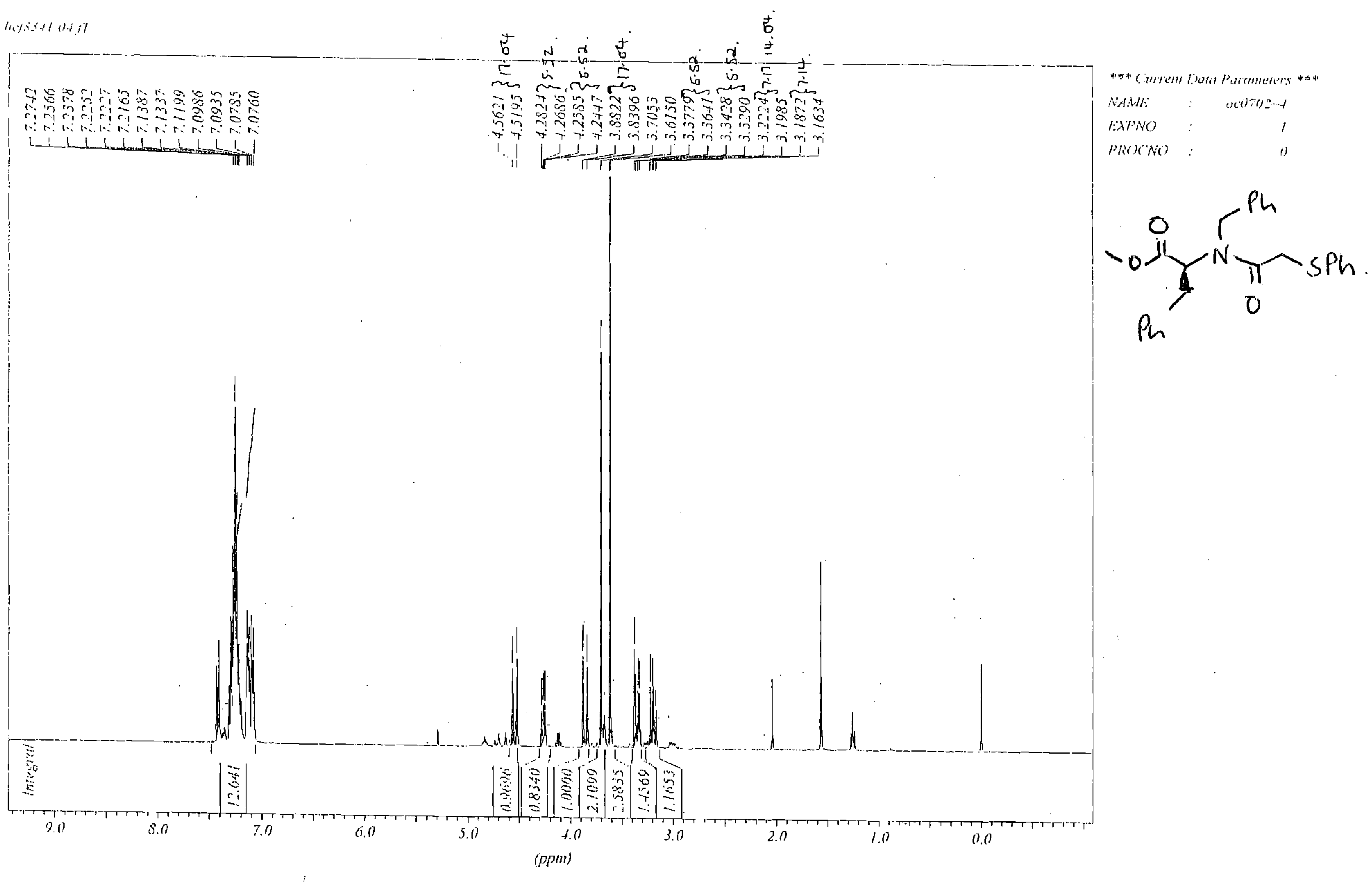

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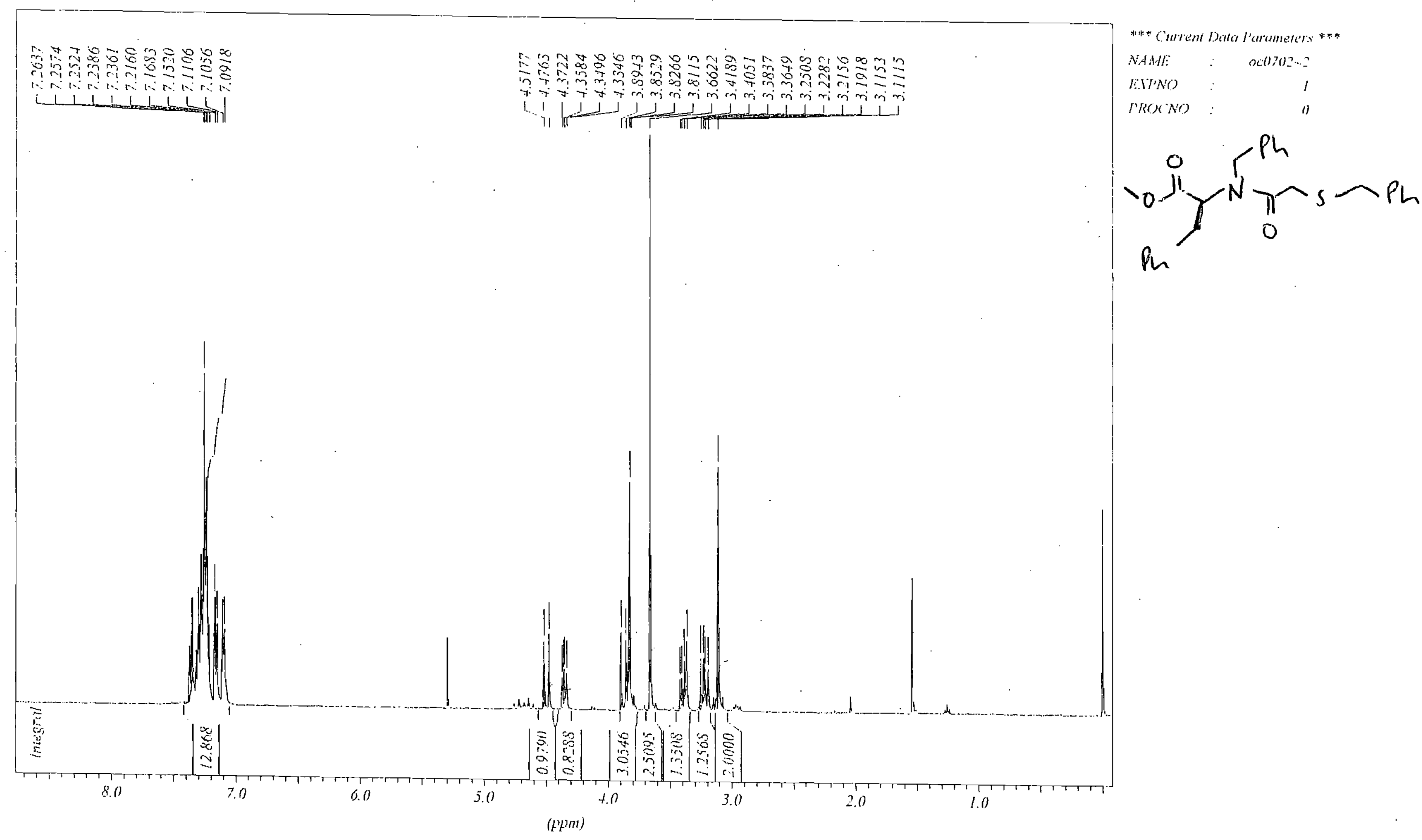

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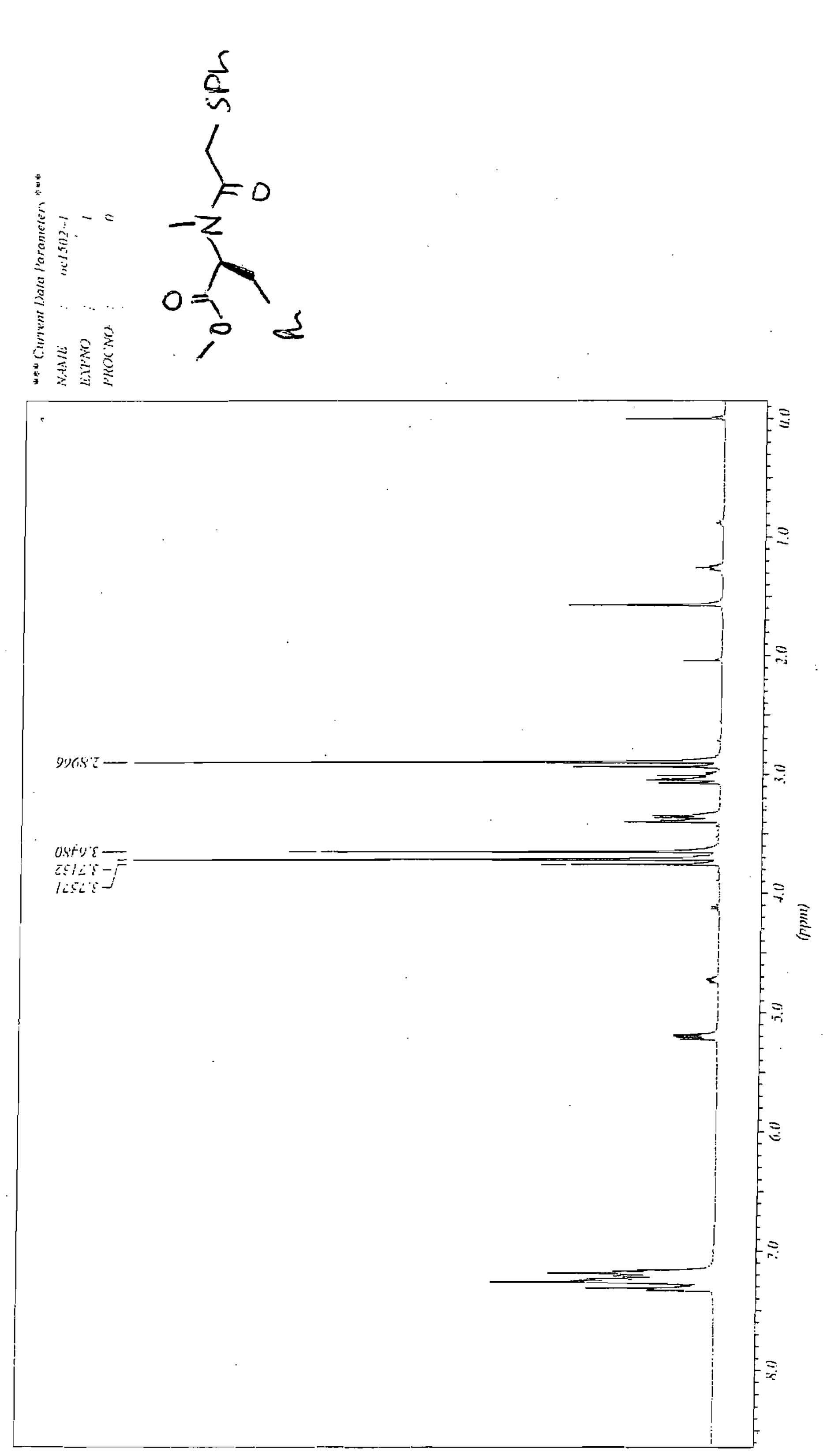

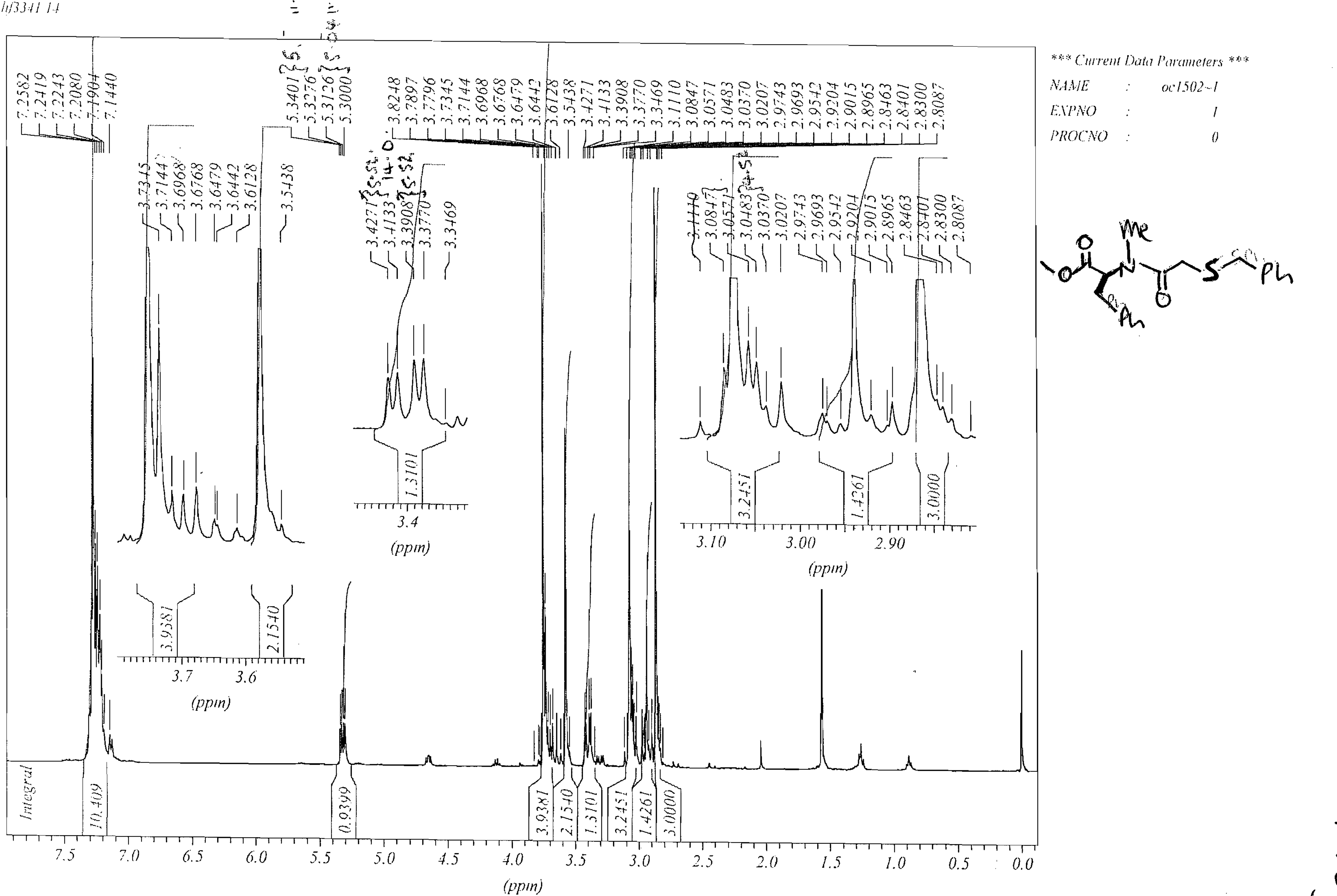

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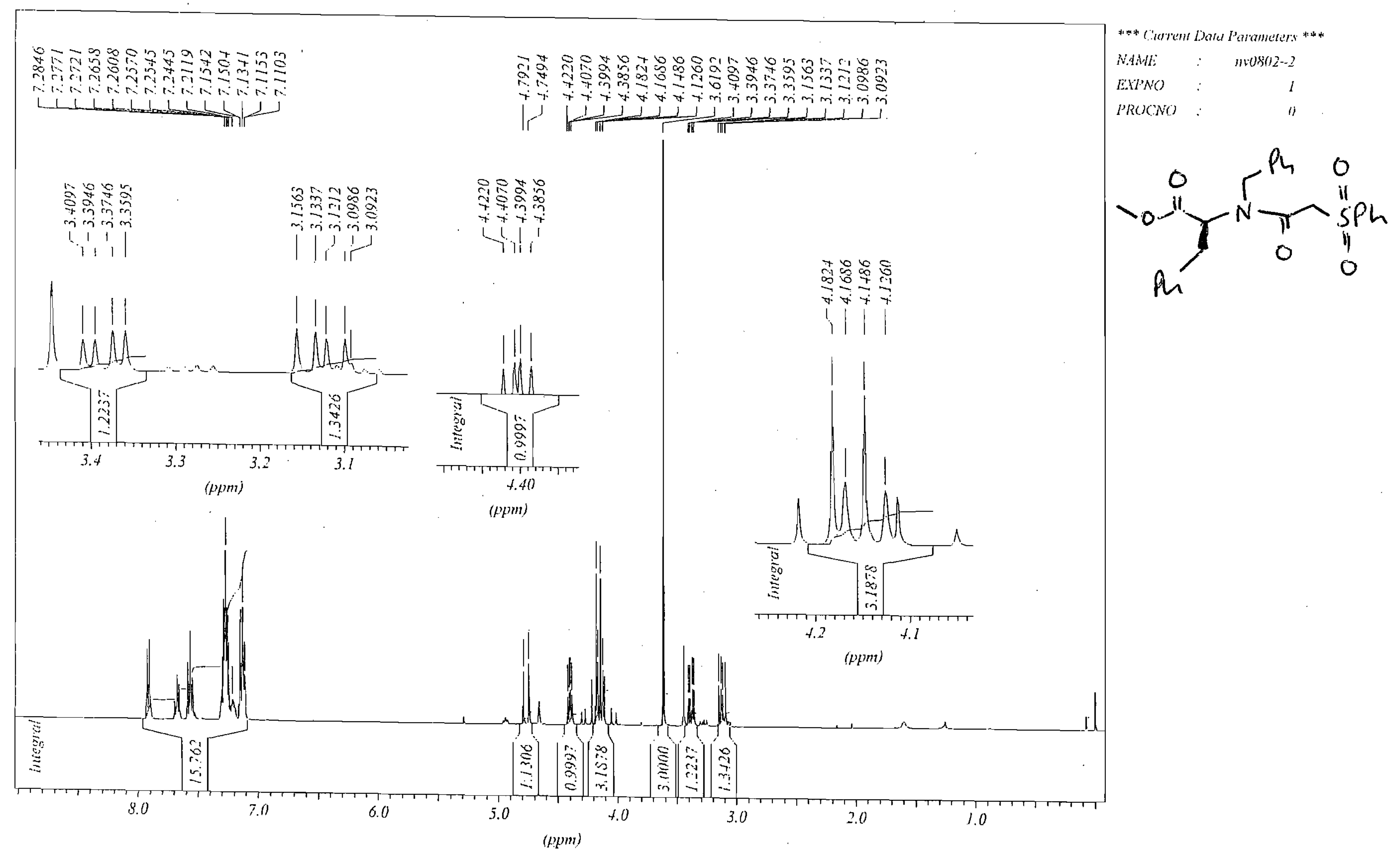

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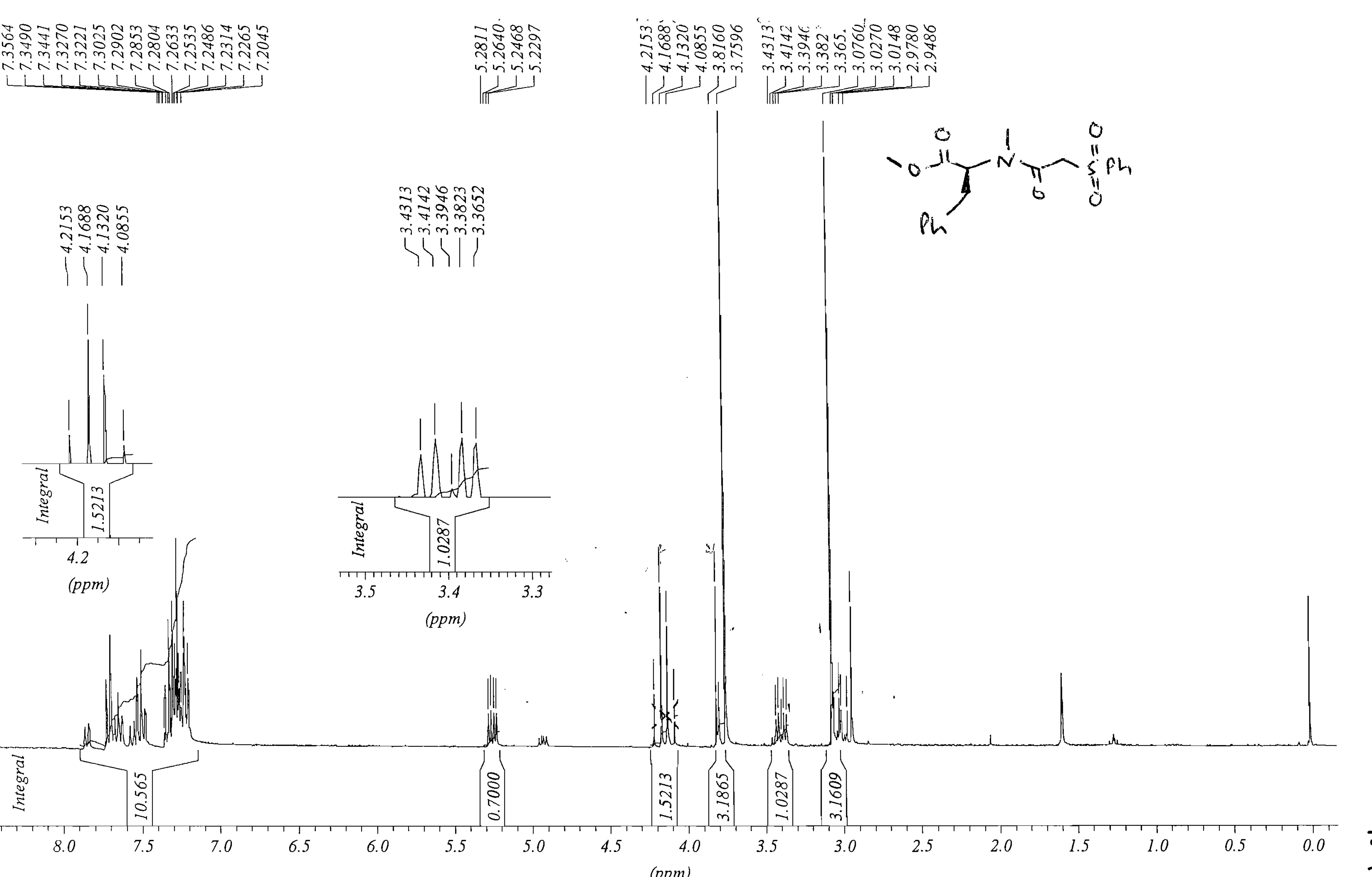

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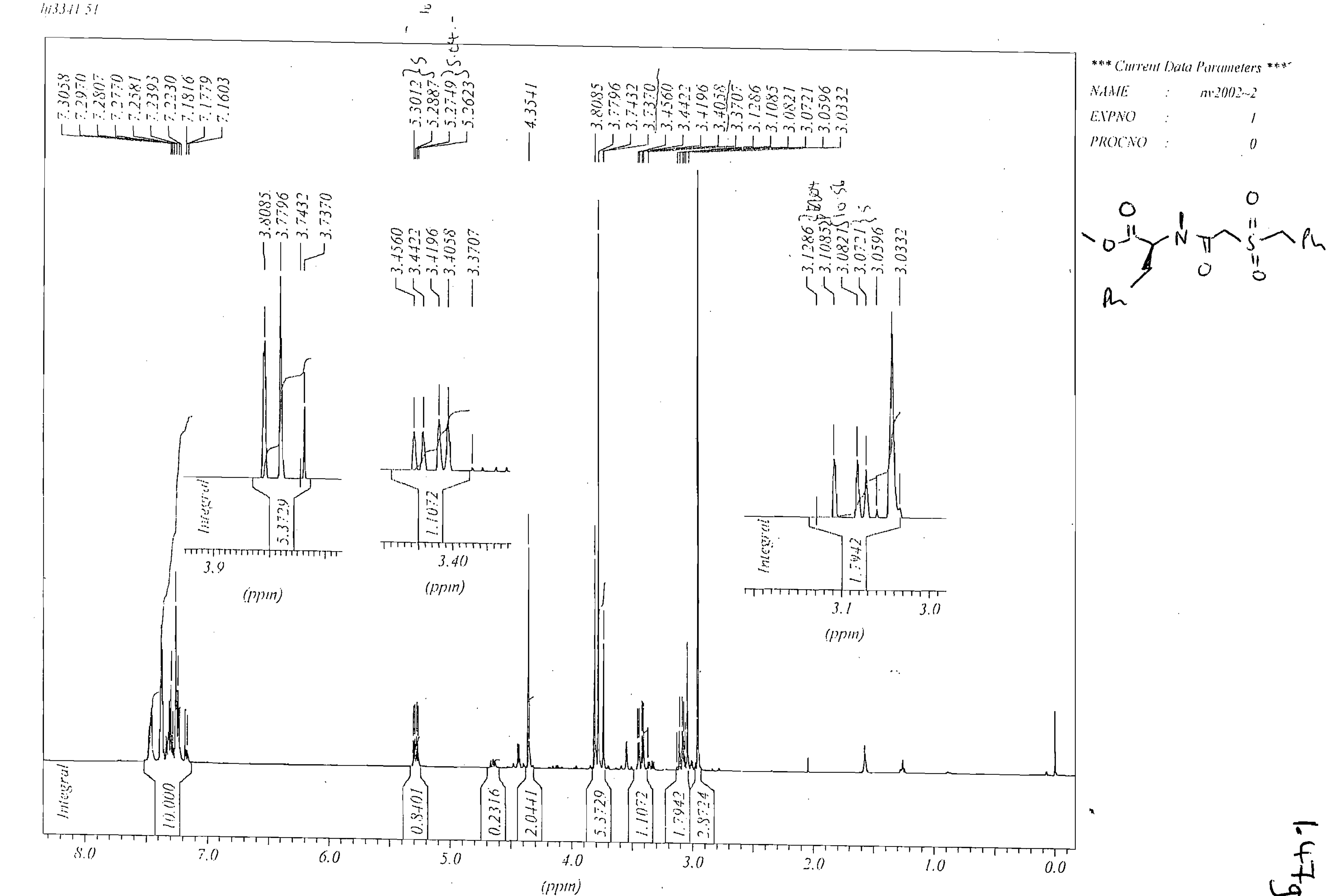

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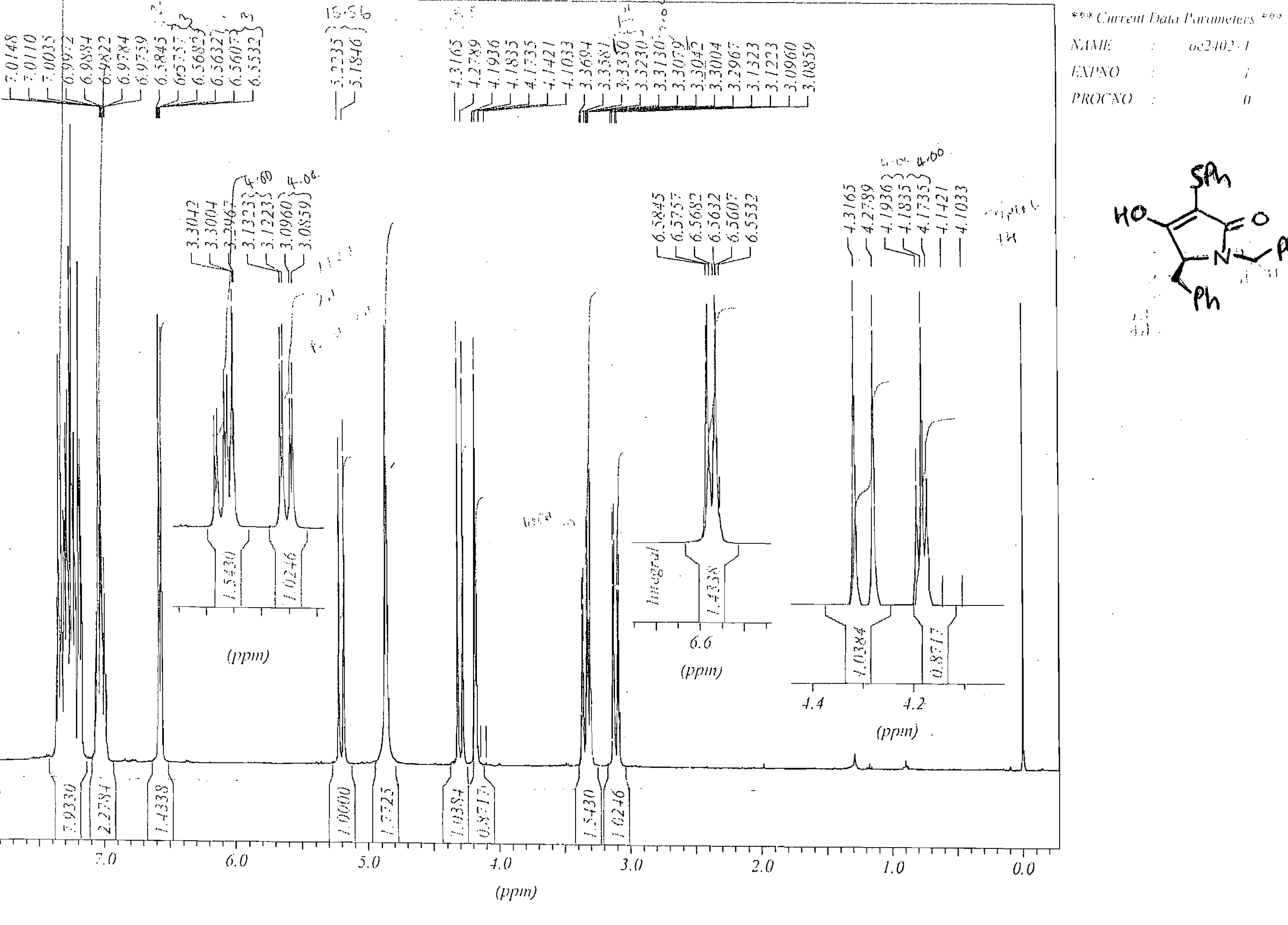
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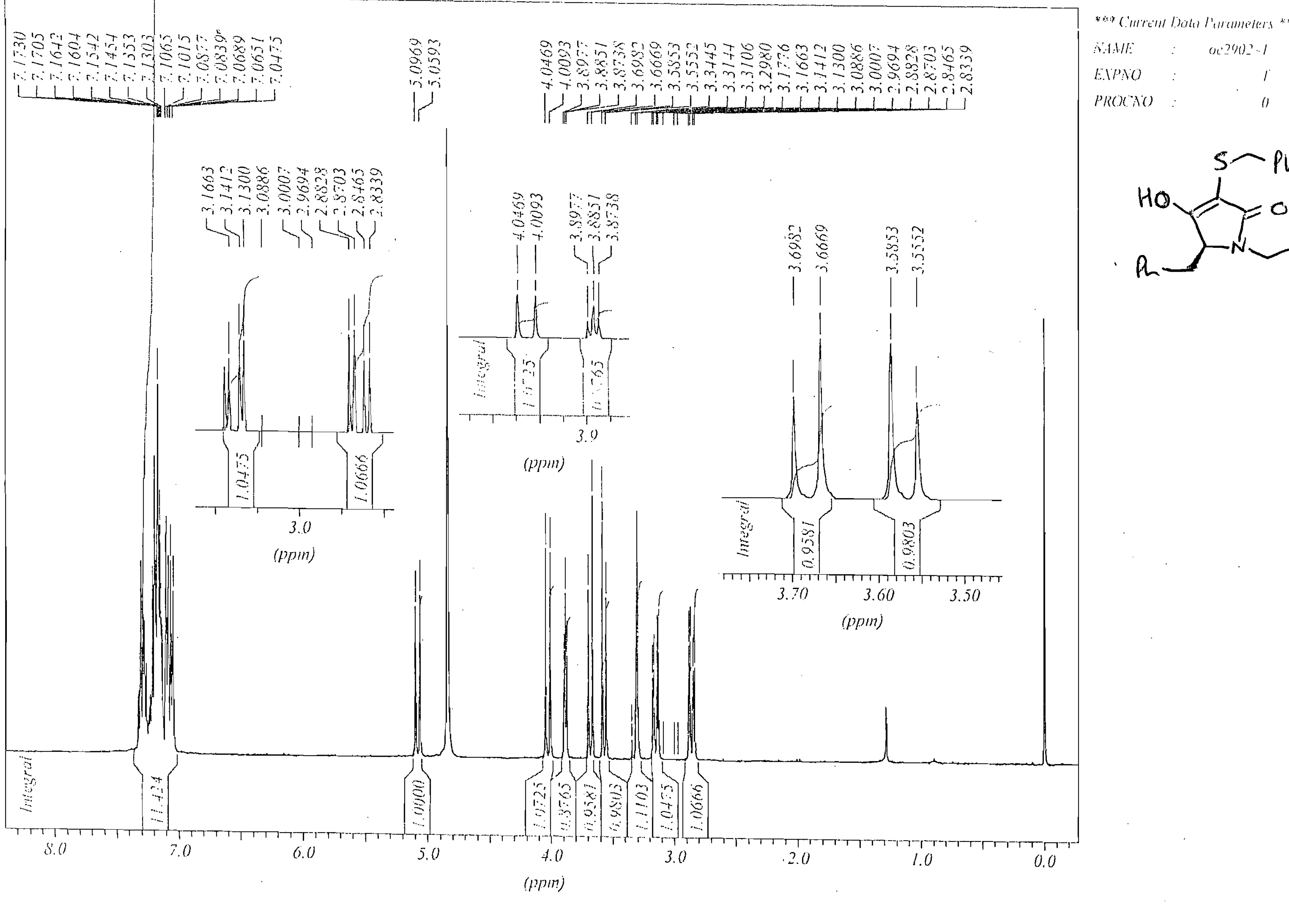

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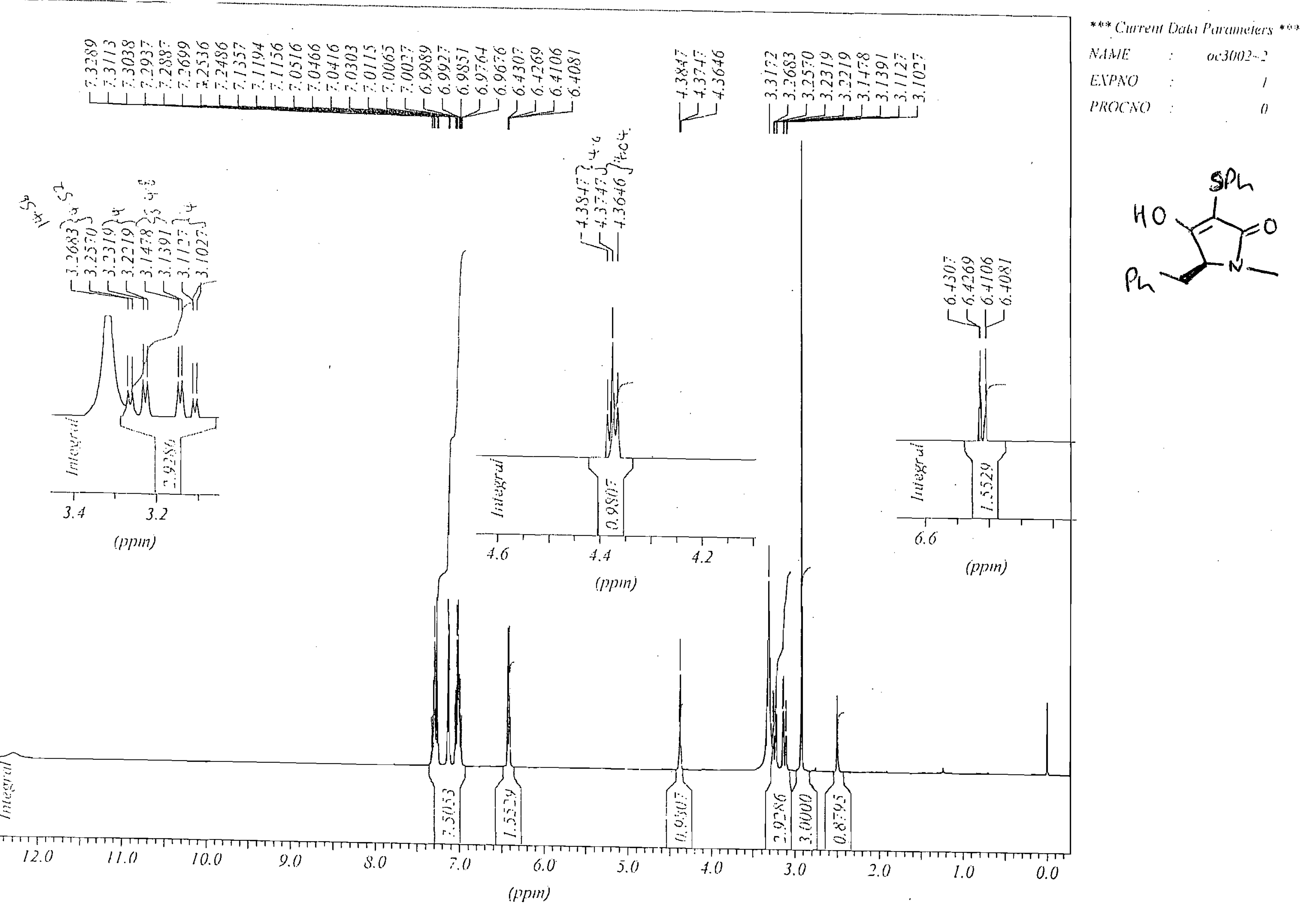

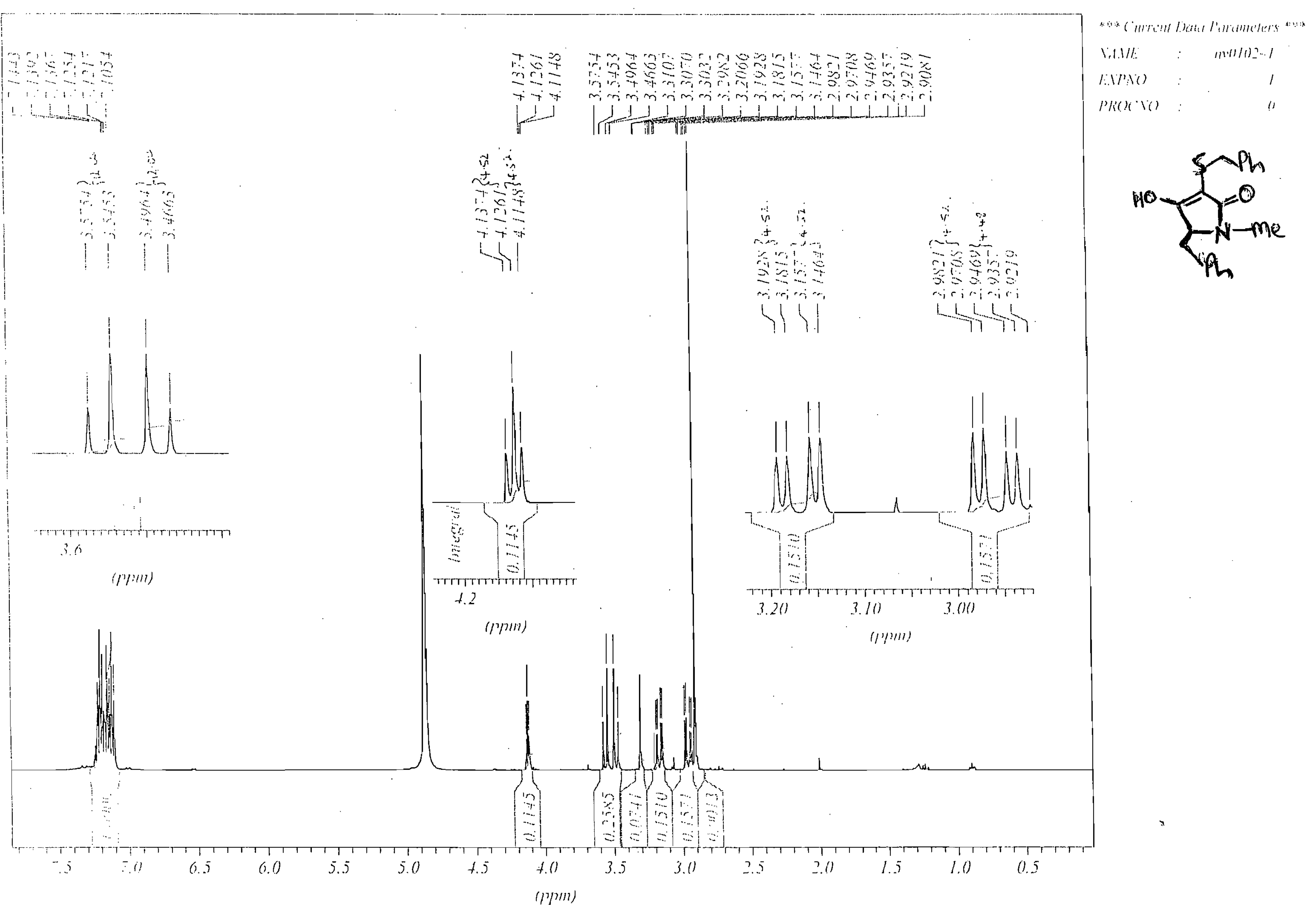

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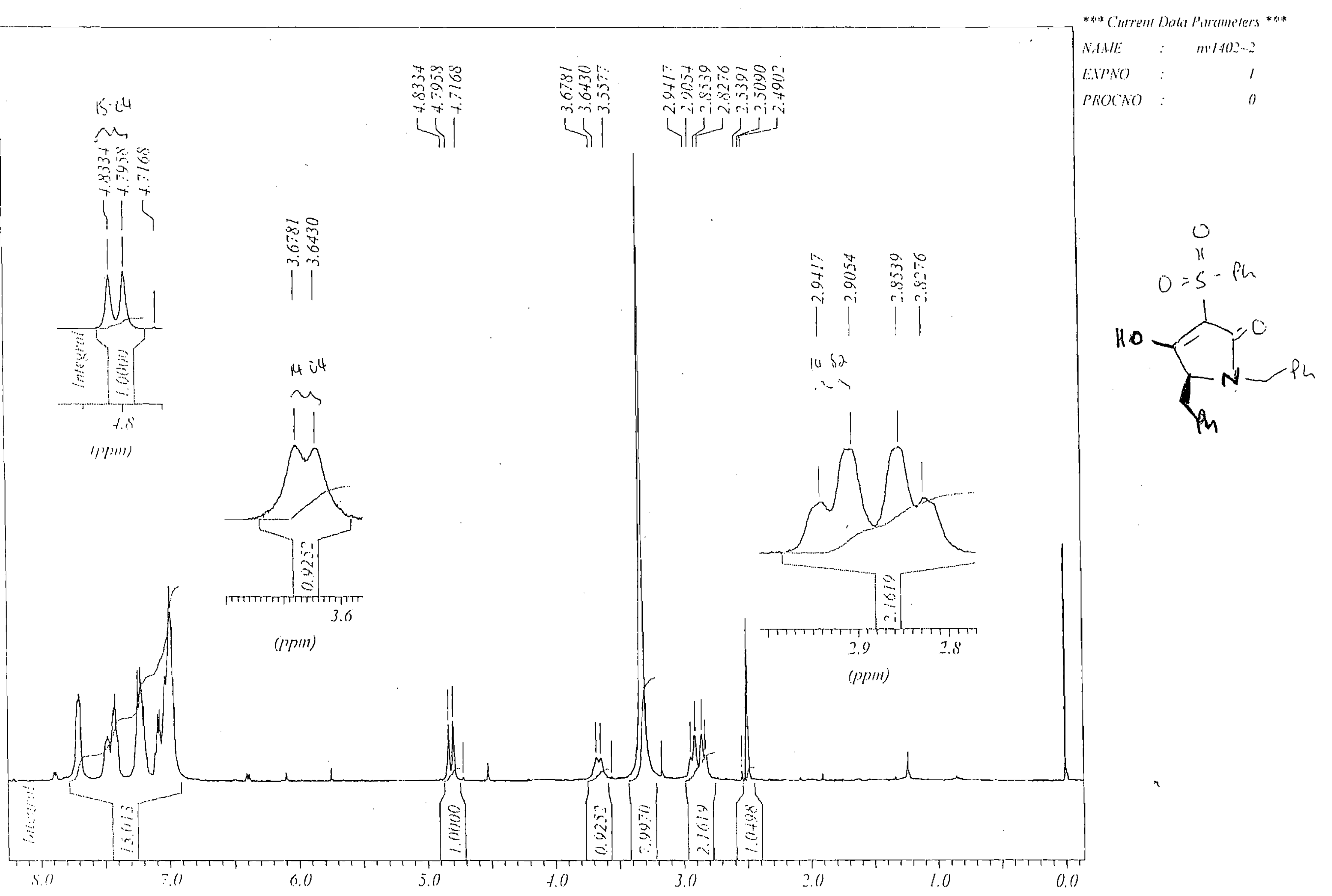

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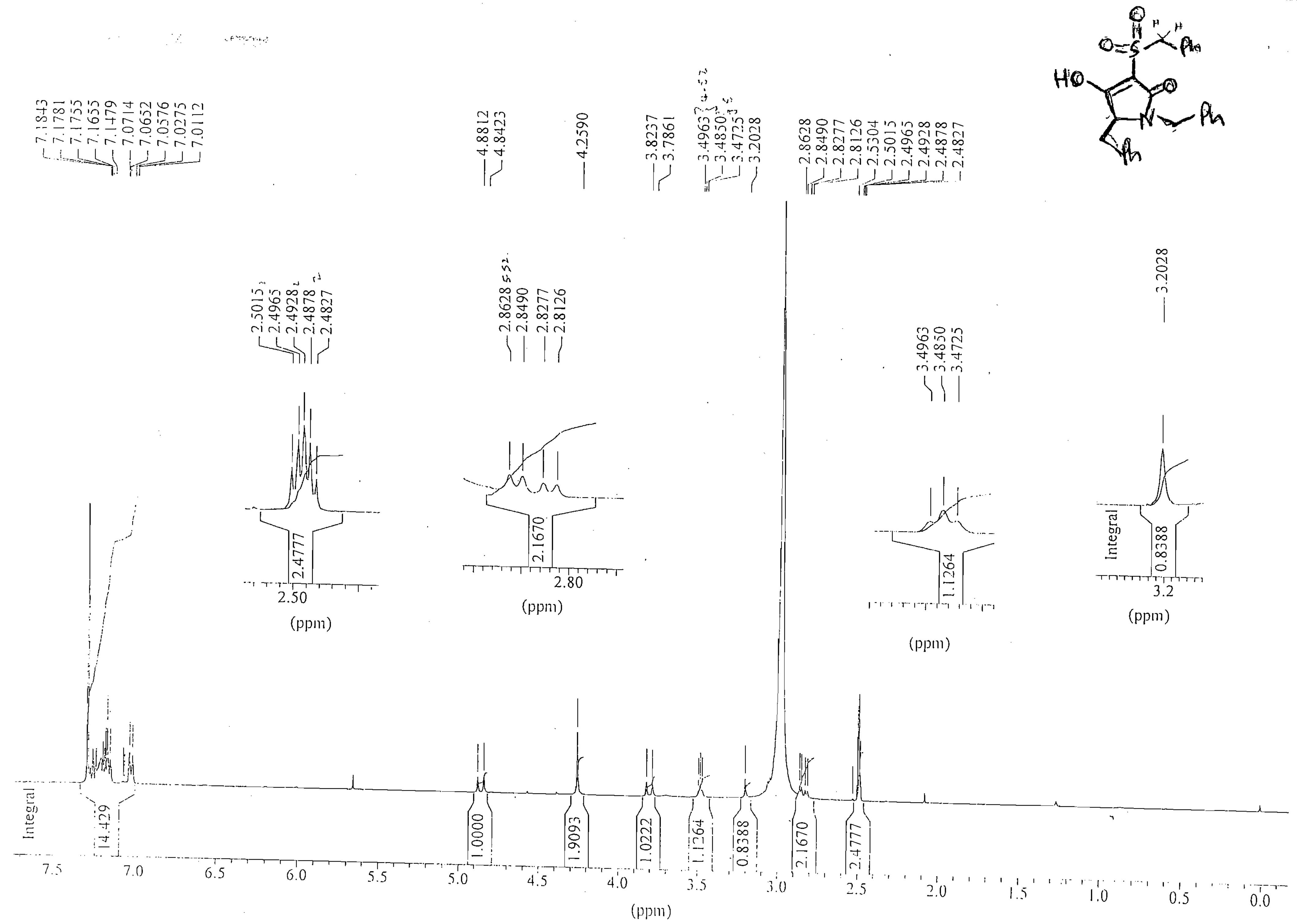

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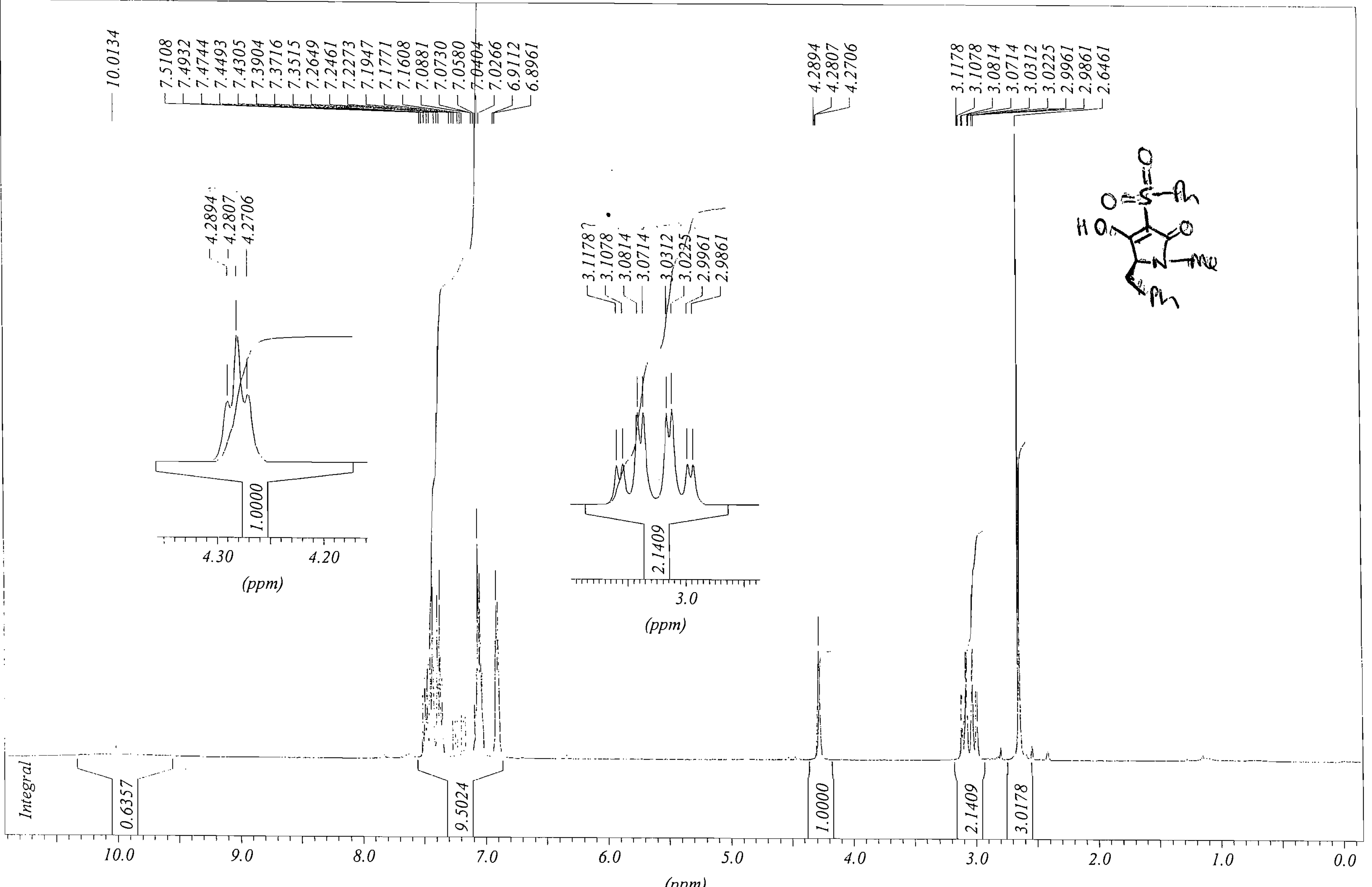
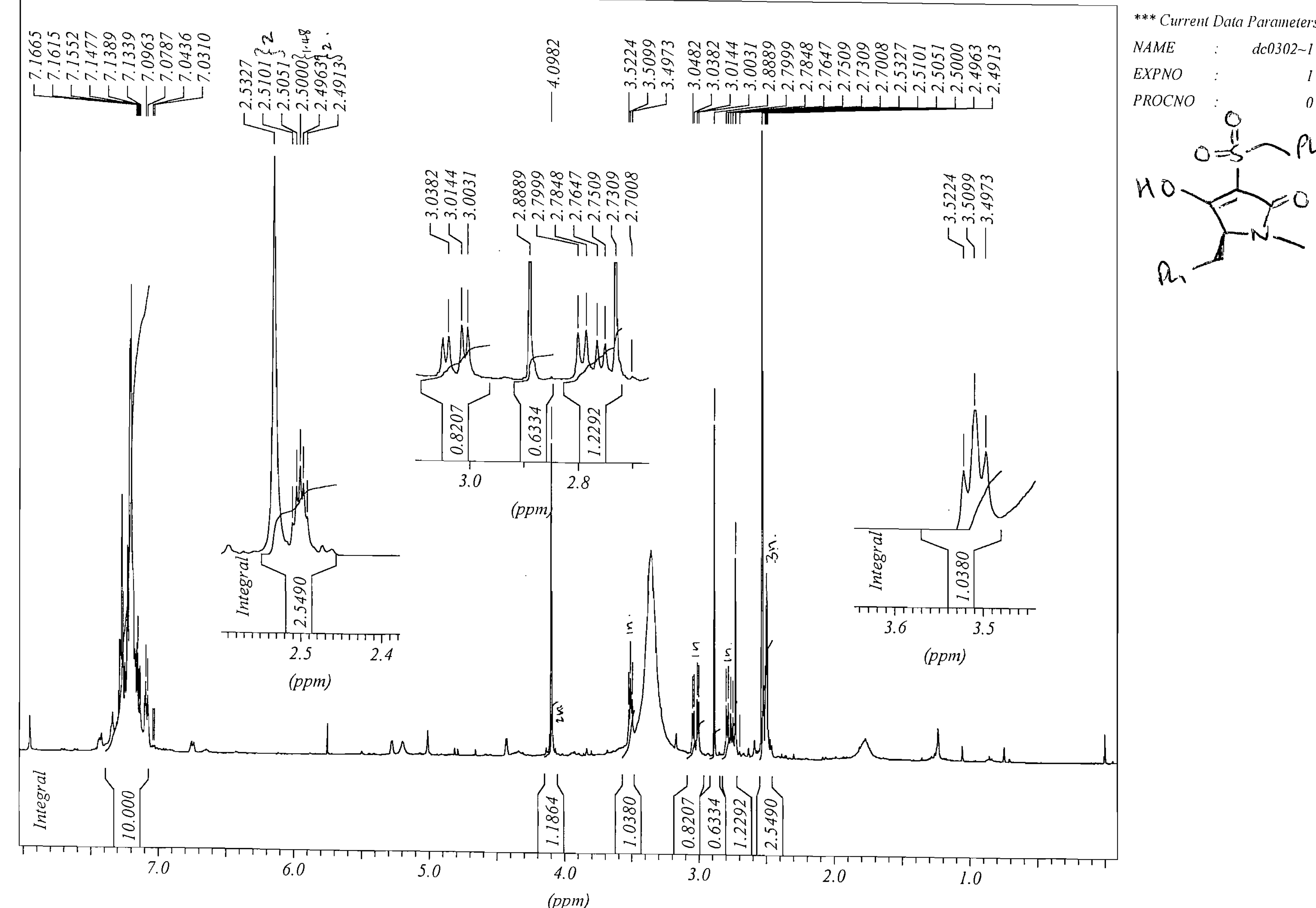
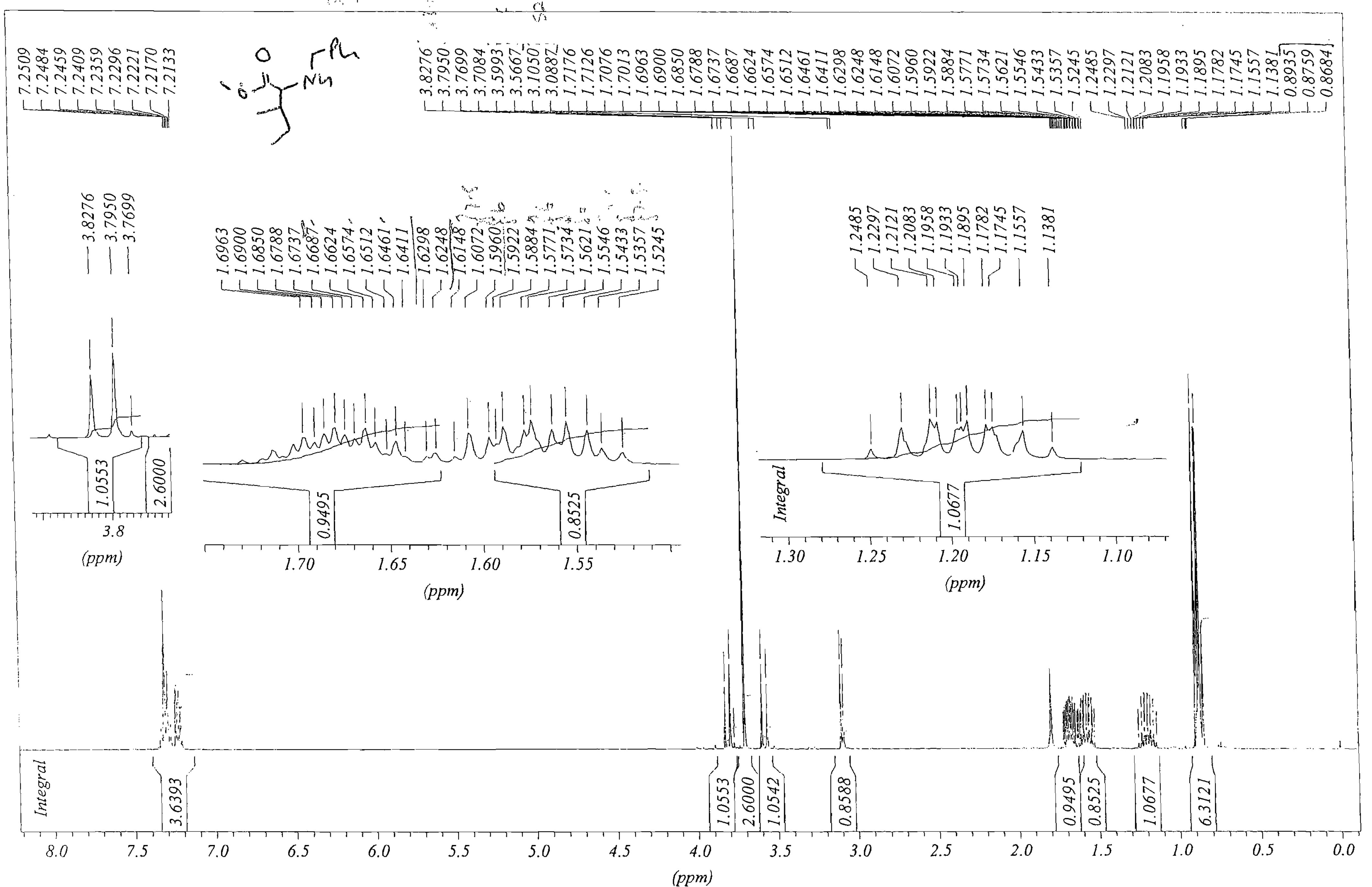
2.0
1.5
1.0
0.5
0.0
(ppm)



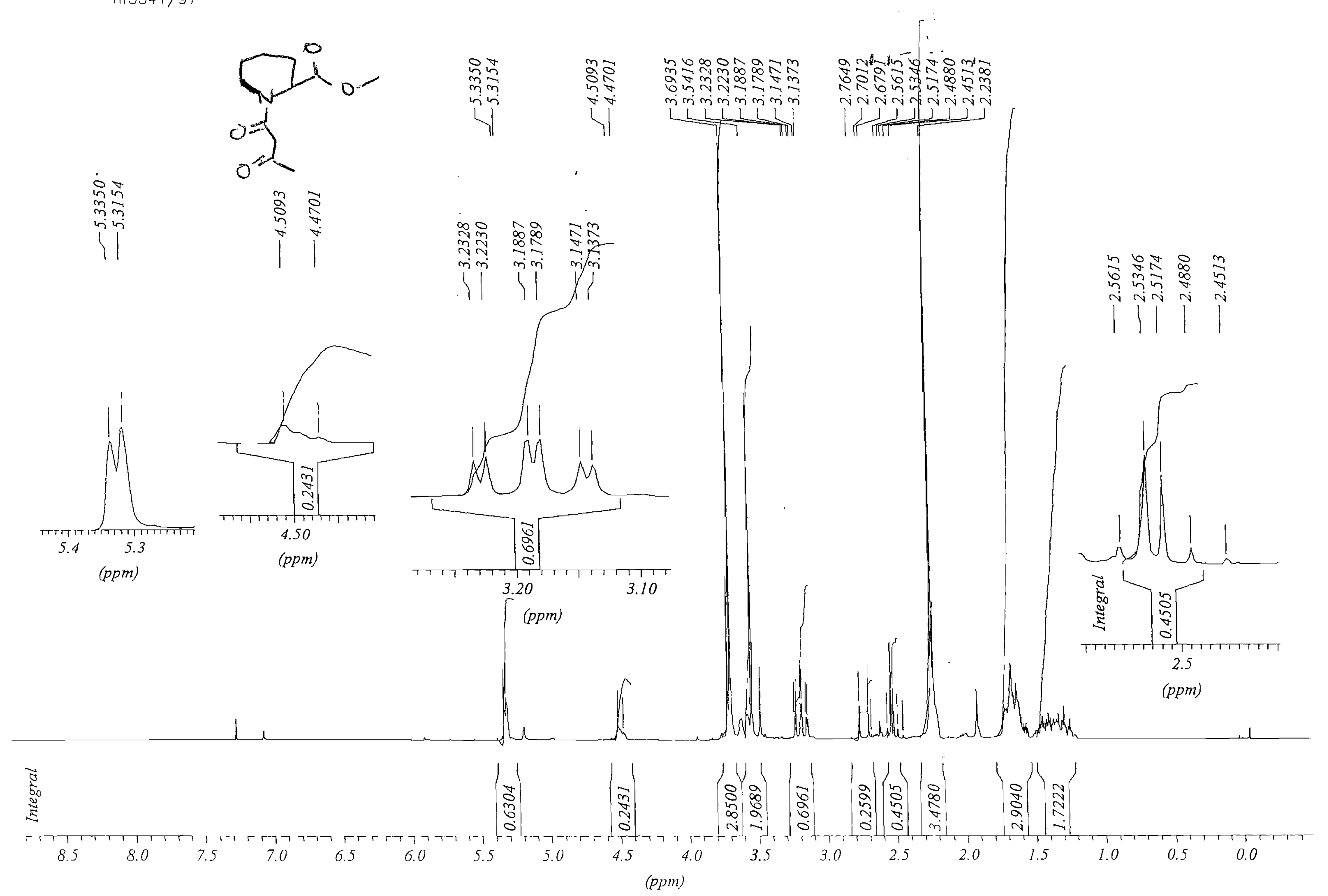
hf3522/14
10.0134
7.5108
7.4932
7.4744
7.4493
7.4305
7.3904
7.3716
7.3515
7.2649
7.2461
7.2273
7.1947
7.1771
7.1608
7.0881
7.0730
7.0580
7.0404
7.0266
6.9112
6.8961
4.2894
4.2807
4.2706
3.1178
3.1078
3.0814
3.0714
3.0312
3.0225
2.9961
2.9861
2.6461
1.0000
4.30
4.20
(ppm)
2.1409
3.0
(ppm)
Integral
0.6357
9.5024
1.0000
2.1409
3.0178
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)
1.49e



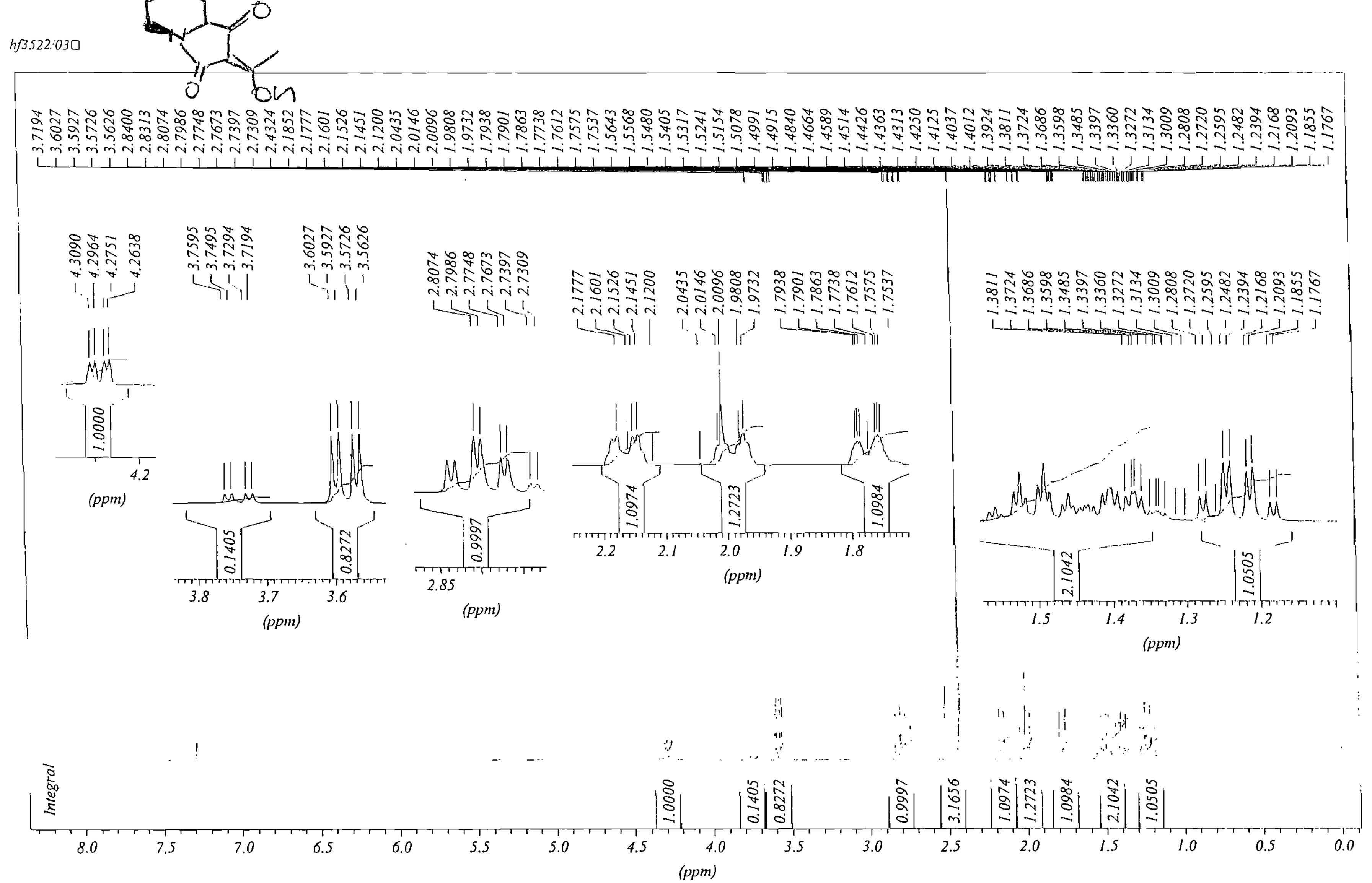
hf3341/55
7.1665
7.1615
7.1552
7.1477
7.1389
7.1339
7.0963
7.0787
7.0436
7.0310
2.5327
2.5101
2.5051
2.5000
2.4963
2.4913
4.0982
3.5224
3.5099
3.4973
3.0482
3.0382
3.0144
3.0031
2.8889
2.7999
2.7848
2.7647
2.7509
2.7309
2.7008
Integral
10.000
1.1864
1.0380
0.8207
0.6334
1.2292
2.5490
(ppm)
*** Current Data Parameters ***
NAME : dc0302~1
EXPNO : 1
PROCNO : 0



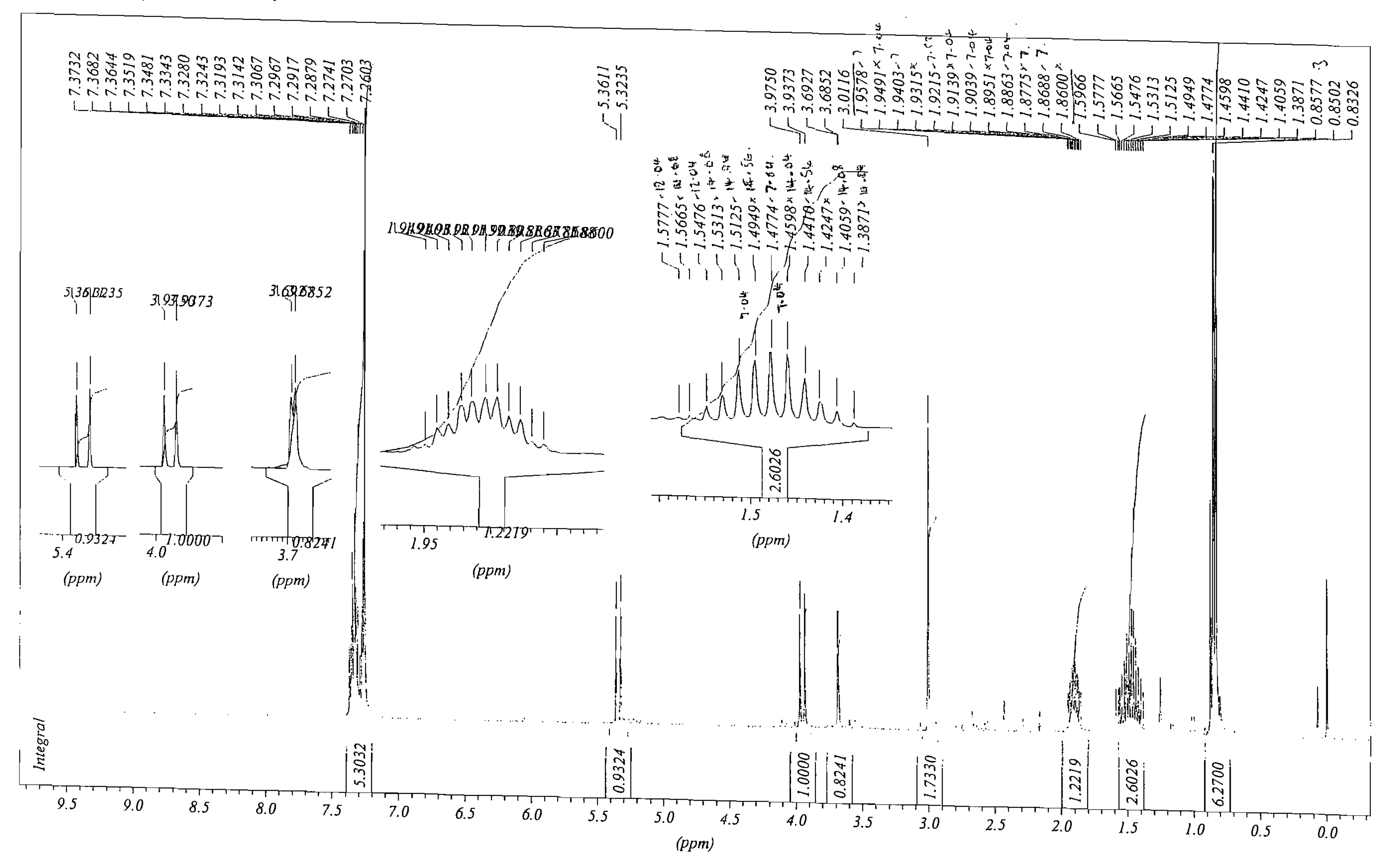
hf3341/73/2
7.2509
7.2484
7.2459
7.2409
7.2359
7.2296
7.2221
7.2170
7.2133
3.8276
3.7950
3.7699
3.7084
3.5993
3.5667
3.1050
3.0887
1.7176
1.7126
1.7076
1.7013
1.6963
1.6900
1.6850
1.6788
1.6737
1.6687
1.6624
1.6574
1.6512
1.6461
1.6411
1.6298
1.6248
1.6148
1.6072
1.5960
1.5922
1.5884
1.5771
1.5734
1.5621
1.5546
1.5433
1.5357
1.5245
1.2485
1.2297
1.2121
1.2083
1.1958
1.1933
1.1895
1.1782
1.1745
1.1557
1.1381
0.8935
0.8759
0.8684
1.0553
2.6000
3.8
(ppm)
0.9495
0.8525
1.70
1.65
1.60
1.55
(ppm)
Integral
1.0677
1.30
1.25
1.20
1.15
1.10
(ppm)
Integral
3.6393
1.0553
2.6000
1.0542
0.8588
0.9495
0.8525
1.0677
6.3121
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)
1.54c



hf3341/97
5.3350
5.3154
4.5093
4.4701
3.6935
3.5416
3.2328
3.2230
3.1887
3.1789
3.1471
3.1373
2.7649
2.7012
2.6791
2.5615
2.5346
2.5174
2.4880
2.4513
2.2381
Integral
0.6304
0.2431
2.8500
1.9689
0.6961
0.2599
0.4505
3.4780
2.9040
1.7222
(ppm)

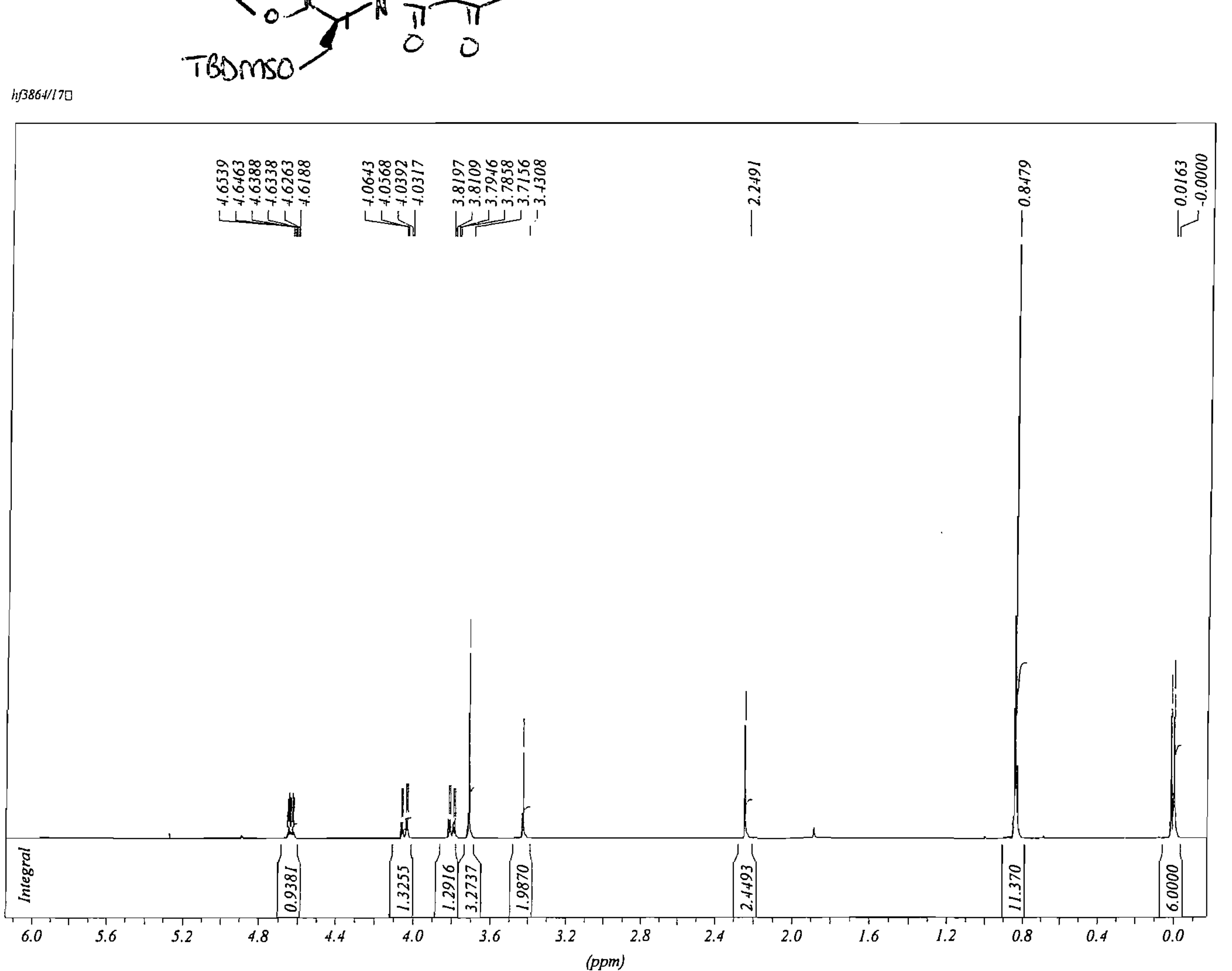


hf3522:030
3.7194
3.6027
3.5927
3.5726
3.5626
2.8400
2.8313
2.8074
2.7986
2.7748
2.7673
2.7397
2.7309
2.4324
2.1852
2.1777
2.1601
2.1526
2.1451
2.1200
2.0435
2.0146
2.0096
1.9808
1.9732
1.7938
1.7901
1.7863
1.7738
1.7612
1.7575
1.7537
1.5643
1.5568
1.5480
1.5405
1.5317
1.5241
1.5154
1.5078
1.4991
1.4915
1.4840
1.4664
1.4589
1.4514
1.4426
1.4363
1.4313
1.4250
1.4125
1.4037
1.4012
1.3924
1.3811
1.3724
1.3686
1.3598
1.3485
1.3397
1.3360
1.3272
1.3134
1.3009
1.2808
1.2720
1.2595
1.2482
1.2394
1.2168
1.2093
1.1855
1.1767
4.3090
4.2964
4.2751
4.2638
1.0000
4.2
(ppm)
3.7595
3.7495
3.7294
3.7194
3.6027
3.5927
3.5726
3.5626
0.1405
0.8272
3.8
3.7
3.6
(ppm)
2.8074
2.7986
2.7748
2.7673
2.7397
2.7309
0.9997
2.85
(ppm)
2.1777
2.1601
2.1526
2.1451
2.1200
2.0435
2.0146
2.0096
1.9808
1.9732
1.7938
1.7901
1.7863
1.7738
1.7612
1.7575
1.7537
1.0974
1.2723
1.0984
2.2
2.1
2.0
1.9
1.8
(ppm)
1.3811
1.3724
1.3686
1.3598
1.3485
1.3397
1.3360
1.3272
1.3134
1.3009
1.2808
1.2720
1.2595
1.2482
1.2394
1.2168
1.2093
1.1855
1.1767
2.1042
1.0505
1.5
1.4
1.3
1.2
(ppm)
Integral
1.0000
0.1405
0.8272
0.9997
3.1656
1.0974
1.2723
1.0984
2.1042
1.0505
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)

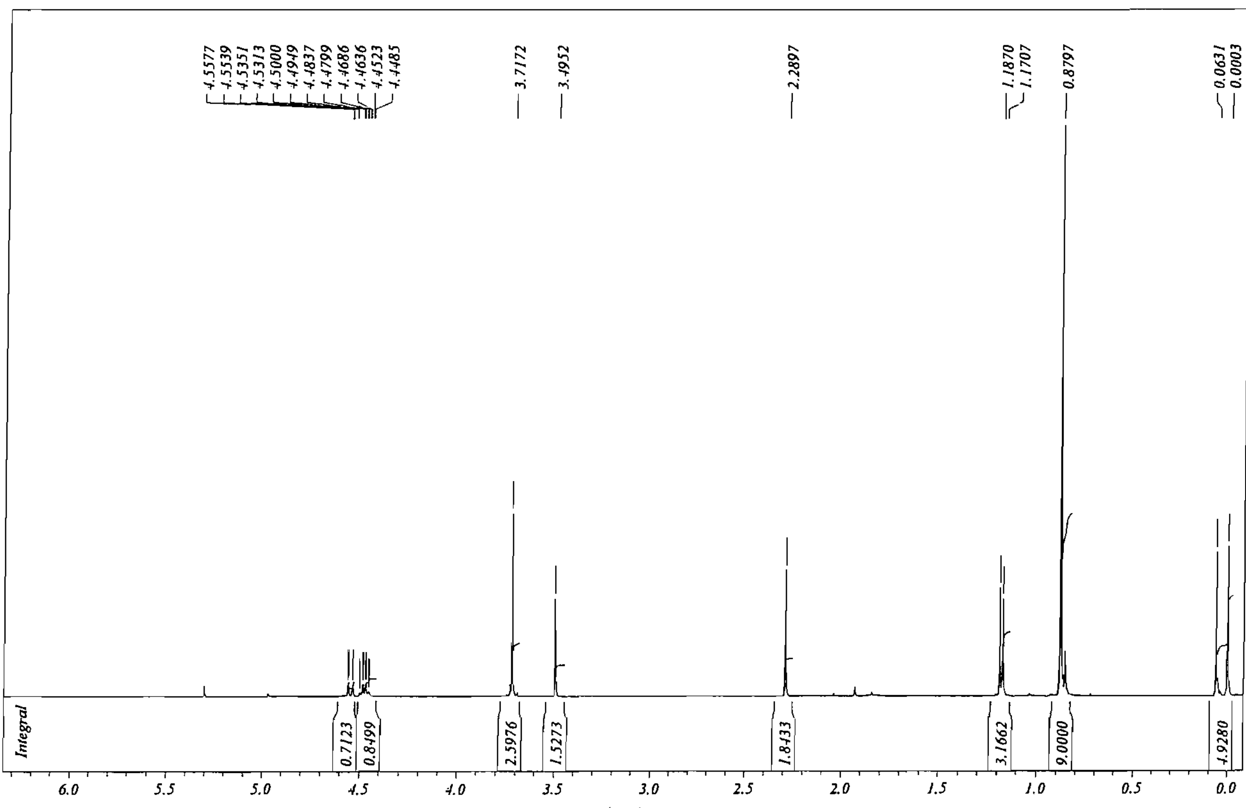


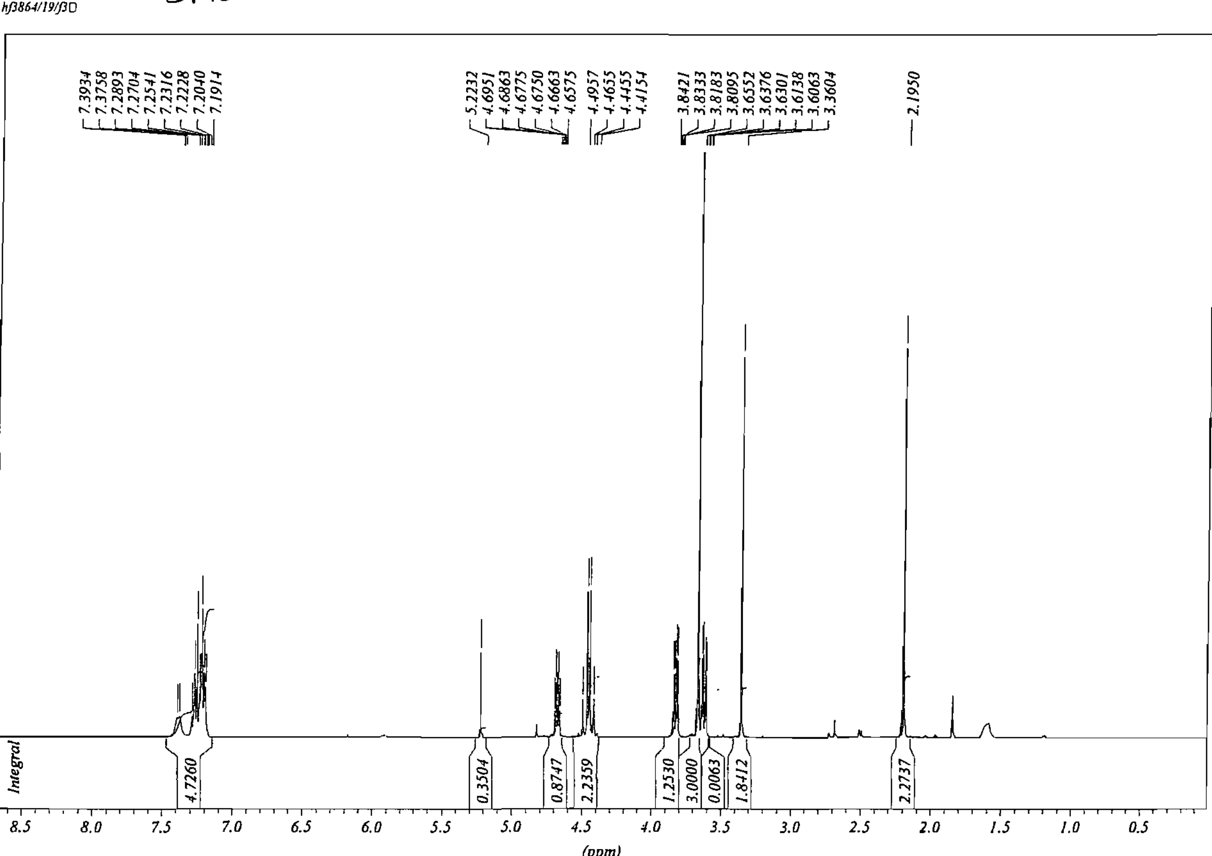
1.58a

1.64a



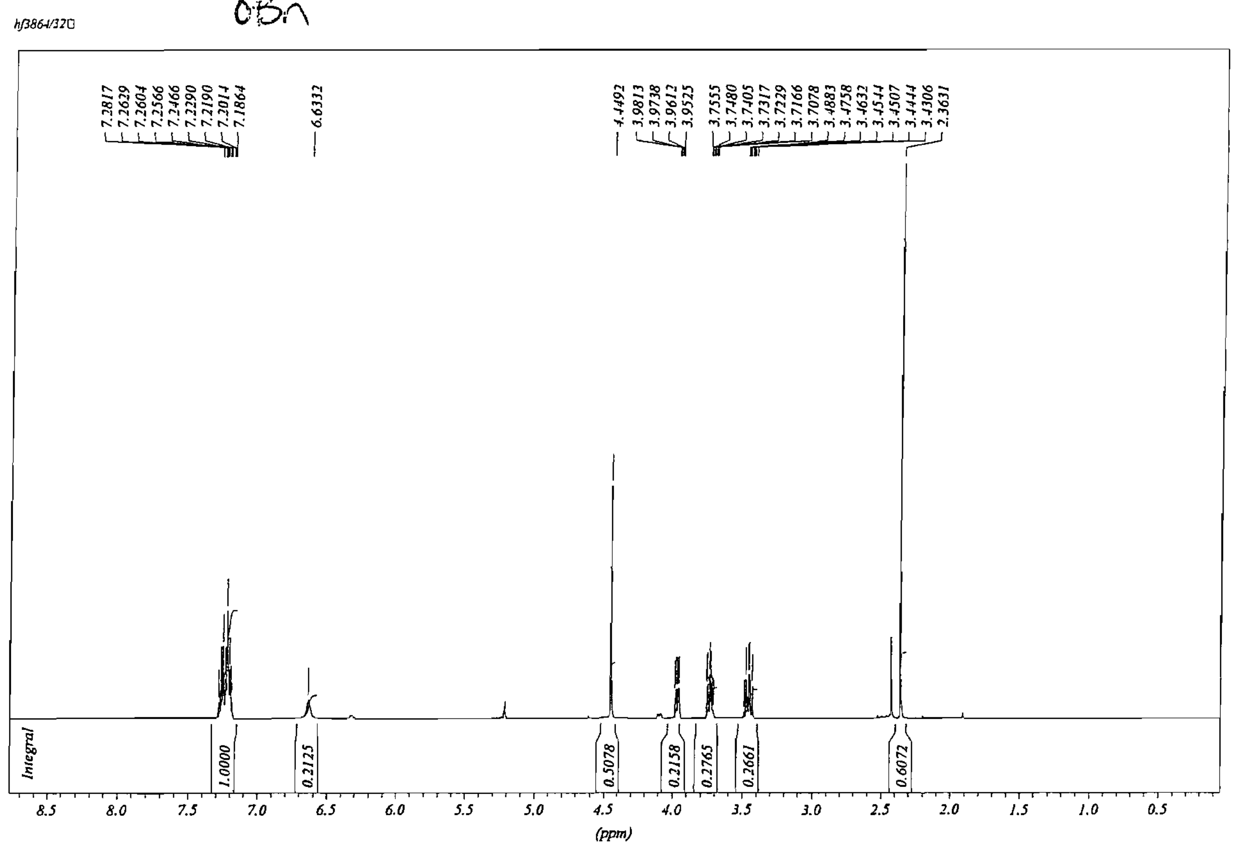
1.646.

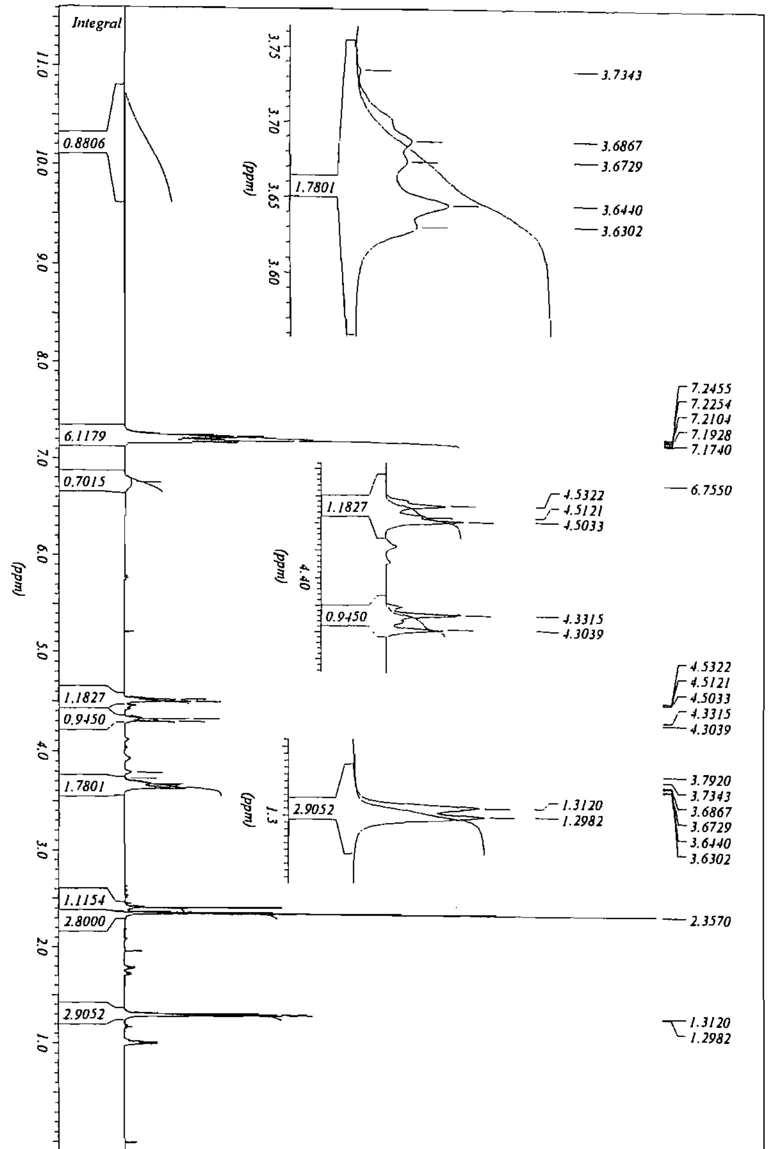




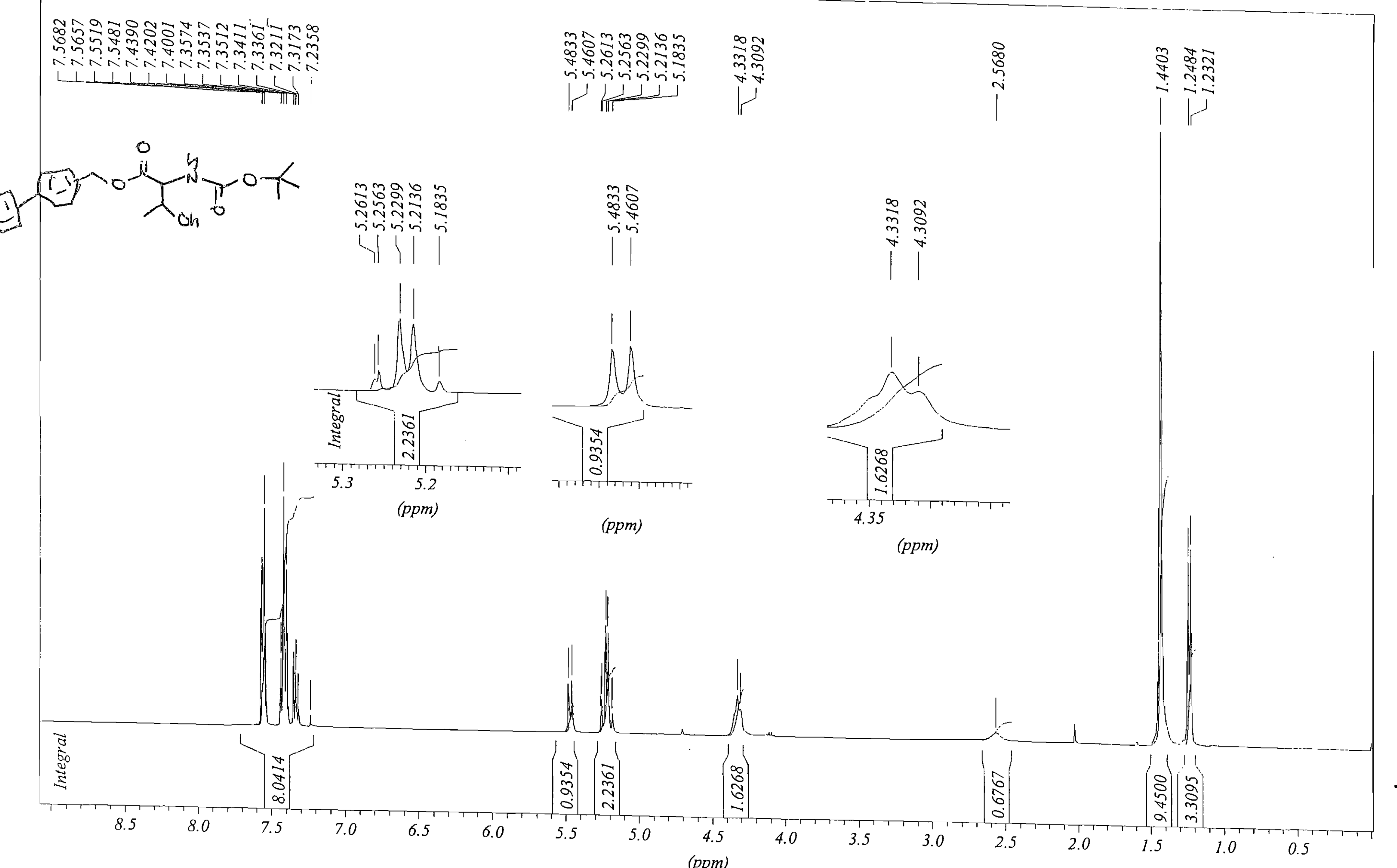
1.64c

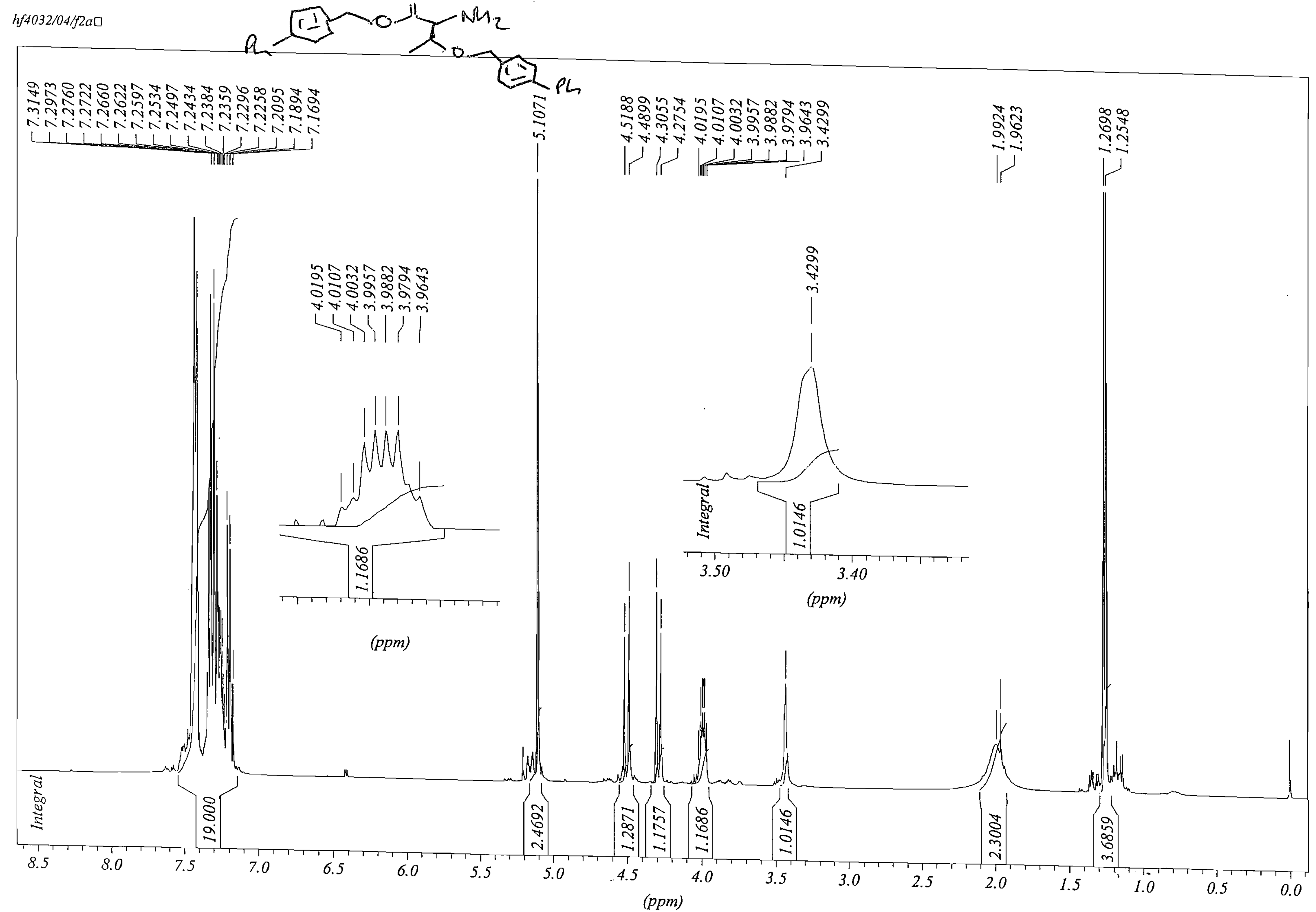
1.66c





1.66d


hf3864/71
7.5682
7.5657
7.5519
7.5481
7.4390
7.4202
7.4001
7.3574
7.3537
7.3512
7.3411
7.3361
7.3211
7.3173
7.2358
5.4833
5.4607
5.2613
5.2563
5.2299
5.2136
5.1835
4.3318
4.3092
2.5680
1.4403
1.2484
1.2321
Integral
2.2361
0.9354
1.6268
8.0414
0.6767
9.4500
3.3095
(ppm)
1.77.



hf4032/04/f2a
Integral
19.000
2.4692
1.2871
1.1757
1.1686
1.0146
2.3004
3.6859
7.3149
7.2973
7.2760
7.2722
7.2660
7.2622
7.2597
7.2534
7.2497
7.2434
7.2384
7.2359
7.2296
7.2258
7.2095
7.1894
7.1694
5.1071
4.5188
4.4899
4.3055
4.2754
4.0195
4.0107
4.0032
3.9957
3.9882
3.9794
3.9643
3.4299
1.9924
1.9623
1.2698
1.2548
1.1686
(ppm)
Integral
1.0146
3.50
3.40
3.4299
(ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)
NH2
Ph
1.78

hf4032/06

Integral

20.156
1.9217
0.9734
1.0331
1.0591
0.8115
1.6652
2.8042
3.4000

(ppm)

8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

Inset (ppm): 4.70; 0.9734
4.6881
4.6808
4.6576
4.6503

Inset (ppm): 4.30 4.20 4.10; 1.0591; 0.8115
4.2797
4.2406
4.2077
4.1907
4.1821
4.1687
4.1614
4.1480
4.1407
4.1273
4.1199

7.3094
7.2862
7.2740
7.2679
7.2630
7.2582
7.2533
7.2472
7.2399
7.2252
7.2204
7.2155
7.2106
7.1850
7.1679
7.1582

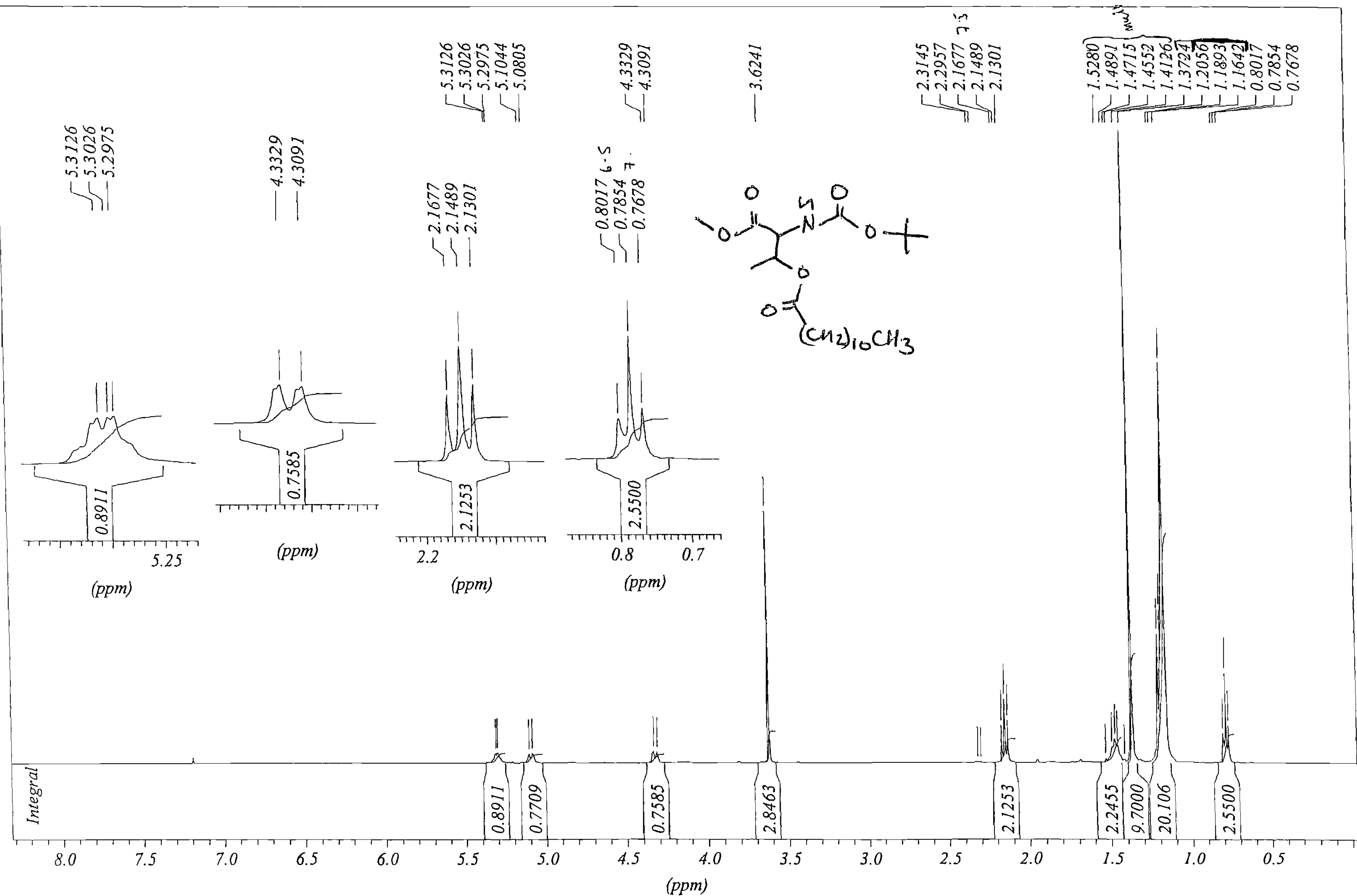
5.0965

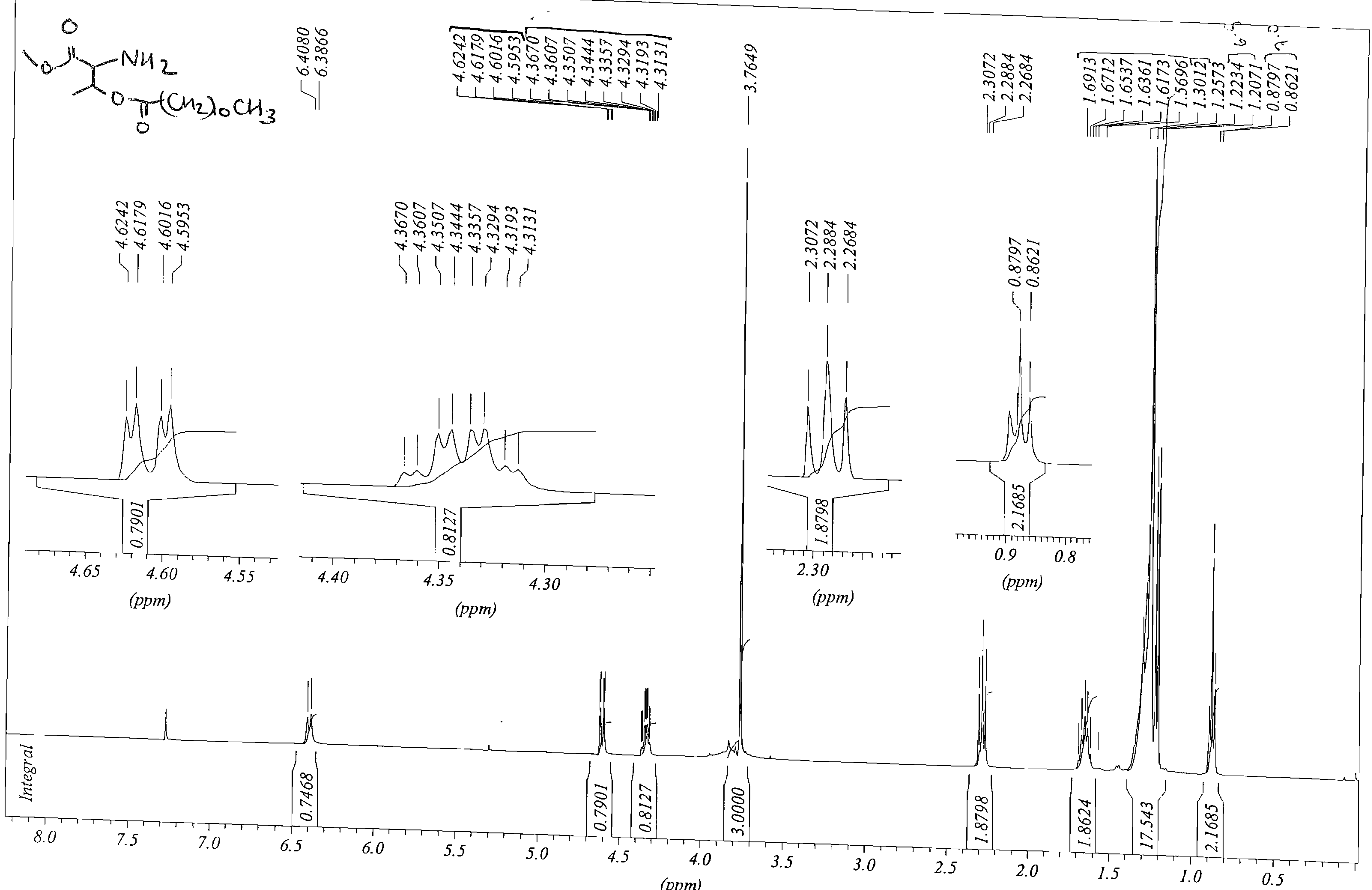
4.6881
4.6808
4.6576
4.6503
4.5101
4.4699
4.4406
4.2797
4.2406
4.2077
4.1907
4.1821
4.1687
4.1614
4.1480
4.1407
4.1273
4.1199
3.4079

2.1900

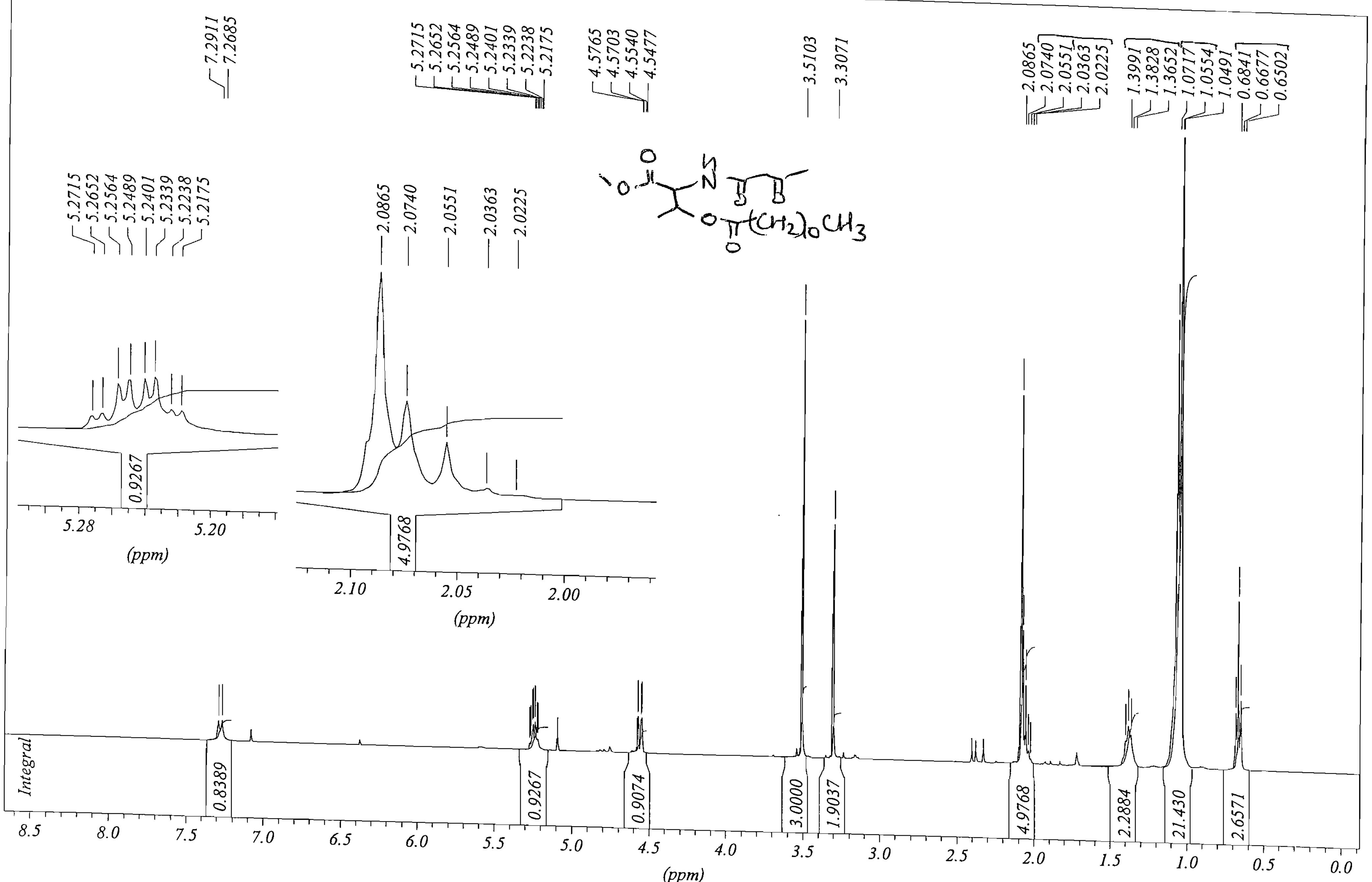
1.1963
1.1744

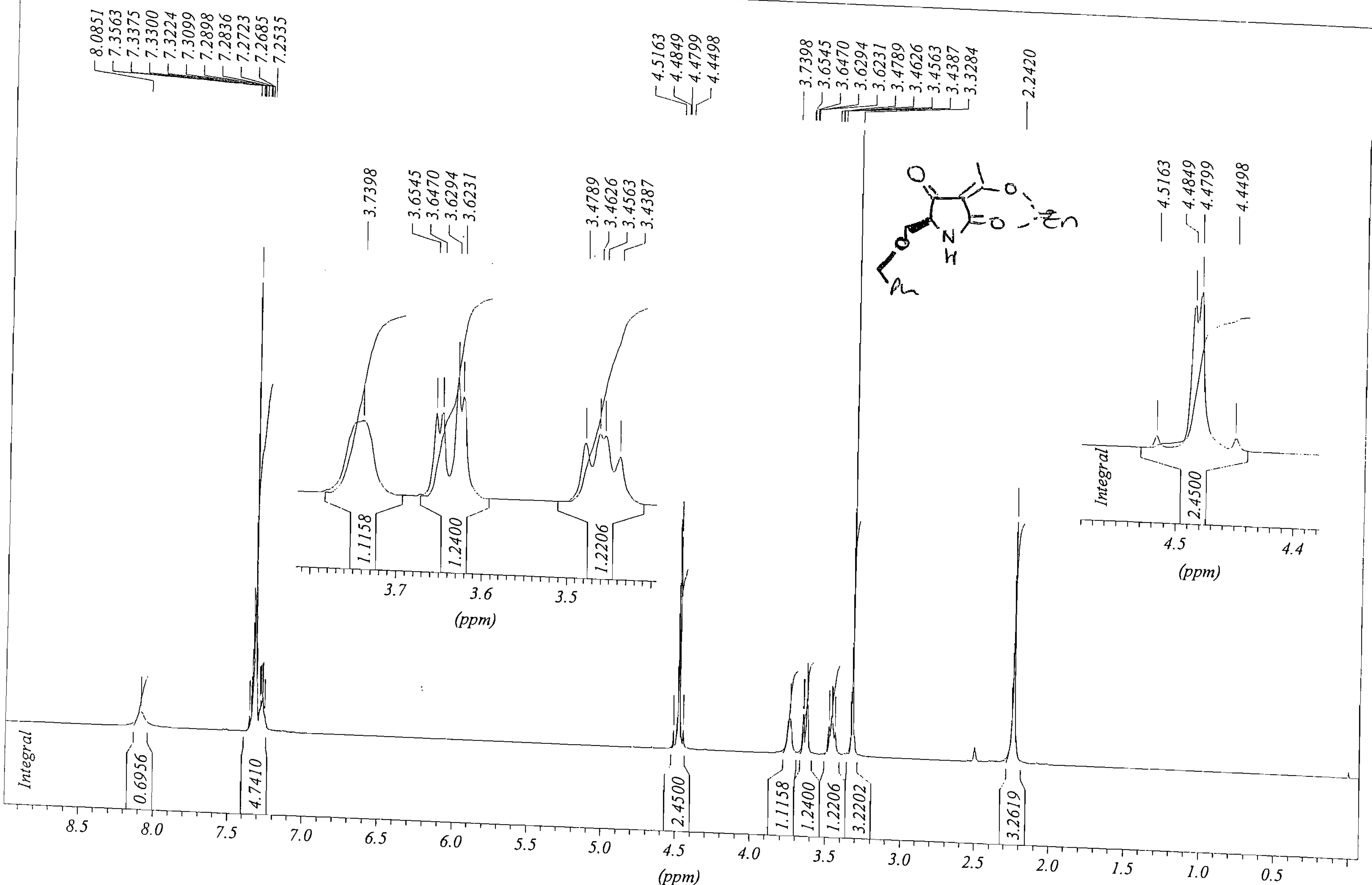
1.79


hf3864/93/f1
Integral
(ppm)
5.3126
5.3026
5.2975
5.1044
5.0805
4.3329
4.3091
3.6241
2.3145
2.2957
2.1677
2.1489
2.1301
1.5280
1.4891
1.4715
1.4552
1.4126
1.3724
1.2056
1.1893
1.1642
0.8017
0.7854
0.7678
0.8911
0.7709
0.7585
2.8463
2.1253
2.2455
9.7000
20.106
2.5500
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
5.25
2.2
0.8
0.7
(CH2)10CH3

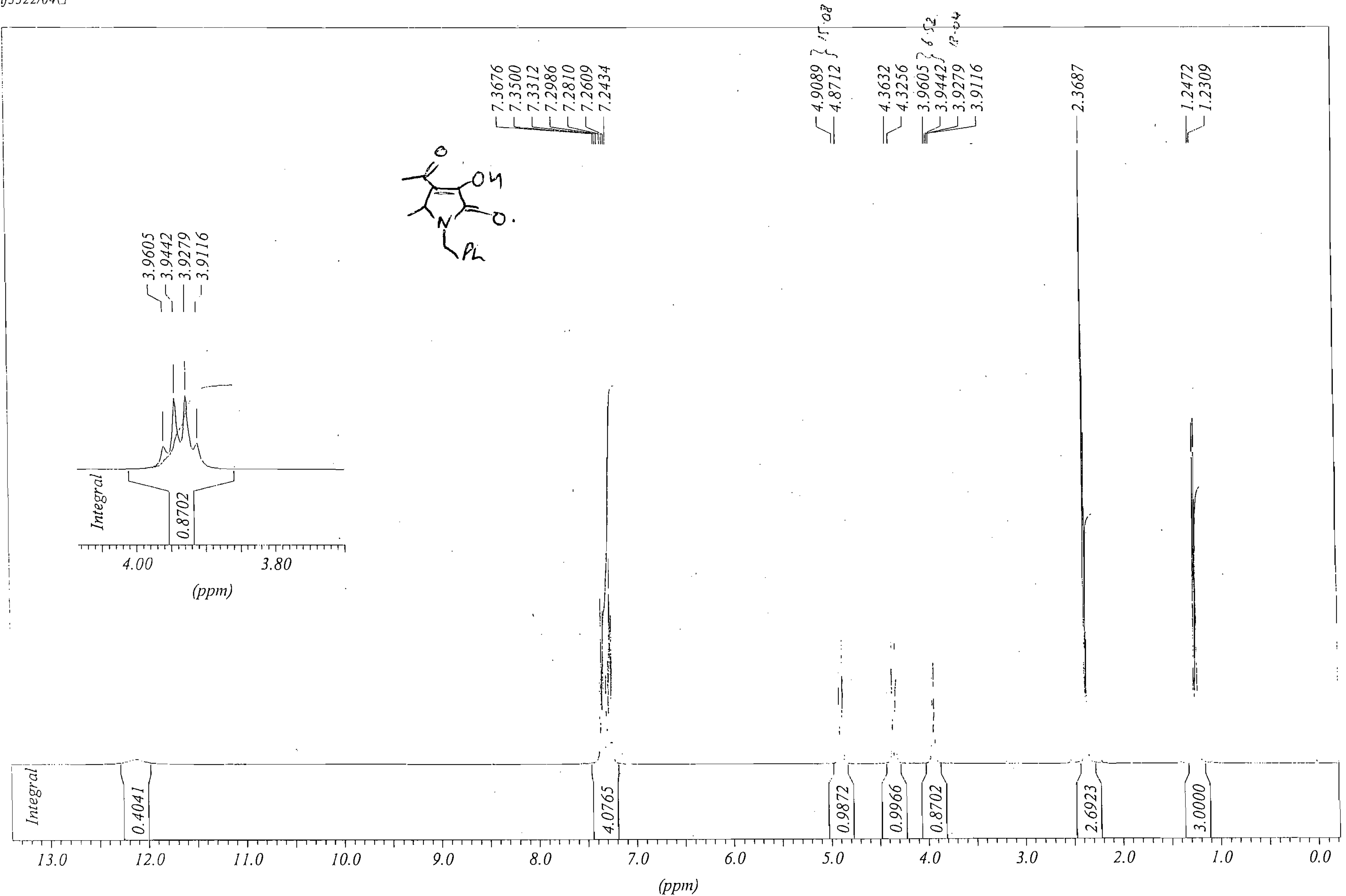


hf3864/96/f2
Integral
(ppm)
0.7468
0.7901
0.8127
3.0000
1.8798
1.8624
17.543
2.1685
6.4080
6.3866
4.6242
4.6179
4.6016
4.5953
4.3670
4.3607
4.3507
4.3444
4.3357
4.3294
4.3193
4.3131
3.7649
2.3072
2.2884
2.2684
1.6913
1.6712
1.6537
1.6361
1.6173
1.5696
1.3012
1.2573
1.2234
1.2071
0.8797
0.8621


hf3864/98/f3
7.2911
7.2685
5.2715
5.2652
5.2564
5.2489
5.2401
5.2339
5.2238
5.2175
4.5765
4.5703
4.5540
4.5477
3.5103
3.3071
2.0865
2.0740
2.0551
2.0363
2.0225
1.3991
1.3828
1.3652
1.0717
1.0554
1.0491
0.6841
0.6677
0.6502
0.9267
5.28
5.20
(ppm)
4.9768
2.10
2.05
2.00
(ppm)
Integral
0.8389
0.9267
0.9074
3.0000
1.9037
4.9768
2.2884
21.430
2.6571
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)
1.84



hf3864/41
8.0851
7.3563
7.3375
7.3300
7.3224
7.3099
7.2898
7.2836
7.2723
7.2685
7.2535
4.5163
4.4849
4.4799
4.4498
3.7398
3.6545
3.6470
3.6294
3.6231
3.4789
3.4626
3.4563
3.4387
3.3284
2.2420
Integral
0.6956
4.7410
2.4500
1.1158
1.2400
1.2206
3.2202
3.2619
(ppm)



2.17a

ly3522/17

Integral

0.5072

9.9986

1.0411

1.0484

1.0070

0.8352

1.0796

3.0000

(ppm)

12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0

0.8352

1.0796

(ppm)

3.4 3.2 3.0

3.3185
3.3097
3.2833
3.2746
3.1767
3.1303
3.1203
3.0952
3.0851
3.0111

1.0070

(ppm)

4.2 4.1

4.2079
4.1991
4.1903

11.9627

7.3740
7.3565
7.3527
7.3376
7.3126
7.3013
7.2950
7.2875
7.2799
7.2774
7.2649
7.2473
7.2411
7.2348
7.2298
7.2185
7.2009
7.1959
7.1909
7.1808
7.1746
7.1645
7.1595
7.1558

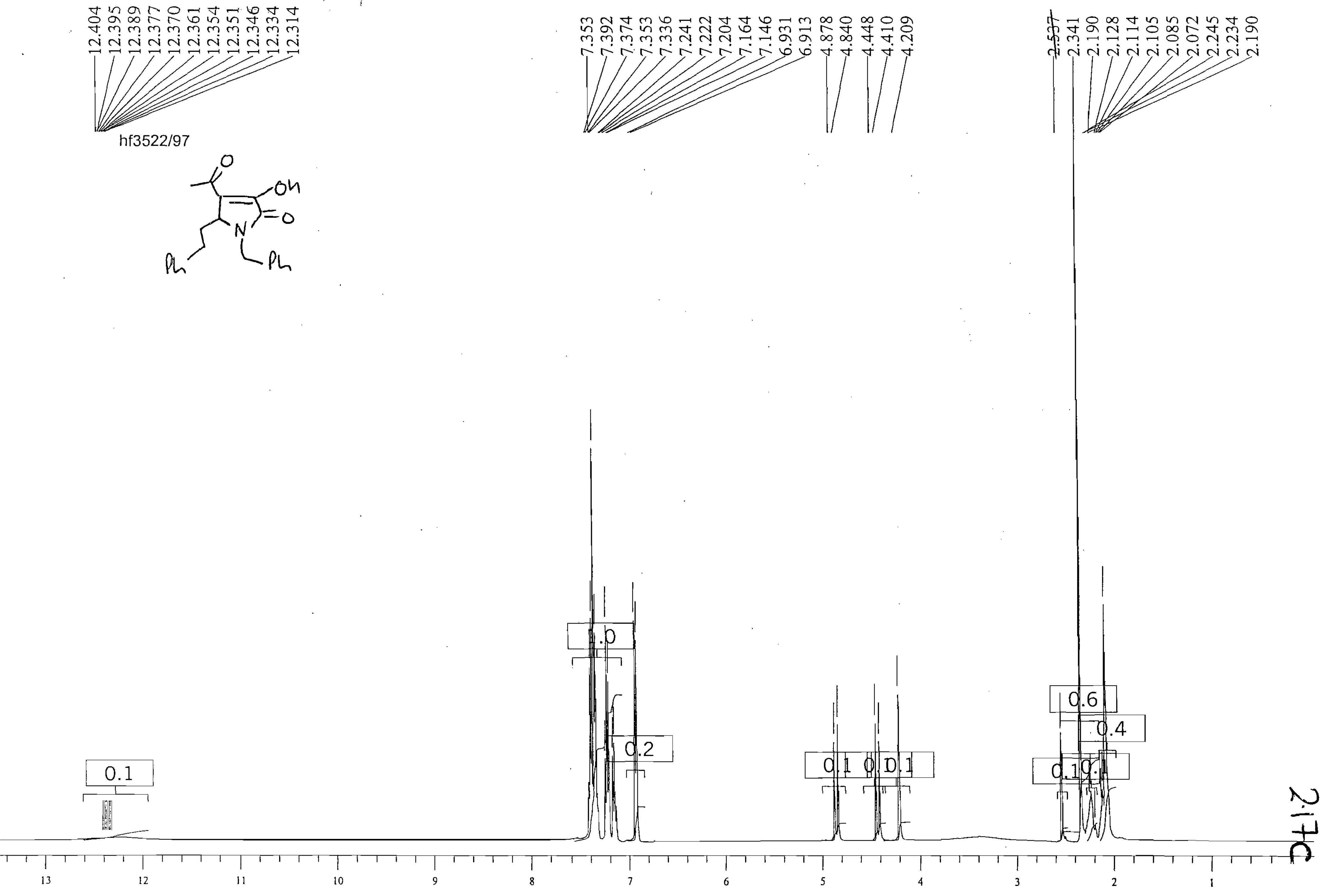
6.8911
6.8873
6.8710

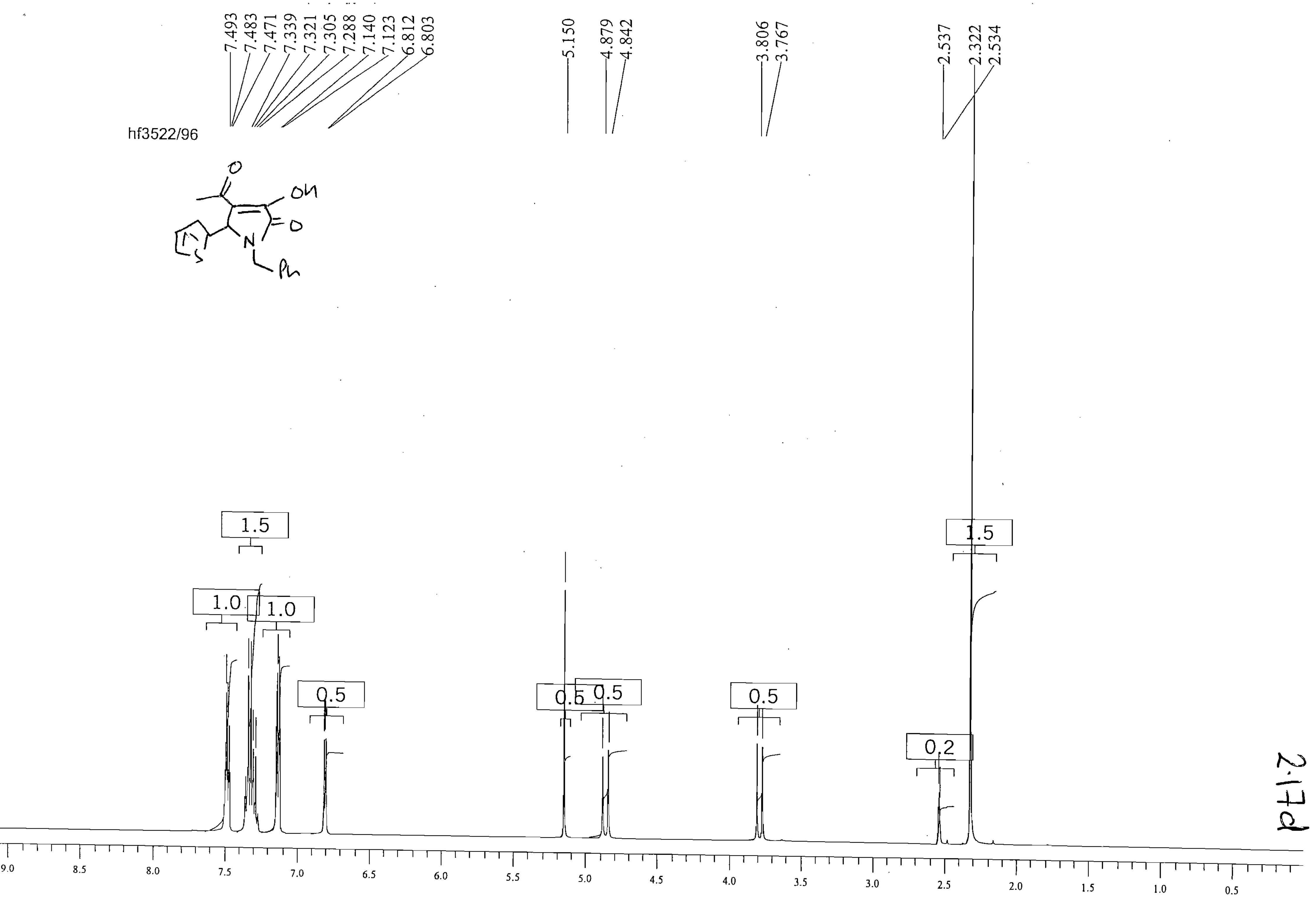
5.0584
5.0207

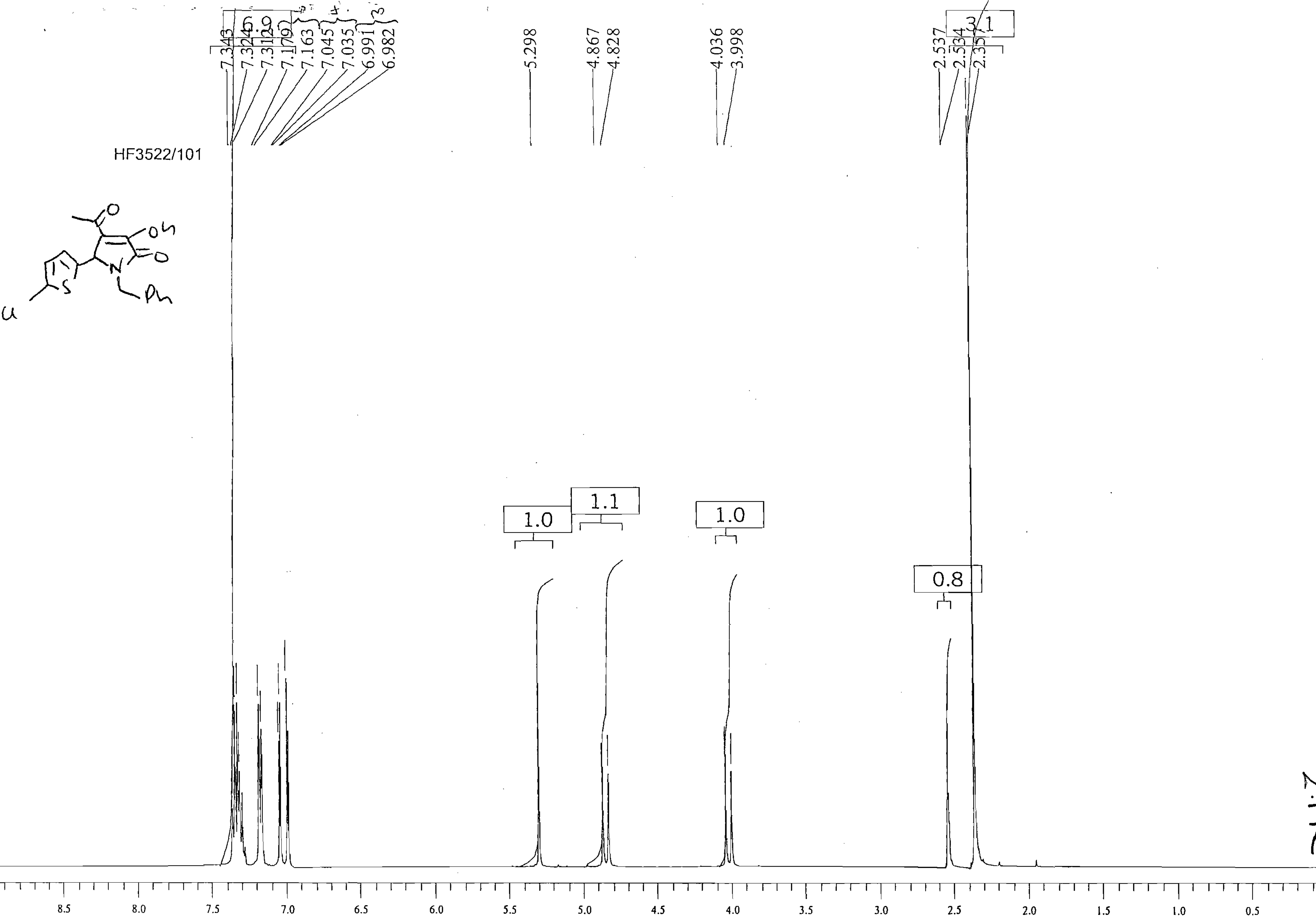
4.4776
4.4399
4.2079
4.1991
4.1903

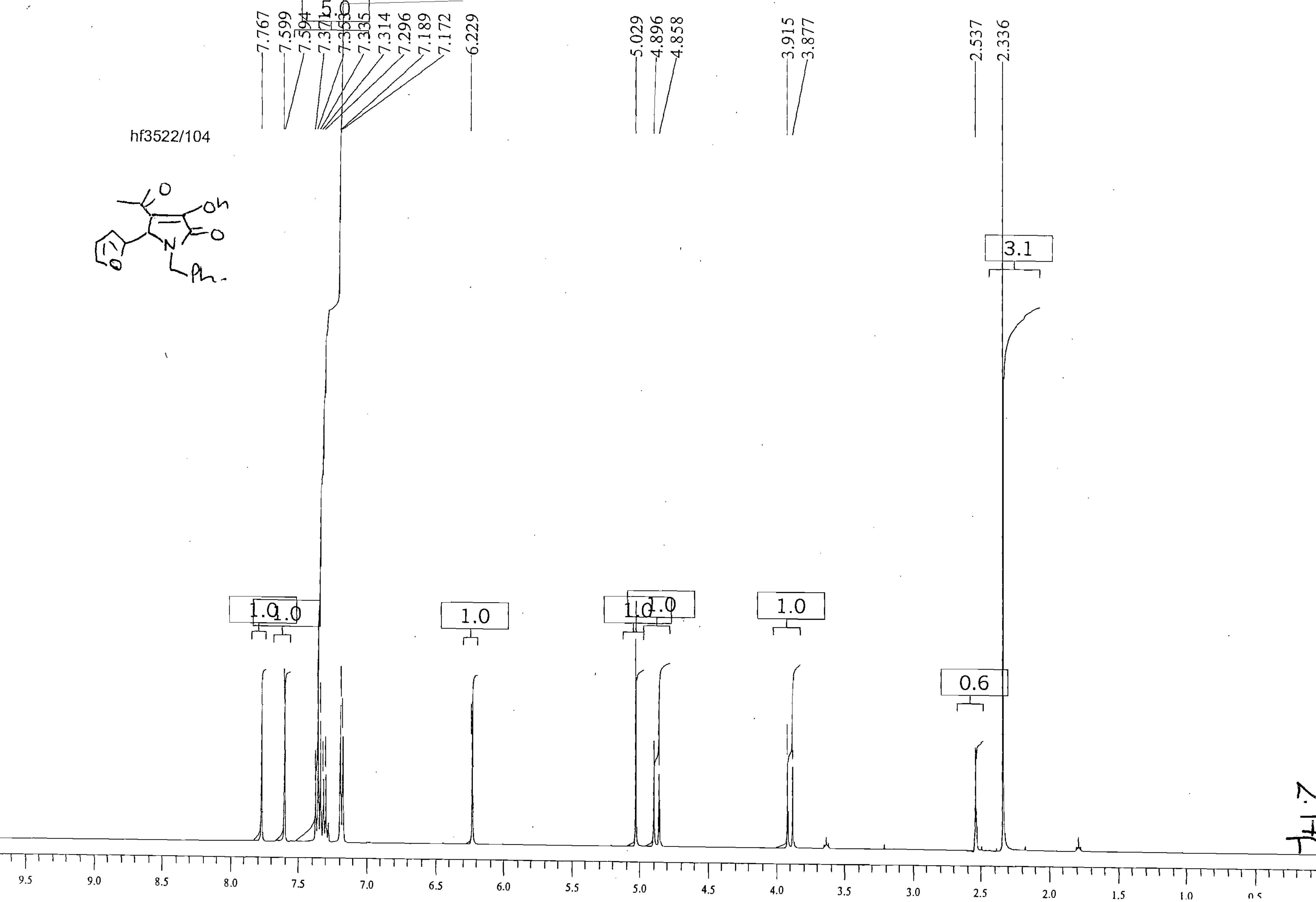
3.3185
3.3097
3.2833
3.2746
3.1767
3.1303
3.1203
3.0952
3.0851
3.0111
2.3199

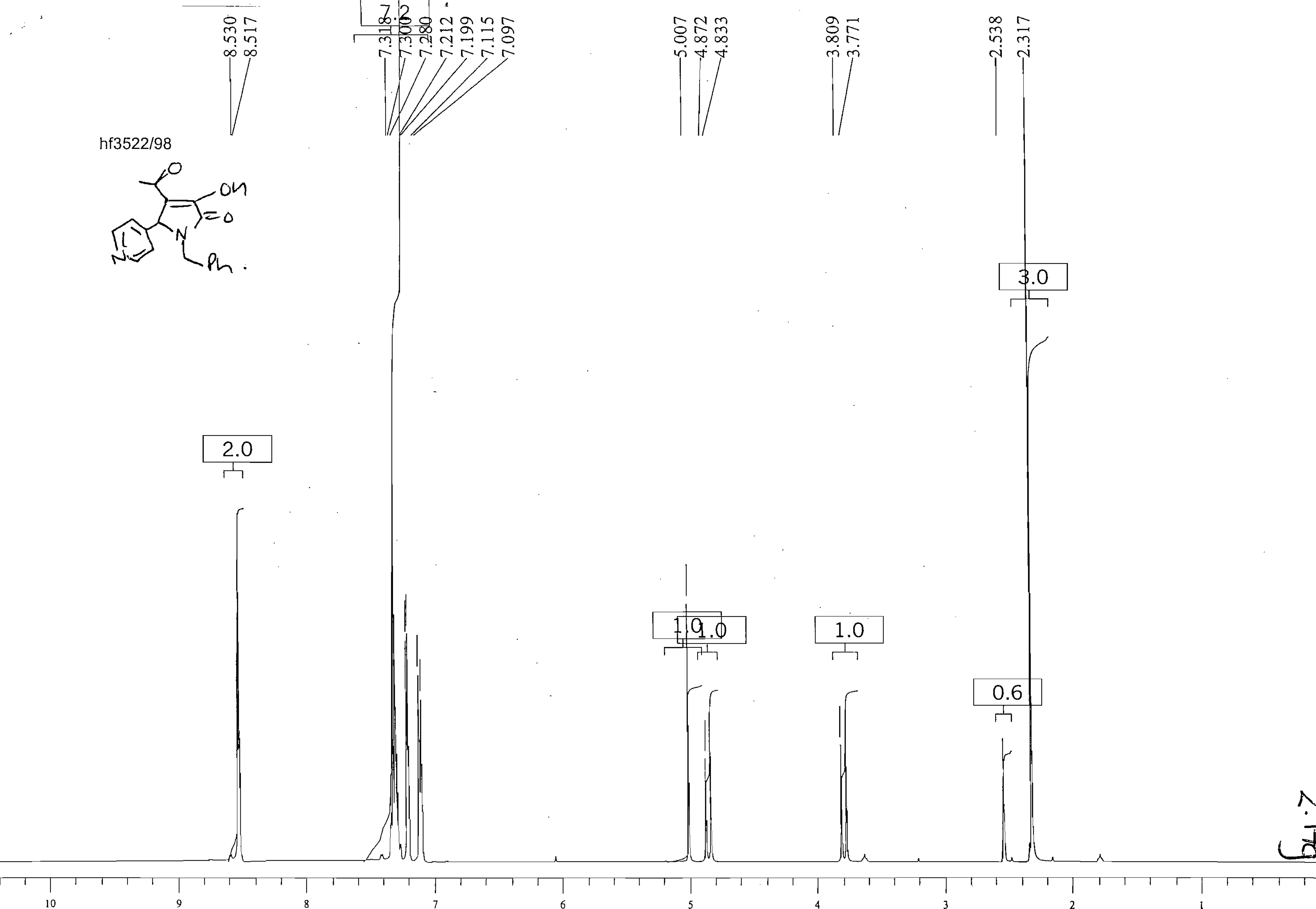
2.176

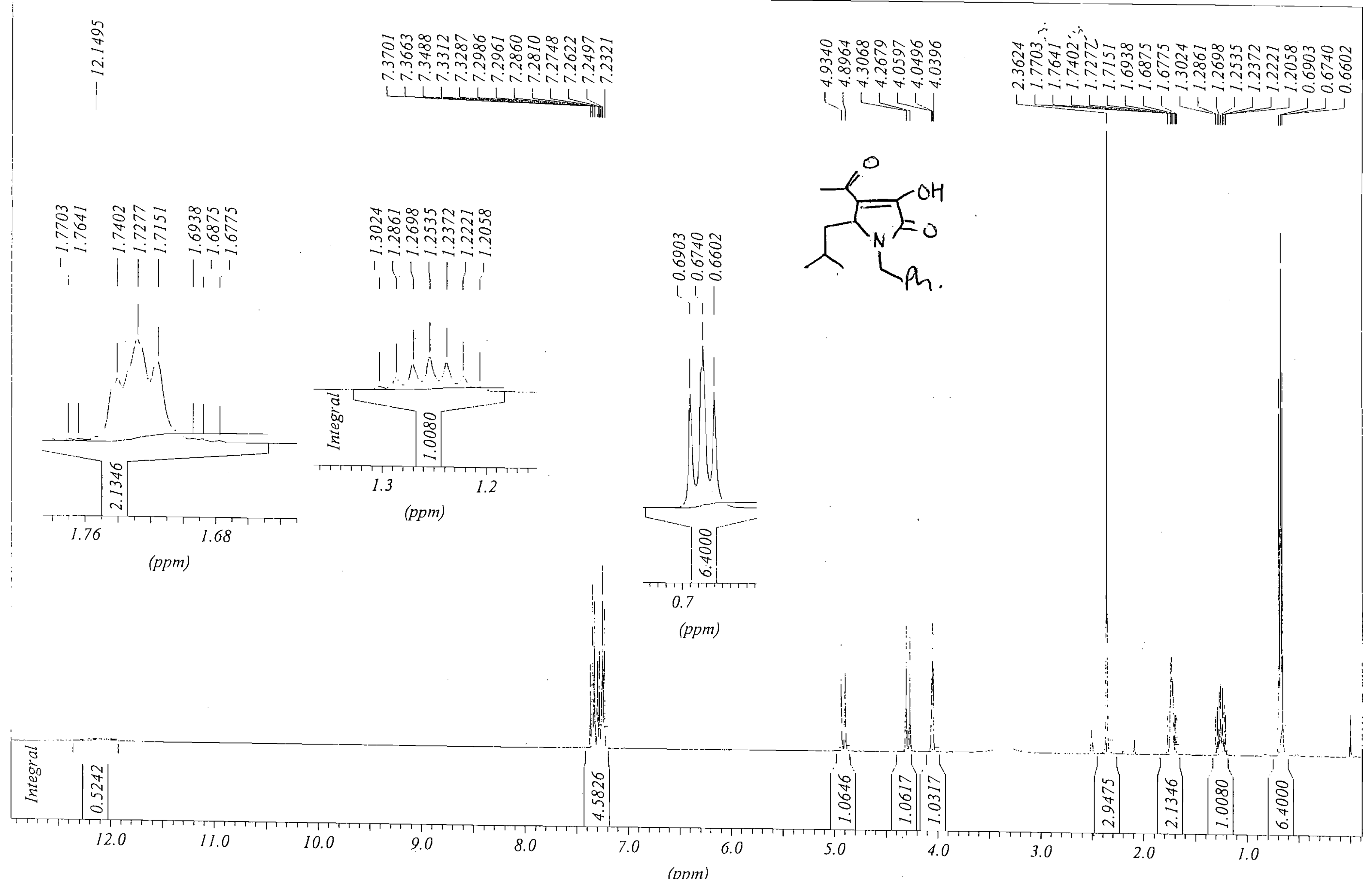

12.404
12.395
12.389
12.377
12.370
12.361
12.354
12.351
12.346
12.334
12.314
hf3522/97
7.353
7.392
7.374
7.353
7.336
7.241
7.222
7.204
7.164
7.146
6.931
6.913
4.878
4.840
4.448
4.410
4.209
2.537
2.341
2.190
2.128
2.114
2.105
2.085
2.072
2.245
2.234
2.190
0.1
0.2
0.6
0.4
13
12
11
10
9
8
7
6
5
4
3
2
1
2·17C


hf3522/96
7.493
7.483
7.471
7.339
7.321
7.305
7.288
7.140
7.123
6.812
6.803
5.150
4.879
4.842
3.806
3.767
2.537
2.322
2.534
1.5
1.0
1.0
0.5
0.5
0.5
0.5
0.2
1.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.17d


HF3522/101
7.343
7.324
7.312
7.179
7.163
7.045
7.035
6.991
6.982
5.298
4.867
4.828
4.036
3.998
2.537
2.534
2.357
6.0
3.1
1.0
1.1
1.0
0.8
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


hf3522/104
7.767
7.599
7.594
7.371
7.353
7.335
7.314
7.296
7.189
7.172
6.229
5.029
4.896
4.858
3.915
3.877
2.537
2.336
1.0
1.0
5.0
1.0
1.0
1.0
1.0
0.6
3.1
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


8.530
8.517
7.318
7.300
7.280
7.212
7.199
7.115
7.097
5.007
4.872
4.833
3.809
3.771
2.538
2.317
hf3522/98
OH
N
Ph.
7.2
3.0
2.0
1.0
1.0
1.0
0.6
2.17g
10
9
8
7
6
5
4
3
2
1



hf3522/18
Integral
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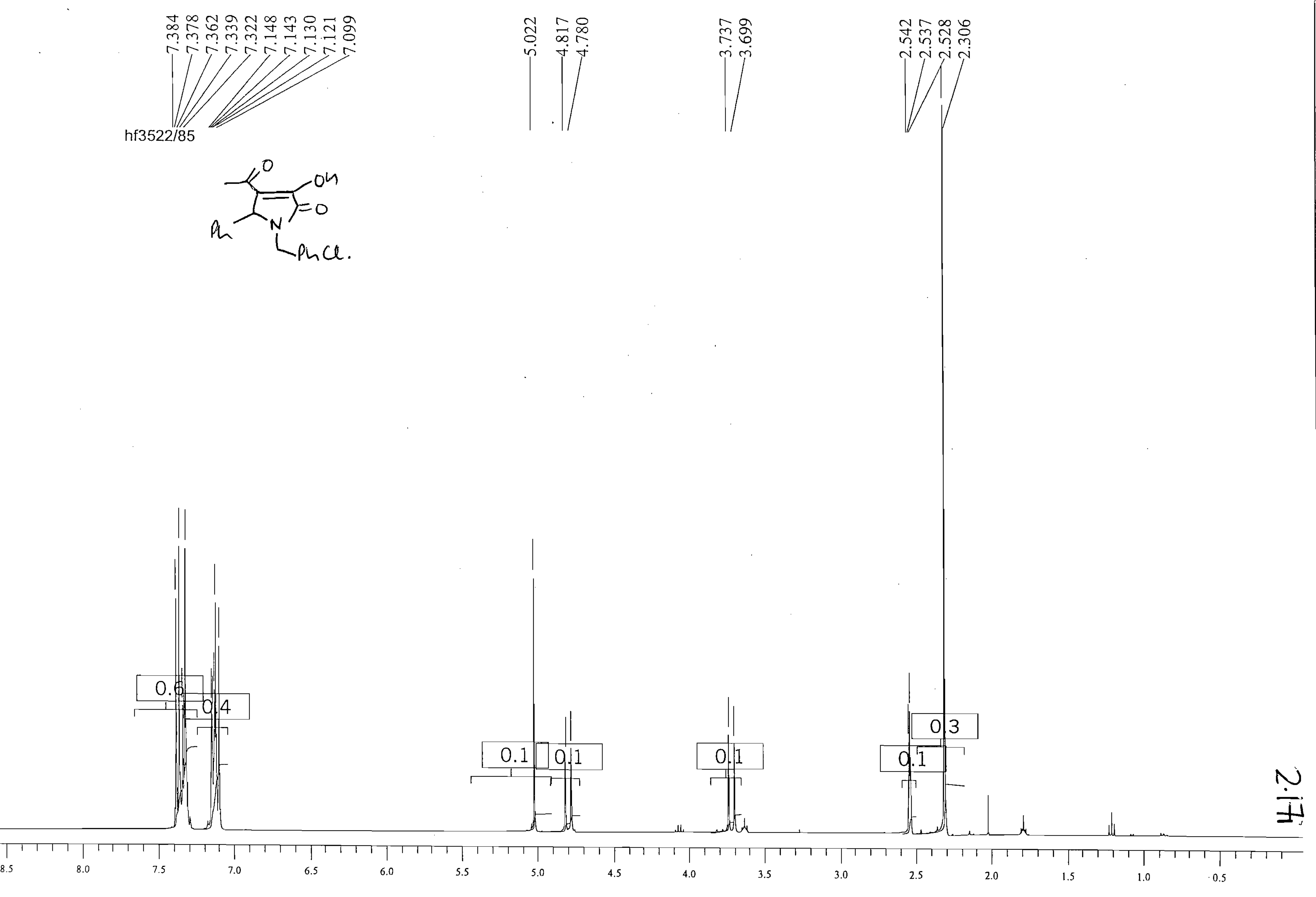
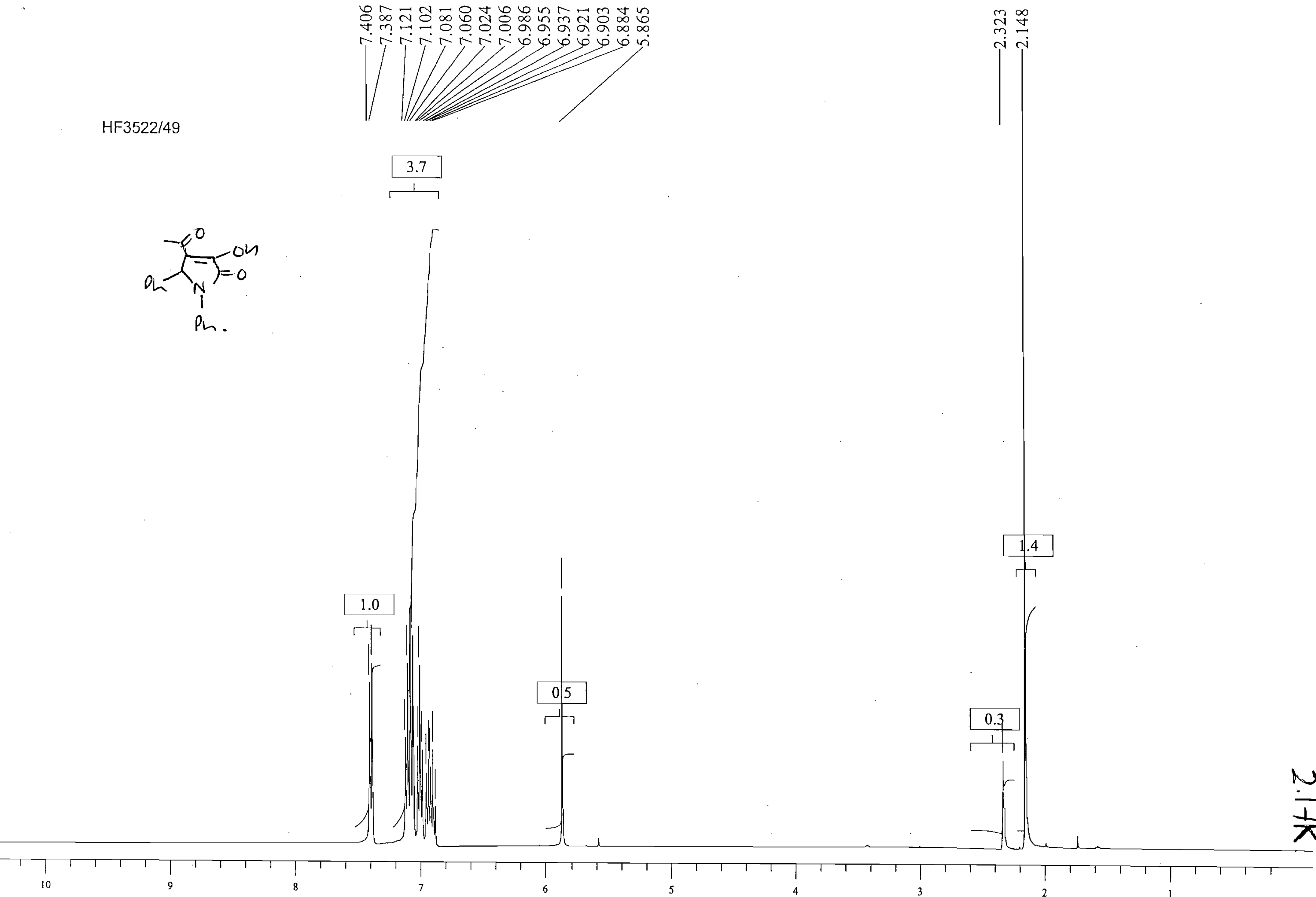
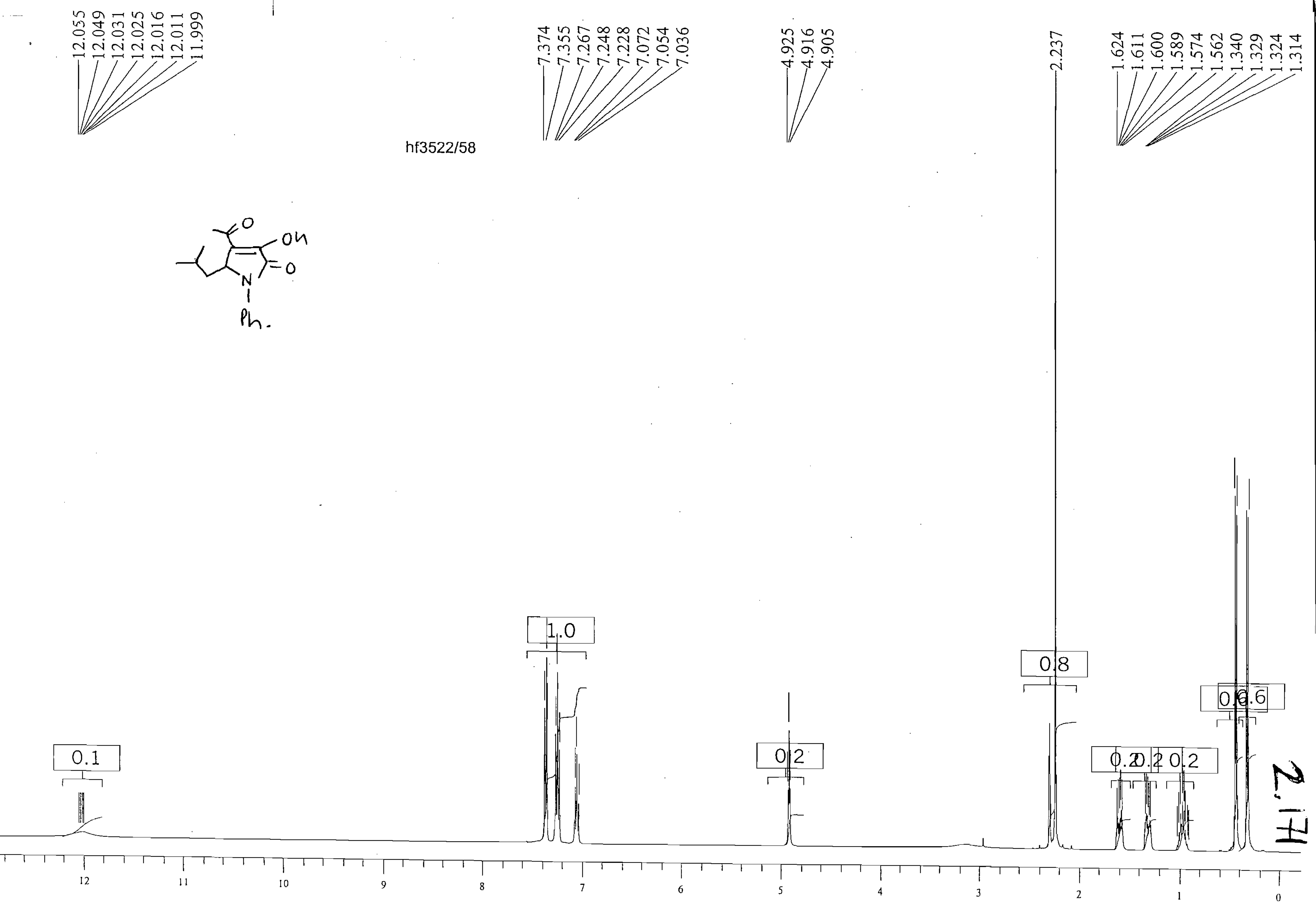
1.0646
1.0617
1.0317
2.9475
2.1346
1.0080
6.4000
(ppm)
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
12.1495
7.3701
7.3663
7.3488
7.3312
7.3287
7.2986
7.2961
7.2860
7.2810
7.2748
7.2622
7.2497
7.2321
4.9340
4.8964
4.3068
4.2679
4.0597
4.0496
4.0396
2.3624
1.7703
1.7641
1.7402
1.7277
1.7151
1.6938
1.6875
1.6775
1.3024
1.2861
1.2698
1.2535
1.2372
1.2221
1.2058
0.6903
0.6740
0.6602
1.76
1.68
1.3
1.2
0.7
OH
N
Ph.
2.17h


7.384
7.378
7.362
7.339
7.322
7.148
7.143
7.130
7.121
7.099
5.022
4.817
4.780
3.737
3.699
2.542
2.537
2.528
2.306
hf3522/85
0.6
0.4
0.1
0.1
0.1
0.3
0.1
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


HF3522/49
7.406
7.387
7.121
7.102
7.081
7.060
7.024
7.006
6.986
6.955
6.937
6.921
6.903
6.884
5.865
2.323
2.148
3.7
1.0
0.5
0.3
1.4
OH
O
N
Ph
Ph.
2.17K
10
9
8
7
6
5
4
3
2
1


12.055
12.049
12.031
12.025
12.016
12.011
11.999
hf3522/58
7.374
7.355
7.267
7.248
7.228
7.072
7.054
7.036
4.925
4.916
4.905
2.237
1.624
1.611
1.600
1.589
1.574
1.562
1.340
1.329
1.324
1.314
0.1
1.0
0.2
0.8
0.2
0.2
0.2
0.6
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7
6
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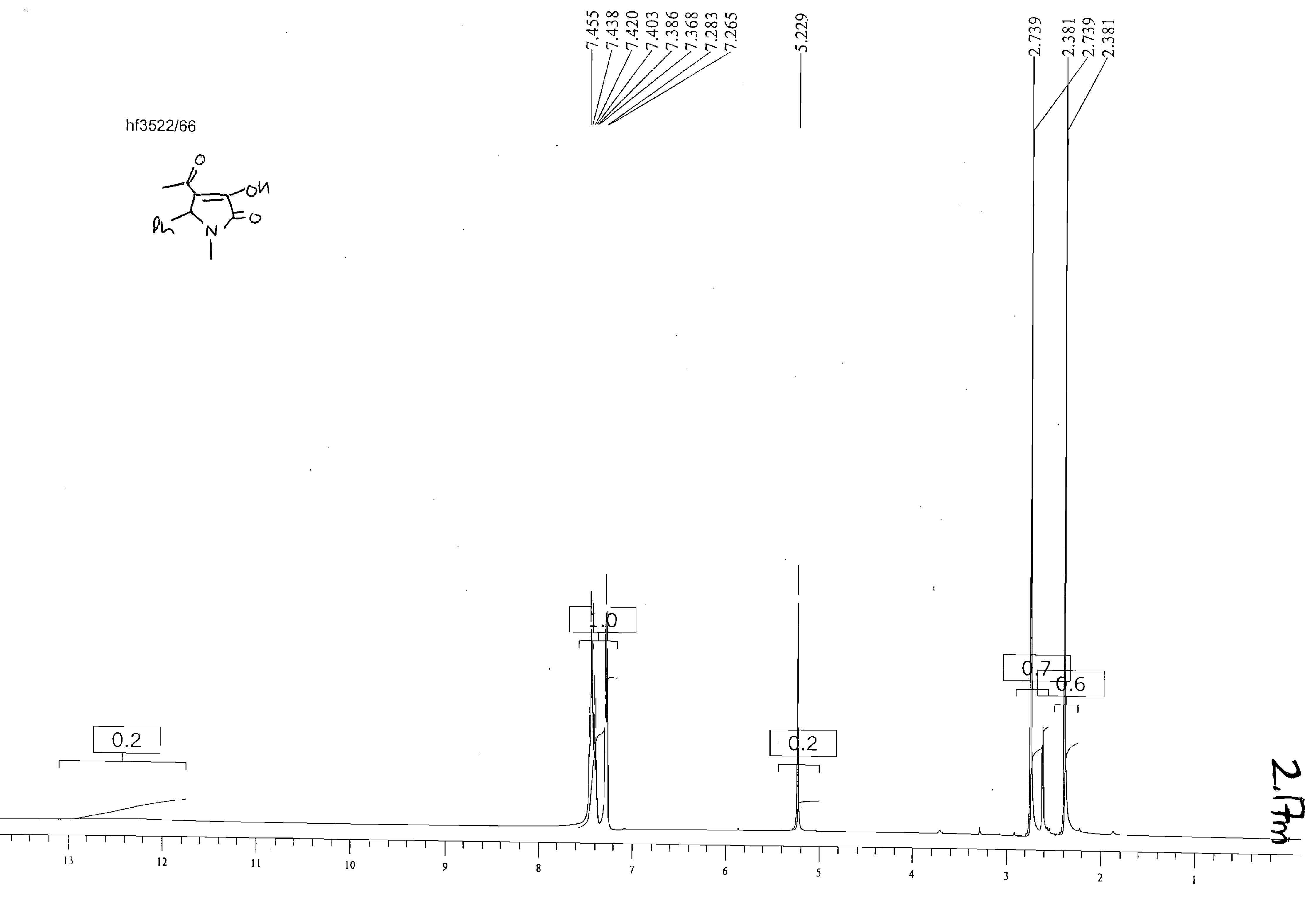
3
2
1
0



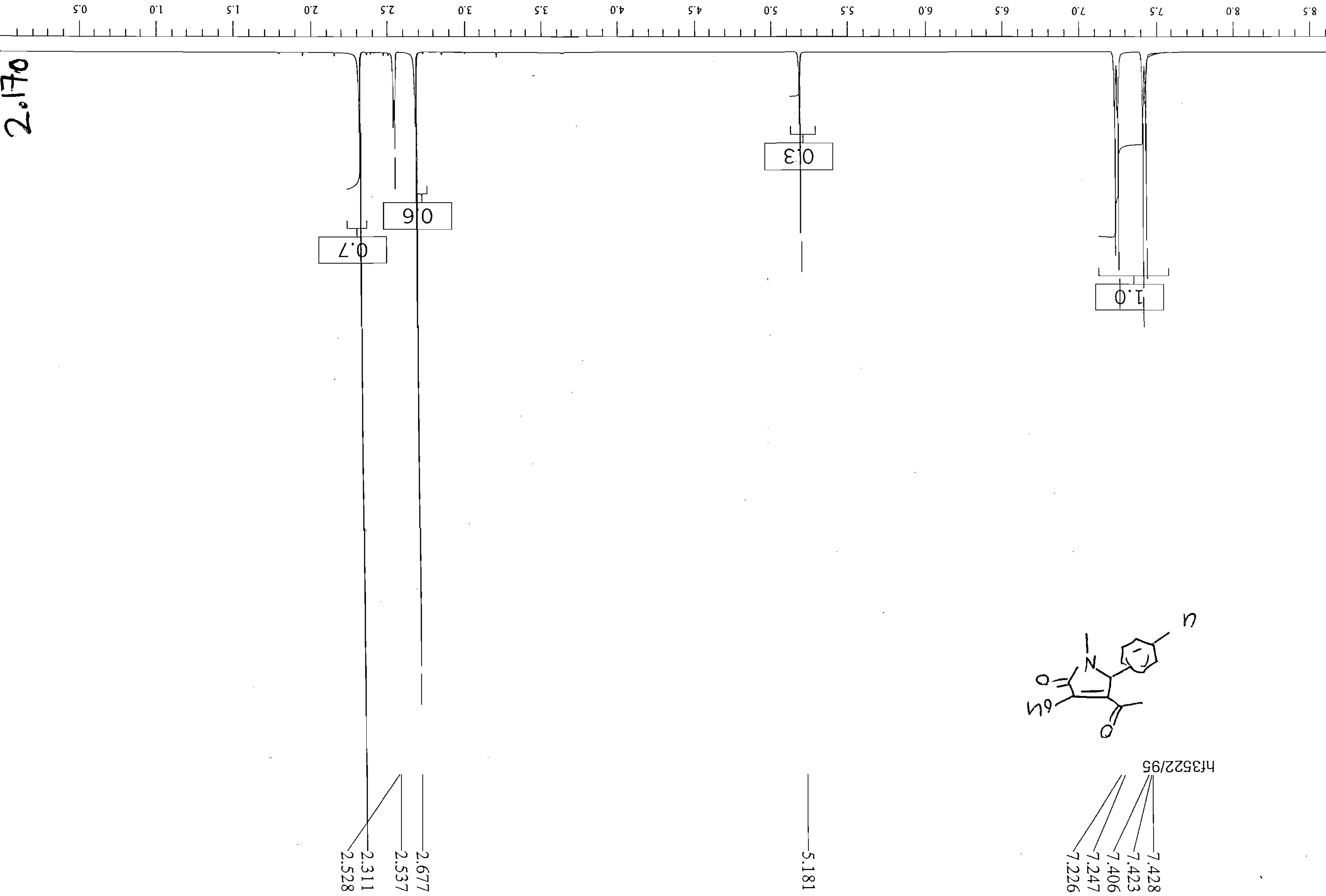
hf3522/66
Ph
N
OH
O
O
7.455
7.438
7.420
7.403
7.386
7.368
7.283
7.265
5.229
2.739
2.381
2.739
2.381
0.2
1.0
0.2
0.7
0.6
13
12
11
10
9
8
7
6
5
4
3
2
1
2.17m

2.17n

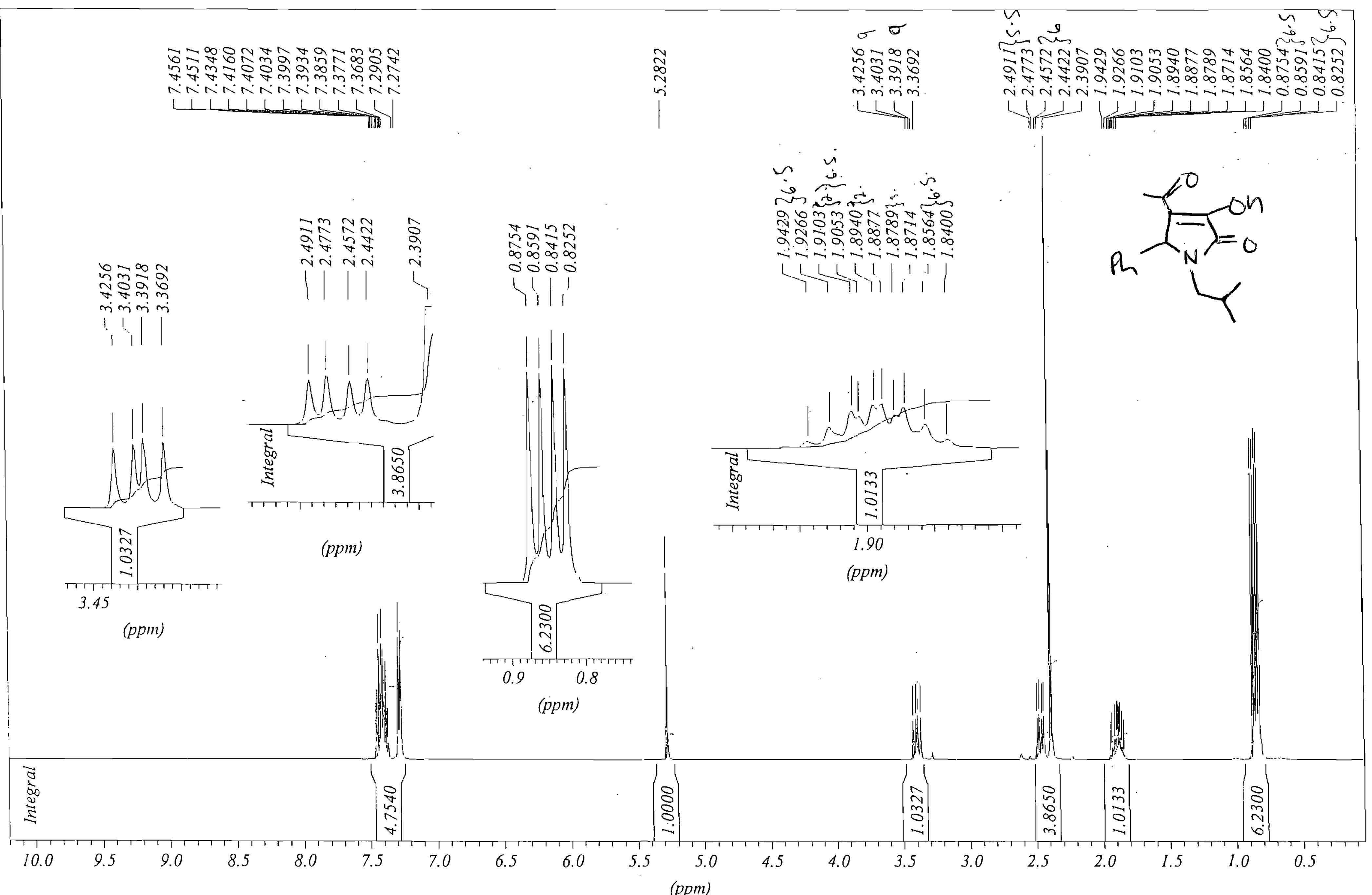
hf3522/94

7.122
7.102
6.923
6.901
5.107
3.766
2.659
2.300

16 14 12 10 8 6 4 2 0 -2

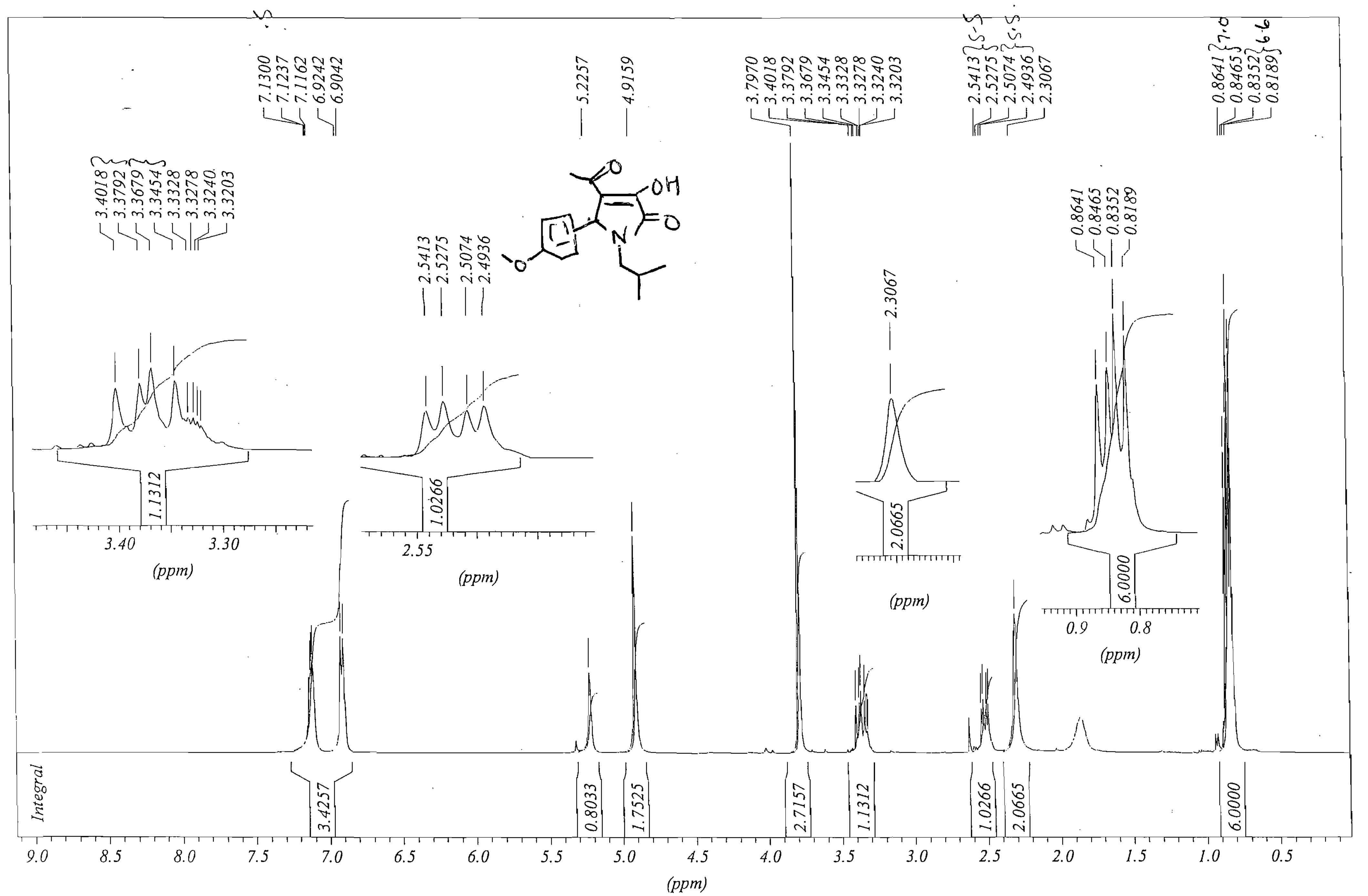


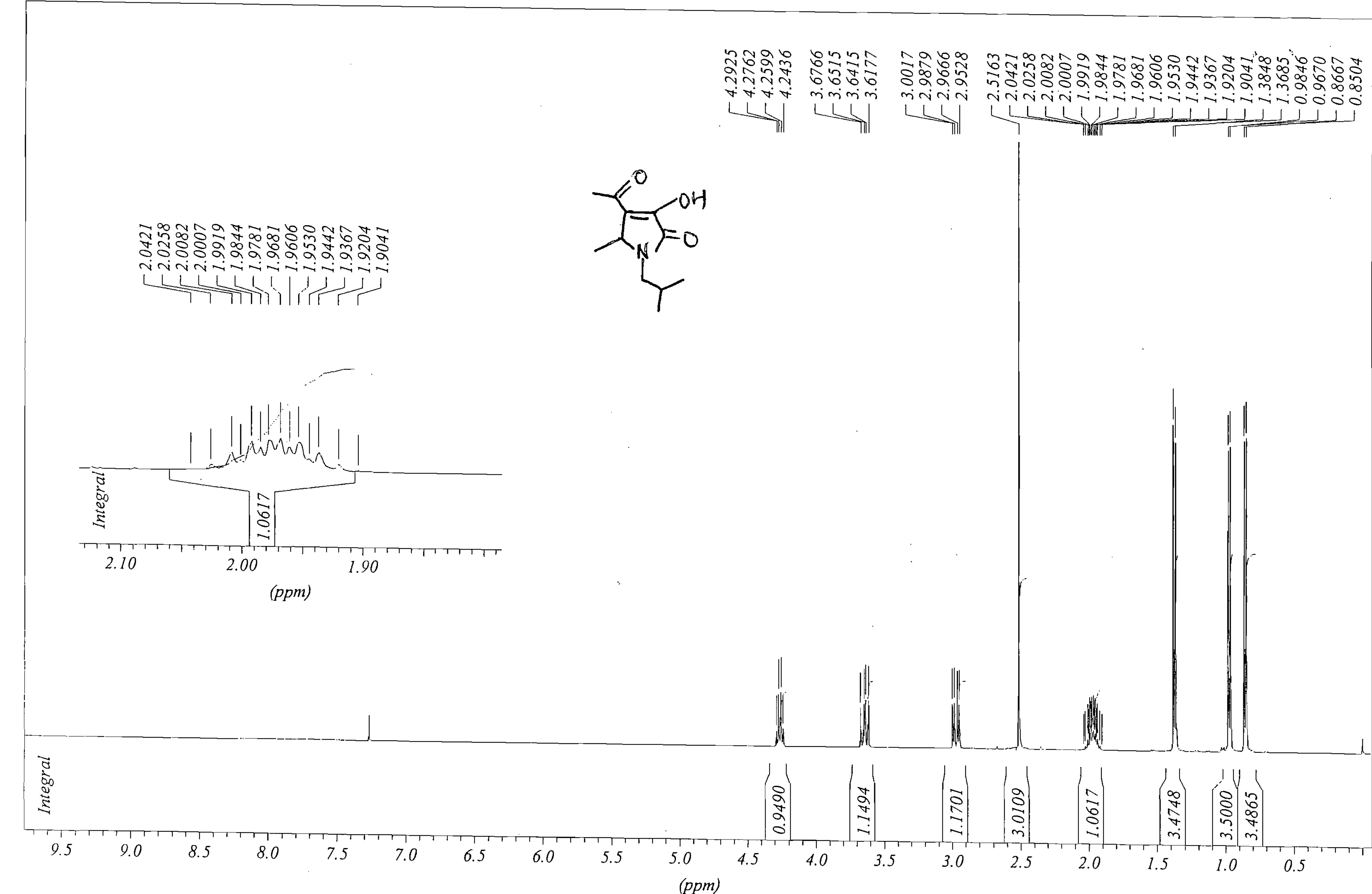
2.170
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
0.7
0.6
0.3
1.0
2.528
2.311
2.537
2.677
5.181
7.226
7.247
7.406
7.423
7.428
hf3522/95



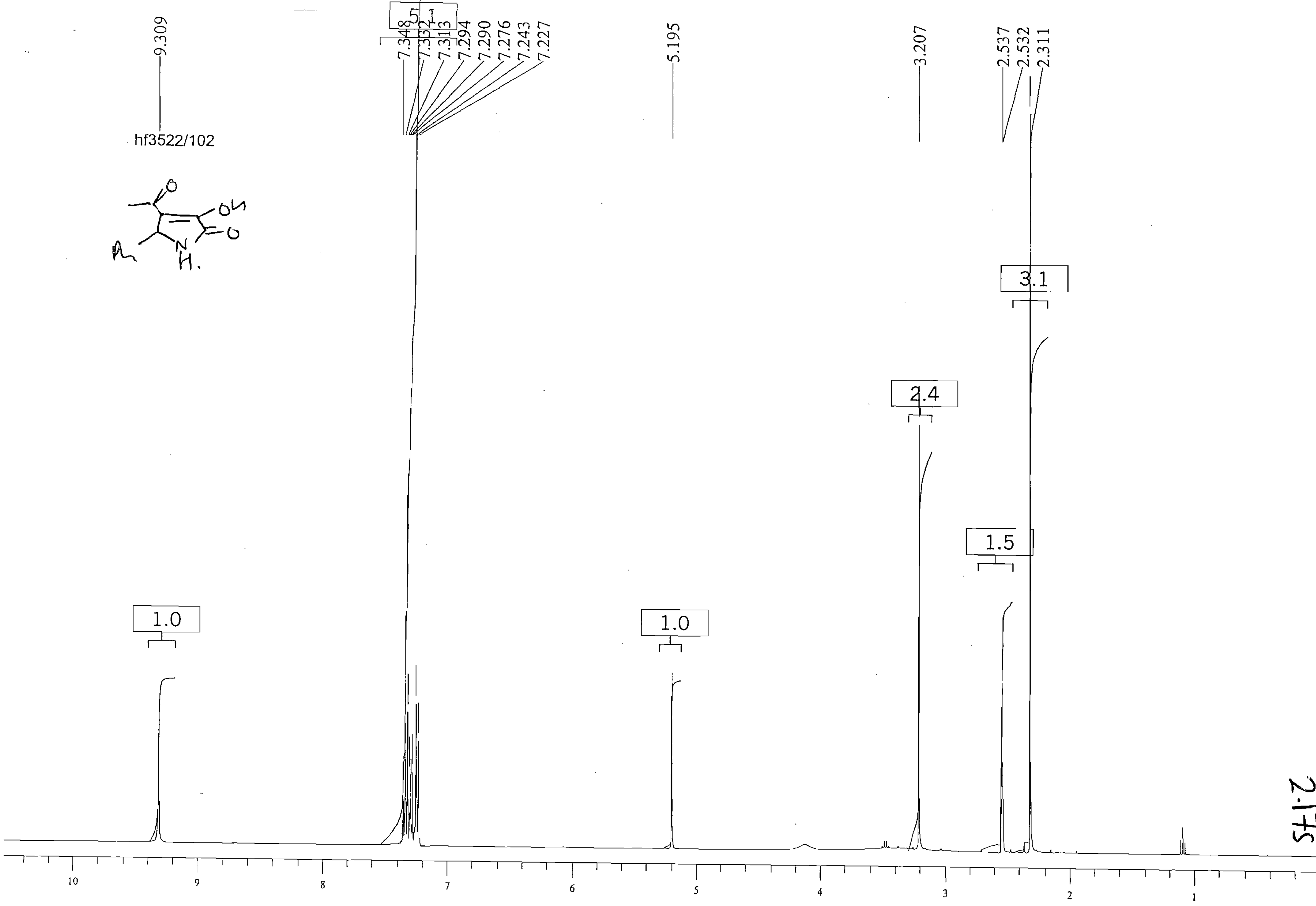
hf3522/19
7.4561
7.4511
7.4348
7.4160
7.4072
7.4034
7.3997
7.3934
7.3859
7.3771
7.3683
7.2905
7.2742
5.2822
3.4256
3.4031
3.3918
3.3692
2.4911
2.4773
2.4572
2.4422
2.3907
1.9429
1.9266
1.9103
1.9053
1.8940
1.8877
1.8789
1.8714
1.8564
1.8400
0.8754
0.8591
0.8415
0.8252
Integral
1.0327
3.45
(ppm)
3.8650
6.2300
0.9
0.8
1.0133
1.90
4.7540
1.0000
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.17p

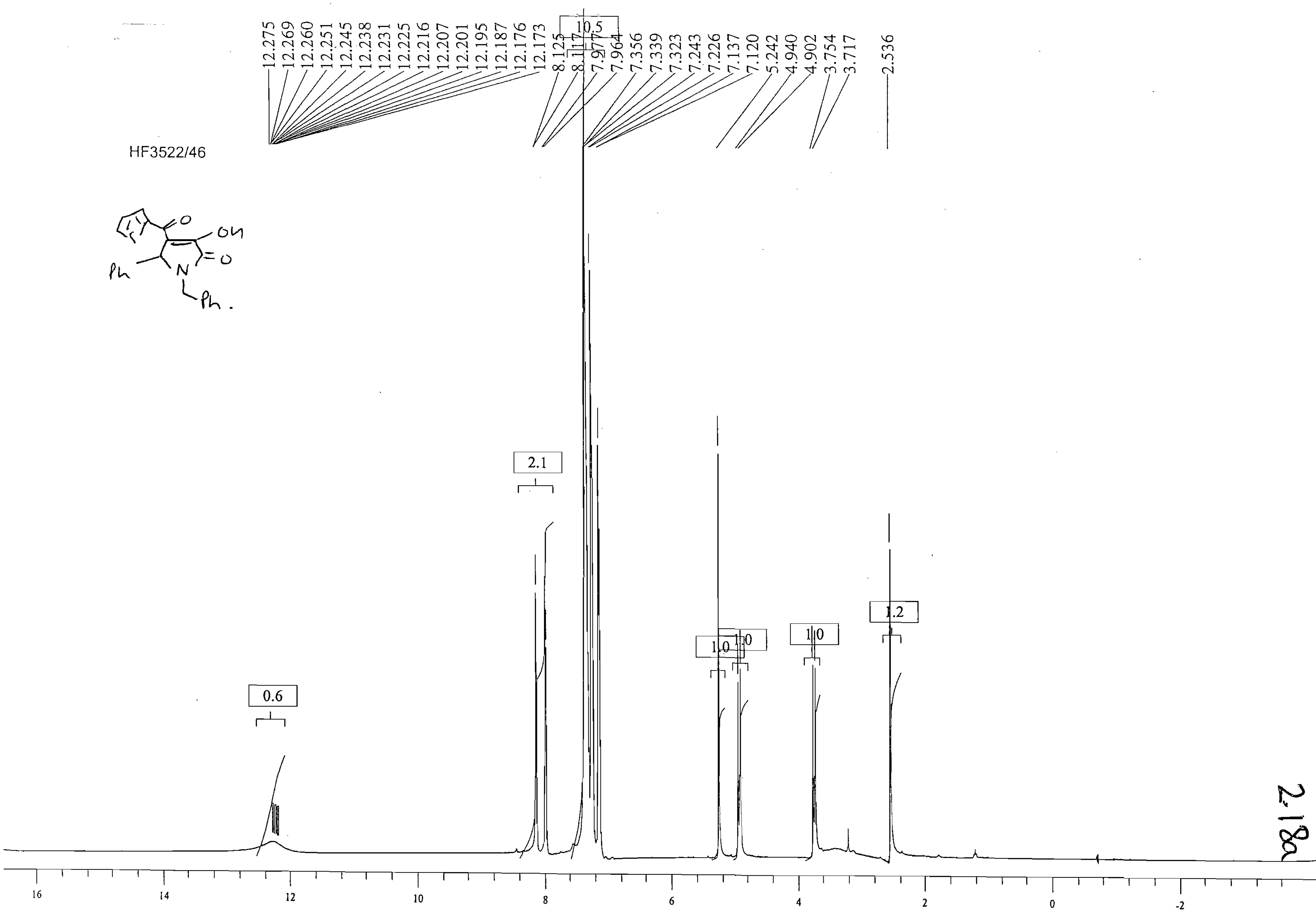
hf3522/22□

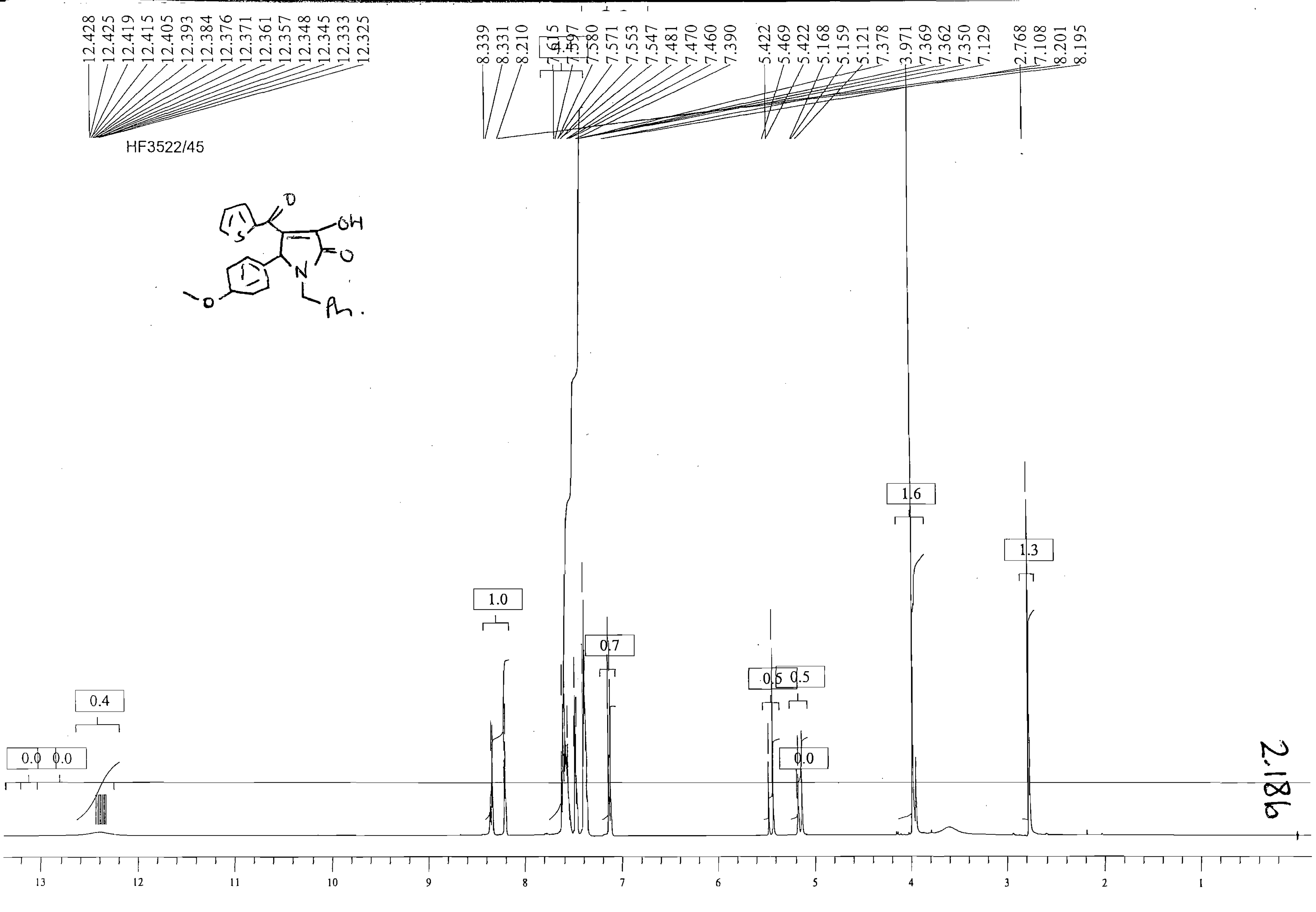




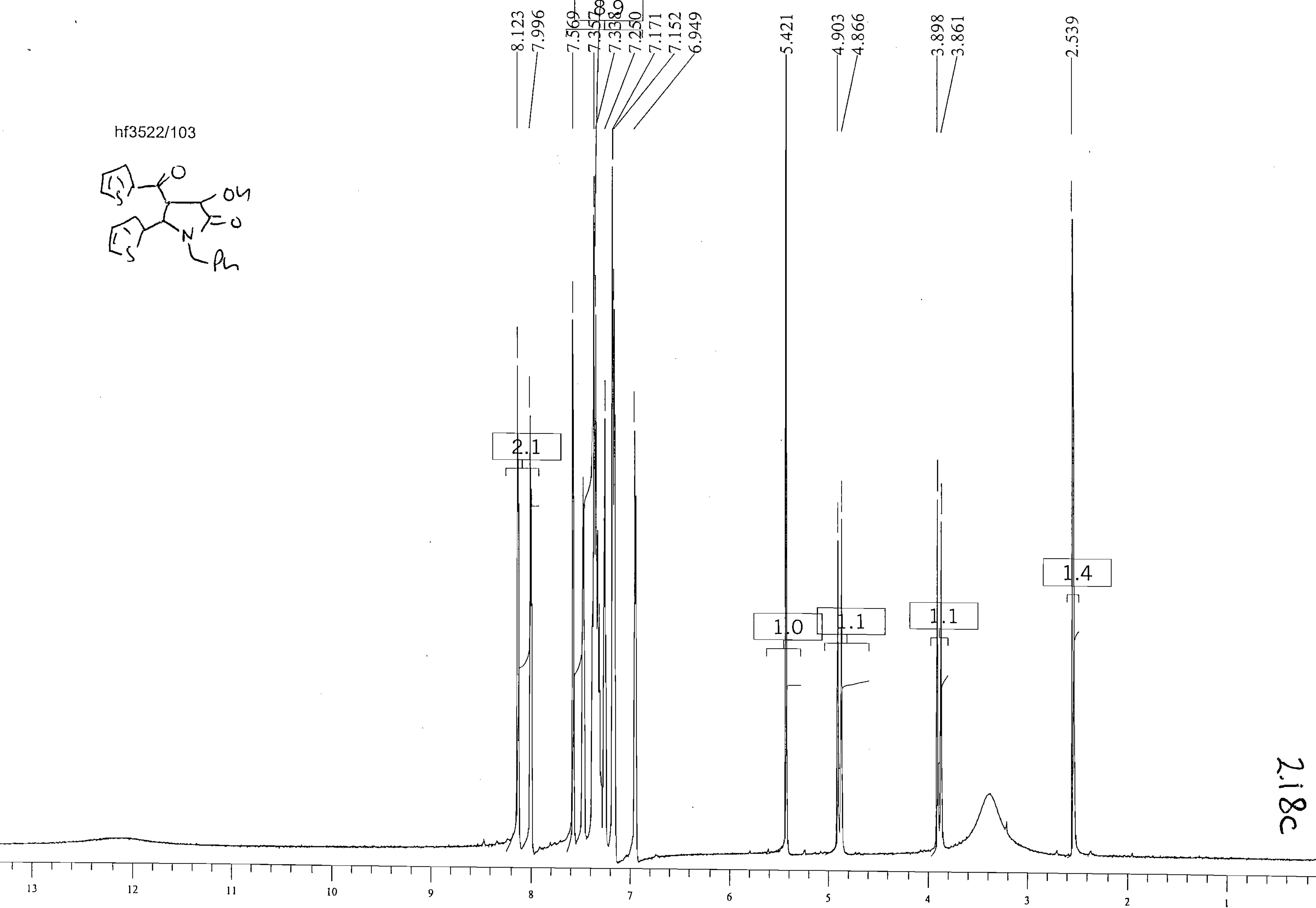
HF3522/23
2.0421
2.0258
2.0082
2.0007
1.9919
1.9844
1.9781
1.9681
1.9606
1.9530
1.9442
1.9367
1.9204
1.9041
Integral
1.0617
2.10
2.00
1.90
(ppm)
OH
O
N
O
4.2925
4.2762
4.2599
4.2436
3.6766
3.6515
3.6415
3.6177
3.0017
2.9879
2.9666
2.9528
2.5163
2.0421
2.0258
2.0082
2.0007
1.9919
1.9844
1.9781
1.9681
1.9606
1.9530
1.9442
1.9367
1.9204
1.9041
1.3848
1.3685
0.9846
0.9670
0.8667
0.8504
Integral
0.9490
1.1494
1.1701
3.0109
1.0617
3.4748
3.5000
3.4865
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)
2.17r

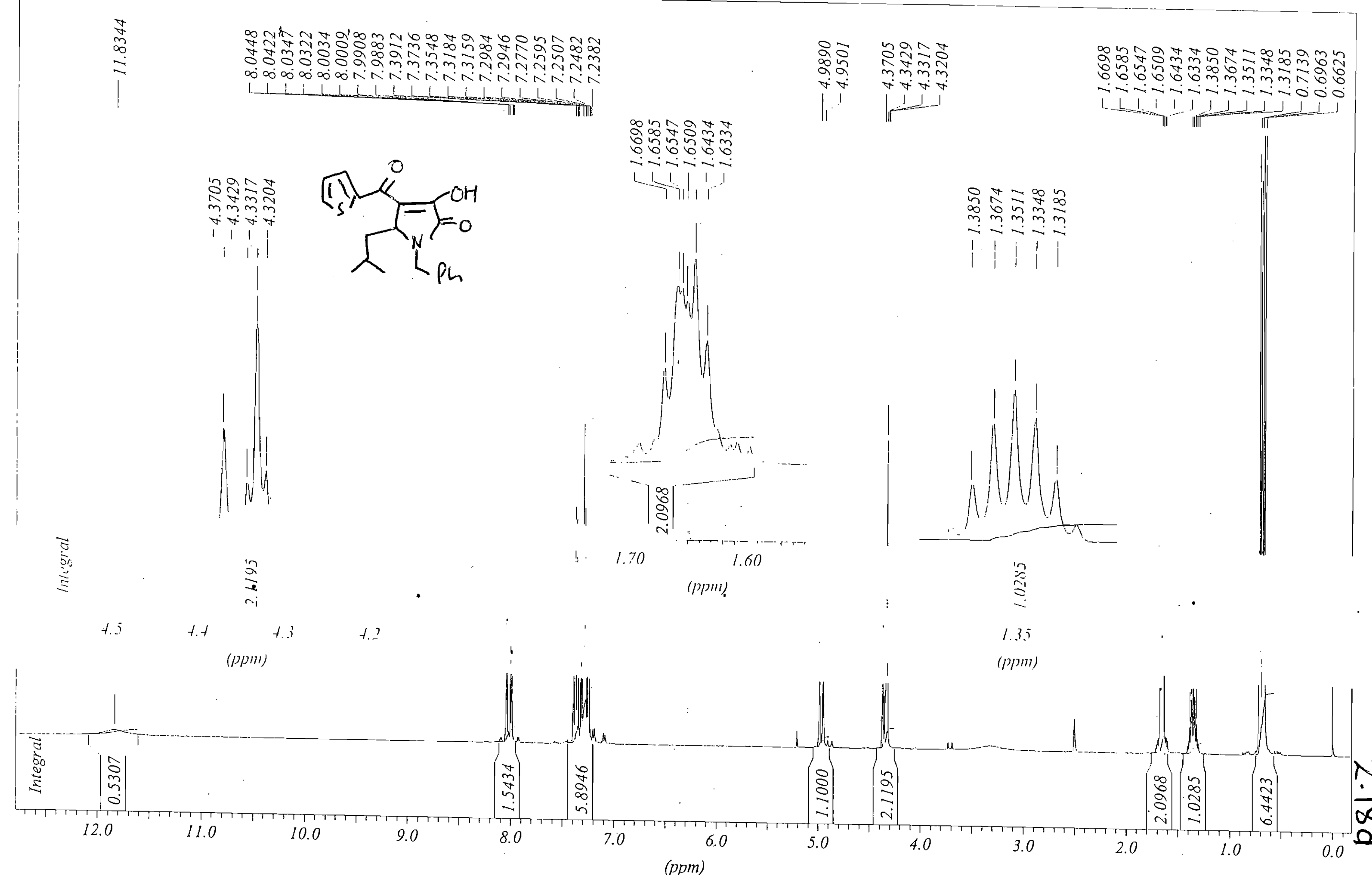

hf3522/102
9.309
7.348
7.332
7.313
7.294
7.290
7.276
7.243
7.227
5.195
3.207
2.537
2.532
2.311
1.0
5.1
1.0
2.4
1.5
3.1
10
9
8
7
6
5
4
3
2
1
2.175


HF3522/46
OH
O
O
N
Ph
Ph.
12.275
12.269
12.260
12.251
12.245
12.238
12.231
12.225
12.216
12.207
12.201
12.195
12.187
12.176
12.173
8.125
8.117
7.977
7.964
7.356
7.339
7.323
7.243
7.226
7.137
7.120
5.242
4.940
4.902
3.754
3.717
2.536
10.5
2.1
0.6
1.0
1.0
1.0
1.2
16
14
12
10
8
6
4
2
0
-2
2.18a



HF3522/45
12.428
12.425
12.419
12.415
12.405
12.393
12.384
12.376
12.371
12.361
12.357
12.348
12.345
12.333
12.325
8.339
8.331
8.210
7.615
7.597
7.580
7.571
7.553
7.547
7.481
7.470
7.460
7.390
5.422
5.469
5.422
5.168
5.159
5.121
7.378
3.971
7.369
7.362
7.350
7.129
2.768
7.108
8.201
8.195
0.0
0.0
0.4
1.0
0.7
0.5
0.5
0.0
1.6
1.3
13
12
11
10
9
8
7
6
5
4
3
2
1
OH
O
N
S
2.186


2.18c
hf3522/103
OH
O
N
Ph
S
S
8.123
7.996
7.569
7.357
7.338
7.250
7.171
7.152
6.949
5.421
4.903
4.866
3.898
3.861
2.539
2.1
8.9
1.0
1.1
1.1
1.4
13
12
11
10
9
8
7
6
5
4
3
2
1


hf3522/47
11.8344
8.0448
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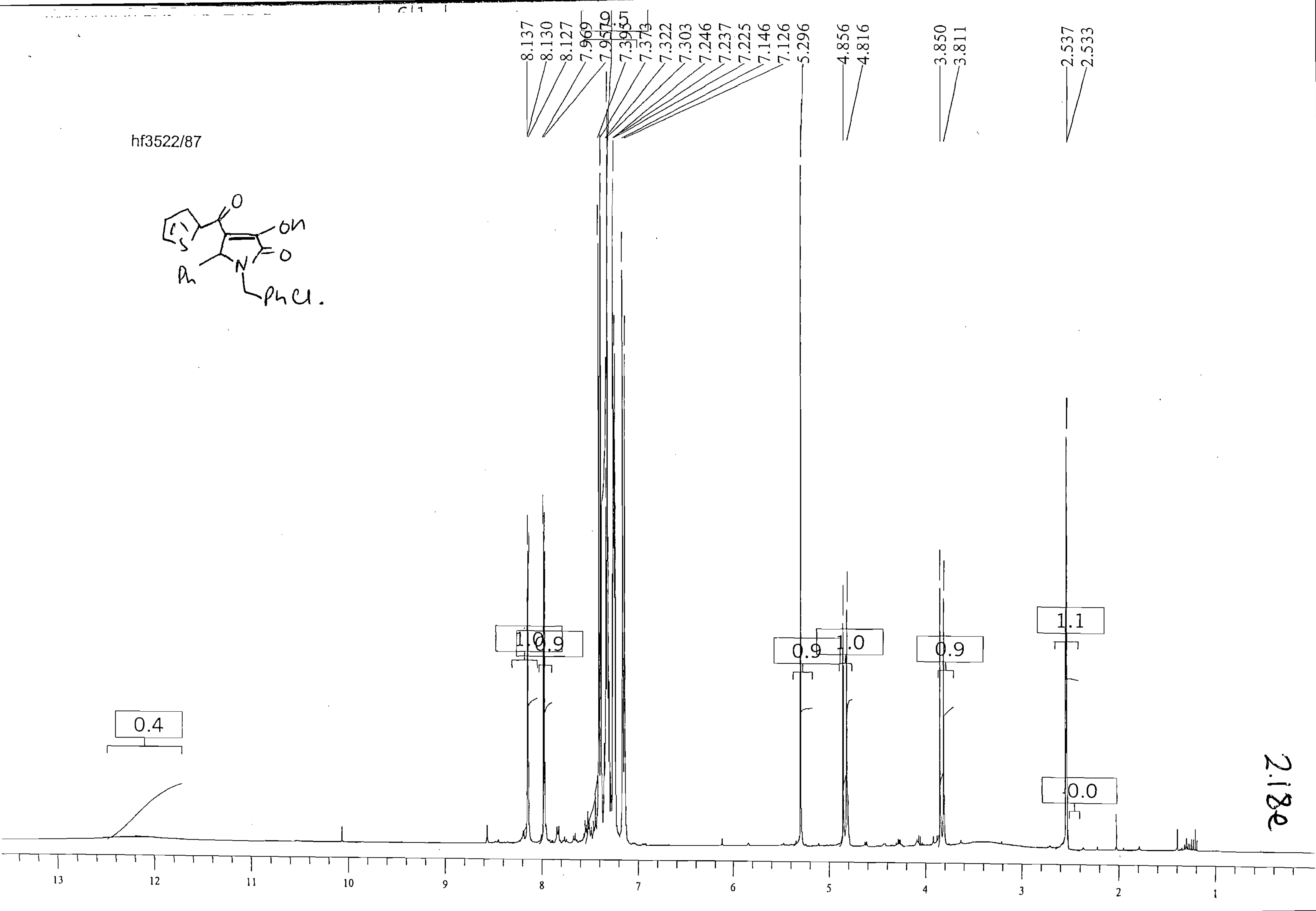
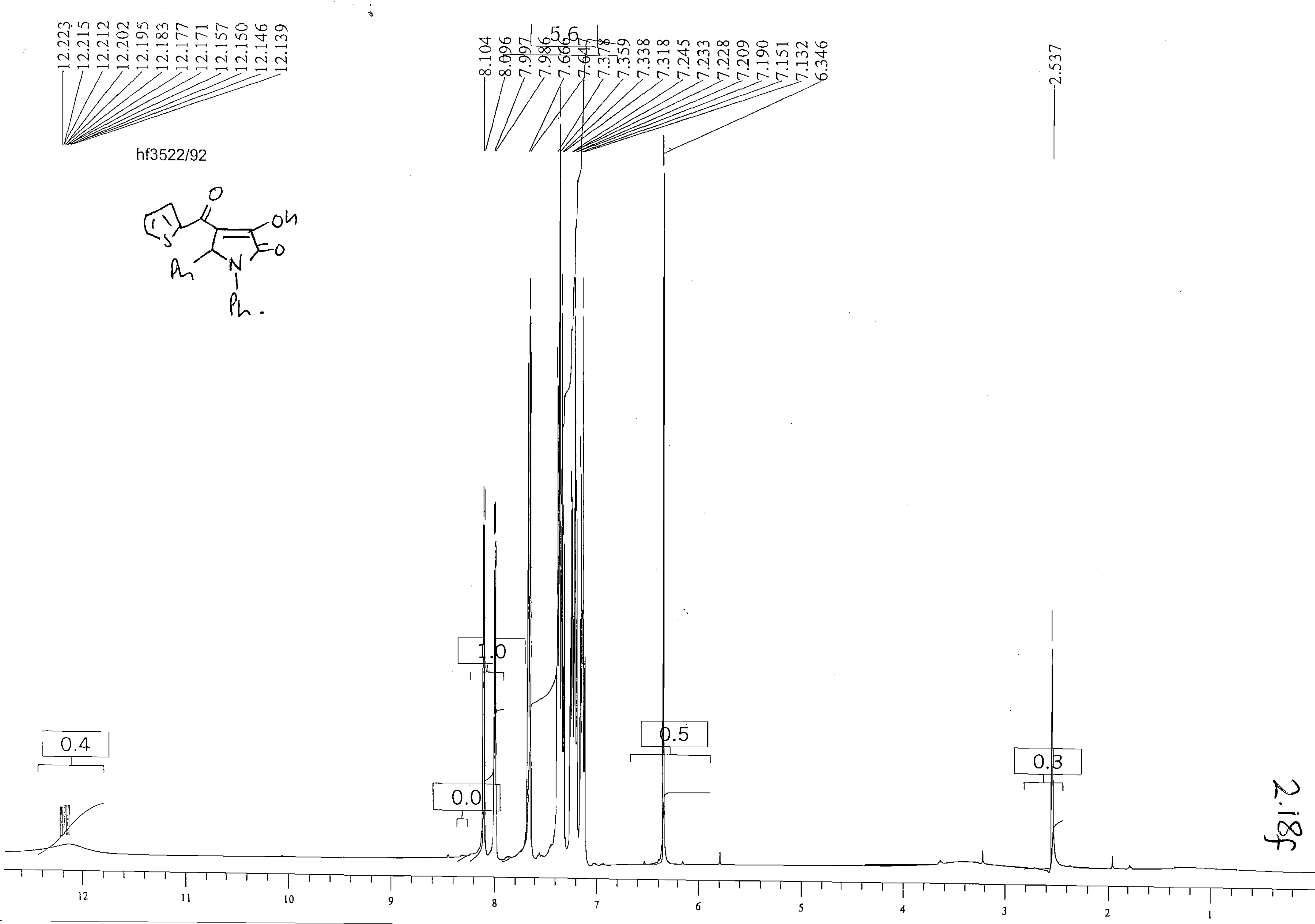
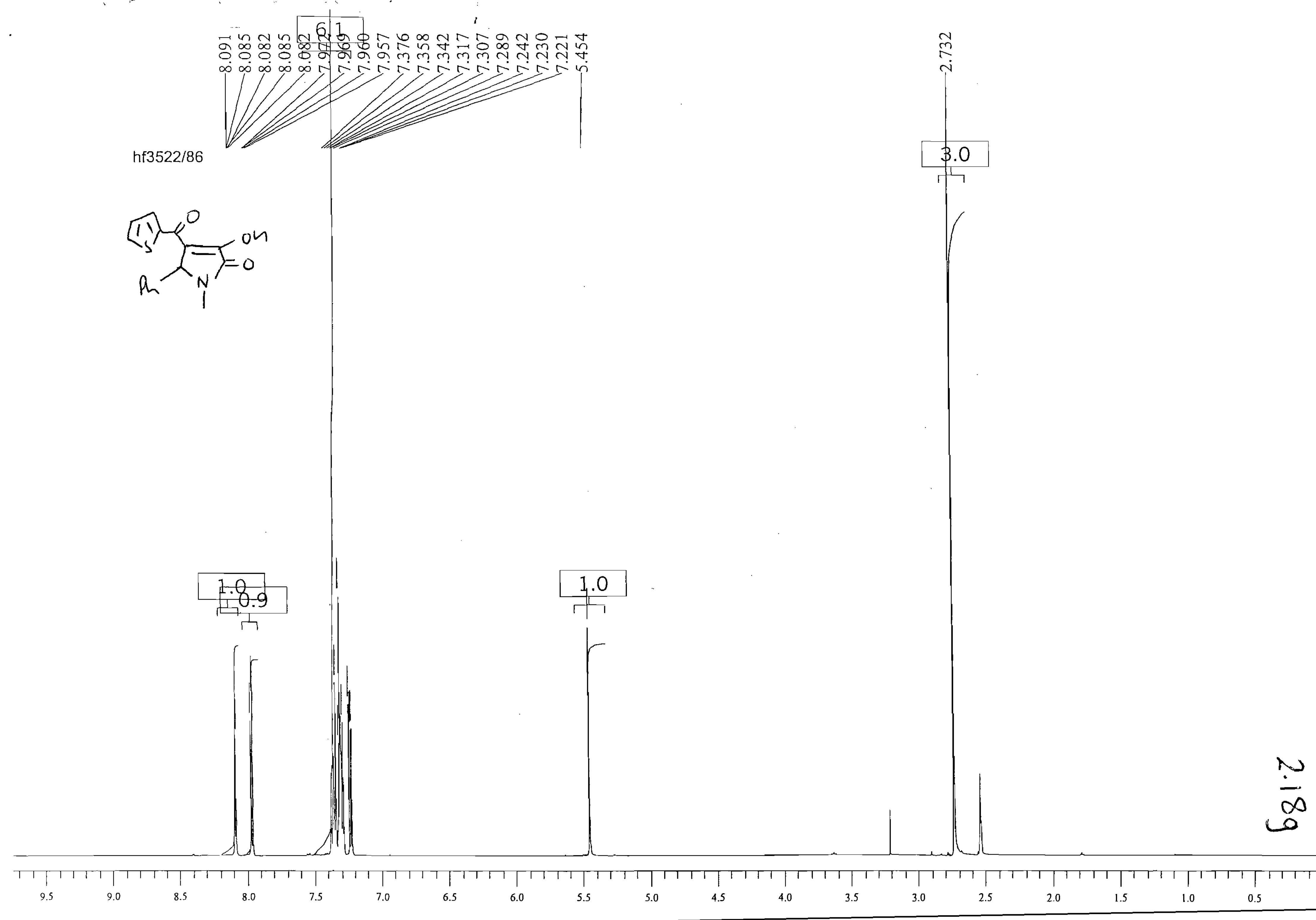
8.0347
8.0322
8.0034
8.0009
7.9908
7.9883
7.3912
7.3736
7.3548
7.3184
7.3159
7.2984
7.2946
7.2770
7.2595
7.2507
7.2482
7.2382
4.9890
4.9501
4.3705
4.3429
4.3317
4.3204
1.6698
1.6585
1.6547
1.6509
1.6434
1.6334
1.3850
1.3674
1.3511
1.3348
1.3185
0.7139
0.6963
0.6625
4.3705
4.3429
4.3317
4.3204
OH
Ph
1.6698
1.6585
1.6547
1.6509
1.6434
1.6334
1.3850
1.3674
1.3511
1.3348
1.3185
Integral
2.1195
2.0968
1.70
1.60
(ppm)
1.0285
4.5
4.4
4.3
4.2
(ppm)
1.35
(ppm)
Integral
0.5307
1.5434
5.8946
1.1000
2.1195
2.0968
1.0285
6.4423
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)
2.18d



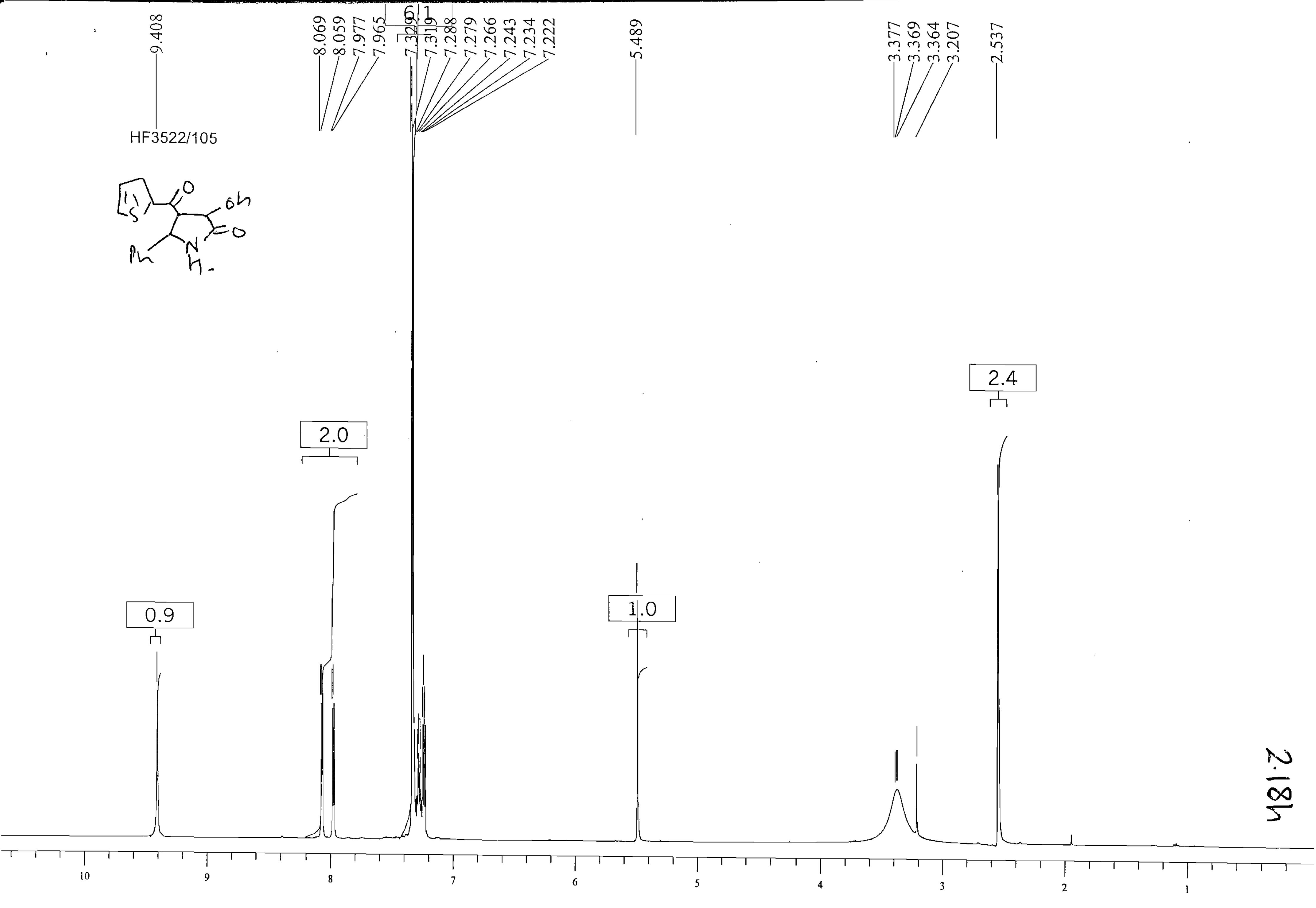
hf3522/87
8.137
8.130
8.127
7.969
7.957
7.395
7.373
7.322
7.303
7.246
7.237
7.225
7.146
7.126
5.296
4.856
4.816
3.850
3.811
2.537
2.533
0.4
1.0
0.9
0.9
1.0
0.9
1.1
0.0
13
12
11
10
9
8
7
6
5
4
3
2
1
2.18e



12.223
12.215
12.212
12.202
12.195
12.183
12.177
12.171
12.157
12.150
12.146
12.139
hf3522/92
8.104
8.096
7.997
7.986
7.666
7.647
7.378
7.359
7.338
7.318
7.245
7.233
7.228
7.209
7.190
7.151
7.132
6.346
2.537
5.6
1.0
0.4
0.0
0.5
0.3
12
11
10
9
8
7
6
5
4
3
2
1
2.18f



hf3522/86
8.091
8.085
8.082
8.085
8.082
7.972
7.969
7.960
7.957
7.376
7.358
7.342
7.317
7.307
7.289
7.242
7.230
7.221
5.454
2.732
6.1
3.0
1.0
0.9
1.0
OH
O
O
N
S
Ph
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.189



HF3522/105
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8.069
8.059
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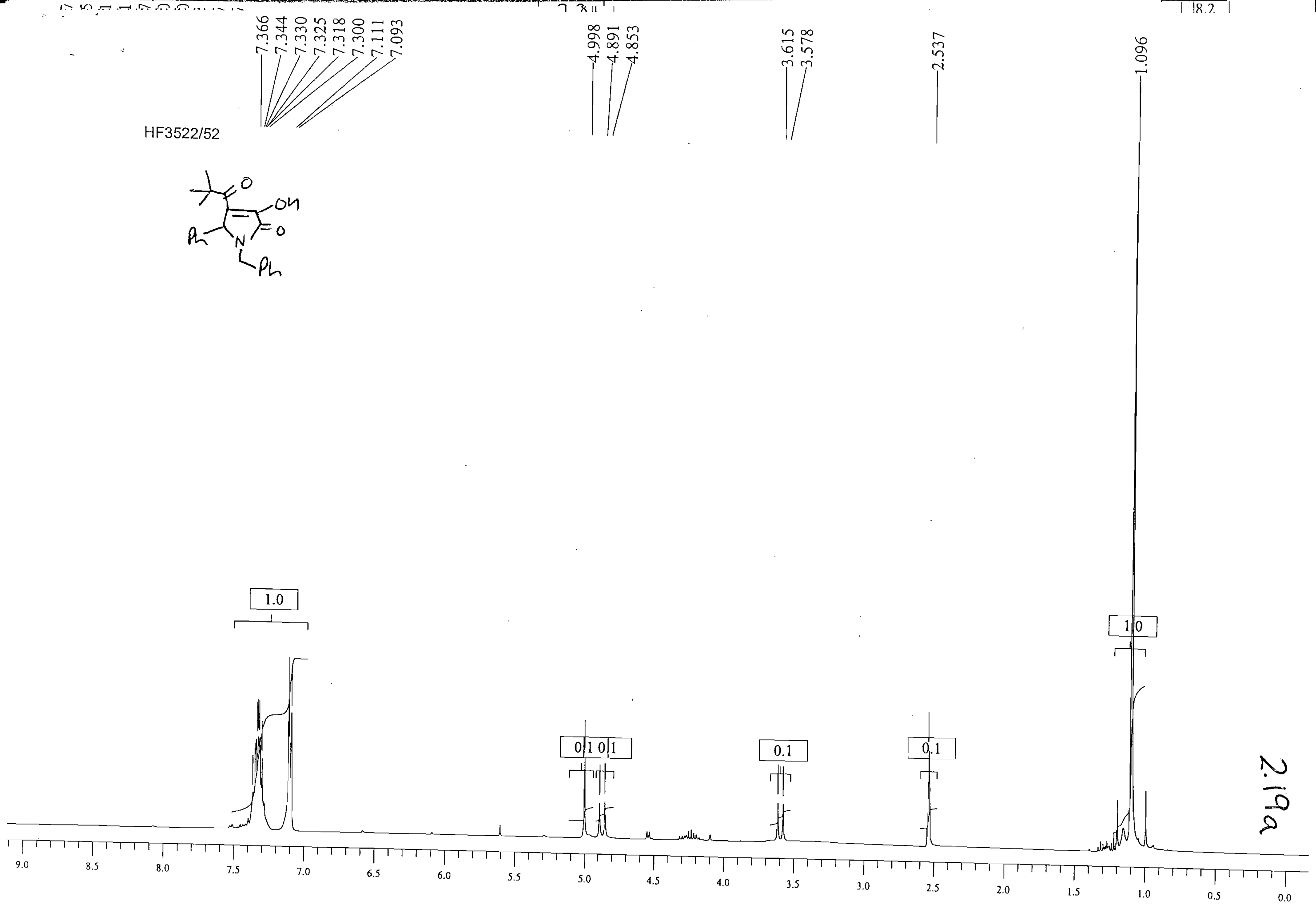
7.965
7.329
7.319
7.288
7.279
7.266
7.243
7.234
7.222
5.489
3.377
3.369
3.364
3.207
2.537
0.9
2.0
6.1
1.0
2.4
10
9
8
7
6
5
4
3
2
1


HF3522/52
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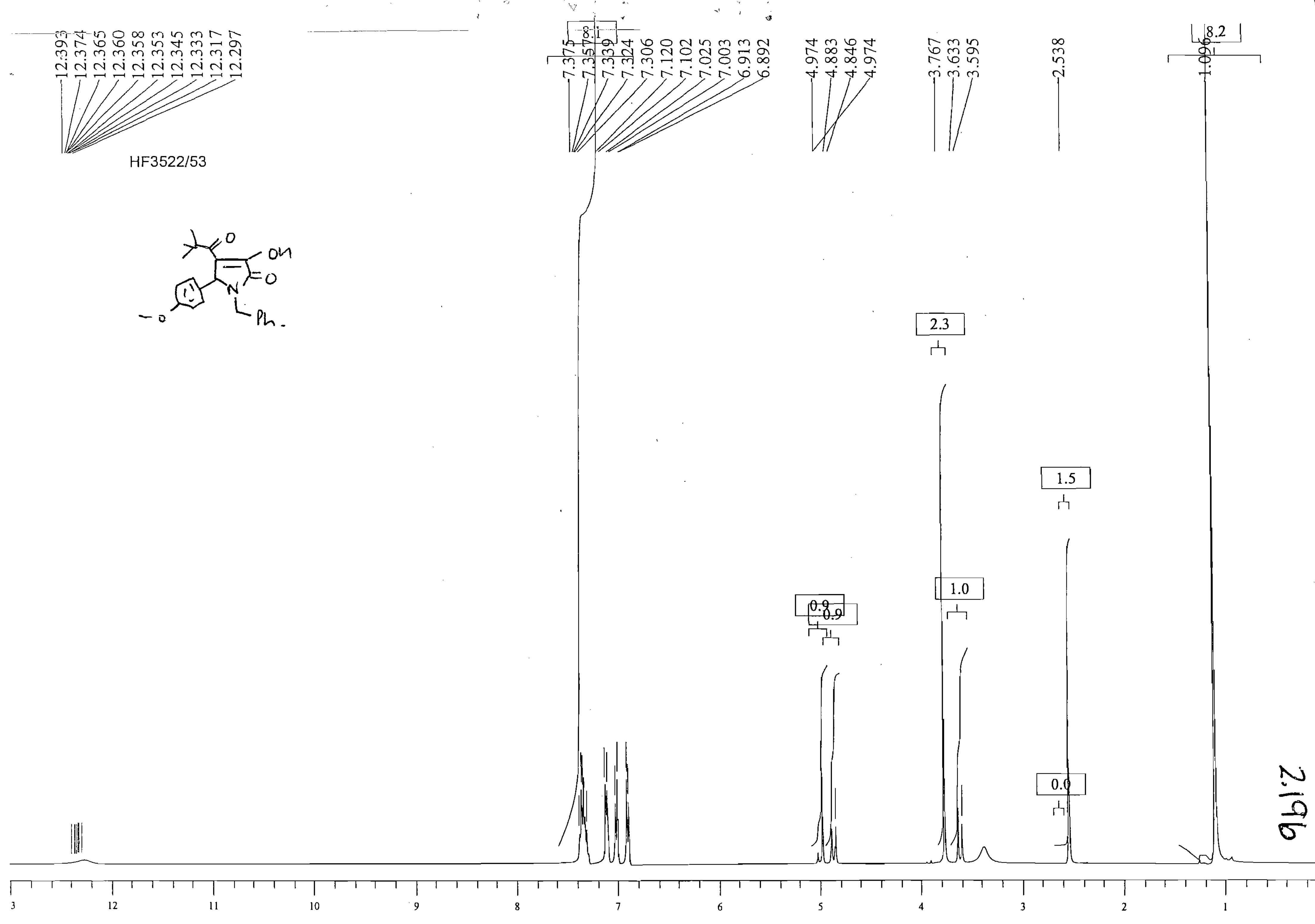
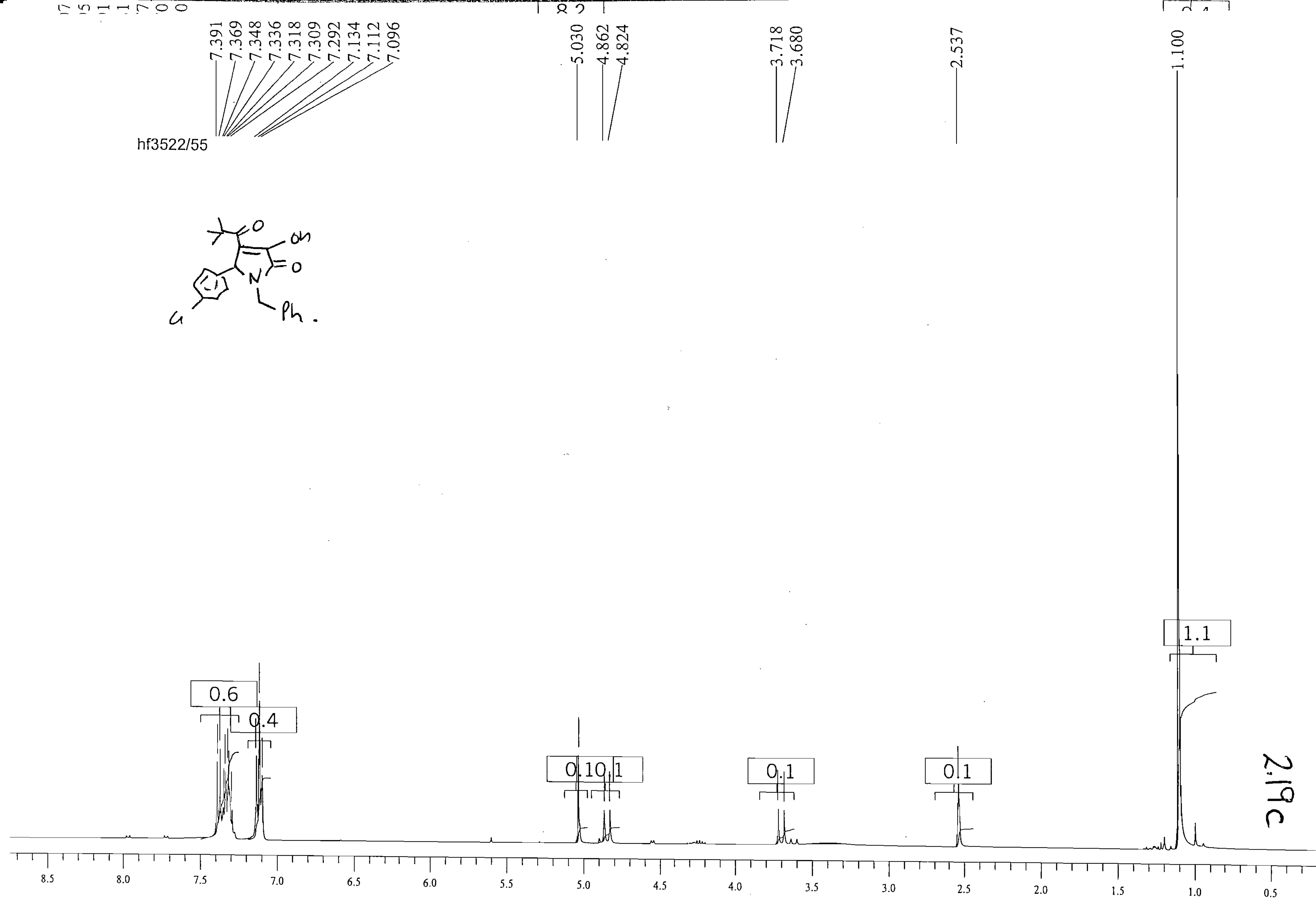
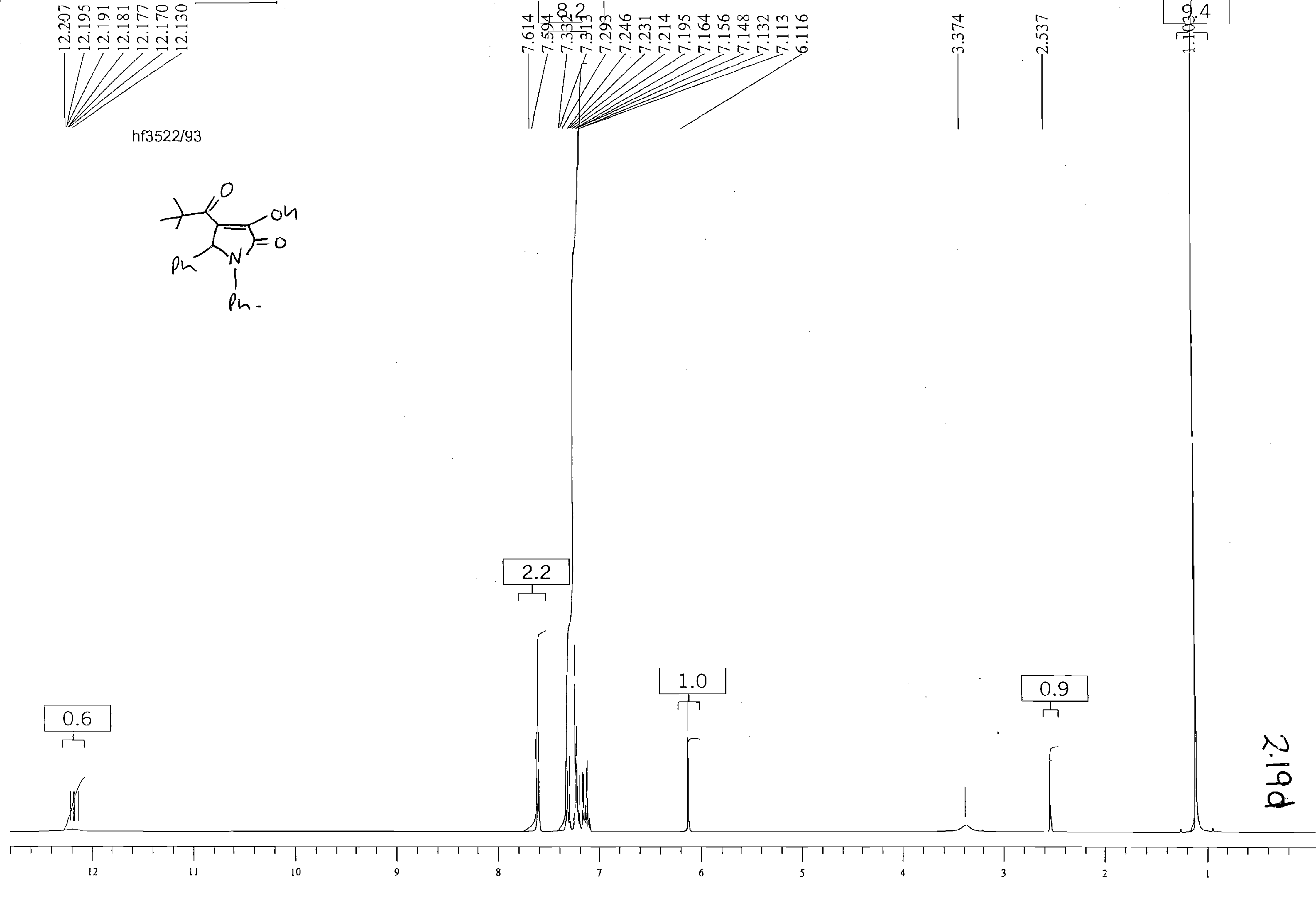
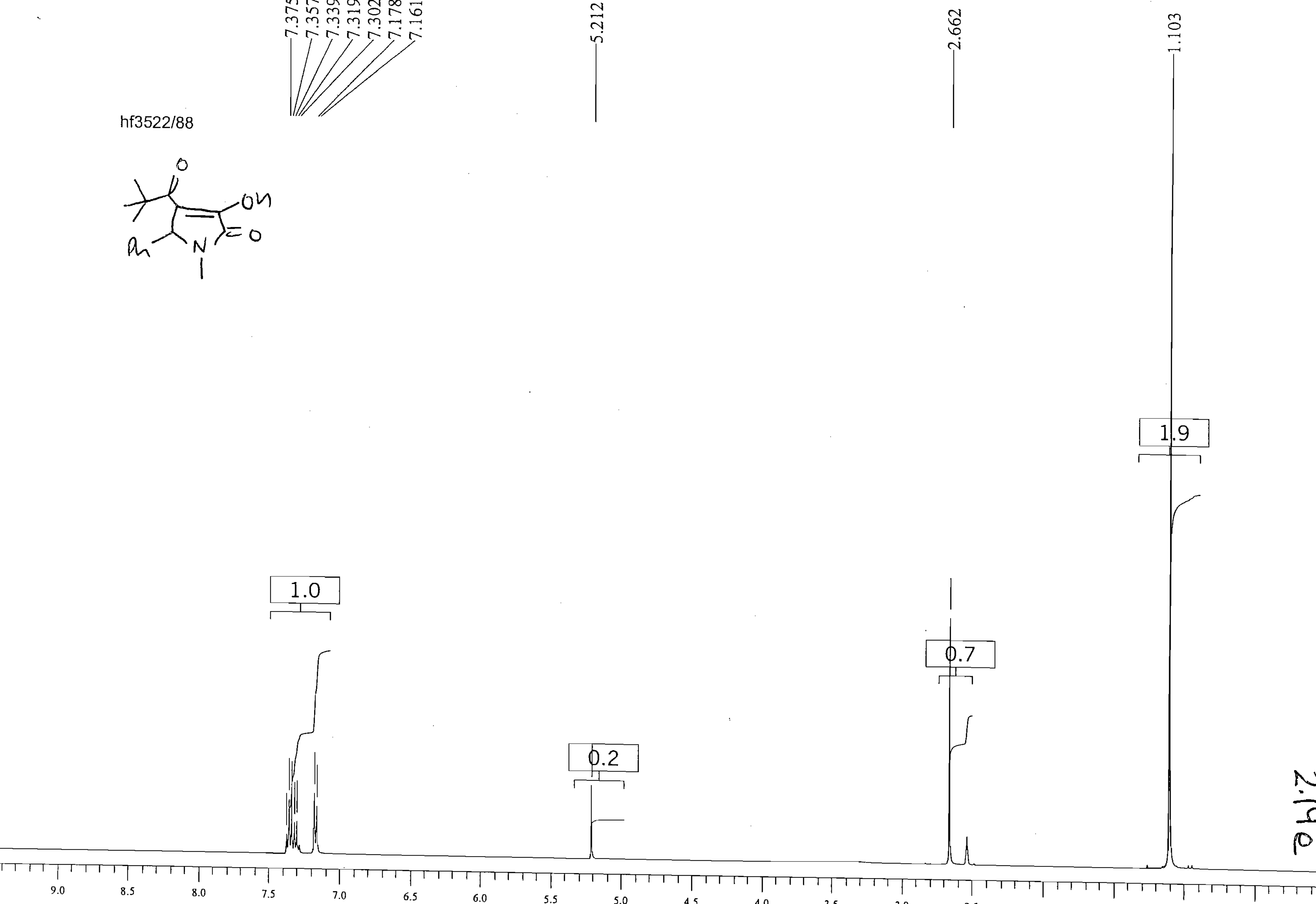
7.344
7.330
7.325
7.318
7.300
7.111
7.093
4.998
4.891
4.853
3.615
3.578
2.537
1.096
1.0
0.1 0.1
0.1
0.1
1.0
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
2.19a


12.393
12.374
12.365
12.360
12.358
12.353
12.345
12.333
12.317
12.297
HF3522/53
7.375
7.357
7.339
7.324
7.306
7.120
7.102
7.025
7.003
6.913
6.892
8.1
4.974
4.883
4.846
4.974
3.767
3.633
3.595
2.538
1.096
8.2
OH
O
N
Ph
2.3
1.5
1.0
0.9
0.9
0.0
3
12
11
10
9
8
7
6
5
4
3
2
1


hf3522/55
7.391
7.369
7.348
7.336
7.318
7.309
7.292
7.134
7.112
7.096
5.030
4.862
4.824
3.718
3.680
2.537
1.100
0.6
0.4
0.1
0.1
0.1
1.1
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
2.19c



hf3522/93
12.207
12.195
12.191
12.181
12.177
12.170
12.130
7.614
7.594
7.332
7.313
7.293
7.246
7.231
7.214
7.195
7.164
7.156
7.148
7.132
7.113
6.116
3.374
2.537
1.103
0.6
2.2
8.2
1.0
0.9
9.4
12
11
10
9
8
7
6
5
4
3
2
1



7.375
7.357
7.339
7.319
7.302
7.178
7.161
5.212
2.662
1.103
hf3522/88
1.0
0.2
0.7
1.9
2.19e
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

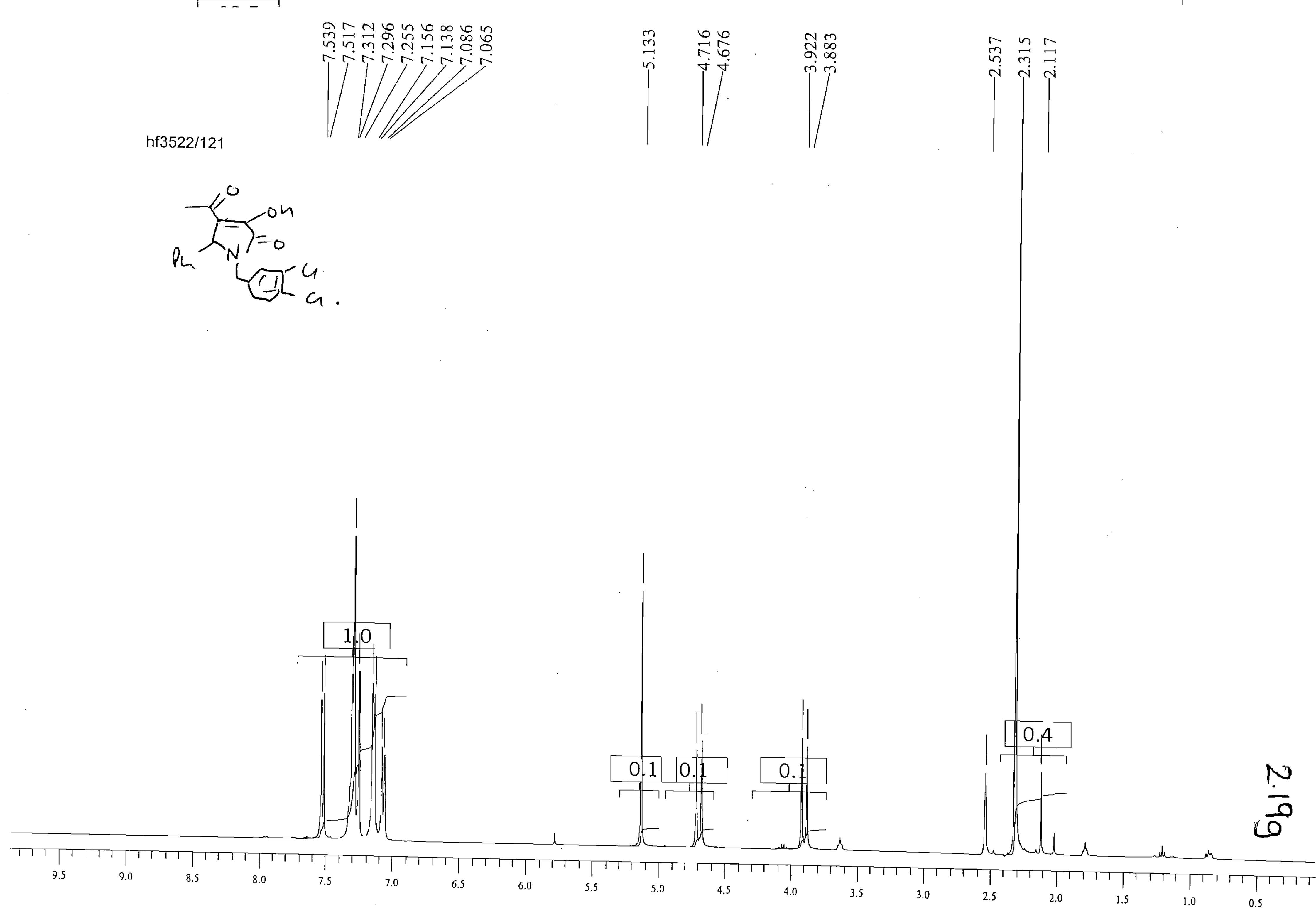
HF3522.106

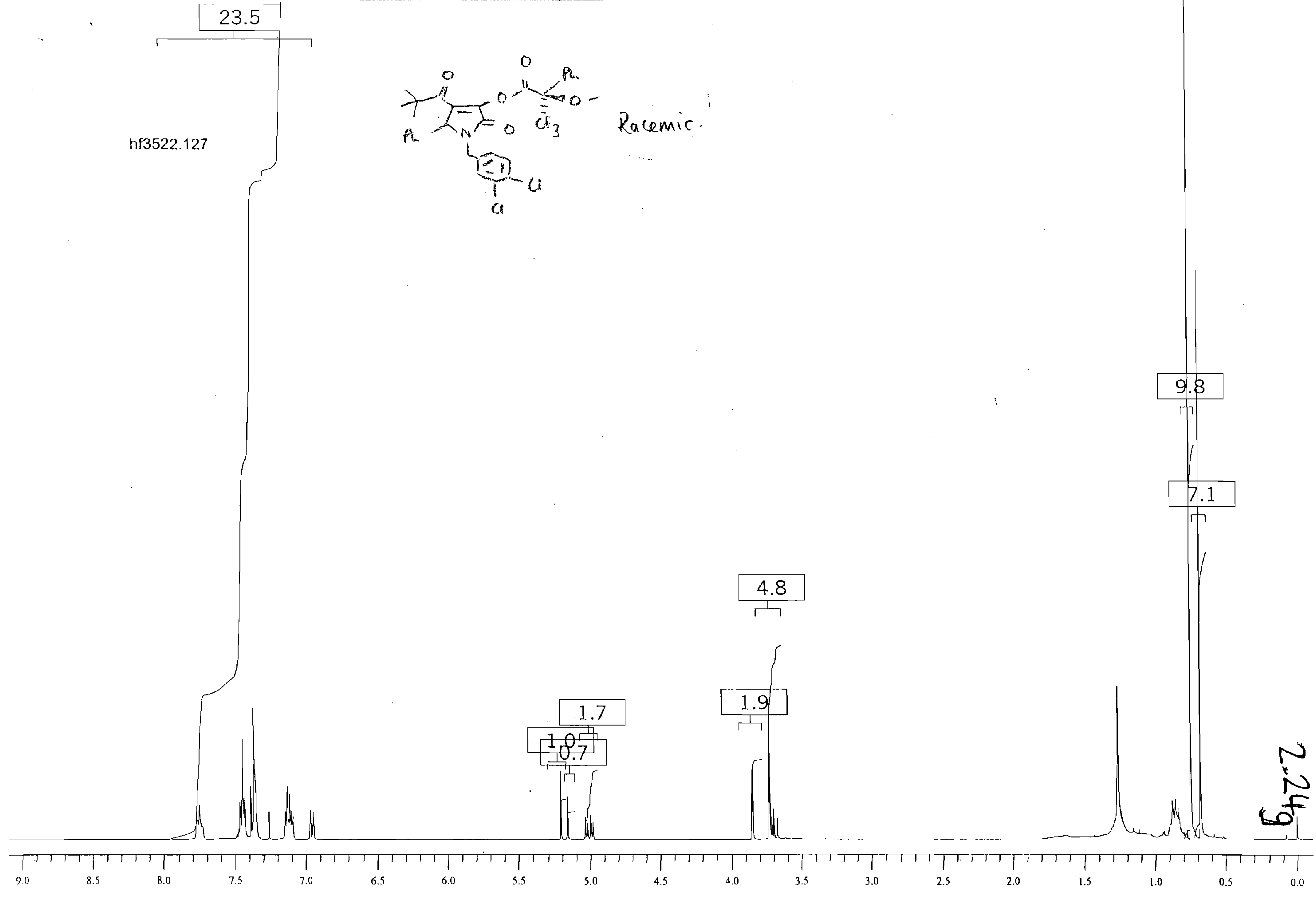
2.19f

11.871
11.867
9.287
7.327
7.308
7.285
7.268
7.209
7.189
5.251
3.366
2.536
1.105

1.0
5.2
1.0
0.8
0.8
9.5

12 11 10 9 8 7 6 5 4 3 2 1


hf3522/121
7.539
7.517
7.312
7.296
7.255
7.156
7.138
7.086
7.065
5.133
4.716
4.676
3.922
3.883
2.537
2.315
2.117
1.0
0.1
0.1
0.1
0.4
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
2.19g

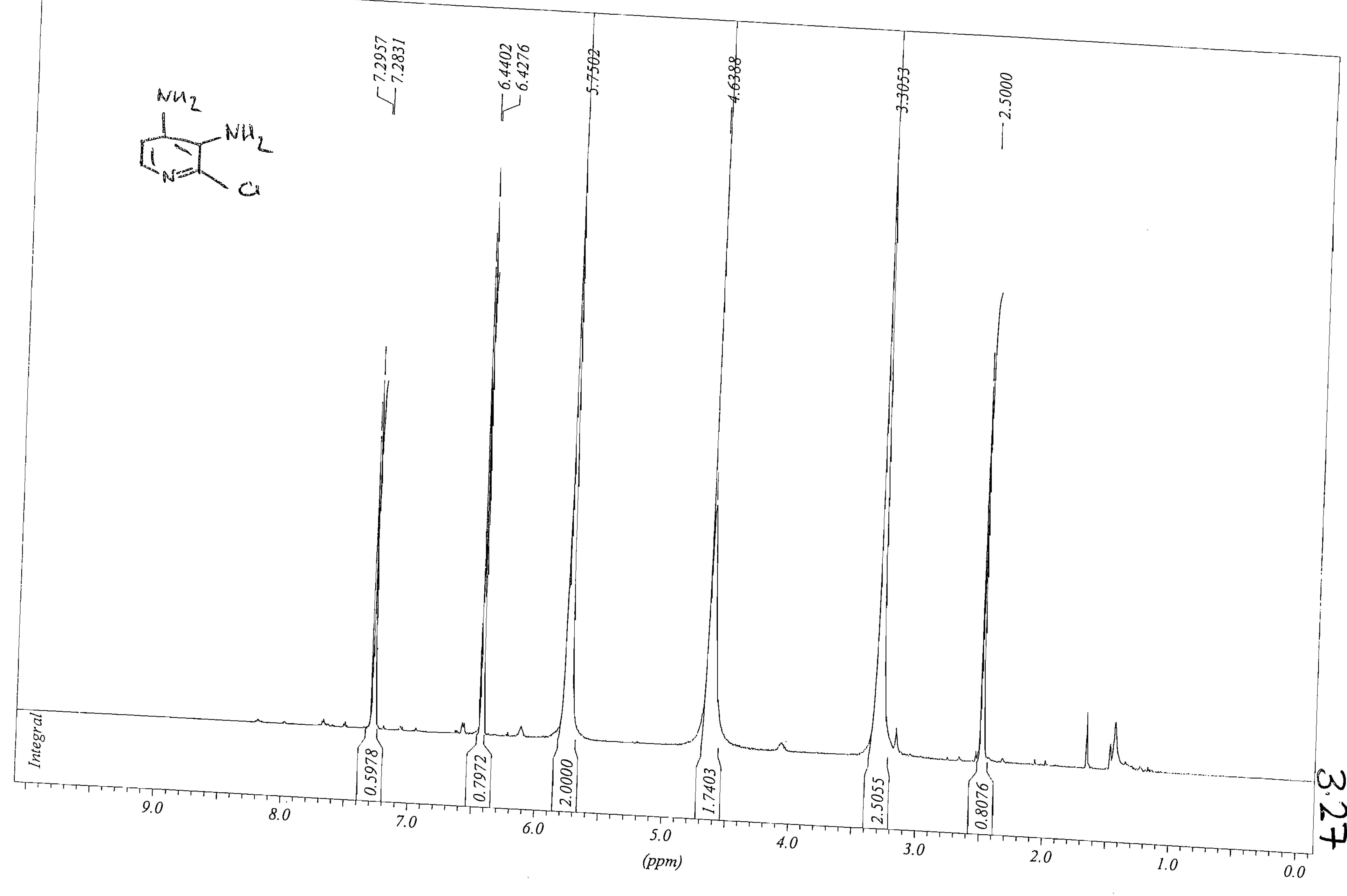


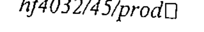
23.5
hf3522.127
Racemic
9.8
7.1
4.8
1.7
1.0
0.7
1.9
2.24g
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

hf 4148/02/2nd

3.26

hf4032/28





11.3726

7.7974
7.7849

7.1326
7.1201

6.7550

3.1686

2.4987
2.4950

HN NH2 N N Cl

Integral

0.6457 0.7995

8.0 7.8 7.6 7.4 7.2

(ppm)

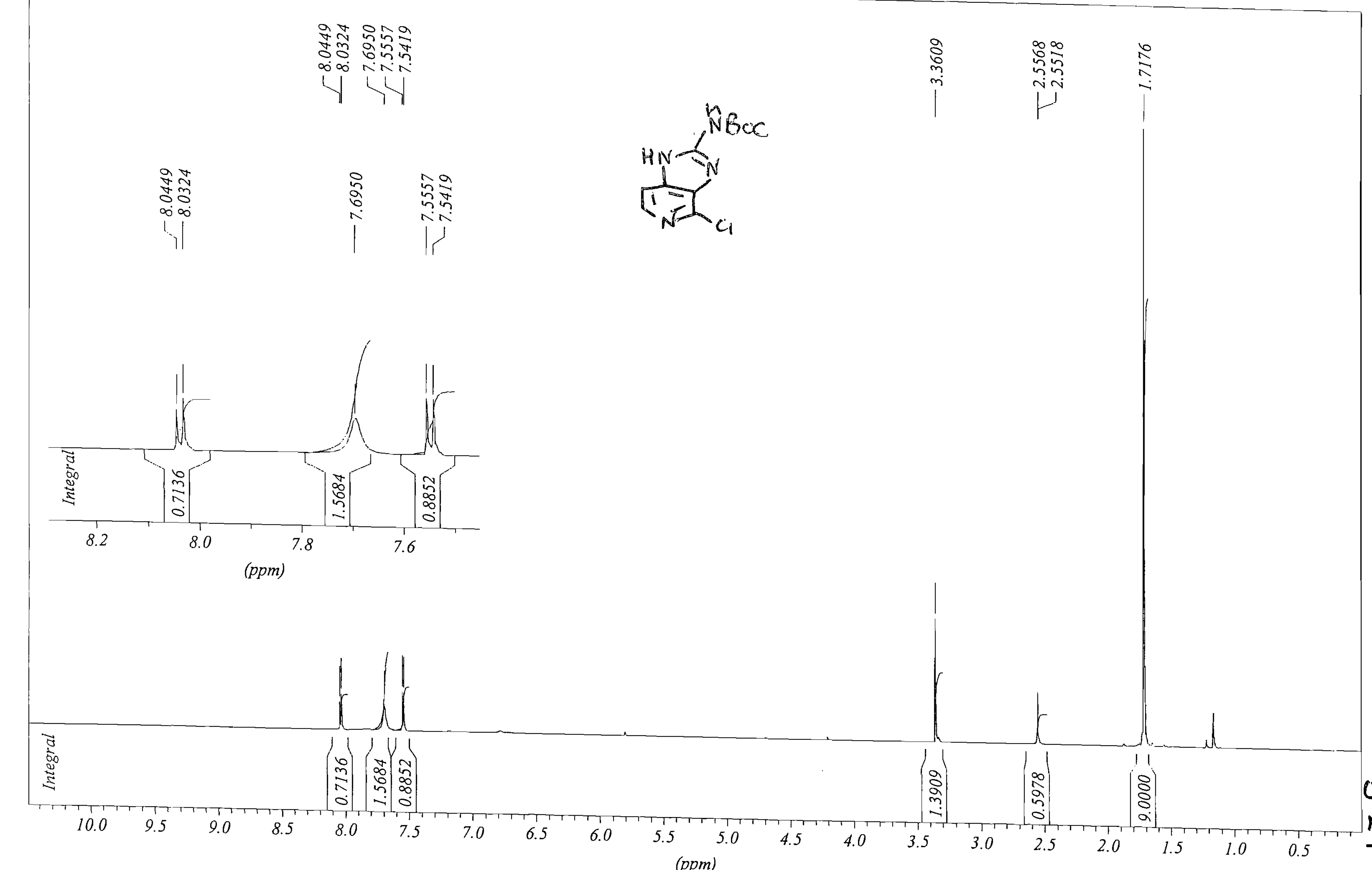
Integral

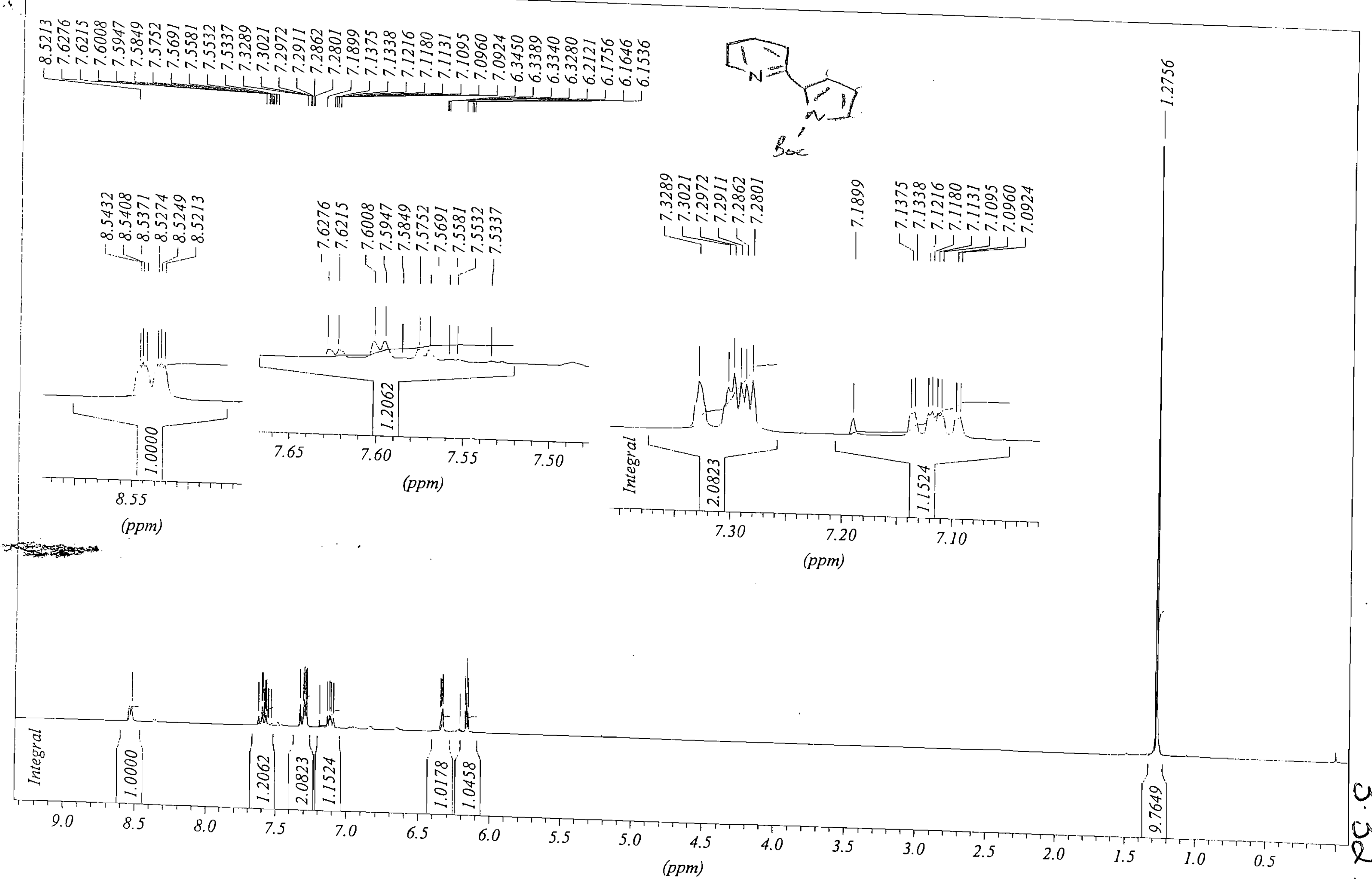
0.3907 0.6457 0.7995 2.0000 1.4458 0.0039 0.5110

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

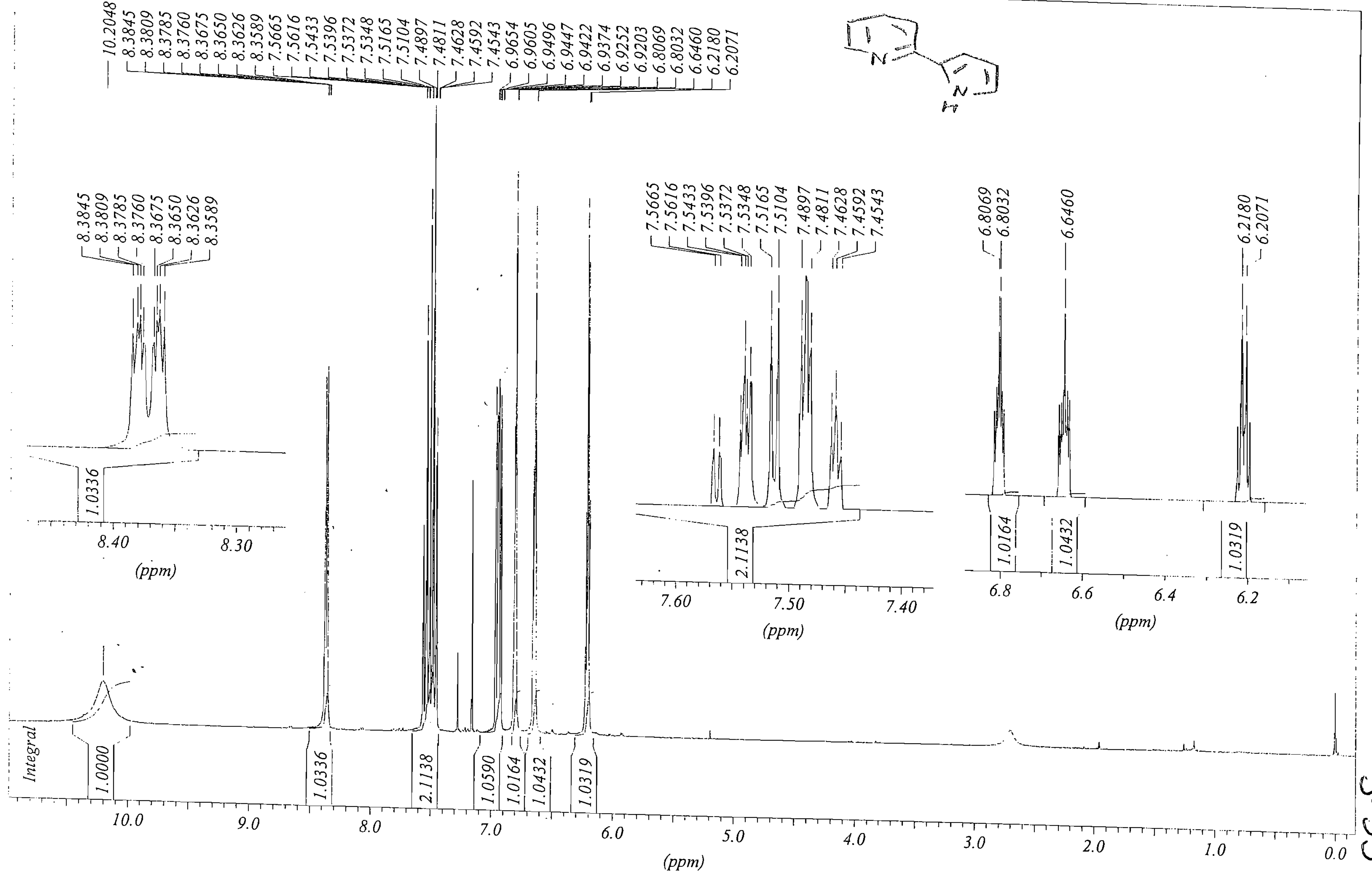
(ppm)

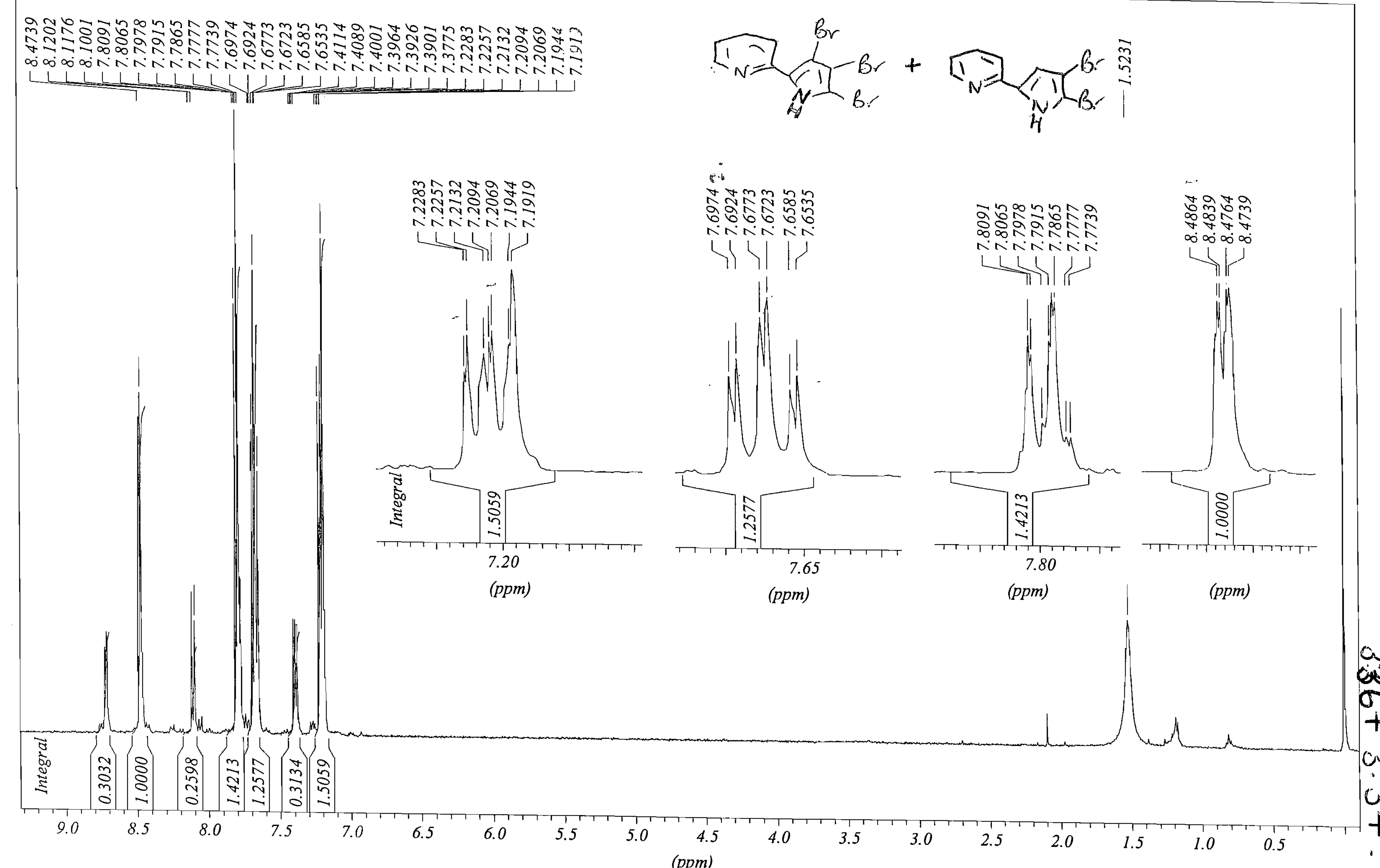
3.28


hf4032/44/f1
8.0449
8.0324
7.6950
7.5557
7.5419
3.3609
2.5568
2.5518
1.7176
HN
N
NBoc
N
Cl
Integral
0.7136
1.5684
0.8852
1.3909
0.5978
9.0000
(ppm)
3.29



hf4032/95/f4
8.5213
7.6276
7.6215
7.6008
7.5947
7.5849
7.5752
7.5691
7.5581
7.5532
7.5337
7.3289
7.3021
7.2972
7.2911
7.2862
7.2801
7.1899
7.1375
7.1338
7.1216
7.1180
7.1131
7.1095
7.0960
7.0924
6.3450
6.3389
6.3340
6.3280
6.2121
6.1756
6.1646
6.1536
1.2756
8.5432
8.5408
8.5371
8.5274
8.5249
8.5213
1.0000
8.55
(ppm)
7.6276
7.6215
7.6008
7.5947
7.5849
7.5752
7.5691
7.5581
7.5532
7.5337
1.2062
7.65
7.60
7.55
7.50
(ppm)
7.3289
7.3021
7.2972
7.2911
7.2862
7.2801
7.1899
7.1375
7.1338
7.1216
7.1180
7.1131
7.1095
7.0960
7.0924
Integral
2.0823
1.1524
7.30
7.20
7.10
(ppm)
Integral
1.0000
1.2062
2.0823
1.1524
1.0178
1.0458
9.7649
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)
Boc
N
3.32.


hf4032/95/f3
10.2048
8.3845
8.3809
8.3785
8.3760
8.3675
8.3650
8.3626
8.3589
7.5665
7.5616
7.5433
7.5396
7.5372
7.5348
7.5165
7.5104
7.4897
7.4811
7.4628
7.4592
7.4543
6.9654
6.9605
6.9496
6.9447
6.9422
6.9374
6.9252
6.9203
6.8069
6.8032
6.6460
6.2180
6.2071
1.0336
2.1138
1.0164
1.0432
1.0319
Integral
1.0000
1.0590
(ppm)
3.35


hf4032/100
8.4739
8.1202
8.1176
8.1001
7.8091
7.8065
7.7978
7.7915
7.7865
7.7777
7.7739
7.6974
7.6924
7.6773
7.6723
7.6585
7.6535
7.4114
7.4089
7.4001
7.3964
7.3926
7.3901
7.3775
7.2283
7.2257
7.2132
7.2094
7.2069
7.1944
1.5231
Integral
1.5059
7.20
(ppm)
1.2577
7.65
1.4213
7.80
1.0000
8.4864
8.4839
8.4764
0.3032
0.2598
0.3134
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

hf4148/20/f2

HNBoc

NH_2

N

Cl

8.1901

7.6896

7.6758

6.5869

6.5731

6.1178

3.3179

2.5000

1.4400

Integral

0.0806

0.1064

7.7

7.6

6.60

(ppm)

(ppm)

Integral

0.0719

0.0806

0.1064

0.2078

0.1440

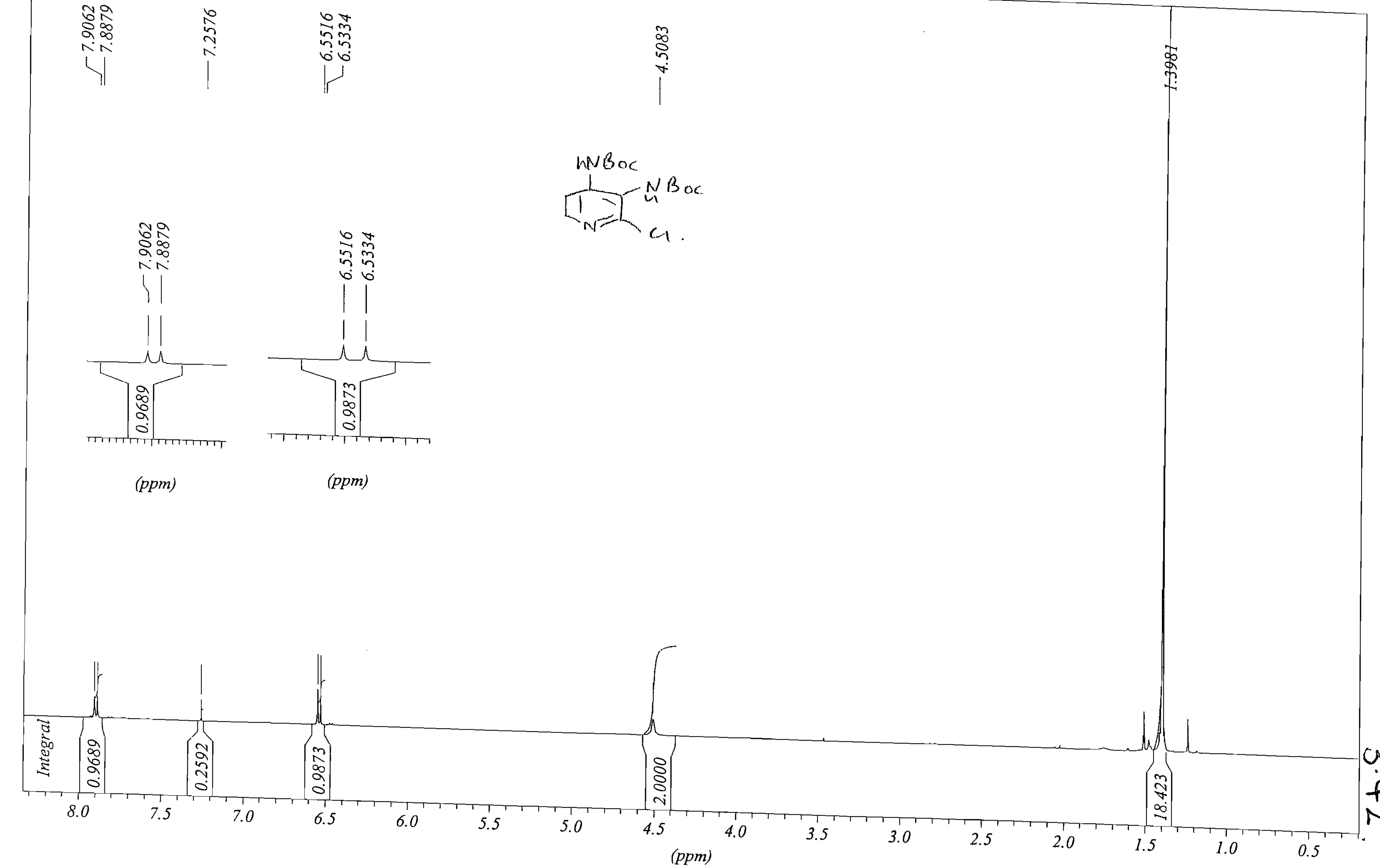
0.0352

1.0000

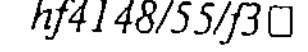
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

(ppm)

3.41



hf4148/22/f3 - 300 MHz.
7.9062
7.8879
7.2576
6.5516
6.5334
4.5083
1.3981
NNBoc
NBoc
Cl.
0.9689
0.9873
(ppm)
Integral
0.2592
2.0000
18.423
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
3.42



hf4148/55/f3

9.5298
9.4546
8.1838
8.1700
8.1487
8.1349

NHAc
NHAc
H
N
Cl

3.3694
2.5076
2.5038
2.1701
2.0936

8.1838
8.1700
8.1487
8.1349
5.5
5.5
Integral
1.5399
8.20 8.10
(ppm)

Integral
1.0000
0.9530
1.5399
4.7918
2.6833
3.1485
3.4489

10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
(ppm)

3.43

Integral

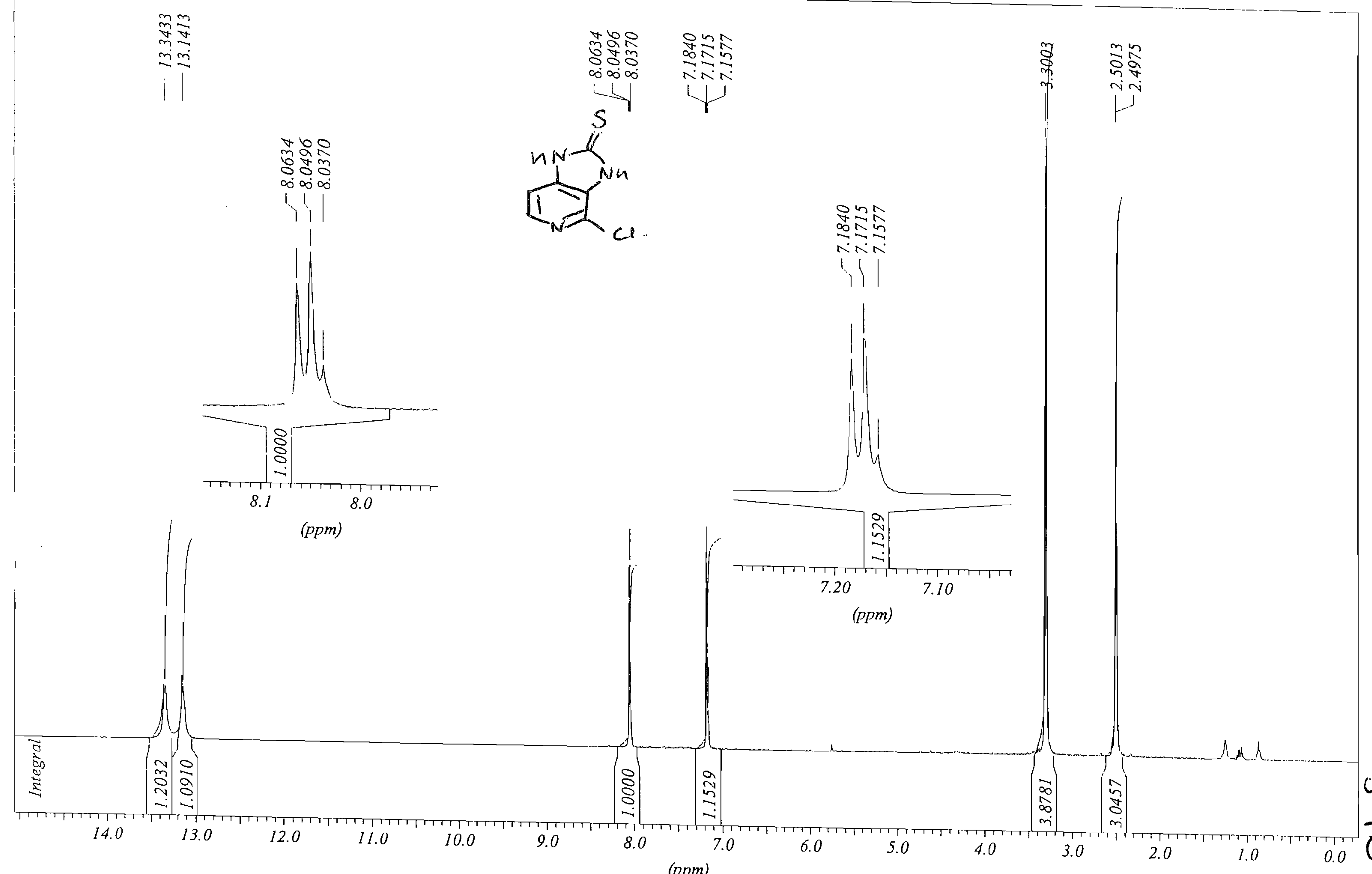
2.3645
1.0000
1.0948
4.0361
2.9833

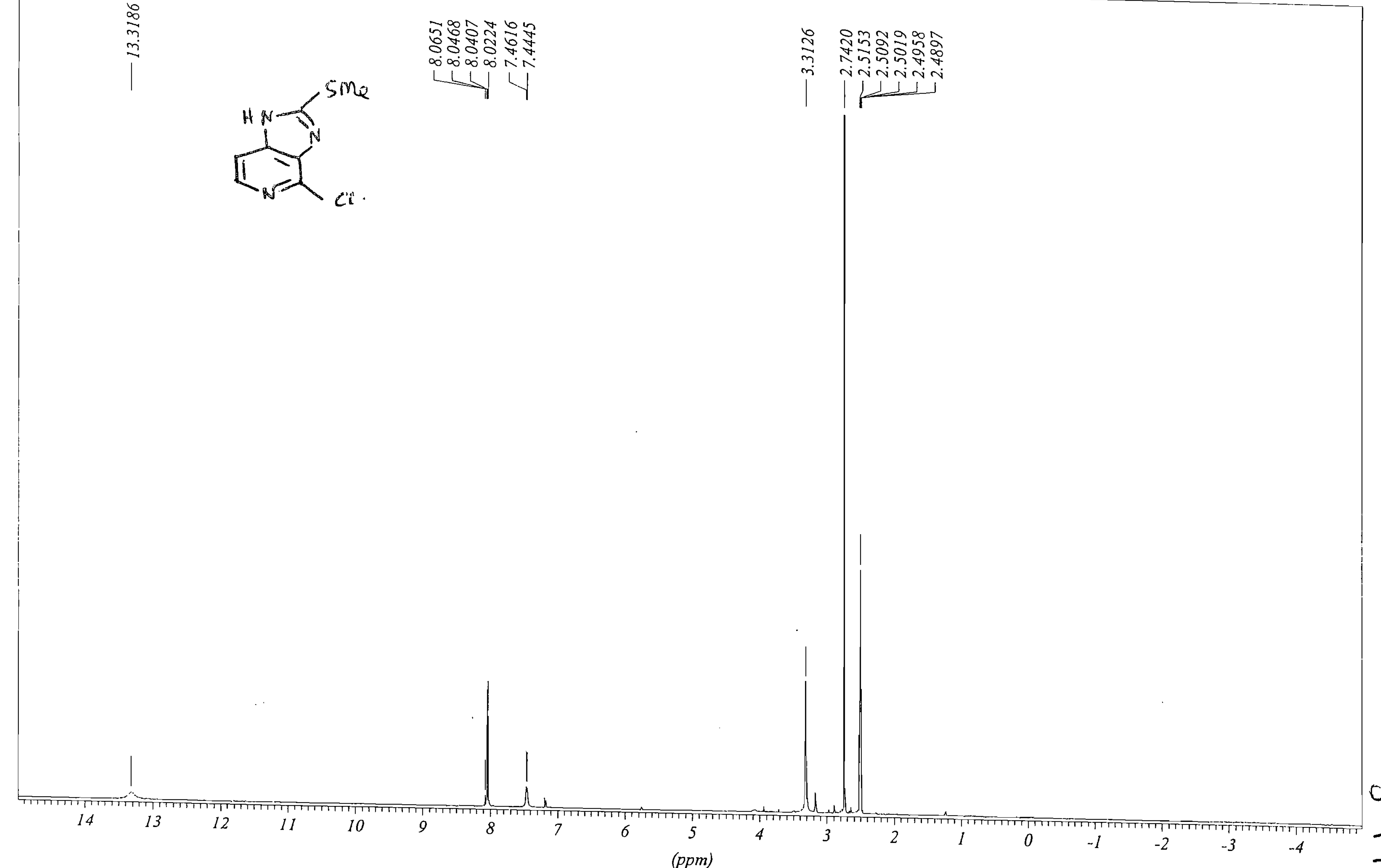
(ppm)
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0

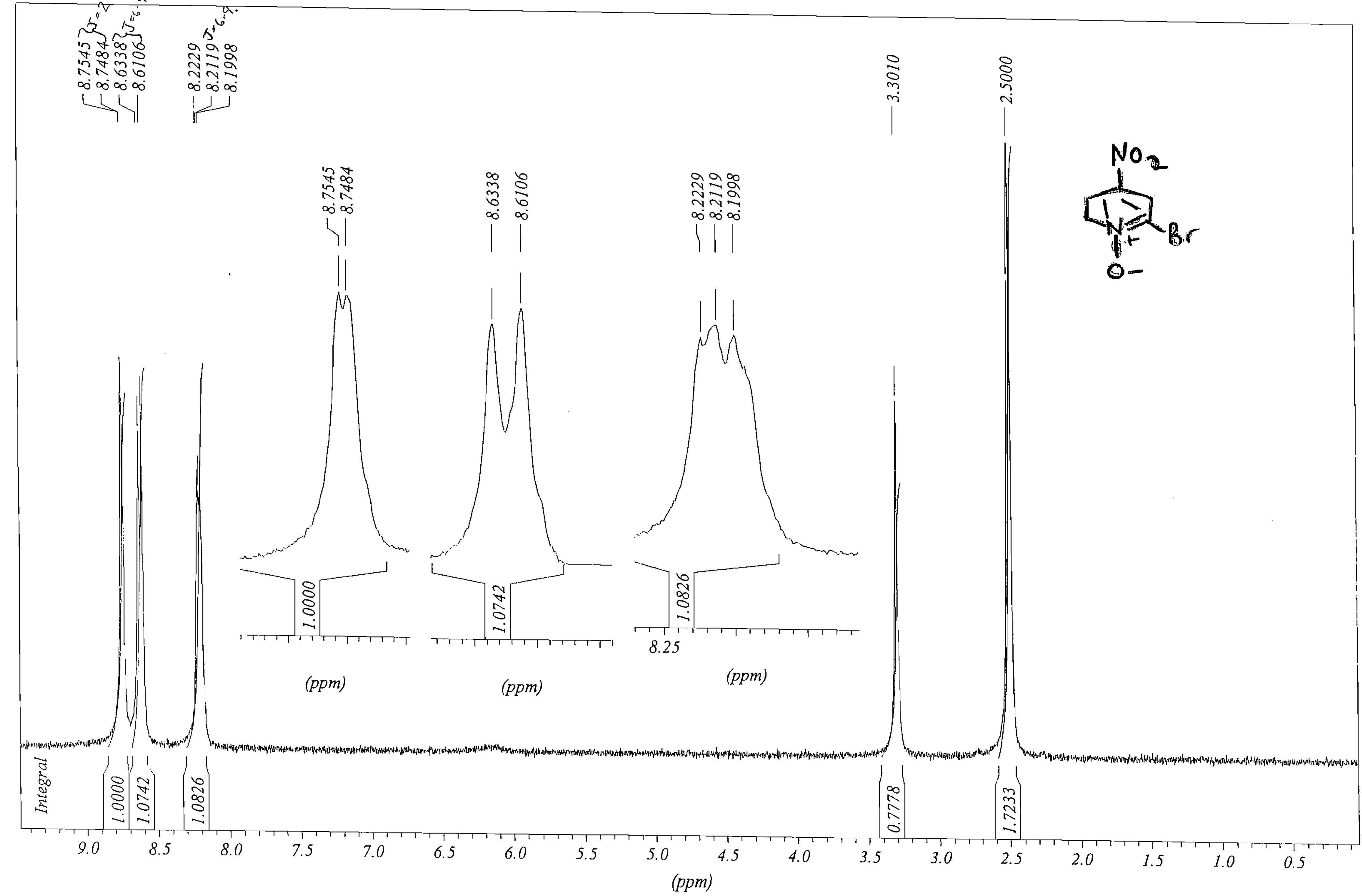
8.00 7.95 7.90 7.85 (ppm)
1.0000
7.9304
7.9179
7.9116

11.3864
7.9304
7.9179
7.9116
7.0134
7.0009
3.2966
2.5088
2.5038
2.4987
2.4950
2.4900

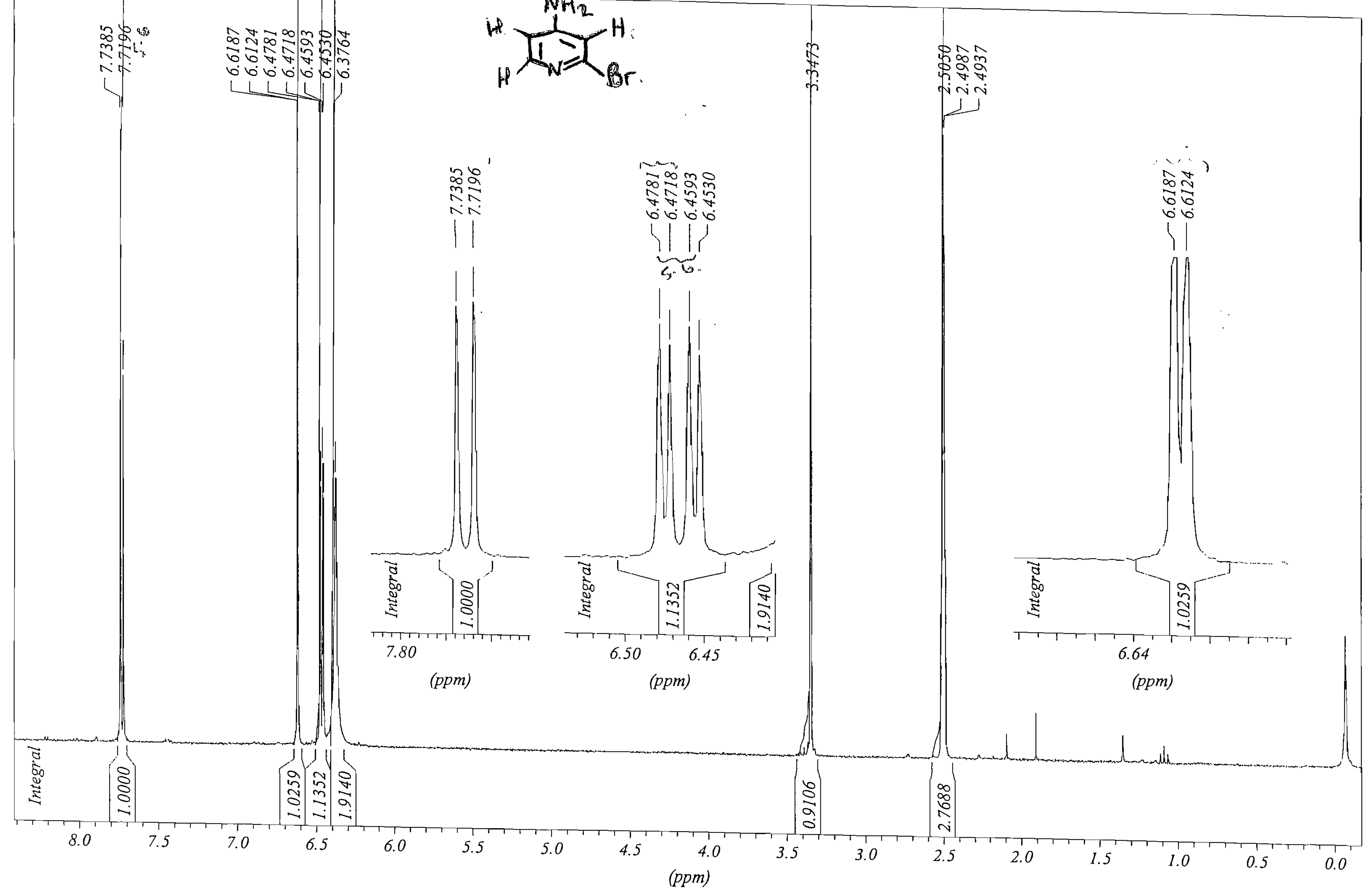
3.44


hf4032/11/sol
13.3433
13.1413
8.0634
8.0496
8.0370
7.1840
7.1715
7.1577
3.3003
2.5013
2.4975
S
NH
NH
N
Cl
1.0000
8.1
8.0
(ppm)
1.1529
7.20
7.10
(ppm)
Integral
1.2032
1.0910
1.0000
1.1529
3.8781
3.0457
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
0.0
(ppm)
3.46

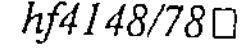

hf4032/72/f1
13.3186
8.0651
8.0468
8.0407
8.0224
7.4616
7.4445
3.3126
2.7420
2.5153
2.5092
2.5019
2.4958
2.4897
HN
N
SMe
N
Cl
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
(ppm)
3.47.



3.55



hf4148/69
7.7385
7.7196
6.6187
6.6124
6.4781
6.4718
6.4593
6.4530
6.3764
NH2
H
N
Br
3.3473
2.5050
2.4987
2.4937
Integral
1.0000
1.1352
1.9140
1.0259
0.9106
2.7688
7.80
6.50
6.45
6.64
(ppm)
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
3.56



hf4148/78

3.53

hf4286/bromonitro/isomer 400MHz

3.58

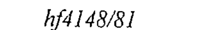
8.7621
8.0283
7.1100
3.3053
2.5000
2.4962

NH_2
O_2N
N
Br

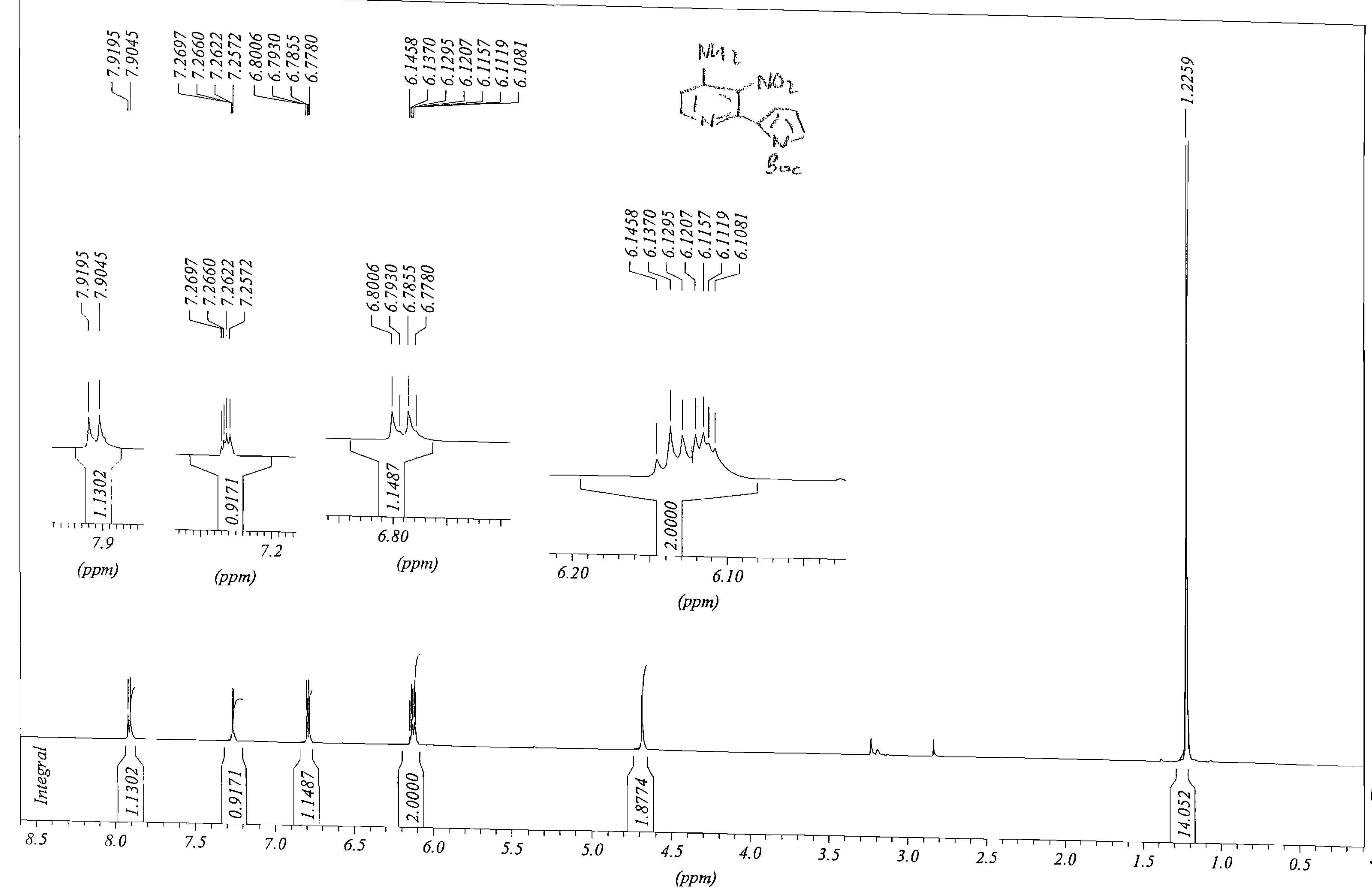
Integral
0.3127
2.0000
0.6863
0.3065
0.2612

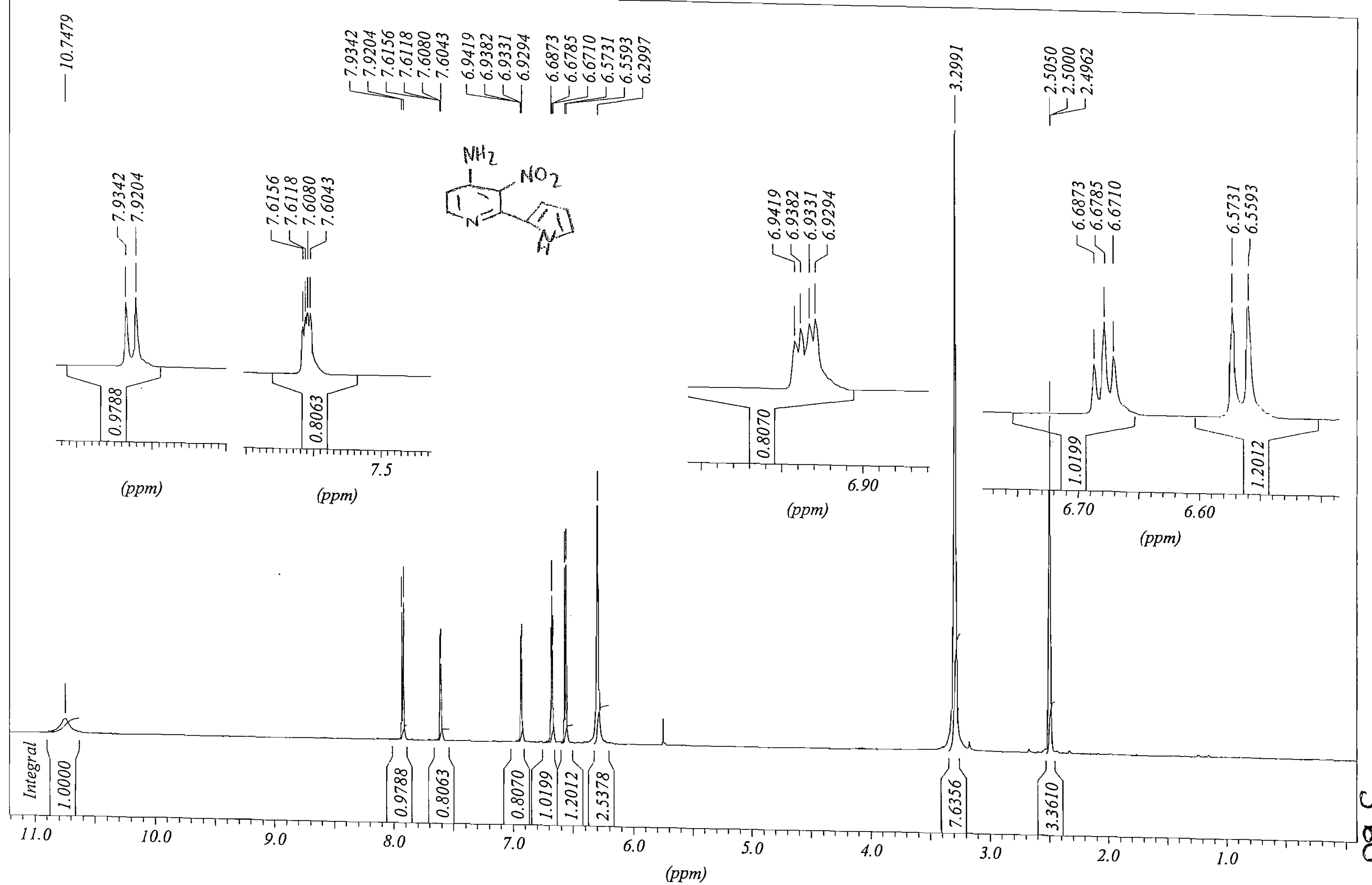
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

(ppm)

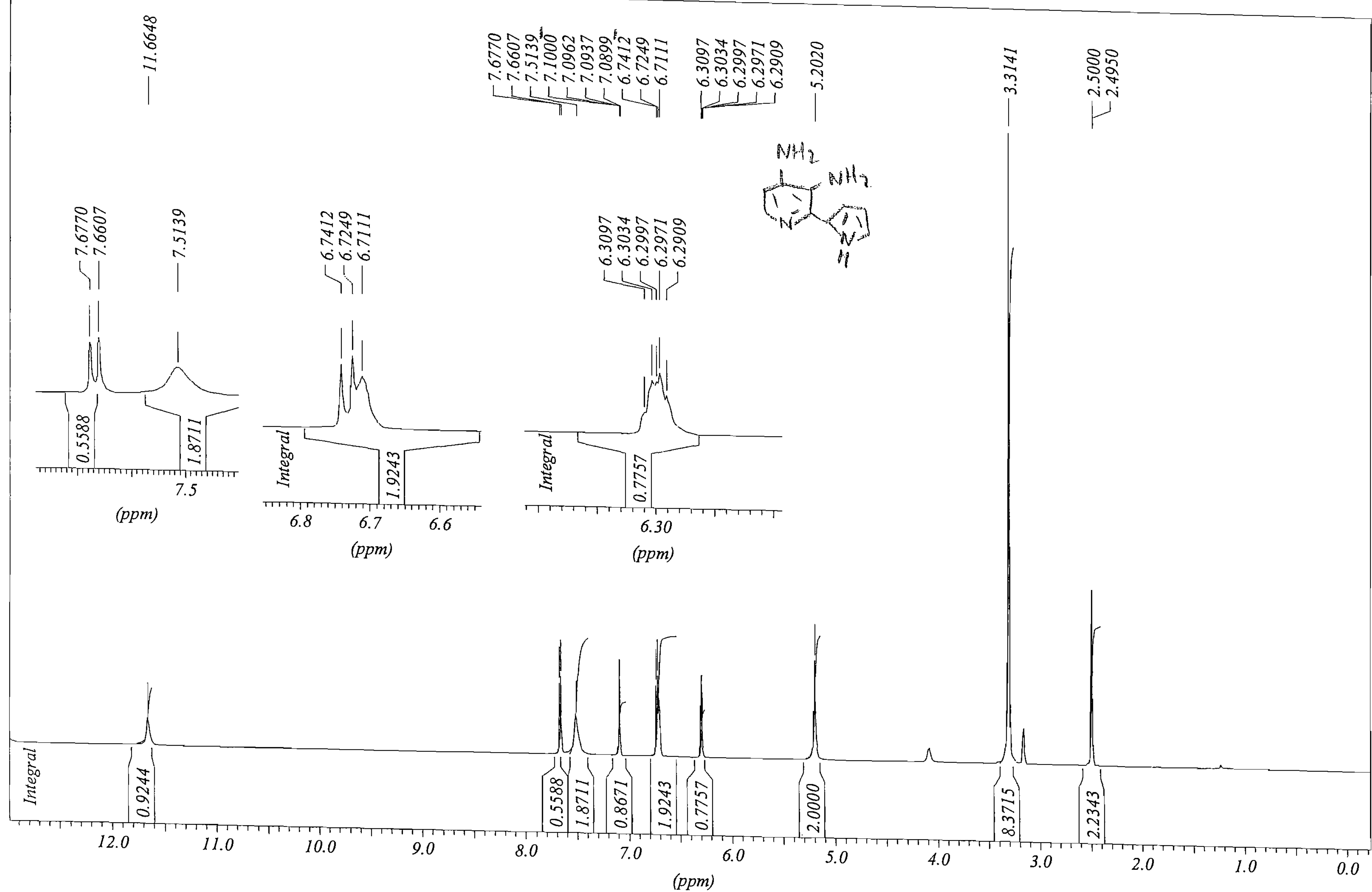


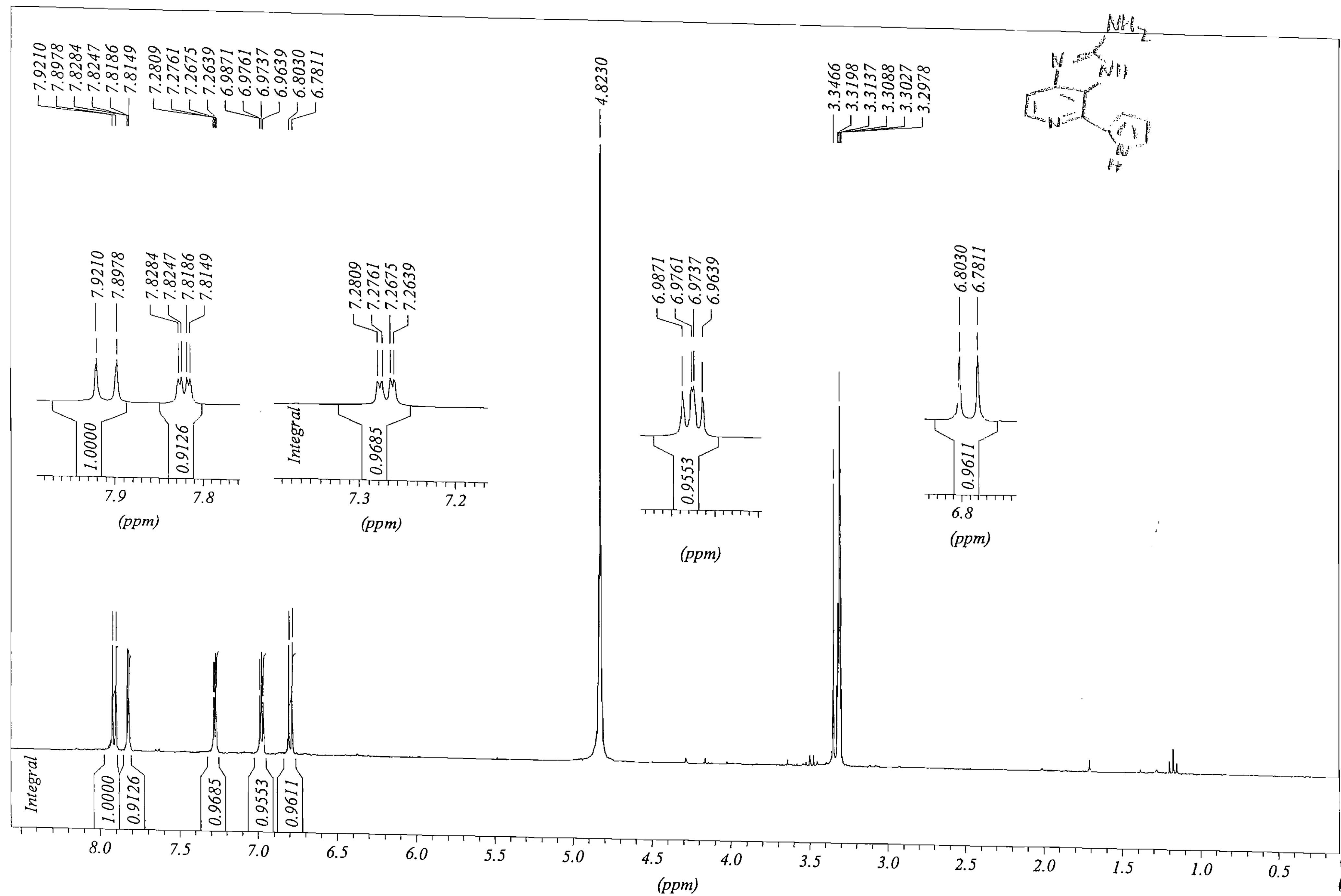
3.48

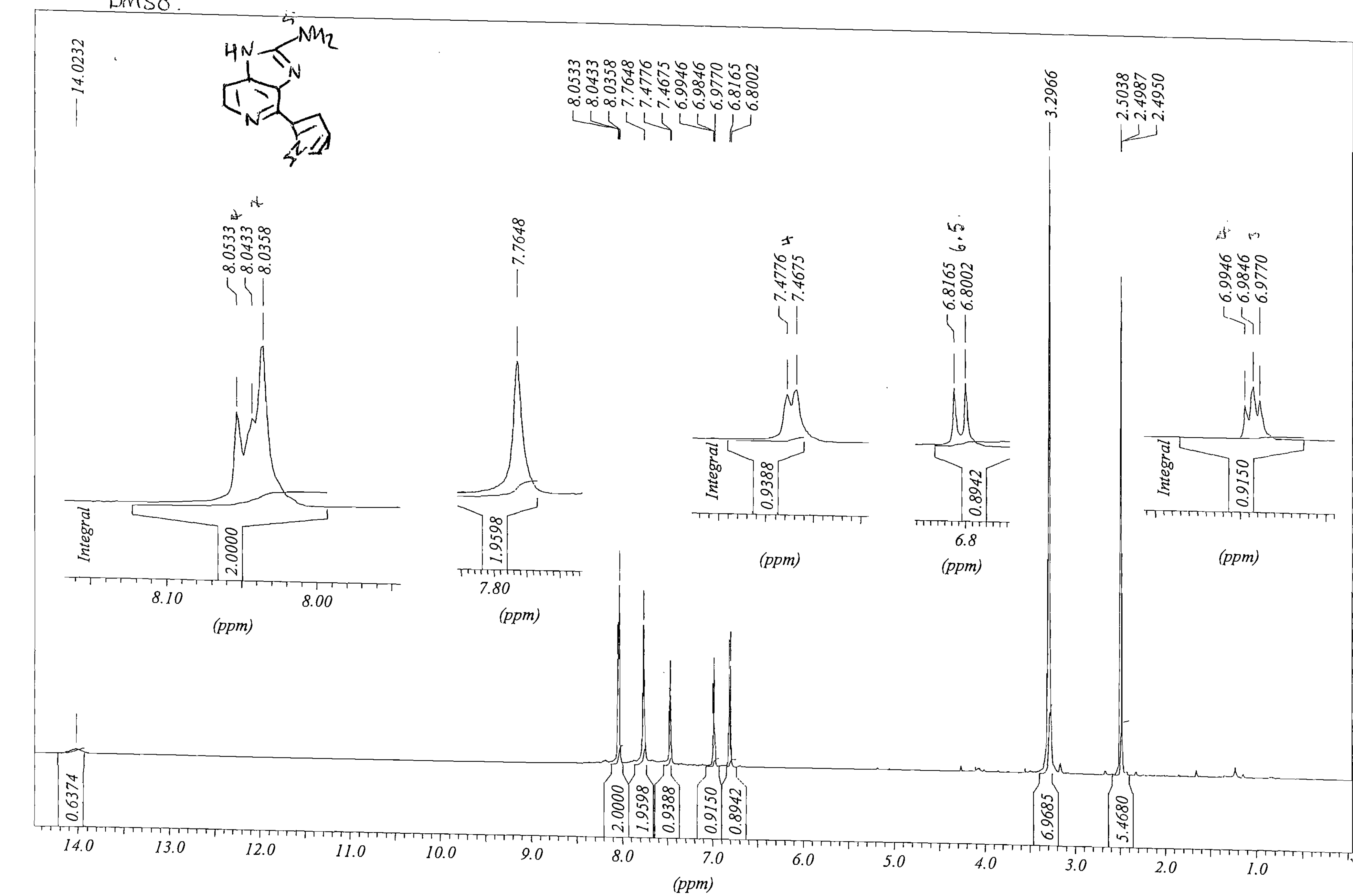

hf4148/46/f2a
400MHz
7.9195
7.9045
7.2697
7.2660
7.2622
7.2572
6.8006
6.7930
6.7855
6.7780
6.1458
6.1370
6.1295
6.1207
6.1157
6.1119
6.1081
1.2259
NH2
NO2
N
Boc
1.1302
0.9171
1.1487
2.0000
1.8774
14.052
Integral
(ppm)
3.59

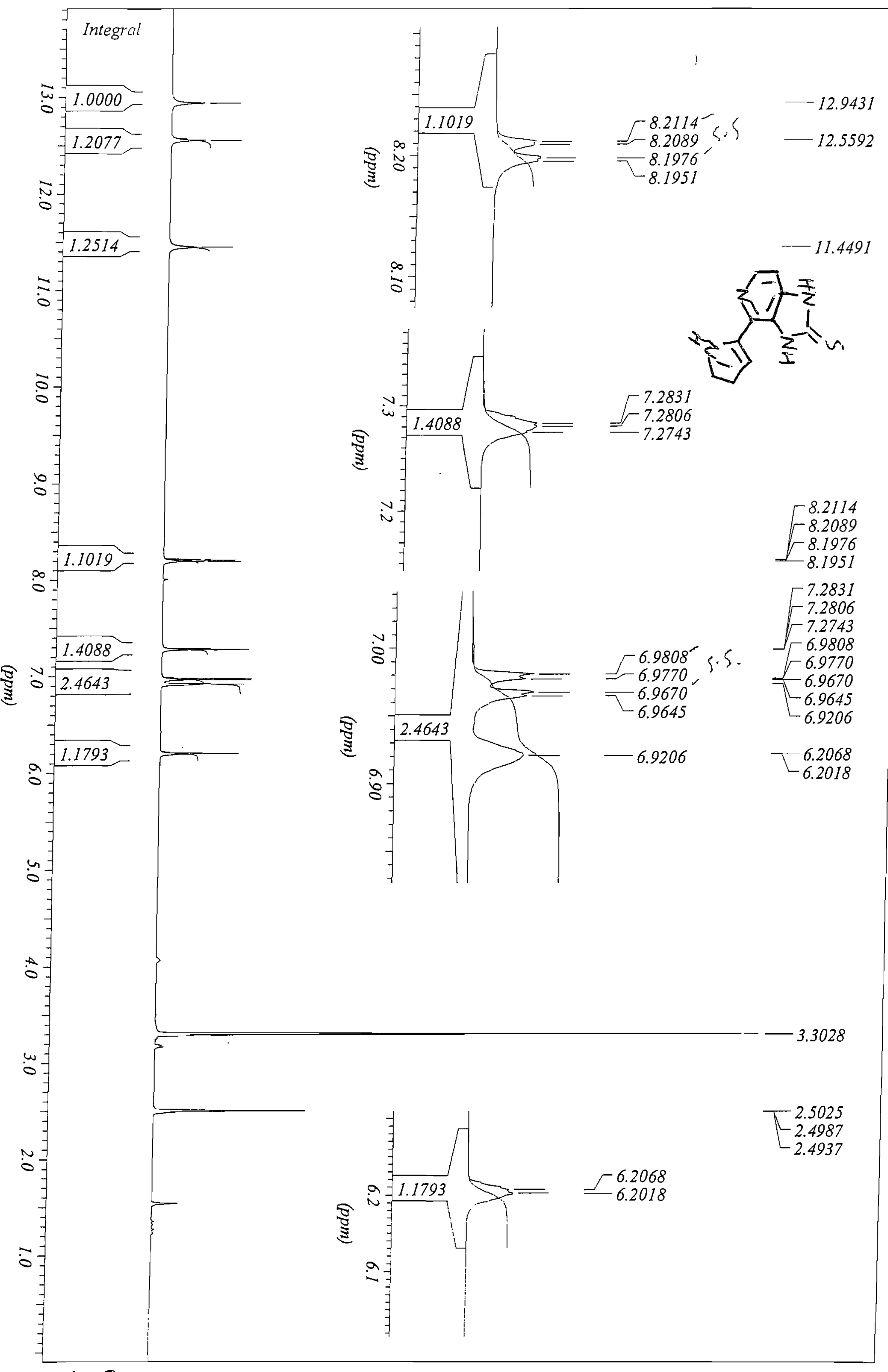


hf4148/77
10.7479
7.9342
7.9204
7.6156
7.6118
7.6080
7.6043
6.9419
6.9382
6.9331
6.9294
6.6873
6.6785
6.6710
6.5731
6.5593
6.2997
3.2991
2.5050
2.5000
2.4962
NH2
NO2
N
0.9788
0.8063
0.8070
1.0199
1.2012
7.5
6.90
6.70
6.60
(ppm)
Integral
1.0000
2.5378
7.6356
3.3610
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
3.60

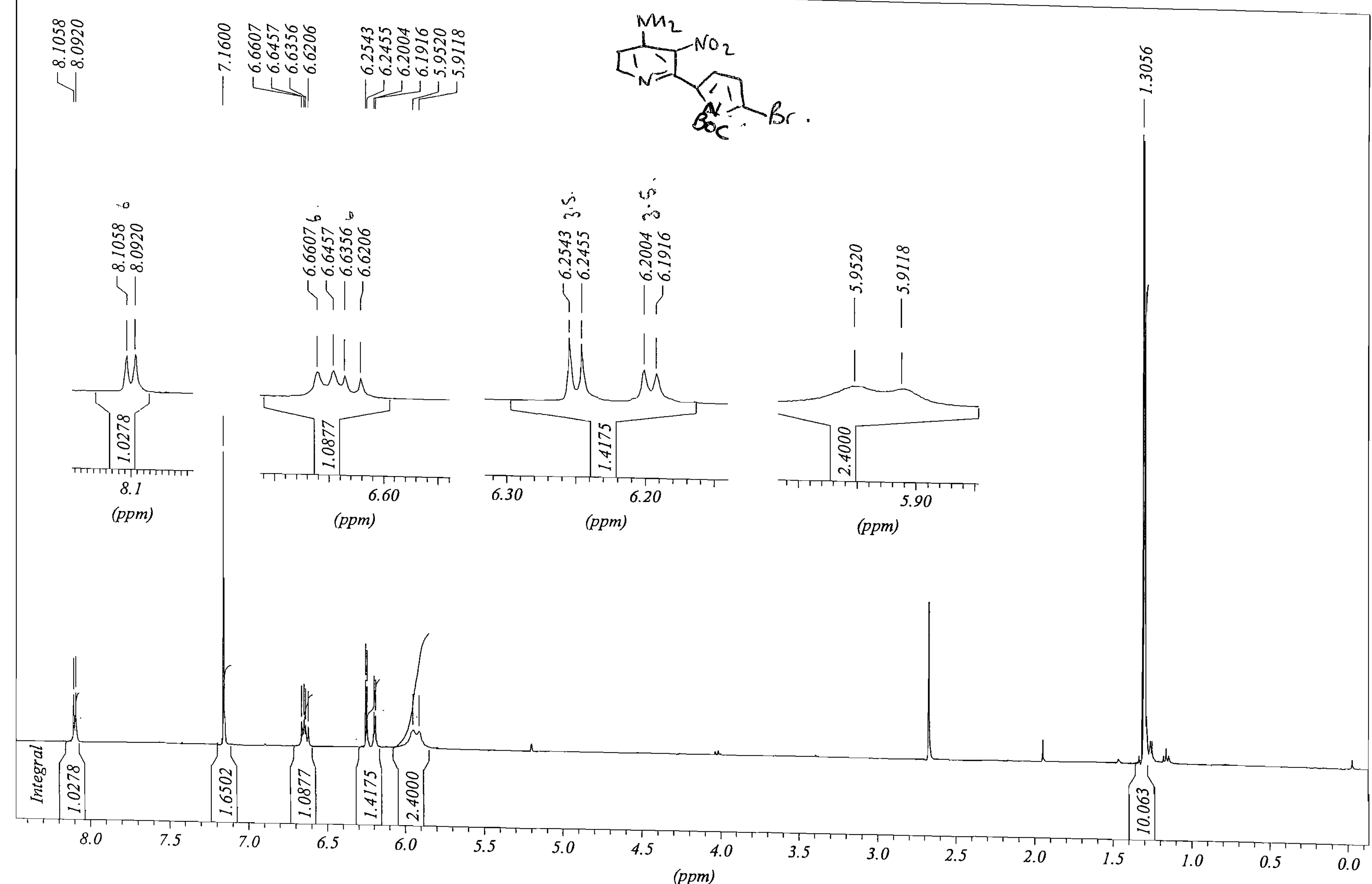

hf4286/14
11.6648
7.6770
7.6607
7.5139
7.1000
7.0962
7.0937
7.0899
6.7412
6.7249
6.7111
6.3097
6.3034
6.2997
6.2971
6.2909
5.2020
3.3141
2.5000
2.4950
NH2
NH2
0.5588
1.8711
7.5
(ppm)
Integral
1.9243
6.8
6.7
6.6
0.7757
6.30
Integral
0.9244
0.8671
2.0000
8.3715
2.2343
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3.67


hf4286/guan/recryst -- 400 MHz MeOD.
7.9210
7.8978
7.8284
7.8247
7.8186
7.8149
7.2809
7.2761
7.2675
7.2639
6.9871
6.9761
6.9737
6.9639
6.8030
6.7811
4.8230
3.3466
3.3198
3.3137
3.3088
3.3027
3.2978
NH2
N
NH
Integral
1.0000
0.9126
0.9685
0.9553
0.9611
(ppm)
3.68

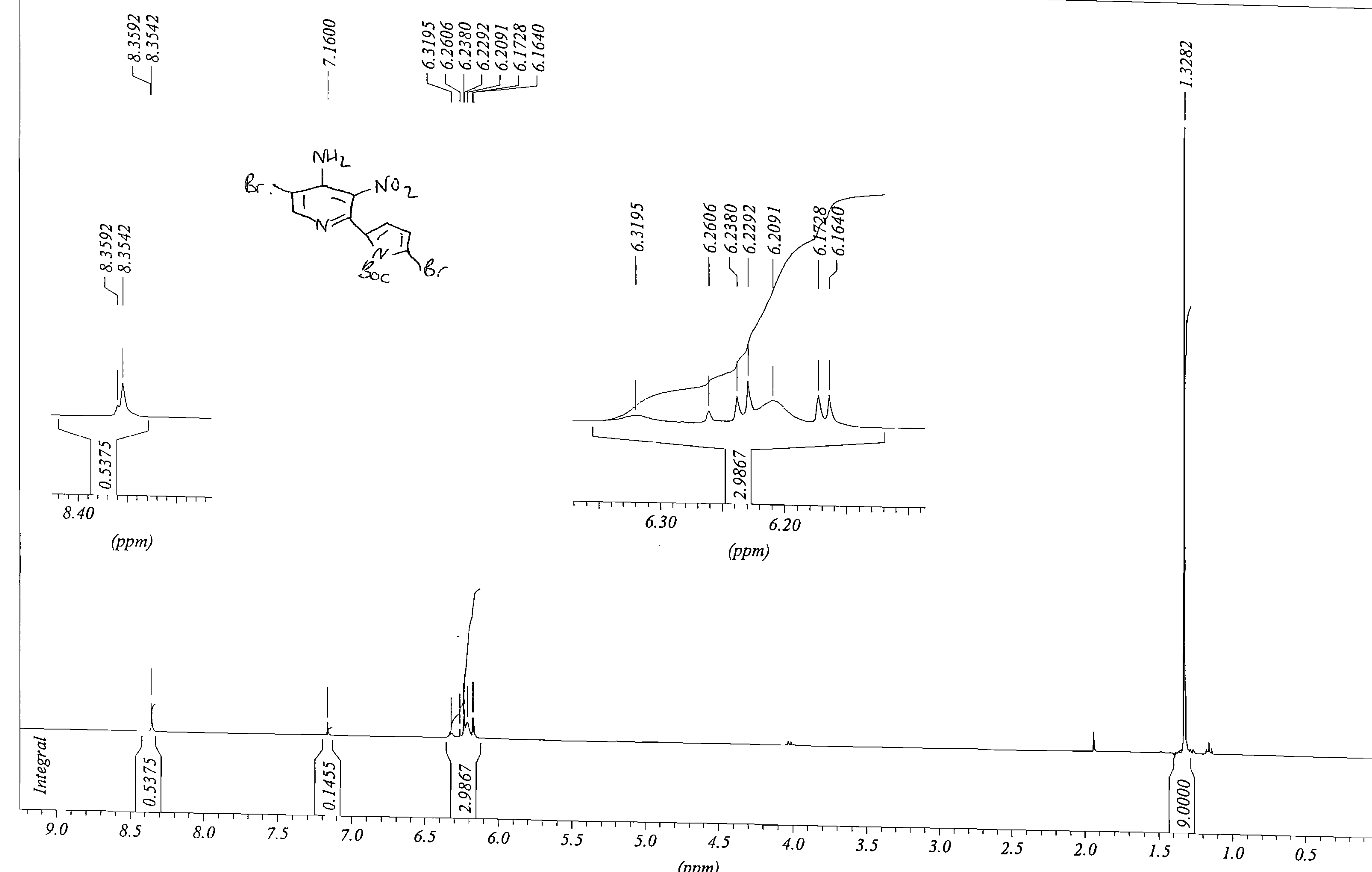

hf4286/43 guan
DMSO.
14.0232
8.0533
8.0433
8.0358
7.7648
7.4776
7.4675
6.9946
6.9846
6.9770
6.8165
6.8002
3.2966
2.5038
2.4987
2.4950
Integral
2.0000
1.9598
0.9388
0.8942
0.9150
0.6374
6.9685
5.4680
(ppm)
3.68 (DMSO)



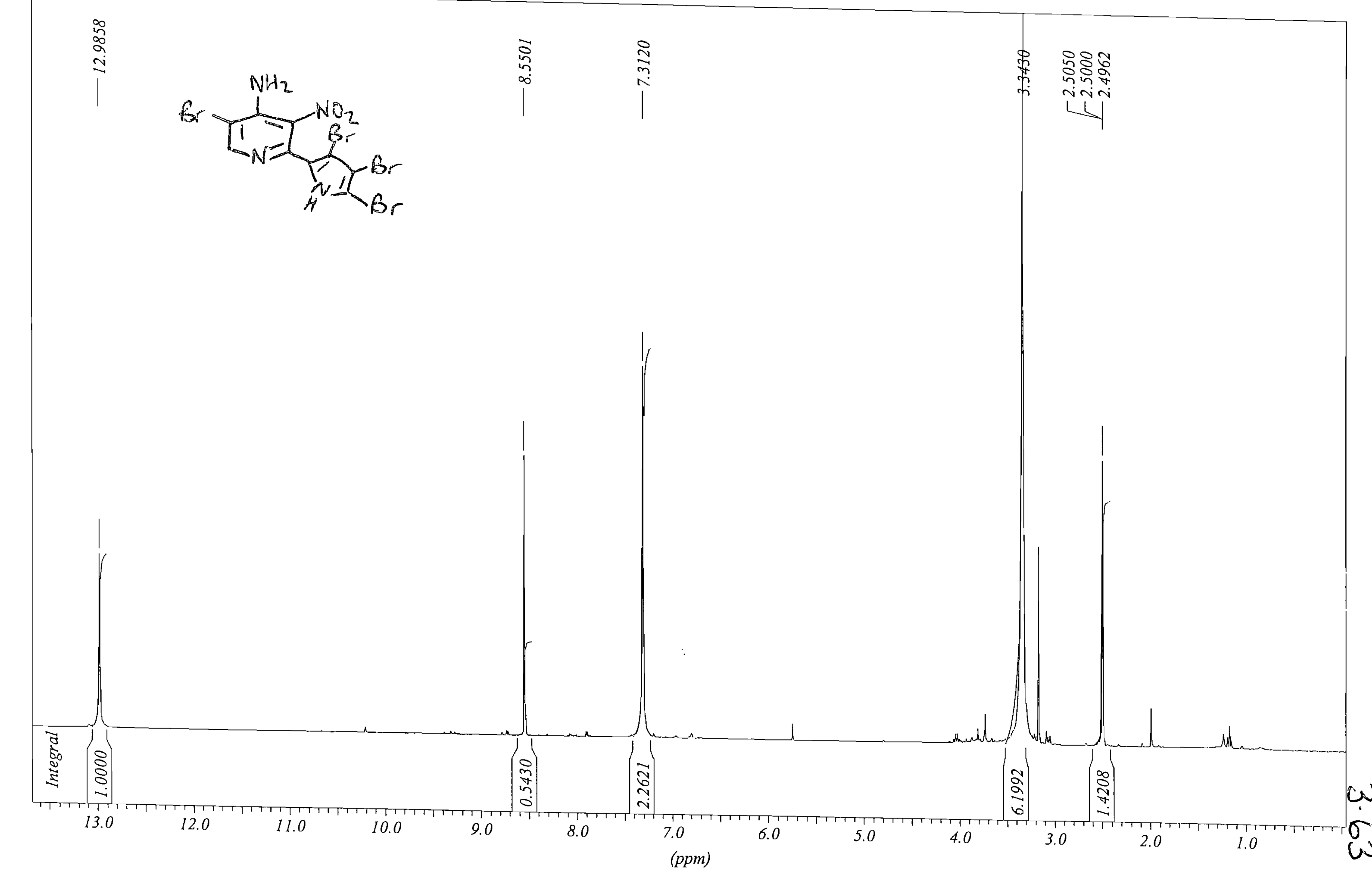
Integral
1.0000
1.2077
1.2514
1.1019
1.4088
2.4643
1.1793
12.9431
12.5592
11.4491
8.2114
8.2089
8.1976
8.1951
7.2831
7.2806
7.2743
6.9808
6.9770
6.9670
6.9645
6.9206
6.2068
6.2018
3.3028
2.5025
2.4987
2.4937
(ppm)
hf4286/25/1



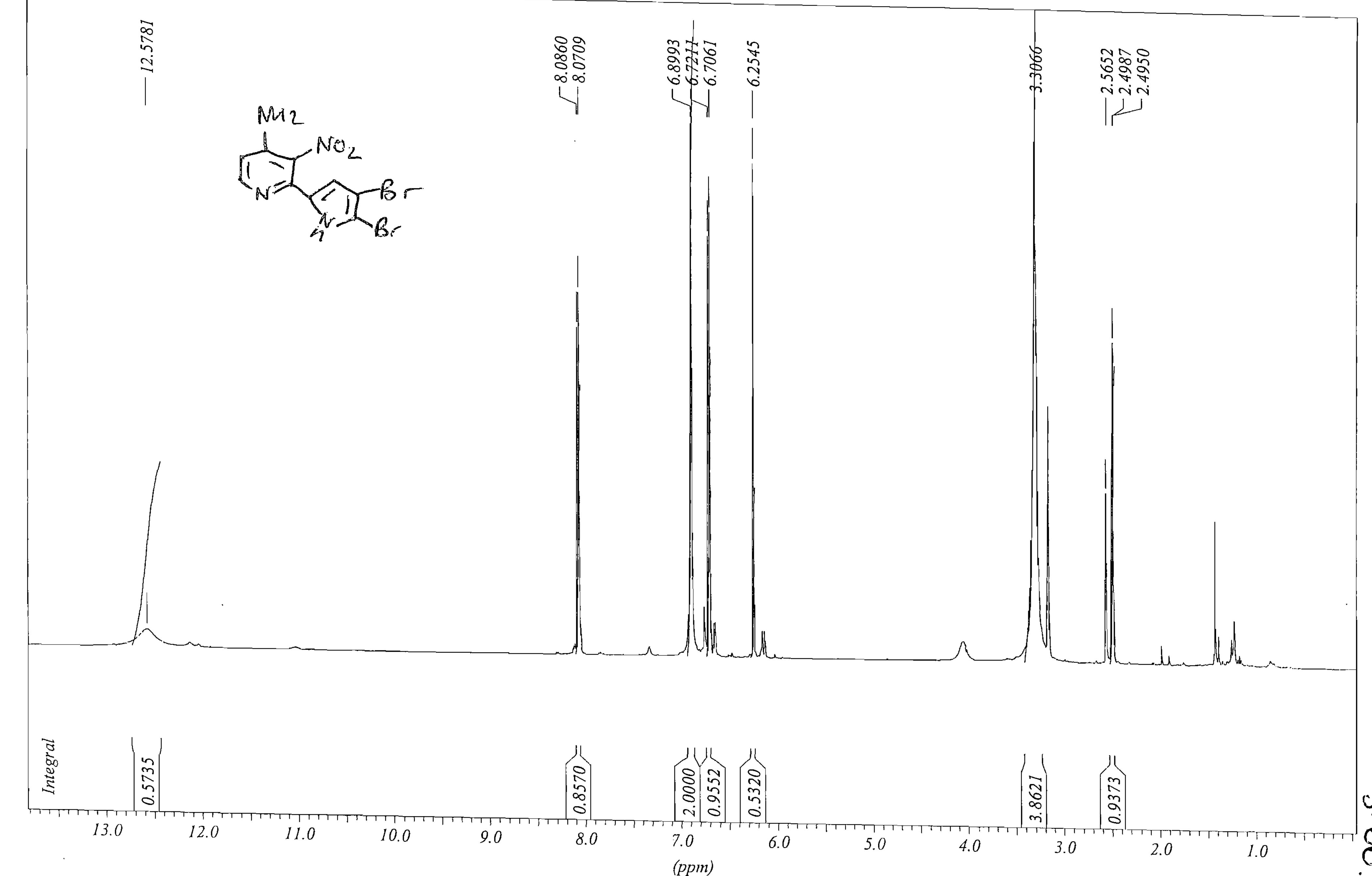
hf4148/93/2/monobr
– 400MHz
8.1058
8.0920
7.1600
6.6607
6.6457
6.6356
6.6206
6.2543
6.2455
6.2004
6.1916
5.9520
5.9118
1.3056
NH2
NO2
N
Boc
Br.
Integral
1.0278
1.6502
1.0877
1.4175
2.4000
10.063
(ppm)
3.61



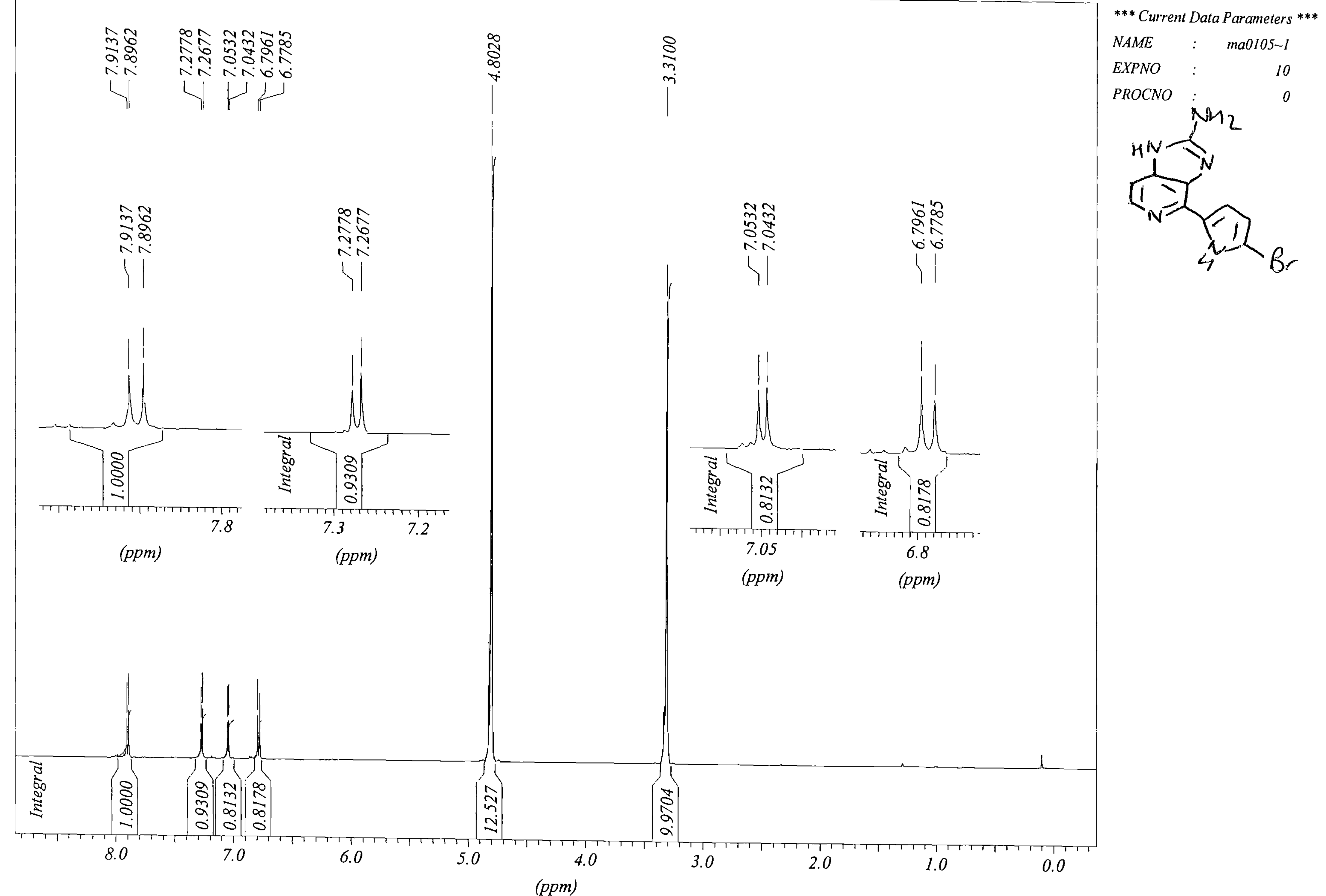
hf4148/93/f1/dibr
400 MHz.
8.3592
8.3542
7.1600
6.3195
6.2606
6.2380
6.2292
6.2091
6.1728
6.1640
1.3282
NH2
Br
NO2
N
Boc
0.5375
8.40
(ppm)
2.9867
6.30
6.20
Integral
0.1455
9.0000
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
3.62

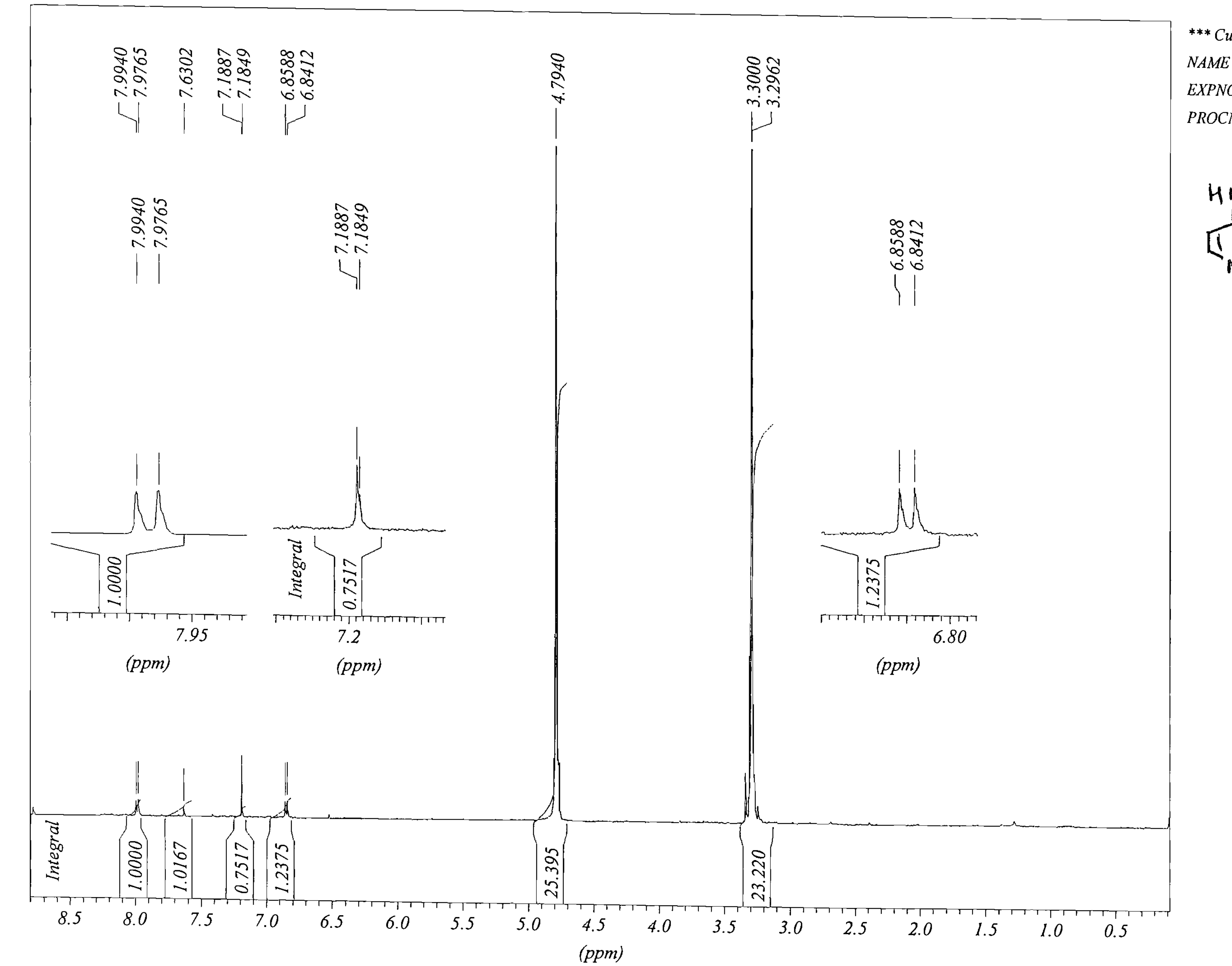


hf4286/09/1
3·63
12.9858
8.5501
7.3120
3.3430
2.5050
2.5000
2.4962
NH2
NO2
Br
N
H
Integral
1.0000
0.5430
2.2621
6.1992
1.4208
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)



hf4286/10/2
12.5781
8.0860
8.0709
6.8993
6.7211
6.7061
6.2545
3.3066
2.5652
2.4987
2.4950
NO_2
Br
Br
N
Integral
0.5735
0.8570
2.0000
0.9552
0.5320
3.8621
0.9373
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)
3.66


hf4286/44/hplc(2)
TFA SALT.
7.9137
7.8962
7.2778
7.2677
7.0532
7.0432
6.7961
6.7785
4.8028
3.3100
Integral
1.0000
0.9309
0.8132
0.8178
12.527
9.9704
7.8
7.3
7.2
7.05
6.8
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)
*** Current Data Parameters ***
NAME : ma0105~1
EXPNO : 10
PROCNO : 0
NH2
HN
N
S
Br
3.76.



*** Current Data Parameters ***

NAME : ma0105~2

EXPNO : 10

PROCNO : 0

3·1

hf4286/49/1+2□ MeOD 400MHz · FREE BASE

7.8774
7.8636
7.1160
7.1059
6.8199
6.8099
6.5853
6.5715
4.8028
3.3427
3.3075
3.3038
3.3000

N NH₂ NH N N Br

Integral 1.0000 (ppm) 7.8774 7.8636

Integral 1.0355 (ppm) 7.1160 7.1059

0.8436 6.75 (ppm) 6.8199 6.8099

0.7967 6.60 (ppm) 6.5853 6.5715

Integral 1.0000 1.0355 0.8436 0.7967 5.0922 1.3858

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)

5·76 (FREE B)

hf4286/45/fo

3.75.

hf4286/46/f1 — MeOH.

7.5934
7.5784
7.5733

6.7191
6.7141
6.7028
6.6978

4.7133
4.7082

3.2493
3.2443
3.2180
3.2142
3.2104
3.2054
3.2017
3.1966

NH_2, NH_2, Br, Br, Br, N, N

7.5934
7.5784
7.5733

Integral 1.1797

7.65 7.60 7.55 7.50
(ppm)

6.7191
6.7141
6.7028
6.6978

J=6.5.

1.0000

6.76 6.72 6.68
(ppm)

Integral 1.1797 1.0000 12.571 8.0192

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)