Identification of Renin Inhibiting Natural Products from *Nauclea latifolia*

By

Anthony Agomuoh

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the Master of Science degree.

Department of Biology
Master of Science in Bioscience, Technology and Policy Program
The University of Winnipeg
Winnipeg, Manitoba, Canada
June, 2011

THE UNIVERSITY OF WINNIPEG

FACULTY OF GRADUATE STUDIES
*****
COPYRIGHT PERMISSION

Identification of Renin Inhibiting Natural Products From *Nauclea latifolia*

By

Anthony Agomuoh

A thesis submitted to the Faculty of Graduate Studies, The University of Winnipeg in partial fulfillment of the requirement of the degree

MASTER OF SCIENCE

Abstract

Natural products, particularly plants have been an important source of food and medicine throughout the history of mankind. High blood pressure has become a growing health burden all over the world. There is an obvious need to discover better therapeutic agents to tackle this problem. Exploitation of natural products is one important option. This thesis describes the results obtained from the phytochemical, and bioassay studies, on *Nauclea latifolia* of Nigerian origin that are summarized as follows:

Chemical studies on the crude methanolic extract of the stem/bark of *N. latifolia* resulted in the isolation of seven new indole alkaloids, latifoliamide A-G (**46-52**), along with one known alkaloid, angustoline (**45)**. The structures of compounds (**45-52**) were elucidated using extensive NMR and mass spectral studies. Compounds (**45-52**) were evaluated for their *in vitro* renin inhibitory activity. Compounds (**45, 46, 49-51**) were found to exhibit a wide range of anti-renin activity, while **47** and **52** were inactive in the bioassays. Based on these bioactivity data, it appears that previously reported anti-hypertensive property of this plant in the literature might be due to the presence of these bioactive alkaloids.

Acknowledgements

First of all, I am immeasurably grateful to Almighty God for his faithfulness. I wish to thank my supervisor, Dr. Athar Ata for his untiring efforts and assistance towards the completion of my thesis. My gratitude also goes to the Department of Biology for giving me the opportunity to pursue the Bioscience programme. I am particularly grateful to my committee members, Dr. Sara Good-Avilla, and Dr. Civetta, Alberto for their assistance throughout the thesis work.

I thank the staff of Chemistry Dept., particularly Ramin Vakili for their technical support. I am grateful to the Faculty of Graduate studies of University of Winnipeg for providing me financial support. Finally, I am indebted to my wife Jane, and my wonderful and lovely kids for their moral support, and encouragement throughout the duration of my studies.

"Every simple plant remedy is blessed and gifted by GOD and its Handmaiden nature to such an extent, that according to it's own nature and way, it has the power to heal, strengthen, allay pain, cool, warm up, purge, and sweat"- Heironymus Bock, Kreuterbuch

TABLE OF CONTENTS

LIST OF TABLES **Page**

LIST OF FIGURES **Page**

LIST OF SCHEMES **Page**

LIST OF ABBREVIATIONS

^{0}C	Degrees centigrade
µg	Microgram
µg/Ml	Microgram per milliliter
µL	Microlitre
µM	Micomolar
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
1D-NMR	One-dimensional nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
2D-NMR	Two-dimensional nuclear magnetic resonance
Å	Angstrom
ACE	Angiotensin converting enzyme
ACEIs	Angiotensin converting enzyme inhibitors
A I	Angiotensin one
A II	Angiotensin two
ARBs	Angiotensin receptor blockers
$CDCl_{3d}$	Deuterated chloroform
$CHCl_3$	Chloroform
δ	Chemical shift value.
COSY	Correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethylsulfoxide

d	Doublet
dd	Doublet-doublet
DRIs	Direct renin inhibitors
EtOAc	Ethyl acetate
FDA	Food and drug administration
$FIU.min^{-1}$	Fluorescence intensity unit per min
g	Grams
GST	Gluthatione-s-transferase
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond connectivity
HREIMS	High resolution electron impact mass spectroscopy
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
IC_{50}	Concentration of inhibitor required to inhibit a target by 50%
J	Coupling constant
Kg	Kilograms
m	Multiplet
nM	Nanometer
MeOH	Methanol
Mg	Milligrams
MHz	Mega hertz
mL	Milliliter

M	Molar
Na+	Sodium ion
NaCl	Sodium chloride
nM	Nanomolar
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
Prep. TLC	Preparatory thin-layer chromatography
RAS	Renin angiotensin system
R*f*	Retention factor
TLC	Thin-layer chromatography
Tris-HCL	2-Amino-2-hydroxymethyl-1, 3-propanediol hydrochloride
UV	Ultraviolet

CHAPTER 1

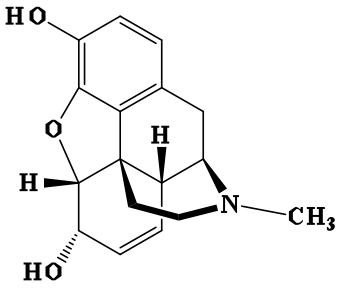
Natural Products Chemistry

1.1 NATURAL PRODUCTS: HISTORICAL BACKGROUND

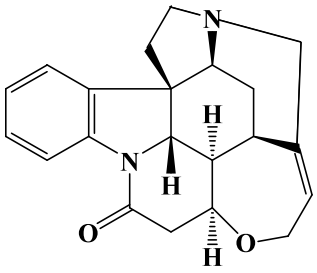
Natural products are organic compounds produced by living organisms, including plants, marine organisms, and microorganisms, for their survival. These compounds exhibit various biological activities that make them an important source of lead compounds in drug discovery program. Plant natural products have provided a significant number of lead compounds to the pharmaceutical industry. Recent estimates indicate that approximately 60% of anti-cancer and anti-infective agents available on the market are of natural product origin. Furthermore, 25% of these pharmaceutical agents are of plant origin[1].

For a long time, humans have used plant products to cure disease[2,3]. The Chinese, Indians, and North African cultures have documented the use of plants as medicines[4]. One of the earliest known written documents on herbs is a 4000 year old "Sumerian" clay tablet that records remedies for the cure of various illnesses[3]. The Western world has utilized some of this ancient information in discovering many of the currently used pharmaceuticals. For instance, William Withering studies of foxglove between 1775 and 1785 as treatment of dropsy (heart failure) set the standard for pharmaceutical chemistry[3]. Over the years, traditional use of natural products had been intertwined with many challenges[5] particularly in developing countries, due to several factors, including lack of standardization, attribution of results to magic and witchcraft etc. However, it was not until early 1800's that the active principles in medicinal plants were identified, which

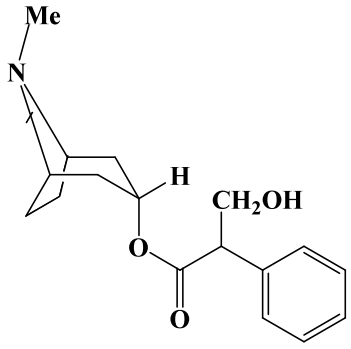
provided a new direction to the pharmaceutical industry for obtaining lead compounds from plants. In 1826, E. Merck identified morphine[1] (**1**), as the first commercially available natural product as pain killer from *Papaver somniferum*. Similarly, other natural products like strychnine (**2)**, atropine (**3)**, and colchicine **(4)** were discovered[1].



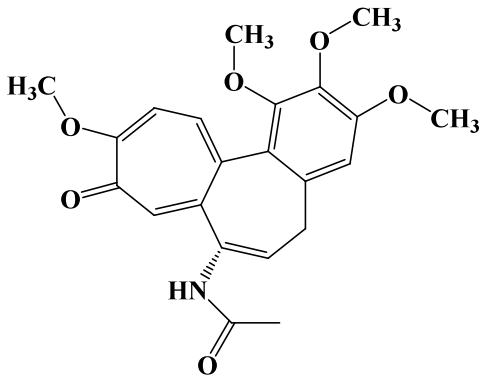
1



2



3



4

With the development of new chromatographic techniques, and chemical methods to elucidate the structures of organic compounds, the active substances of many plants have been isolated by the western world[6]. Plants provide structural diversity in producing natural products, and this diversity makes plants an interesting source to explore for their new bioactive lead compounds. In some Asian and African countries, nearly 80% of the population depends on traditional medicine for primary health care[7]. It has been estimated that approximately over 250,000 flowering plant species are present world wide. Nearly half of these are found in the tropical forests. These plants continue to be a rich source of natural products, providing chemists with a rich endowment for the discovery of useful drugs, and nutraceuticals[8]. The search for new biologically active compounds is most often based on hints coming from ethnobotany, which prompts phytochemical investigation of the particular plant[9]. For the most part, the discovery of new lead bioactive natural products stem from knowledge that their extracts are used to treat one or more diseases. The most bioactive extracts are then subjected to bioassay-directed investigation for the identification of bioactive lead compounds[10]. Natural product scaffolds have also been well recognized as being 'privileged' structures in terms of their ability to provide novel lead compounds with potential biomedical application. Such scaffolds are being used as cores of compound libraries made by combinatorial chemistry[11]. Natural products serve not only as source of important pharmaceutical leads, but also help to reveal their novel physiological aspects. For example, digoxin **(5)**, isolated from foxglove showed the role of sodium-potassium-ATPase in the maintenance of membrane resting potential; morphine **(1)** explained the role of receptors affected by

endogenous opioids; muscarine (**6**), nicotine (**7**) and tubocurarane (**8**) helped to explore the different types of acetylcholine receptors[12,13].

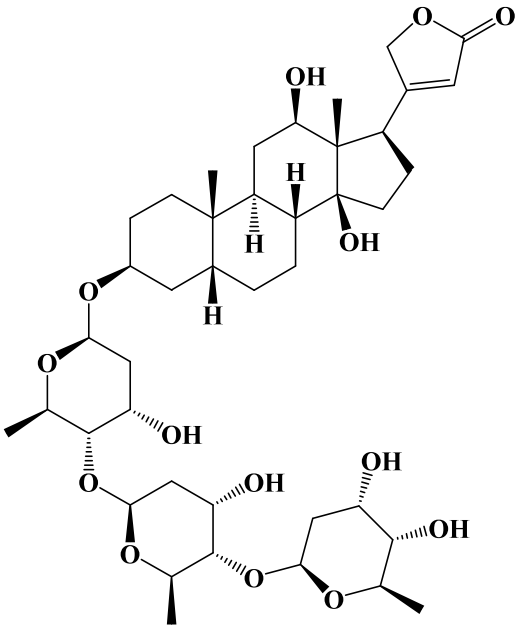
An increased awareness and patronage of natural products by people in different parts of the world has resulted in a tremendous increase in the amount of plant products in the international market, making natural products a huge and potential source of revenue for both the developing nations, as well as the developed nations.

1.2 NATURAL PRODUCTS AND DRUG DISCOVERY

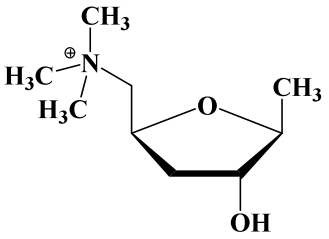
Natural products have been one of the major providers of lead drug molecules[1]. The use of natural products as an important source of active ingredients for the treatment of various medical conditions is historical. This ranges from ethno-botanical applications[7], to the modern drug research and development[35]

1.2.1 Natural Products as Anti-infective Agents

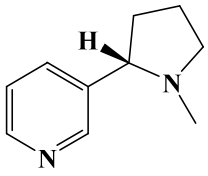
World wide, infectious disease is the number one cause of death, accounting for approximately one-half of all deaths in tropical countries[14]. For instance, approximately 60 % of all deaths in children between five and fourteen years of age in India from 2001 to 2003 were due to infectious diseases[15].



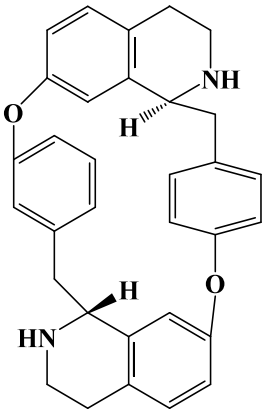
5



6



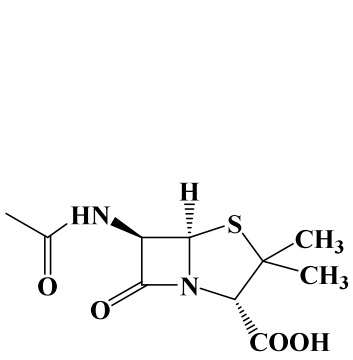
7



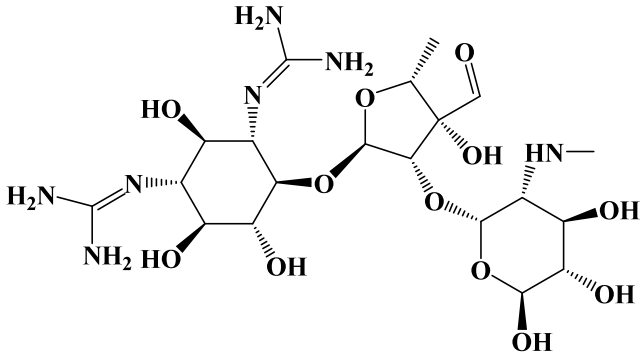
8

It is estimated that infectious diseases are the underlying cause of death in 8% of the deaths occurring in the US[15]. The 2004 WHO global burden of disease update indicates that death resulting from infectious and parasitic diseases amounted to 16.2% of the total causes of death in 2004[16].

The first real breakthrough in the field of anti-infective pharmaceuticals was the discovery of penicillin (**9)**, in 1928. Later on**,** the discovery of streptomycin (**10)**, helped in the treatment of tuberculosis.



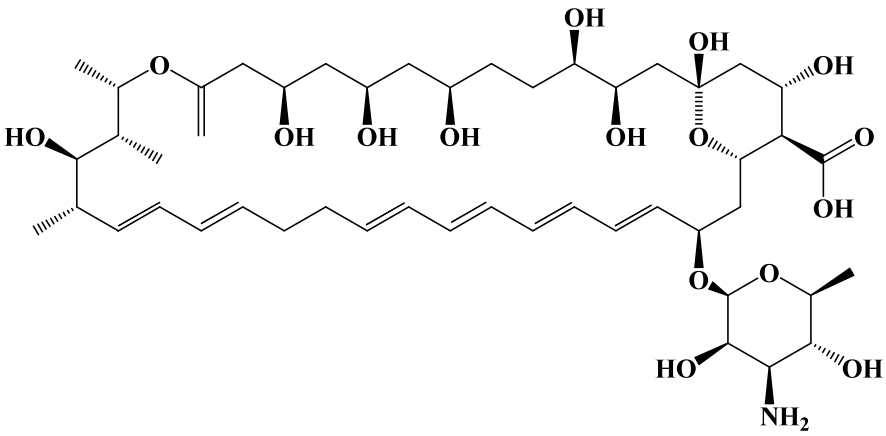
9



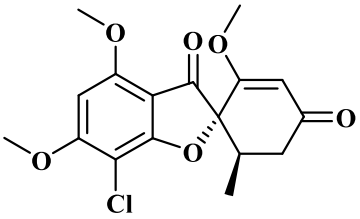
10

Out of the 90 antibiotics that became commercially available in the United States, or were approved world wide, from 1982 to 2002, approximately 79% of these were of natural product origin[17]. The screening for potent antimicrobials has become more intense in recent times due to the growing level of drug resistance to many antibiotics. For instance, ethanolic extracts of 78 traditional medicinal plants used for infectious disease treatment in India have shown anti-bacterial and anti-fungal activity at very low concenteration[18]. Most currently used antifungal drugs are also derived from natural products. Examples include polyenes, like nystatin **(11)**, and griseofulvin (**12)**, derived from some species of *Streptomyces*. Echinocandins (**13)** are synthetic derivative of papulacandins, isolated from a strain of *Papularia sphaerosperma*. Cephalosporins (14),

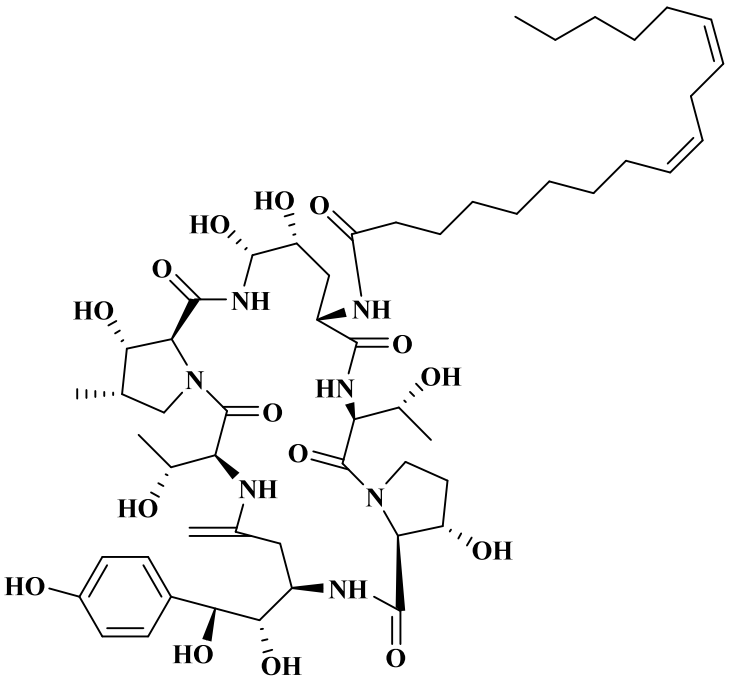
are bactericidal group of antibiotics, first isolated from cultures of Cephalosporium acremonium, and interfere with the synthesis of peptidoglycan layer of bacteria cell wall.



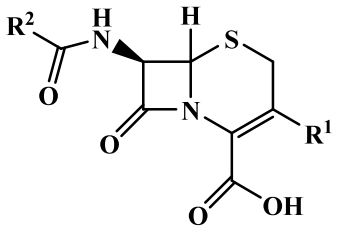
11



12



13

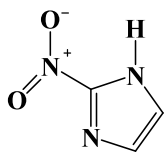


14

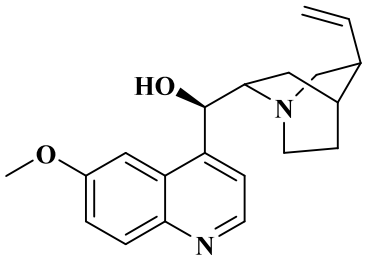
Although the azoles generally are considered to be synthetic in origin, their drug prototype pathway could be traced back to the *Streptomyces* metabolite azomycin (**15**)[19]**.**

1.2.2 Natural Products as Anti-parasitic Agents

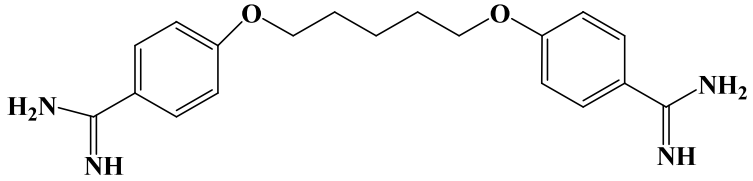
Parasitic infestation is one of the major health concerns in both developing and developed world. Malaria is the most important health problem affecting people in the Southern hemisphere. The World Health Organization 2011 "World Malaria Report", estimates that approximately 225 million malaria episodes occurred in 2009 leading to approximately 781, 000 deaths[20]. The discovery of quinine (**16**) from *Cinchona succiruba* (Rubiaceae) and its subsequent development as an anti-malarial drug represented a milestone in the history of anti-parasitic drugs from nature for the treatment of all parasitic diseases. Early studies on plant products were followed by an era of organic chemistry that led to the development of the diamidines, like pentamidine (**17)** and nitro heterocyclic anti-protozoal drugs like metronidazole (**18**)[21].



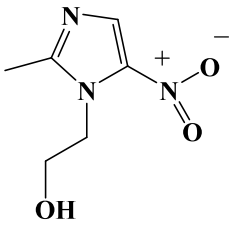
15



16



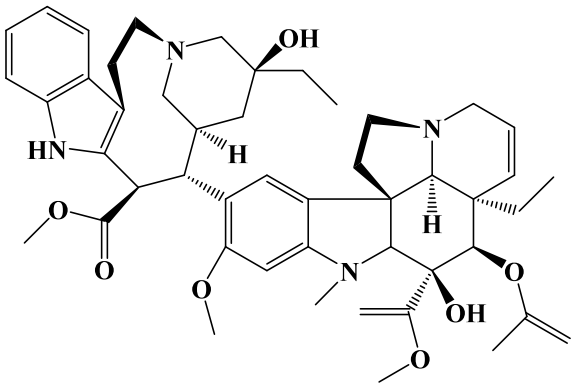
17



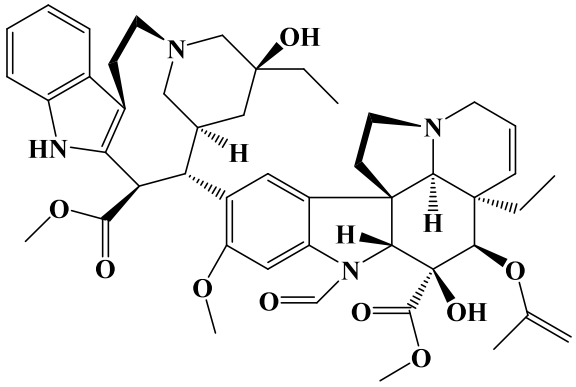
18

1.2.3 Natural products as Anti-cancer Agents

Cancer is the second leading cause of death in the United States, and other developed countries; one out of every four deaths is from cancer. It has also been predicted that as lifestyles changes in developing countries, there would likely be a significant rise in the incidence of cancers that have previously been associated with developed countries, (such as breast, colon, prostate and lung cancers),[22] in addition to those that are already common. Some of the most effective cancer treatments to date are natural products or compounds derived from natural products. The search for anti-cancer agents from plant sources started in the early 1950s with the discovery and development of the vinca alkaloids, vinblastin (**19)** and vincristine (**20**)[23], originally derived from the periwinkle plant *Catharanthus roseus,*



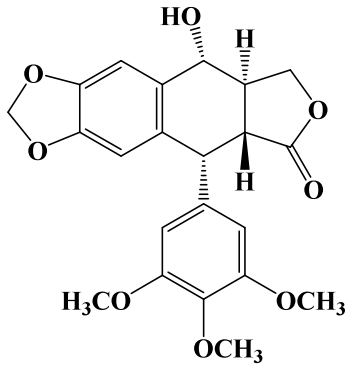
19



20

and the isolation of the cytotoxic podophyllotoxins (**21**)[24], a non-alkaloid ligan extracted from Podophyllum species. These discoveries prompted the National Cancer Institute, USA to initiate an extensive world-wide plant collection programme in 1960[25]. Of the 92 anti-cancer drugs commercially available prior to 1983 in the US, and among world wide approved anti-cancer drugs between 1983 and 1994, 60% are of natural product origin[25]. As many as 84% of pediatric oncology patients, 50% of breast cancer patients and 37% of prostate cancer patients use complementary and alternative medicine, including predominantly herbal approaches[26]. Plant secondary metabolites also show promise for

the cancer chemoprevention, which has been defined as "the use of non-cytotoxic nutrients or pharmacological agents to enhance physiological mechanisms that protect the organism against mutant clones of malignant cells[27]. A number of reviews[28,29-35] on compounds with anti-cancer activities from natural sources are present in the literature. These are important sources of information that would help identify those compounds that would need further exploration.



21

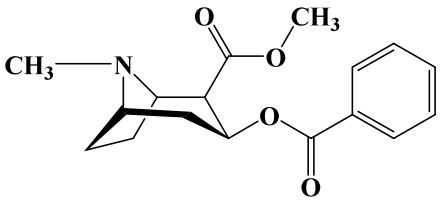
1.2.4 Role of Natural Products as Anti-viral Agents

Viral infections constitute one of the most serious health concerns among humans. The HIV infection has become a pandemic and has proved to be one of the longest infections in history to defy modern medical cure. The medical and economic impact particularly in developing countries has been devastating. As of December 2009, more than 208 countries worldwide have reported laboratory confirmed cases of pandemic influenza H1N1, including at least 11,516 deaths[36]. Consequently, there has been considerable effort over the last 20 years invested into antiviral drug discovery, especially in the field of HIV[37]. Natural product is one important area where scientists continue to

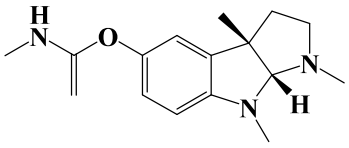
explore for anti-infective agents. For instance, betulinic acid, a triterpene of natural origin, isolated from various plants, has shown various biologic activities, including anti-HIV properties[38].

1.2.5 Role of Natural Products in the Treatment of Neurological conditions

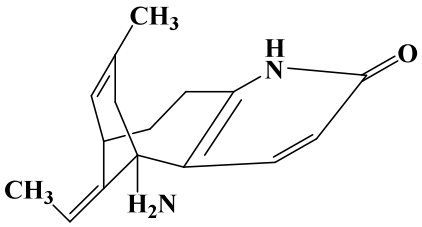
The opiate alkaloids, obtained from *Papaver somniferum*, are one of the earliest, but still relevant central nervous system (CNS) groups of natural product drugs. Other compounds include ergot from the fungus, *Claviceps purpurea*, cocaine, (**22)** from the South American plant, *Erythroxylon coca*, and the anticholinesterase, physostigmine (**23)** from the Nigerian plant, *Physostigma venenosum*[39]. Recently, newer drugs have been added to this group. They include: huperzine (**24)** from the club moss, *Huperzia serrata*, and galantamine (**25)** from the plant, *Galanthus nivalis*, used to treat Alzheimer's disease. Also, epibatidine (**26)** from the Ecuadorian frog, *Epipedobates tricolor*, used as a lead compound for the development of drugs for pain relief[40-42].



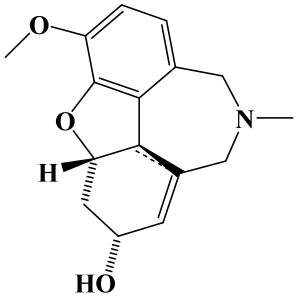
22



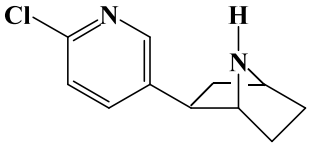
23



24



25



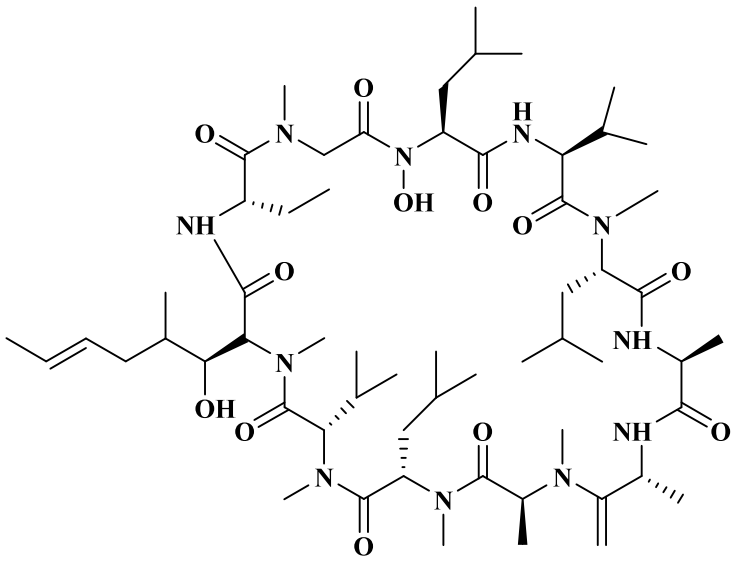
26

1.2.6 Role of Natural Products in Immunological, inflammatory and related disease Conditions

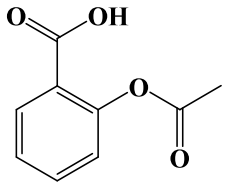
The area of immunology and inflammatory conditions has not been left out in the impact of natural products. The need for organ transplant in individuals with end stage organ conditions, or acute organ damage had witnessed tremendous set back primarily due to antigenic variation between the donor and recipient. For this to be minimized one has to look for the best possible match, preferably an identical twin, otherwise the chances of rejection is be high. The discovery of cyclosporine (**27)**, from the fungus *Tolypocladium inflatum,* and other immunosuppresants[43-47], helped in no small means to address this issue.

The discovery of aspirin (**28)** in 1890s has, and continues to make a tremendous impact in different medical conditions particularly cardiovascular conditions, as an anti-

inflammatory, and as an analgesic[47]. It has equally served as a lead drug in the discovery of other anti-inflammatory agents[48], through the discovery of cyclooxygenase isozymes COX-1 and 2[49].



27



28

1.3 NATURAL PRODUCT AND MODERN BIOTECHNOLOGY

Man has been applying the principles of biotechnology throughout history, ranging from the domestication of animals and plants, to the propagation and preservation of desired traits. However, the field of biotechnology was revolutionized following the discovery of gene splicing and gene cloning in 1973 by Stanley Cohen and his colleagues. Consequently, modern biotechnology has and continues to make impact in virtually all aspects of human life. Since the origin of life, plants have been providing man with various needs such as food, and medicine for the treatment of ailments. However, the astronomical increase in the human population and his activities, have resulted in dwindling natural resources. A great number of different herbs and trees have

gone extinct, and many, are already endangered[50]. Many plants cannot be propagated through vegetative methods; plants raised through seeds carry multiple traits and display variations in their growth patterns, and yields. Many are affected by a lot of modifiable environmental factors and are prone to many disease conditions. These, no doubt pose a serious threat to the survival of mankind. In addition, the classical plant-based pharmaceutical drug production approach has been hampered by low yields, lack of reproducibility, and the high complexity of plant extracts[51].

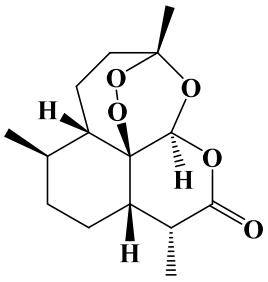
The emerging field of biotechnology promises to help offer a reasonable solution to some of the problems outlined above[52]. Biotechnological tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as *in-vitro* regeneration and genetic transformations. It can also be harnessed for production of secondary metabolites using plants as bioreactors[53]. Some modern techniques that could be of benefit in preserving plant biodiversity and enhancing optimal drug development include:

1. Conservation: This involves the storage of plant genetic material in liquid nitrogen for future revival and propagation. This is particularly important in endangered plants and those already facing extinction.

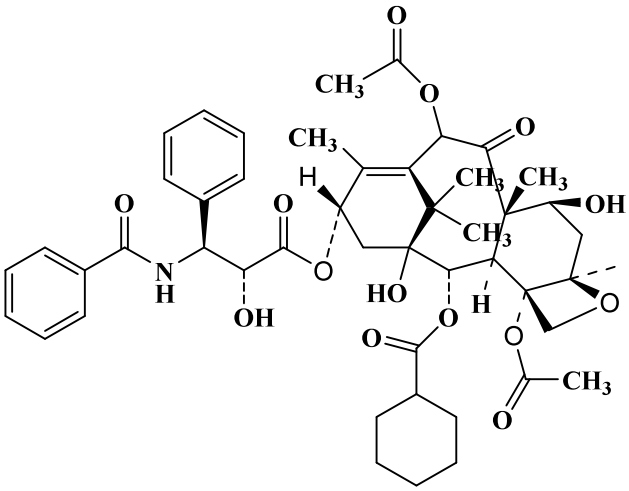
2. Micro propagation: This is another bio-technique which involves the raising of multiple plants having the same genetic makeup. This is especially useful for plants in high demand, or for those that are slow to cultivate naturally[54]. Biotechnology enables the transfer, manipulation and alteration of plant genetic information. Thus, the cultivation of desirable traits allows for functioning improvements in medicinal and aromatic plants, such as enhanced medicinal actions or nutritional content[55].

3. Tissue Culture: Through this technique, isolated plant tissues or organs could be monitored under controlled conditions and bioengineered to produce desired plant secondary metabolites[56,57].

4. Combinatorial biosynthesis: The combination of genes from different organisms for the production of new and desired metabolites. Combinatorial biosynthesis is a new tool in the generation of novel natural products, and for the production of rare and expensive natural products such as Artemisinin (**29)**, Pacilitaxel (**30)**, and morphine (**1**)[58].



29



30

These techniques no doubt have different setbacks[59], however, the possibilities of overcoming these difficulties is very high judging from the pace of science and technological development in the 21st century.

1.4 NATURAL PRODUCTS AND CARDIOVASCULAR CONDITIONS

Cardiovascular disease (diseases affecting the heart and blood vessels), is one of the leading causes of death and disabilities, particularly in the developed world[60].

Hypertension (High blood pressure) has been described as a major risk factor in different cardiovascular diseases, stroke, death, as well as an important cause of end stage kidney disease. The prevalence of hypertension among adult population globally, was estimated to be 26% in 2000[61]. It has been estimated that this prevalence will increase by 24% in developed countries, and 80% in developing countries by 2025[61].

1.4.1 The Role of Renin Angiotensin System in Blood pressure control

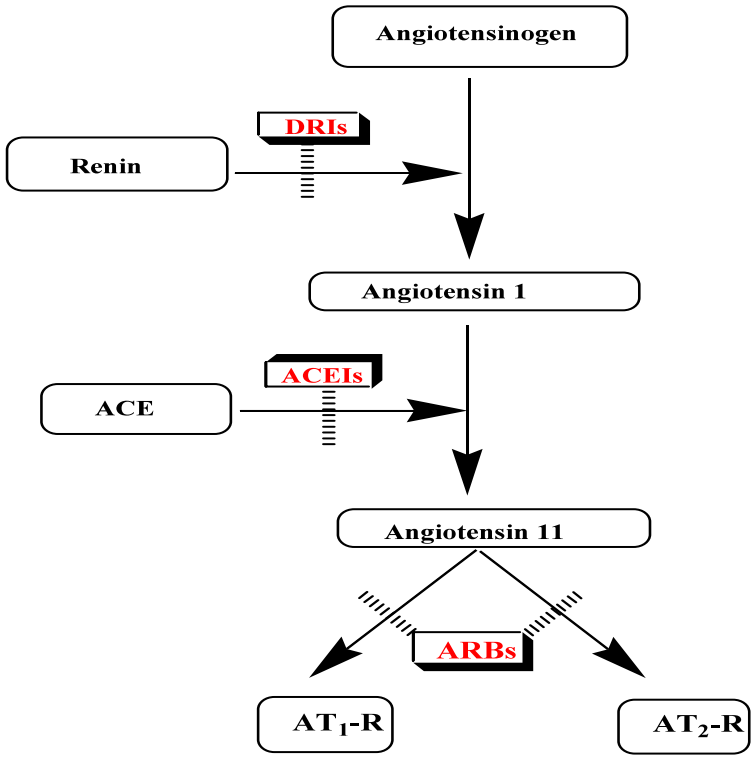
The renin-angiotensin system (RAS) has been recognized as the main physiological modulator of blood pressure[62]. The RAS is a hormonal pathway that maintains the water and electrolyte balance of the body and consequently the blood pressure. Low blood volume in the body stimulates the juxtaglomerular cells of the kidney to produce the enzyme renin. Renin converts angiotensinogen, a glycoprotein produced by the liver cells, to angiotensin I. This is the rate limiting step in the pathway. Angiotensin converting enzyme (ACE), produced by the surface covering of the lungs and kidney, converts angiotensin I, to angiotensin II. Angiotensin II has a multiple stimulatory effect in different organ systems of the body. These actions are modulated through the binding of Angiotensin II to either of its receptors $AT\text{-}_1$ or $AT\text{-}_2$[63]. Some of these effects include: constriction of blood vessels; stimulation of aldosterone production by the adrenal gland (aldosterone causes increase in salt and water reabsorption by the kidney); secretion of anti-diuretic hormone (ADH) by the pituitary gland (which decreases water removal from the body), etc. The overall effect is an increase in blood pressure. Therefore, hyperactivity of the RAS system from any source would ultimately result in high blood pressure with its negative impact on important organs of the body, like heart, kidney, brain etc.

Skeggs, and his colleagues in 1957 postulated 3 possible approaches to pharmacologically inhibit the renin-angiotensin system: 1) Inhibition of renin, 2) inhibition of angiotensin converting enzyme (ACE), 3) Interference with binding of angiotensin II to its receptors. Since renin is the initial and rate-limiting substance," these authors observed, "the first approach would be the most likely to succeed[64,65], to overcome the aforementioned problems.

Thus, the discovery of the RAS has made it possible for scientist to design drugs that target the major enzymes, and sites that are strategic in this pathway, ultimately helping in the control of blood pressure, and indirectly preventing the development of complications like renal failure, diabetes, heart failure, etc.

1.4.2 RAS as an Important Drug Target

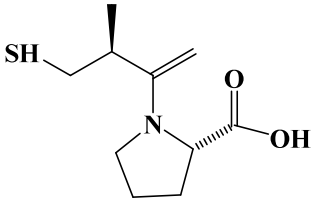
As shown in scheme 1, the RAS system provides three basic points whose blockade could provide a means of lowering blood pressure: Direct renin inhibition (DRI); Angiotensin converting enzyme inhibition (ACEI); and Angiotensin receptor blockade (ARI).



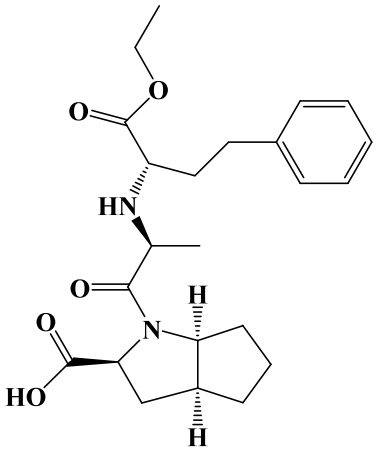
Scheme 1.1 Sites of Possible blockade of the RAS cascade

Attempts to block renin, the rate-limiting step in the production of Angiotensin II, using monoclonal antibodies, started in the early 1950s[66]. However, the development of clinically effective renin inhibitors has remained elusive because of difficulties in identifying suitable agents with the required combination of high affinity for renin's active site and sufficient bioavailability to permit chronic oral administration[67]. Today, about ten angiotensin converting enzyme (ACE) drug inhibitors, including captopril **(31)**, ramipril **(32)** etc, and seven angiotensin receptor blocker (ARB) drugs, including candesartan **(33)**, lorsartan **(34)**, are in use as antihypertensive agents. However, after more than 50 years of discovering renin and its strategic role in the RAS pathway, only one drug, aliskiren **(35)**, was approved by FDA in 2007 as a direct renin inhibitor (DRI).

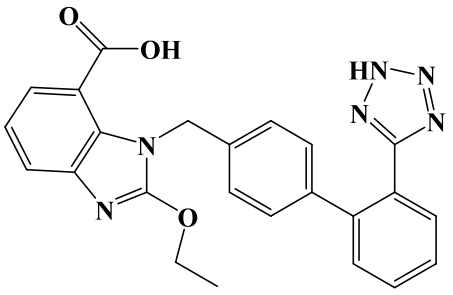
It is also important to note that many of these drugs are synthetically derived. The need to discover more DRIs particularly from natural sources is therefore imperative.



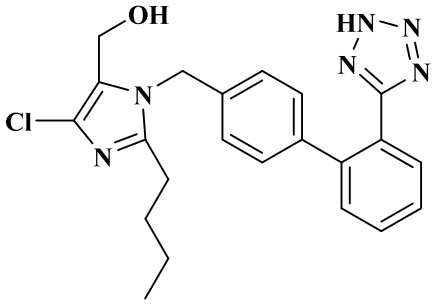
31



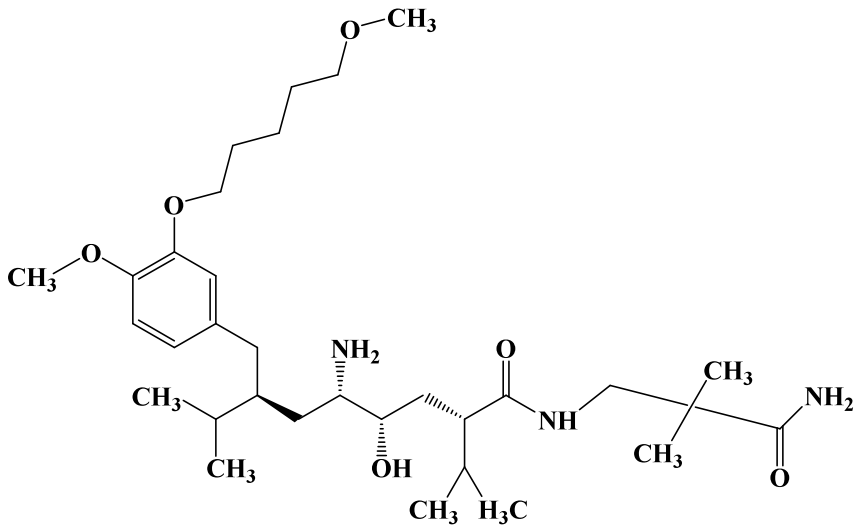
32



33



34



35

1.5 MEDICINAL PLANTS AND EMERGING DRUG TARGETS.

The 20th century has witnessed a monumental advancement in the field of science and technology. Consequently, life and its activities have been modified or even changed. The field of medicine is one area that has witnessed a continuous change. Drug administration and usage is now target specific, and target directed. This is due to the identification of specific molecular targets whose deficiency, activity or excess leads to the development, or transmission of a disease condition. With the completion of human genome project in 2003, and the subsequent sequencing of the genome of many plants and animals, pharmacogenomics has become a new face of drug design and application[68,69].

While different types of drug targets continue to be discovered, including multi target approaches, enzyme targets has and will continue to be a major source of drug target for many conditions, whether therapeutic or diagnostic. Enzymes catalyze multistep

chemical reactions and achieve phenomenal rate accelerations by matching protein and substrate chemical groups in the transition state. Inhibitors that take advantage of these chemical interactions are among the most potent and effective drugs known[70,71]. Recently, three new enzyme targets have been validated by FDA approval of new enzyme inhibitor drugs. These include mitogen-activated protein kinase, renin, and dipeptidyl peptidase IV[71]. The unique structures and diversity of compounds from natural sources makes them good and interesting candidates for the exploitation of these targets for the identification of different medicines to handle present and emerging health conditions. Many studies targeting different enzymes in the treatment and diagnosis of different medical conditions have been done, some of which include: shikimate-pathway enzymes[72], malaria[73], antibacteria[74], cancer[75,76], and parasitic infestations[77].

Based on the importance of plants in drug discovery process, the present project was designed to isolate natural products from *N. latifolia* and to evaluate them for inhibitory activity against renin enzyme.

1.6 Objectives of the Study

The objectives of this study were:

1. To isolate pure compounds from *N. latifolia*
2. To screen the isolated compounds for anti-renin activity

1.7 REFERENCES

1 Cragg GM, Newman DJ. Antineoplastic agents from natural sources: achievements and future directions. Expert Opin Investig Drugs 2000 ;9(12):2783-2797.

2 Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep 2000 ;17(3):215-234.

3 Kong JM, Goh NK, Chia LS, Chia TF. Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin 2003 ;24(1):7-21.

4 Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry 2001 ;56(3):237-243.

5 Balunas JM, Kinghorn DA. Drug discovery from medicinal plants. Life Sciences 2005; 78(5): 431-441.

6 Saad B, Azaizeh H, Said O. Tradition and perspectives of Arab Herbal medicine: A review. eCAM 2005;2(4):475-479.

7 http://www.who.int/mediacentre/factsheets/fs134/en/

8 Jachak SM, Saklan A. Challanges and opportunities in drug discovery from plants. Curr Sci 2007;92(9):1251-1257.

9 Bankova V. Natural products chemistry in the third millennium. Chem Cent J 2007;1(1).

10 Fansworth NR. Biodiversity. In: Wilson EOE, editor. Washington DC: National Academy Press; 1988. p. 83-97.

11 Leach AR, Hann MM. The in silico world of virtual libraries. Drug Discovery today 2000;5(8):326-336.

12 Roth GJ, Majerus PW. The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. J Clin Invest 1975 ;56(3):624-632.

13 Loewi O, Navratil E. Humoral transfer of heat-nerve action X. Fate of the vagus substance. Pfugers Arch ges Physiol 1926;214:678-688.

14 Hotez PJ, Remme JH, Buss P, Alleyne G, Morel C, Breman JGB. Combating tropical infectious diseases: Report of the disease control priorities in developing countries project. Clin Infect Dis 2004;38(6):871-878.

15 Morris KS, Bassani DG, Awasthi S, Kumar R, Shet A, Suraweera W, Jha P. Diarrhea, pneumonia, and infectious disease mortality in children aged 5 to 14 years in India. PloS ONE 2011; 6(5): e20119.

17 Raja A, Gajalakshmi P, Mohamed M.R. Drugs from the natural bio sources for human disease. Int J Pharmacol 2010; 6(4): 360-363.

16 http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004 update_full.pdf

18 Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol 1997 ;58(2):75-83.

19 Butler MS. Natural products to drugs: natural product derived compounds in clinical trials. Nat Prod Rep 2005 ;22(2):162-195.

20 http://www.who.int/malaria/world_malaria_report_2010/en/index.html

21 Kayser O, Kiderlen AF, Croft SL. Natural products as potential antiparasitic drugs. : Amsterdam: Elsevier Science B.V; 2002. p. 779-848.

22 Rastogi T, Hildesheim A, Sinha R. Opportunities for cancer epidemiology in developing countries. Nat Rev Cancer 2004 ;4(11):909-917.

23 Noble RL. The discovery of the vinca alkaloids--chemotherapeutic agents against cancer. Biochem Cell Biol 1990 ;68(12):1344-1351.

24 Nadkarni MV, Hartwell JL, Maury BP, Leiter J. Components of Podophyllin. XI. Isolation of Two New Compounds from Podophyllum emodi Wall. J Am Chem Soc 1953;75(6):1308-1312.

25 Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol 2005;100(1-2):72-79.

26 Dy GK, Bekele L, Hanson LJ, Furth A, Mandrekar S, Sloan JA, et al. Complementary and alternative medicine use by patients enrolled onto phase I clinical trials. J Clin Oncol 2004 1;22(23):4810-4815.

27 Morse MA, Stoner GD. Cancer chemoprevention: principles and prospects. Carcinogenesis 1993 ;14(9):1737-1746.

28 Schwartsmann G. Anticancer drugs from nature. Medical and pediatric oncology 2001;37(1):79-80.

29 Cragg GM, Newman DJ. Discovery and development of antineoplastic agents from natural sources. Cancer Invest 1999;17(2):153-163.

30 Amador ML, Jimeno J, Paz-Ares L, Cortes-Funes H, Hidalgo M. Progress in the development and acquisition of anticancer agents from marine sources. Ann Oncol 2003 ;14(11):1607-1615.

31 Mayer AMS, Gustafson KR. Marine pharmacology in 2000: antitumor and cytotoxic compounds. Int J Canc 2003;105:291-299.

32 Mayer AMS, Lehmann VK. Marine pharmacology in 1999: Antitumor and cytotoxic compounds. Anticancer Research 2001;21:2489-2500.

33 Nuijen B, Bouma M, Manada C, Jimeno JM, Schellens JH, Bult A, et al. Pharmaceuiticaldevelopment of anticancer agents derived from marine sources. Anti-Cancer Drugs 2000;11(10):793-811.

34 Jirge SS, Chaudhari YS. Marine: the ultimate source of bioactives and drug. IJRAP 2010;1(1):55-62.

35 Mou X, Kesari S, Wen PY, Huang X. Crude drugs as anticancer agents. Int J Clin Exp Med 2011;4(1):17-25.

36 http://www.who.int/csr/don/2009_12_23/en/index.html

37 Vo TS, Kim SK. Potential anti-HIV agents from marine resources: an overview. Mar Drugs 2010 29;8(12):2871-2892.

38 Padia HS, Siragy HM. Renin inhibition as a new strategy to combat cardiovascular disease. Curr Hyp Rev 2007;3:39-43.

39 Carlini EA. Plants and the central nervous system. Biochem Pharmacol Beheav 2003;75(3):501-512.

40 Clement JA, Yoder BJ, Kingston DGI. Natural Products as a Source of CNS-Active Agents. Mini-Reviews in Organic Chemistry 2004;1(2):183-208.

41 Zhu D, Jiang S, Huang M, Lin L, Cordell GA. Huperserratinine from *Huperzia serrata.* Phytochemistry 1994;36(4):1069-1072.

42 Howes MJ, Perry NS, Houghton PJ. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother Res 2003 ;17(1):1-18.

43 Kilic M, Kahan BD. New trends in immunosuppression. Drugs Today (Barc) 2000;36(6):395-410.

44 Archana SJ, Paul R, Tiwari A. Indian medicinal plants: a rich source of natural immuno-modulator. Int J Pharmacol 2011; 7: 198-205.

45 Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res 2003 15;110(5-6):255-258.

46 Kovarik JM. Everolimus: a proliferation signal inhibitor targeting primary causes of allograft dysfunction. Drugs Today (Barc) 2004 ;40(2):101-109.

47 Kiefer W, Dannhardt G. Novel insights and therapeutical applications in the field of inhibitors of COX-2. Curr Med Chem 2004 ;11(24):3147-3161.

48 Brune K, Hinz B. The discovery and development of antiinflammatory drugs. Arthritis and Rheumatism 2004;50(8):2391-2399.

49 Flower RL. Non-steroidal anti-inflamatory drugs: back to the future. Rheumatology 1999;38(8):693-696.

50 Tripathi L, Tripathi JN. Role of Biotechnology in medicinal plants. Trop J Pharrm Res 2003;2(2):243-253.

51 Rischer H, OKsman-Caldentey KM. Plant Genetic Resources: Characterization and Utilization. Biotechnological utilization of plant genetic resources for the production of phytopharmaceuticals. 2005;3(3):83-89.

52 Batawa P, Ghosh SK, Bhandari P, Singh B, Ghosh DP, Ahuja PS, Mondal TK. Micropropagation of an elite line of Picrorhiza scrophulariiflora Pennell, an endangered high valued medicinal plant of the Indo-China Himalayan region. Med Aro Pla Sci Biotech 2010; 4(1): 1-7.

53 Iliana I. Optimization of flavonoid production in cell cultures of Astragalus missouriensis Nutt. (Fabaceae). Pharmacognosy magazine 2009;5(18):92-97.

54 Sharanappa P, Rai VR. Micropropagation of Thalictrum dalzellii hook. Through rhizome buds. J Phytol 2011; 3(5): 51-55.

55 DiCosmo F, Misawa M. Plant cell and tissue culture: alternatives for metabolite production. Biotechnol Adv 1995;13(3):425-453.

56 Ammar MN, Taha HS, Hefnawy MS, Afifi AH. Biotecnological and phytochemical studies on Sabal yapa Becc. growing in Egypt. J Am Sci 2011;7(2):14-20.

57 Girjashankar V. Micropropagation of multipurpose medicinal tree acacia auriculiformis. J Med Plants Res 2011;5(3):462-466.

58 Liu C, Zhao Y, Wang Y. Artemisinin: current state and perspectives for biotechnological production of an antimalarial drug. Appl Biotech 2006;72:11-20.

59 Walsh CT. Combinatorial biosynthesis of antibiotics: challenges and opportunities. Chembiochem 2002 ;3(2-3):125-134.

60 Gersh BJ, Sliwa K, Mayosi BM, Yusuf S. Novel therapeutic concepts: the epidemic of cardiovascular disease in the developing world: global implications. Eur Heart J 2010 ;31(6):642-648.

61 Tu K, Chen Z, Lipscombe LL, Canadian Hypertension Education Program Outcomes Research Taskforce. Prevalence and incidence of hypertension from 1995 to 2005: a population-based study. CMAJ 2008 20;178(11):1429-1435.

62 Padia HS, Siragy HM. Renin inhibition as a new strategy to combat cardiovascular disease. Curr Hyp Rev 2007;3:39-43.

63 Christiana R, Gunter W. Renin-Angiotensin-Aldosterone System and Progression of Renal Disease. J Am Soc Nephrol 2006;17:2985-2991.

64 Skeggs LT, Kahn JR, Lentz K, Shumway NP. The preparation, purification, and amino acid sequence of a polypeptide renin substrate. J Exp Med 1957 1;106(3):439-453.

65 Fisher ND, Allan D, Kifor I, Gaboury CL, Williams GH, Moore TJ, et al. Responses to converting enzyme and renin inhibition. Role of angiotensin II in humans. Hypertension 1994 ;23(1):44-51.

66 Galen FX, Devaux C, Atlas S, Guyenne T, Menard J, Corvol P, et al. New monoclonal antibodies directed against human renin. Powerful tools for the investigation of the renin system. J Clin Invest 1984 ;74(3):723-735.

67 Gradman AH, Kad R. Renin inhibition in hypertension. J Am Coll Cardiol 2008;51(5):519-528.

68 Korcsmaros T, Szalay MS, Bode C, Kovacs IA, Csermely P. How to design multi-target drugs: Target search options in cellular networks. Expert openion on drug discovery 2007;2:1-10.

69 Eldar-Finkelman H, Eisenstein M. Peptide inhibitors targeting protein kinases. Curr Pharm Des 2009;15(21):2463-2470.

70 Robertson JG. Enzymes as a special class of therapeutic target: clinical drugs and modes of action. Curr Opin Struct Biol 2007 ;17(6):674-679.

71 Robertson JG. Mechanistic basis of enzyme-targeted drugs. Biochemistry 2005 19;44(15):5561-5571.

72 Fidock DA, Eastman RT, Ward SA, Meshnick SR. Recent highlights in antimalarial drug resistance and chemotherapy research. Trends Parasitol 2008; 24(12):537-544.

73 Grellier P, Depoix D, Schrevel J, Florent I. Discovery of new targets for antimalarial chemotherapy. Parasite 2008 ;15(3):219-225.

74 Johnson KW, Lofland D, Moser HE. PDF inhibitors: an emerging class of antibacterial drugs. Curr Drug Targets Infect Disord 2005 ;5(1):39-52.

75 Bendall LJ. New and emerging drug targets for the treatment of hematological malignancies. Curr Drug Targets 2010 ;11(7):767-768.

76 Li L, Zhou X, Ching WK, Wang P. Predicting enzyme targets for cancer drugs by profiling human metabolic reactions in NCI-60 cell lines. BMC Bioinformatics 2010 8;11:501.

77 Croft SL, Coombs GH. Leishmaniasis--current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol 2003 ;19(11):502-508.

CHAPTER 2

Phytochemical studies on *Nauclea latifolia*

2.1 INTRODUCTION

N. latifolia, (Rubiaceae) is abundant in Africa and Asia. The plant is known by different names in Africa. For instance, it is called ovoro ilu in south east Nigeria. It is a straggling shrub or small tree with spreading branches, and could reach a height of 3.5-5 m. The tree flowers between April and June, and the fruits ripen during the months of July to September. Flowers have sweet scented heads and produce red fruits that resemble a large hard strawberry. The fruit is edible, with many seeds, and pleasant taste. This is one of the important herbal trees used in folk medicine, particularly in different parts of Africa. This plant is reported to treat various diseases that include stomach pains, fever, diarrhea, helminthic infestation, menstrual irregularities, and diabetes melitus[1-8]. In Nigeria, the bark is also used to treat cough, gonorrhea, and wounds[9, 10].

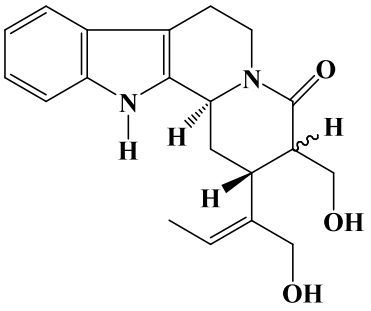
Recent studies have verified the efficacy of *N. latifolia* in some of the above mentioned ethno-medicinal applications. For instance, when albino mice infected with *Plasmodium berghei* parasites were treated with 500 mg kg^{-1} of the crude extract of *N. latifolia* root, 71.15 % suppression of parasitemia, was observed[11]. Aqueous extract infusions and decoctions from stems and roots of *N. latifolia* exhibited *in vitro* inhibitory activity against *Plasmodium falciparum* strains with IC_{50} values of 0.6-7.5 μg ml^{-1}. Abbah *et al.,* also reported that aqueous extract of *N. latifolia* root-back 50-200 mg kg^{-1} per oral, significantly increased the threshold for pain perception in mice[12].

Recent phytochemical studies have shown that majority of the biological activities exhibited by *N. latifolia* are mainly due to the presence of high content of monoterpene

indol alkaloids, tannins, and terpenoids[13,14]. These compounds have been shown to produce different biological activities including anti-parasitic[15,16], anti-helminthic[17], anti-microbial[18-24], anti-diabetic[25], anti-hypertensive[26], and neuropharmacologic activities[27,28].

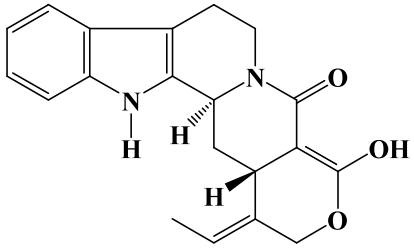
2.1.1 Previously Reported compounds from *Nauclea spp.*

Previous phytochemical studies on the genus *Nauclea* have resulted in the isolation of a number of compounds[29,30]. For instance, five compounds, naucleamides A—E (**36-40)**, monoterpene indole alkaloids were isolated from the bark and wood of *N. latifolia*[13].

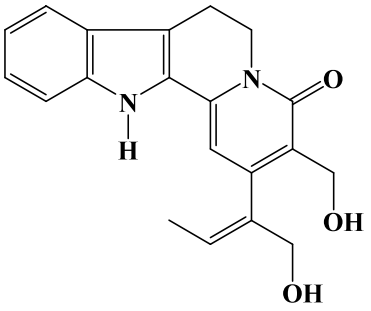


36 H-16α

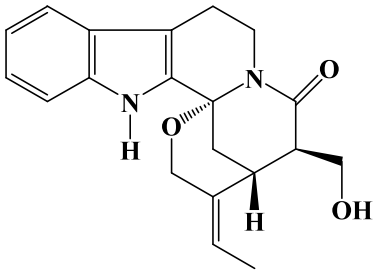
37 H-16β



38

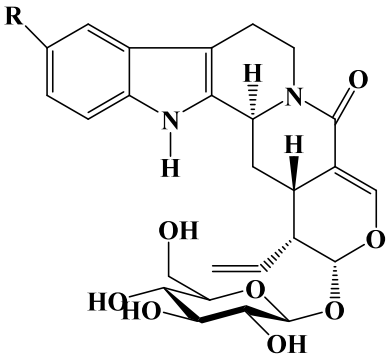


39

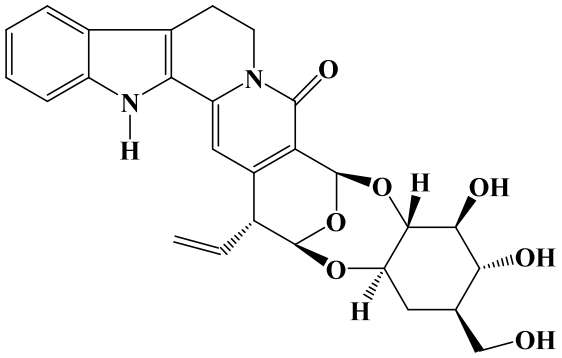


40

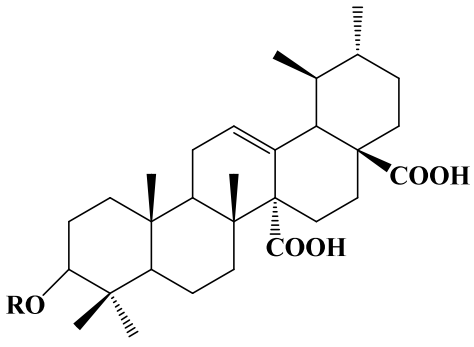
Ata *et al.*, 2009 discovered five known compounds: strictosamide (**41)**, naucleamides A (**36)**, naucleamide F (**42)**, quinovic acid-3-*O*-β-rhamnosylpyranoside (**43)**, and quinovic acid 3-*O*-β-fucosylpyranoside (**44)**, from the crude ethanolic extract of *N. latifolia*. These compounds exhibited GST inhibitory activity with IC_{50} values in the range of 20.5-143.8 μM. Among all the isolated compounds, strictosamide (**41**) was significantly active in this bioassay with an IC_{50} value of 20.5 μM. The bioactivity data of **41,** was more or less comparable to ethacrynic acid, a standard GST inhibitor (IC_{50} = 16 μM)[31].



41



42



43 R= α-Rhamnose

44 R= β- Fucose

Given the wide range of reported biological activities of the crude extracts of this plant, the present project was designed to isolate pure natural products from *N. latifolia*, and to evaluate their renin inhibitory activity, as this plant has been reported to exhibit anti-hypertensive properties[26]. These efforts resulted in the isolation of seven new indole alkaloids, namely: latifoliamede A-D (**46-52)**, along with a known alkaloid, angustoline (**45)**. These compounds (**45-52**), were characterized with the help of extensive NMR and mass spectral studies. In this chapter, isolation and structure elucidation of compounds (**45-52**) are discussed.

2.2 EXPERIMENTAL

2.2.1 General conditions

UV spectra were recorded in methanol on a Shimadzu UV-2501, and Shimadzu UV2UO PC spectrophotometer. IR spectra were acquired on a Varian 1000 FT-IR (Scimitar Series). ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectra (COSY, HSQC, HMBC, NOESY) spectra were recorded on a Bruker Avance 400 MHz spectrometer using deuterated solvents as reference. Mass spectrometer studies were carried out on Varian MAC 112 mass spectrometer. Column chromatography was carried out using 60 Å silica gel (230-400 mesh), as the stationary phase. Thin layer chromatography (TLC), was performed on silica gel 60 F_{254} aluminum-backed TLC plates (Merck, Germany). Developed TLC plates were visualized under UV light using Entela multiband (UV-254/366nm) lamp. All solvents used in the research project were of ACS reagent grade,

and were purchased from Caledon Laboratories Canada. NMR solvents were purchased from Cambridge Isotope Laboratories Canada.

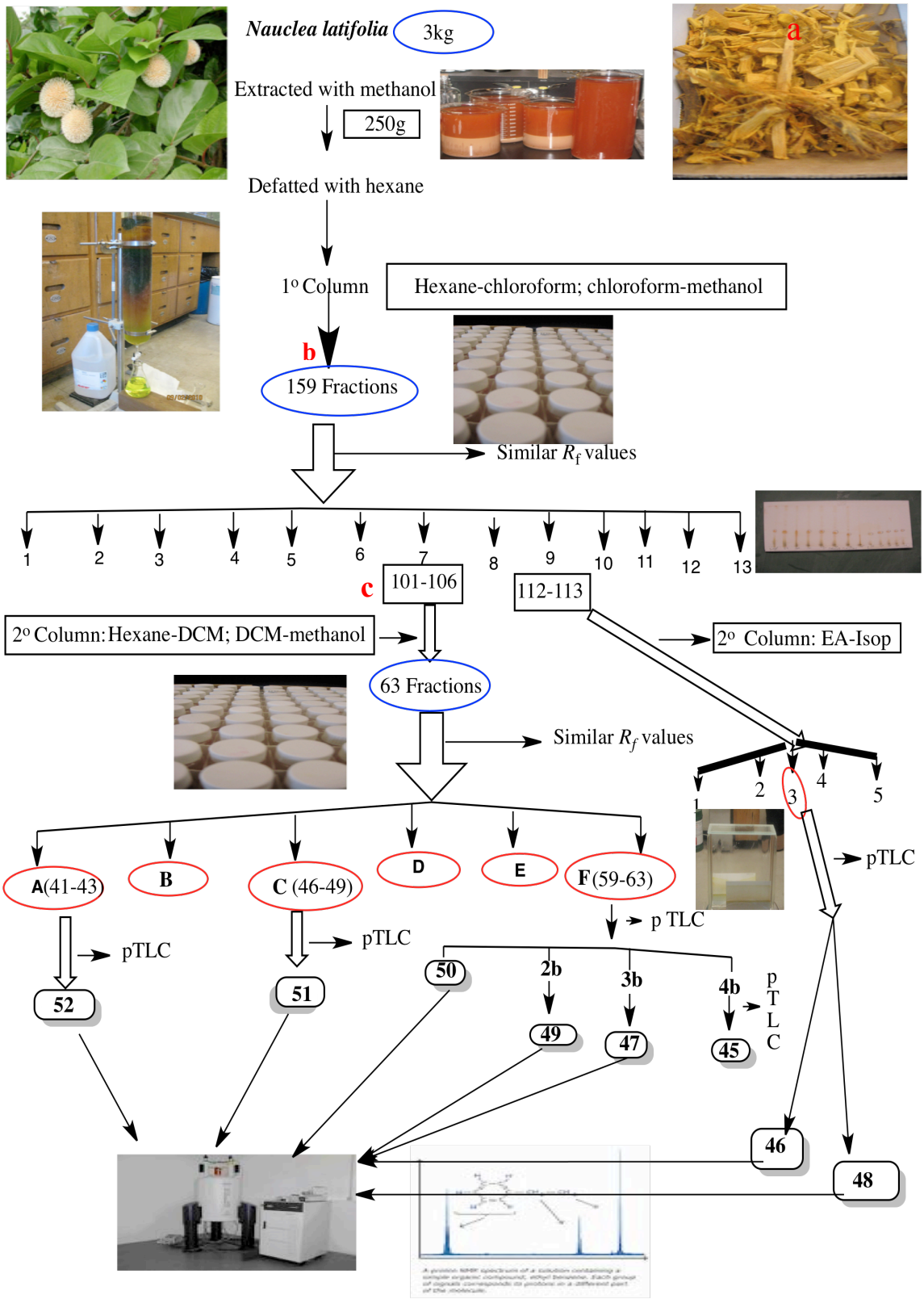
2.2.2 Plant Material

N. latifolia was collected from Mbaise, Imo state, Nigeria in September 2009. This plant was identified by Dr. Biodun Ayodele, Department of Botany, University of Ibadan, Nigeria. A voucher specimen (BDCP037) had already been deposited in the herbarium of the Biotechnology development and conservation program (BDCP) research centre, Nsukka, Nigeria.

2.2.3 Extraction and Isolation of Compounds (45-52)

The stem-bark of *N. latifolia* (3.0 kg) was air dried and cut into smaller pieces (Scheme 2.1a). These small pieces were extracted with methanol for three times at room temperature. This extract was filtered and rotavaped to afford 250 g yellow colored gum. This gum was loaded onto a silica gel column. This column was eluted with hexane-chloroform (0-100), and chloroform-methanol (0-100). This yielded 159 fractions (Scheme 2.1b), which were pooled on the basis of similar R*f* values on analytical TLC, to afford 13 fractions. Combined fractions 101-106 obtained on elution of silica gel column with methanol-chloroform (5:95, 4.98 g), was again loaded onto a silica gel column (secondary column). This column was eluted with hexane-dichloromethane (10:90), and dichloromethane-methanol (99.9:0.1), to afford 63 fractions which were combined on the basis of similar R*f* values into 6 fractions (A-F). Preparatory TLC (prep. TLC) of combined secondary fractions 59-63 (F) (125.7mg) obtained on elution of the secondary column with methanol-dichloromethane (2:98) using methanol-chloroform (7: 93) as a

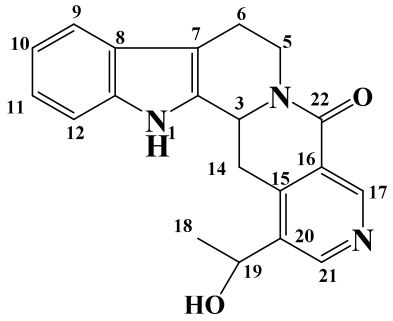
mobile phase yielded compound (**50,** 14.6 mg), and impure bands: 2b(20.9 mg), 3b(13.5mg), 4b(16.4 mg). Compound (**49**), 6.4 mg was purified by the prep. TLC of impure band 2b using isopropanol-chloroform (7:93) as a mobile phase. Band 4b was again subjected to prep. TLC using a mixture of isopropanol-chloroform (6:94), in order to purify compound (**45,** 8.0 mg). The compound (**47),** 3.8 mg, was obtained by the Prep. TLC of band 3b using isopropanol- chloroform (6:94) as mobile phase. Prep TLC of secondary fractions 46-49 (C) obtained on elution of the secondary column, methanol-dichloromethane (2:98), and using isopropanol-chloroform (3:97), yielded compound (**51,** 3.2 mg), while prep. TLC of secondary fraction 41-43(A), using isopropanol-chloroform (3:97), yielded compound (**52**, 2.1 mg). Fractions 112-113 (1.6 g) of the primary column fractions, methanol-chloroform (8: 92) was loaded onto a silica gel secondary column and eluted with ethyl acetate-isopropanol (95:5) as mobile phase. Five fractions were collected. Prep. TLC of fraction 3 (50 mg) obtained on elution of the silica gel column with, ethylacetate-isopropanol (95:5) using methanol-dichloromethane (3.5:96.5) as mobile phase, yielded compounds (**46,** 8.8 mg) and (**48,** 15 mg).



Scheme 2.1 Flow chart of extraction process.

2.3 STRUCTURE ELUCIDATION OF COMPOUNDS (45-52)

2.3.1 Angustoline (45)



45

Compound (**45**) was isolated as a yellow coloured gum. Its IR spectrum displayed intense absorption at 3350 (OH), 2950 (CH), 1631 (amide carbonyl) cm^{-1}. The UV spectrum of **45** showed absorption maxima at 225 and 293 nm. The high-resolution electron-impact mass spectrum (HREIMS) of **45** showed a molecular ion peak, m/z at 333.1475, which was in agreement with molecular formula $C_{20}H_{19}N_3O_2$ (calcd. 333.1477). The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz, **Fig**. A-22) of **45** showed resonances at δ 7.57 (d, *J* = 7.2 Hz), 7.24 (dt, *J* = 8.0, 7.2 Hz), 7.19 (dt, *J* = 8.0, 8.0 Hz) and 7.39 (d, *J* = 8.0 Hz) due to the C-9, C-10, C-11, and C-12 protons respectively. The multiplicity pattern of these signals indicated the presence of *ortho*-substituted benzene moiety in this compound. A combination of ^{1}H- NMR and UV spectral data suggested that compound (**45**) had an indole like structure. The C-5 and C-6 methylene protons resonated at δ 5.35, 3.03 and 3.99, 2.99 respectively. A signal at δ 1.53 was ascribed to the C-18 methyl protons.

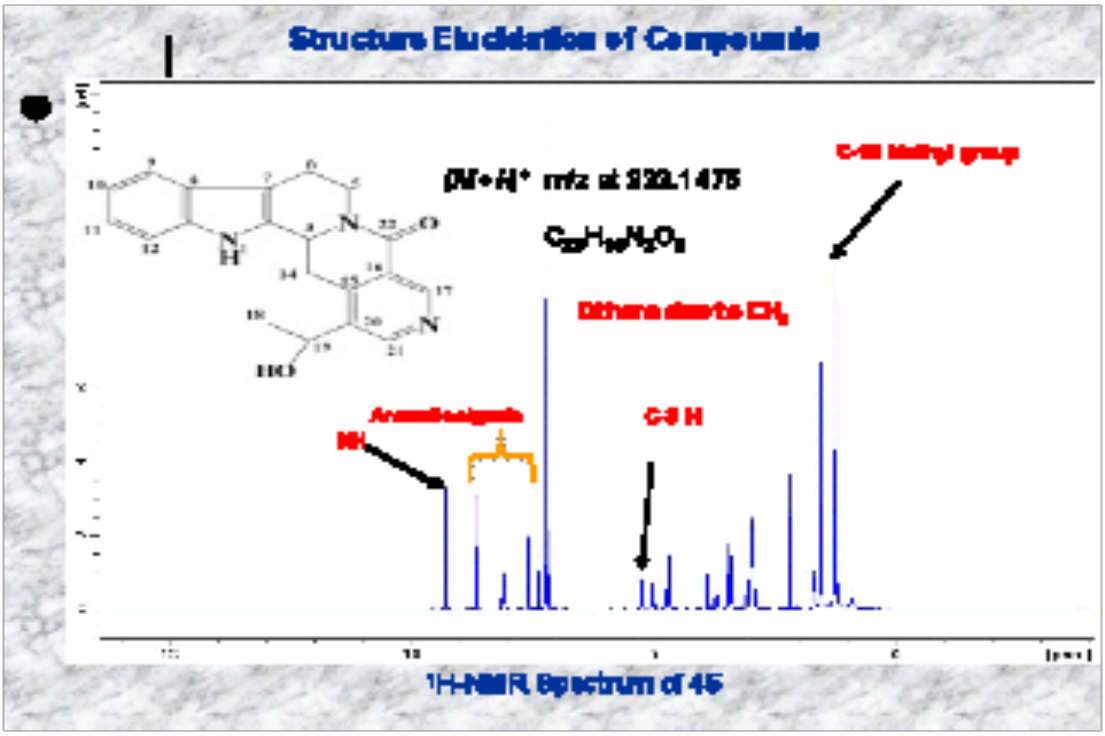


Figure 2.10 ^{1}H-NMR Spectrum of **45**

The COSY-45^{o} spectrum of **45 (Fig**. A-26) showed the presence of four isolated spin systems "a-d". The first spin system "a" was due to the presence of an *ortho*-substituted benzene group. The H-9 (δ 7.57) showed cross-peaks with H-10 (δ 7.24), which in turn exhibited vicinal coupling with H-11 (δ 7.19). The later displayed ^{1}H-^{1}H spin correlations with H-12 (δ 7.39). The second spin system "b" comprised of H-5 (δ 5.35 and 3.03) and H-6 (δ 3.99 and 2.99). Both of these methylene protons exhibited vicinal couplings in the COSY-45^{o} spectrum. The spin system "c" started with H-3 (δ 5.60) that showed cross-peaks with H-14 (δ 3.46 and 1.55). The spin system "d" started with H-18 (δ 1.53) and showed cross-peaks with H-19 (δ 4.70). The spin systems "a-d" are shown in Fig. 2.1.

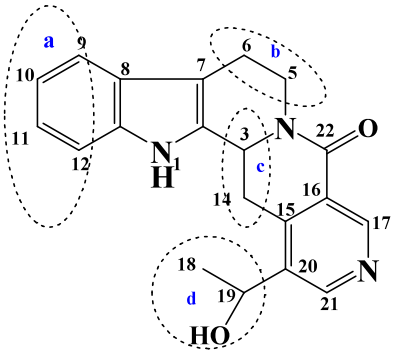


Figure 2.11 Spin system "a-d" of **45,** as deduced from the COSY 45° spectrum.

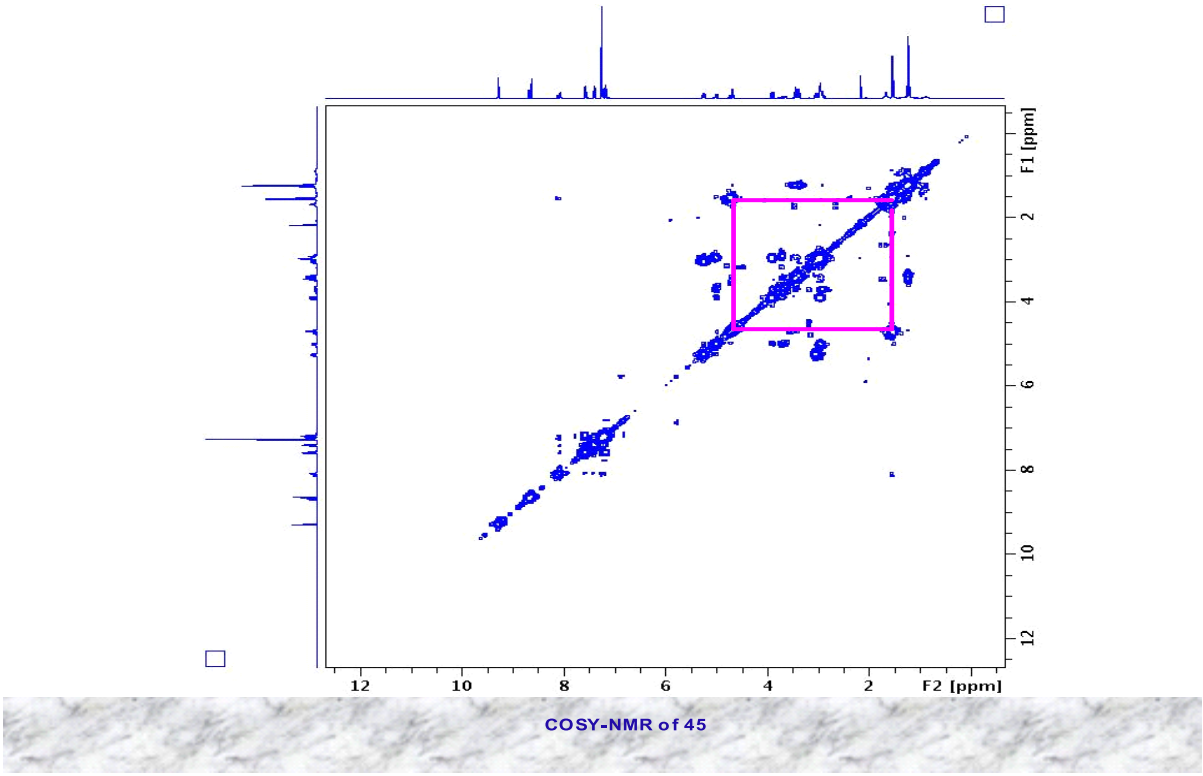


Figure 2.12 COSY 45° spectrum of **45**

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz, **Fig**. A-23) of **45** showed the resonance of all twenty carbons. A combination of DEPT (Figs. A-24, 25) and broad-band ^{13}C-NMR spectral data of **45**, revealed the presence of eight methine, three methylene, one methyl and eight quaternary carbons. Complete ^{1}H and ^{13}C one-bond shift correlations of **45** as determined from HSQC spectrum (**Fig**.A-27), and complete ^{13}C-NMR chemical shift assignment of **45** are shown in Table-2.1

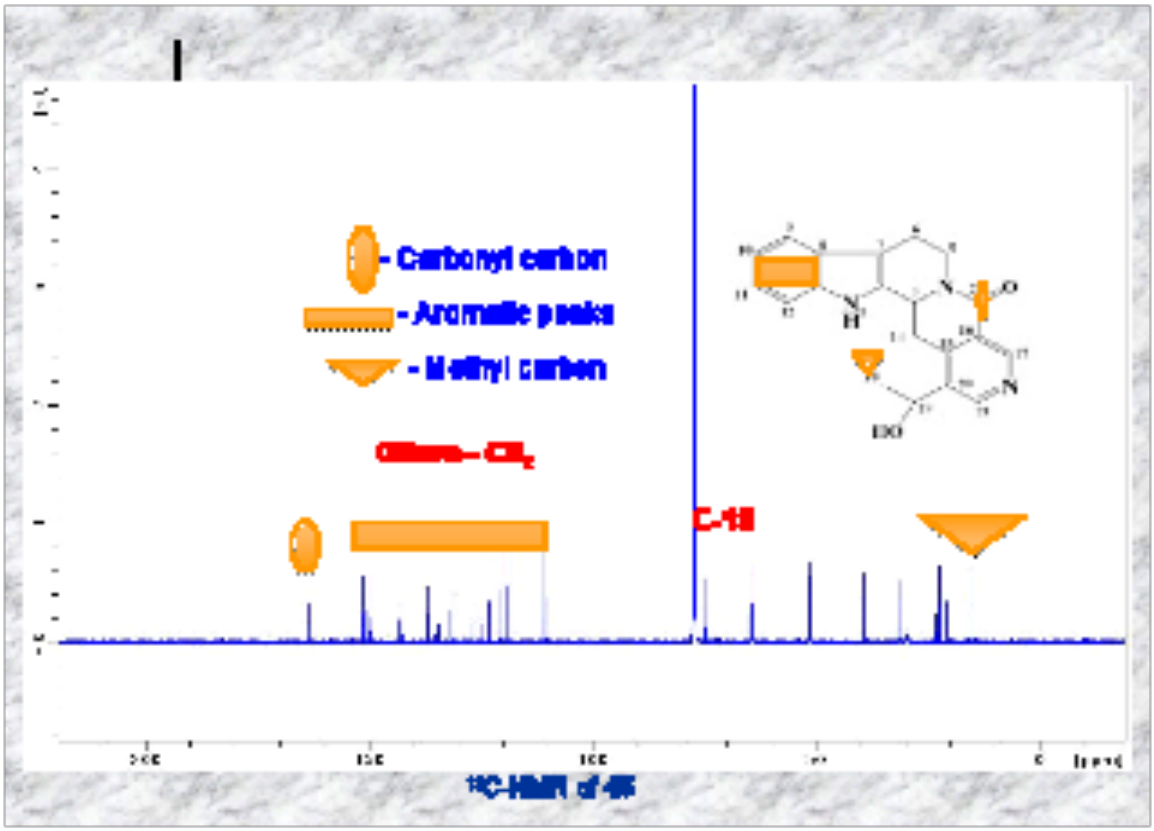


Figure 2.13 ^{13}C-NMR spectrum of **45**

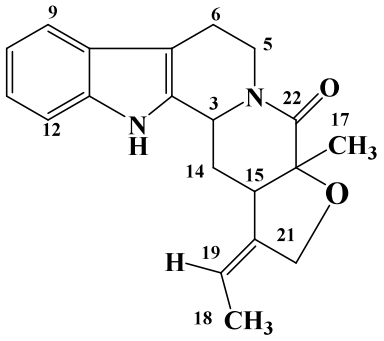
Table 2.1. ^{1}H and ^{13}C-NMR data and $^{1}H/^{13}C$ one-bond shift correlations of **45** as determined by HSQC spectrum

Carbon No.	**^{1}H-NMR**	**^{13}C-NMR**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	131.5 (C)
3.	5.60	51.2 (-CH-)
4.	-	-
5.	5.35	39.4 (CH_2)
	3.03	
6.	3.99	20.8 (CH_2)
	2.99	
7.	-	110.3 (C)
8.	-	126.6 (C)
9.	7.57, d, (7.2)	120.2 (CH)
10.	7.24, dt, (8.0, 7.2)	118.6 (CH)
11.	7.19, dt, (8.0, 8.0)	111.1 (CH)
12.	7.39, d, (8.0)	122.7 (CH)
13.	-	136.6 (C)
14.	3.46	29.7 (CH_2)
	1.55	
15	-	142.0 (C)
16.	-	124.7 (C)
17.	8.60	149.7 (CH)
18.	1.53, d, (6.7)	15.4 (CH_3)
19.	4.70	74.5 (C)
20.	-	136.6 (C)
21.	9.30	150.4 (CH)
22.	-	163.4 (C)

[a]Multiplicity was determined by DEPT spectrum.

The ^{1}H and ^{13}C NMR data of **45** was nearly identical to those of angustoline, reported in the literature[32,33]. Based on these spectral data, compound (**45**) was characterized as angustoline.

2.3.2 Latifoliamide A (46)



46

Latifoliamide A (**46)** was purified as a yellow colored gum. Its IR spectrum displayed intense absorption at 3529 (NH) and 3120 (CH) cm^{-1}. The UV spectrum showed absorption maxima at 221 and 288 nm. Its molecular formula $C_{20}H_{22}N_2O_2$, (calcd. 332.1681), was provided by HREIMS that exhibited molecular ion peak at m/z 332.1678. The ^{1}H-NMR spectrum of **46** ($CDCl_3$, 400 MHz, **Fig**. A-30), showed resonances of *ortho*-substituted benzene group at δ 7.47 (d, *J* =7.6 Hz), 7.13(dd, *J* = 8.0, 7.6 Hz), 7.10 (dd, *J* = 8.0, 7.9 Hz), and 7.31 (d, *J* = 7.9 Hz), due to the C-9, C-10, C-11, and C-12 protons respectively. A combination of ^{1}H-NMR and UV spectral data suggested that compound (**46**) had indole alkaloid like structure. Two resonances in the ^{1}H-NMR spectrum of **46,** δ 4.99, 3.0 and 2.90, 2.70 were due to the C-5 and C-6 methylene protons respectively. The C-18 methyl and C-19 methine protons appeared at δ 1.48 and 5.54, respectively. The COSY-45^o spectrum (**Fig**. A-34) of **46** showed the presence of four isolated spin systems "a-d". The spin system "a" represented an *ortho*-substituted benzene group. In this spin system, H-9 (δ 7.47) showed cross-peaks with H-10 (δ 7.13) which in turn exhibited vicinal coupling with H-11 (δ 7.10). The later displayed ^{1}H-^{1}H

spin correlations with H-12 (δ 7.31). The spin system "b" was due to the H_2-5 (δ 4.99 and 3.00) and H_2 -6 (δ 2.90 and 2.70), as they exhibited vicinal couplings in the COSY spectrum. The third spin system "c" started with H-3 (δ 3.0) that showed cross-peaks with H_2-14 (δ 2.50 and 2.30), which in turn exhibited vicinal coupling with H -15 (δ 2.45). The spin system "d" was due to the cross-peaks between H-19 (δ 5.4) and H_3-18 (δ 1.48). All of these spin systems are labeled in Fig. 2.2.

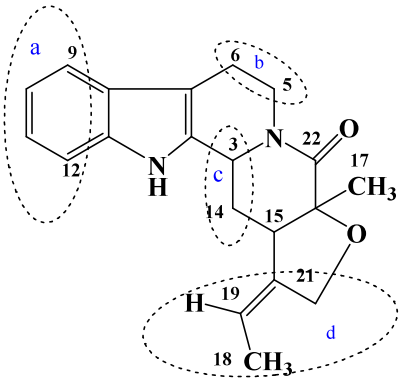


Figure 2.14 Spin system "a-d" of compound (**46**), as deduced from the COSY 45° spectrum.

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz **Fig**. A-31) of **46** showed the resonance of all twenty carbons. A combination of DEPT (**Figs**. A-32, 33) and broad-band ^{13}C-NMR spectrum of **46**, revealed the presence of seven methine, four methylene, two methyl and seven quaternary carbons in **46**. Complete ^{1}H and ^{13}C one-bond shift-correlation of **46** as determined from HSQC (**Fig**. A-35) spectrum and complete ^{1}H, and ^{13}C-NMR chemical shift assignment of **46** are shown in Table-2.2.

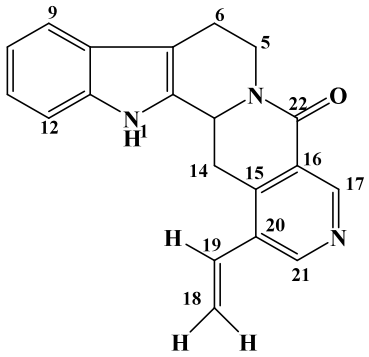
Table 2.2. ^{1}H and ^{13}C-NMR chemical shift assignment of **46** and $^{1}H/^{13}C$ one-bond shift-correlations as determined by HSQC

Carbon No.	**^{1}H-NMR**	**^{13}C-NMR**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	138.2 (C)
3.	3.00	44.2 (CH)
4.	-	-
5.	4.99	
	3.00	46.1 (CH_2)
6.	2.90	
	2.70	29.6 (CH_2)
7.	-	126.8 (C)
8.	-	127.2 (C)
9.	7.47, d (7.6)	126.8 (CH)
10.	7.13, dd, (8.0, 7.6)	120.0 (CH)
11.	7.10, dd, (8.0, 7.9)	122.2 (CH)
12.	7.31, d, (7.9)	111.1 (CH)
13.	-	136.1 (C)
14.	2.50	
	2.30	21.3 (CH_2)
15.	2.45	35.3 (CH)
16.	-	66.1 (C)
17.	1.80	29.4 (CH_3)
18.	1.48, d, (6.9)	20.9 (CH_3)
19.	5.54, d, (7.8)	126.8(C)
20.	-	132.7 (C)
21.	4.10	
	4.20	62.1 (CH_2)
22.	-	173.7 (C)

[a]Multiplicity of each carbon was determined by DEPT spectrum.

These spectral studies led us to propose structure (**46),** for this new alkaloid.

2.3.3 Latifoliamide B (47)



47

Latifoliamide B (**47)** was separated as a yellow colored solid. Its IR spectrum displayed intense absorption at 3276 (NH), 2925 (CH), and 1628 (amide carbonyl) cm^{-1}. The UV spectrum showed absorption maxima at 221 and 293 nm. The HREIMS of **47,** showed molecular ion at m/z 315.1376, that provided its molecular formula $C_{20}H_{17}N_3O$ (calcd. 315.1372)

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz, **Fig**. A-15) of **47** showed the resonance of H-9, H-10, H-11, and H-12 at δ 7.55 (d, *J* = 7.8 Hz), 7.15 (dd, *J* = 7.8, 8.1 Hz), 7.20 (dd, *J* = 8.1, 7.9 Hz) and δ 7.37 (d, *J* = 7.9 Hz), respectively. Furthermore, the coupling constant data suggested the presence of *ortho*-substituted benzene ring in **47**. These ^{1}H-NMR spectral data along with the UV spectra indicated this compound was having indole like structure as those of previously discussed compounds (**45**) and (**46**)**.** The C-5 and C-6 methylene protons resonated at δ 5.21, 2.78, and 4.99 respectively. Two signals at δ 6.85 and 5.55 were due to the H-18 and H_2-19, respectively. Four isolated spin systems "a-d" were obtained by the careful interpretation COSY- 45^o spectrum of **47** (**Fig**. A-18). The first spin system "a" was due to the *ortho*-substituted phenyl group in which H-9 showed cross-peaks with H-10. H-10 exhibited vicinal coupling with H-11. The later displayed

^{1}H-^{1}H spin correlations with H-12 (δ 7.37). The second spin system "b" comprised of H-5 (δ 5.21 and 2.78) and H-6 (δ 4.99). Both of these methylene protons exhibited COSY-45^{o} interactions with each other. The spin system "c" was traced with H-3 (δ 4.55) that showed cross–peaks with H-14 (δ 3.5 and 2.94). The spin system "d" represented H_2-19 (δ 5.55 and 5.66) and H_3-18 (δ 6.85), that exhibited allylic couplings among them. All of the aforementioned spin systems are shown in Fig. 2.3.

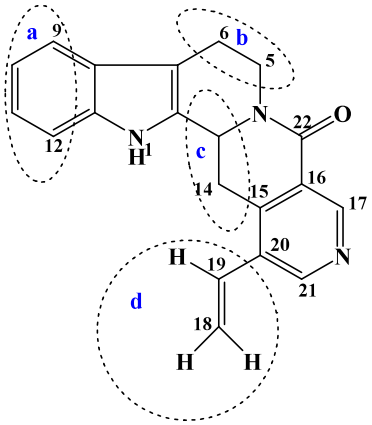


Figure 2.15 Spin system "a-d" of **47** as obtained from COSY-45^{o} spectrum.

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz, **Fig**. A-16) of **47** showed the resonance of all twenty carbons. The DEPT (Fig. A-17) and broad-band ^{13}C-NMR spectral data of this compound, revealed the presence of eight methine, four methylene, and eight quaternary carbons in this compound. Complete ^{1}H/^{13}C one-bond shift correlation of this compound as determined from HSQC spectrum (**Fig**. A-19), as well as complete ^{1}H and ^{13}C-NMR chemical shift assignment of **47** are shown in Table 2.3.

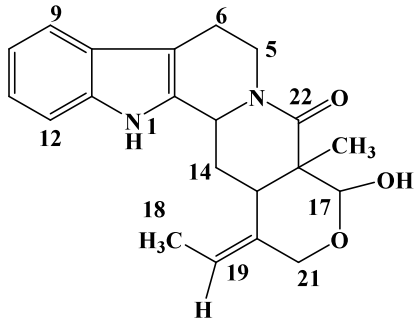
Table 2.3. Complete ^{1}H and ^{13}C-NMR chemical shift assignment and $^{1}H/^{13}C$ one-bond shift correlations of **47** as determined by HSQC

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	126.9 (C)
3.	4.55,	51.4 (CH)
4.	-	-
5.	5.21, d, 1.84	
	2.78	39.5 (CH_2)
6.	4.99	
	21.1 (CH_2)	
7.	-	111.3 (C)
8.	-	124.5 (C)
9.	7.55, d, (7.8)	119.0 (CH)
10.	7.15, dd, (7.8, 8.1)	120.4 (CH)
11.	7.20, dd, (8.1, 7.9)	120.4 (CH)
12.	7.37, d, (7.9)	110.5 (CH)
13.	-	131.7 (C)
14.	3.50, d, (3.96)	
	2.94	32.1 (CH_2)
15.	-	141.6 (C)
16.	-	119.4 (C)
17.	9.22	149.7 (CH)
18.	6.85, dd, (11.3, 17.6)	136.8 (CH)
19.	5.55d (11.3)	123.0 (CH_2)
	5.66, d, (17.6)	
20.	-	142.2 (C)
21.	8.76	150.4 (CH)
22.	-	163.7 (C)

[a]Multiplicity of each carbon was determined by DEPT experiment.

Based on these spectral studies, structure **47,** was assigned to this new alkaloid.

2.3.4 Latifoliamide C (48)



48

Latifoliamide C (**48)** was isolated as a greenish-yellow colored compound. Its IR spectrum displayed intense absorption at 3339 (OH), and 2944 (CH) cm^{-1}. The UV spectrum showed absorption maxima at 224 and 295 nm, indicating the presence of indole moiety. The HREIMS of **48** provided its molecular formula $C_{21}H_{24}N_2O_3$ (m^+ m/z 352.1783, calcd. 352.1787)

The ^{1}H-NMR spectrum ($CDCl_3$ 400 MHz, **Fig**. A-38) of **48** showed signals at δ 7.47 (d, *J* =7.7 Hz), 7.14 (dd, *J* = 7.7, 8.0 Hz), 7.03 (dd, *J* = 8.0, 7.9 Hz) and 7.30 (d, *J* = 7.9 Hz) due to the C-9, C-10, C-11, and C-12 protons of the *ortho*-substituted phenyl groups, respectively. These chemical shift values and UV spectral data were consistent with the previously discussed compounds **45-47,** and indicated the presence of indole alkaloid. Resonances at δ 4.80, 2.90 and 2.80, 2.60 were ascribed to the C-5 and C-6 methylene protons respectively. The C-18 methyl and C-19 methine protons resonated at δ 1.49 and 5.70 respectively. The COSY-45^o spectrum like previously described compounds showed the presence of four partial structures "a-d". The partial structure "a" was due to the presence of an *ortho*-substituted phenyl group as H-9 showed cross-peaks with H-10 that exhibited vicinal couplings with H-11. The later displayed COS-45^o interaction with H-

12 (δ 7.30). The second spin system "b" comprised of H-5 (δ 4.80 and 2.90) and H-6 (δ 2.80 and 2.60), as these protons exhibited COSY-45^{o} interaction with each other. The spin system "c" consisted of H-3 (δ 4.97) that showed cross-peaks with H_2-14 (δ 3.62 and 2.78). The later exhibited vicinal couplings with H-15 (δ 2.76). The spin system "d" was traced with the cross-peaks between H-19 (δ 5.70) and H_3-18 (δ 1.49). Allylic couplings of H_2-21 (δ 4.18, and 3.66), with H-19 were also observed in the COS-45^{o} spectrum of this compound.

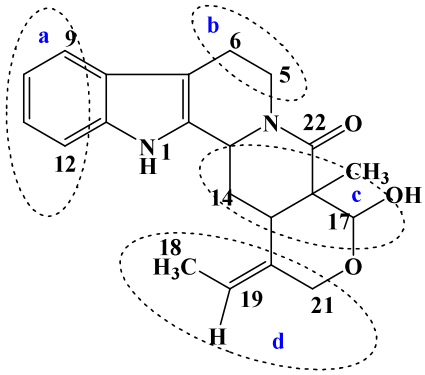


Figure 2.16 Partial structures "a-d" deduced from the COS-45^{o} spectrum of **48**.

The ^{13}C–NMR spectrum ($CDCl_3$, 100 MHz, **Fig**. A-39) of **48,** showed the resonance of all twenty one carbons. A combination of DEPT (**Figs**. A-40, 41) and broad-band ^{13}C-NMR spectra of the alkaloid revealed the presence of eight methine, four methylene, two methyl and seven quaternary carbons in it. The HSQC spectrum (**Fig**. A-42) was used to establish ^{1}H/C^{13} one-bond shift correlations of protonated carbons. In this spectrum, H-3 (δ 4.97) was found to be bonded with C-3 (δ 51.0). Similarly, H-9 (δ 7.47), H-10 (δ 7.14), H-11 (δ 7.03) and H-12 (δ 7.30) exhibited ^{1}H/C^{13} one-bond shift correlations with C-9 (δ 110.0), C-10 (δ 119.7), C-11 (δ 121.7) and C-12 (δ 111.3), respectively. Complete

$^{1}H/C^{13}$ one-bond shift correlations of all hydrogen bearing carbons in **48,** are shown in Table 2.4. Complete ^{1}H and ^{13}C-NMR chemical shift assignments are presented in Table 2.4

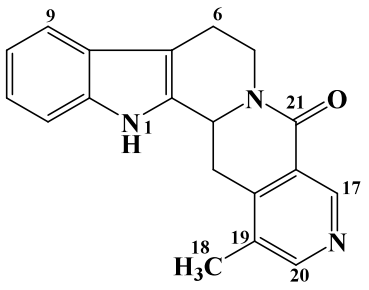
Table 2.4. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of **48** and $^{1}H/^{13}C$ one-bond shift correlations as determined from HSQC spectrum

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	132.7 (C)
3.	4.97	51.0 (CH)
4.	-	-
5.	4.80	
	2.90	92.9 (CH_2)
6.	2.80	
	2.60	21.0 (CH_2)
7.	-	110.5 (C)
8.	-	126.8 (C)
9.	7.47, d, (7.7)	110.0 (CH)
10.	7.14, dd, (7.7, 8.0)	119.7 (CH)
11.	7.03, dd, (8.0, 7.9)	121.7 (CH)
12.	7.30, d, (7.9)	111.3 (CH)
13.	-	136.3(-C-)
14.	3.62	
	2.78	29.5 (CH_2)
15.	2.76	31.1 (CH)
16.	2.76	46.2 (CH)
17.	9.97	92.9 (CH)
18.	1.49, d, (6.9)	12.7 (CH_3)
19.	5.70, q, (6.9)	130.8 (C)
20.	-	138.5 (C)
21.	4.18	
	3.66	65.7 (CH_2)
22.	-	174.0 (C)

[a]Multiplicity of each carbon was determined by DEPT spectrum.

These spectral studies led us to propose structure (**48**) for this new alkaloid.

2.3.5 Latifoliamide D (49)



49

Compound (**49)** was isolated as a yellow colored gum. Its IR spectrum displayed intense absorption at 3319 (NH), 2941(CH), and 1670 (amide carbonyl) cm^{-1}. The UV spectrum of **49** showed absorption maxima at 220 and 289 nm, indicating the presence of indole chromophore. The molecular formula, $C_{19}H_{17}N_3O$ (calcd. 303.1374) by the HREIMS, M+ m/z 303.1377.

The ^{1}H-NMR spectrum (DMSO, 400 MHz, **Fig**. A-9) of **49** showed signals at δ 7.48 (d, *J* = 7.6 Hz), 7.03 (dt, *J* = 7.6, 7.0 Hz), 7.12 (dt, *J* = 8.1, 7.0 Hz) and 7.39 (d, *J* = 8.1 Hz) due to the C-9, C-10, C-11, and C-12 protons. The multiplicity pattern indicated the presence of *ortho*-substituted benzene moiety in **49**. These signals along with the UV spectral data also suggested that compound (**49**) had indole-like structure. The C-5 and C-6 methylene protons resonated at δ 5.05, 2.86 and 3.00, 1.56 respectively. A three proton singlet at δ 2.0 was due to the C-18 methyl proton. The COS-45^o spectrum (**Fig**. A-9) of **49** revealed the presence of three partial structures "a-c". The first partial structure "a" was due to the *ortho*-substituted phenyl moiety. H-9 (δ 7.48) of partial structure "a" showed vicinal couplings with H-10 (δ 7.03) which in turn exhibited vicinal coupling with H-11 (δ 7.12). The later displayed ^{1}H-^{1}H spin correlations with H-12 (δ 7.39). The

second partial structure "b" comprised of H_2-5 (δ 5.05 and 2.86) and H_2-6 (δ 3.00 and 1.56). Both of these methylene protons exhibited vicinal couplings with each other. The third partial structure "c" started with H-3 (δ 5.05) that showed cross-peaks with H_2-14 (δ 3.80 and 2.98).

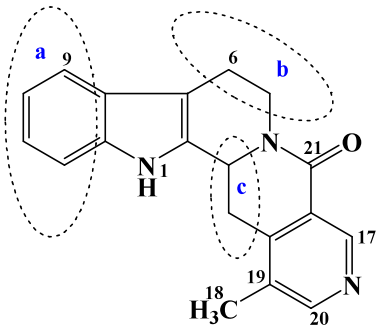


Figure 2.17 Partial structures "a-c" obtained from the COSY 45° spectrum of **49**

The ^{13}C–NMR spectrum (DMSO, 100 MHz, Fig. A-10) of **49** showed the resonance of all nineteen carbons. A combination of DEPT (**Fig**. A-11) and broad-band ^{13}C-NMR spectral data of **49**, suggested the presence of seven methine, three methylene, one methyl and eight quaternary carbons. The HSQC experiment (**Fig**. A-13) was performed to establish ^{1}H and ^{13}C one-bond correlations of all hydrogen-bearing carbons. Complete ^{1}H and ^{13}C one-bond shift correlation of **49** as determined from HSQC spectrum and complete ^{1}H and ^{13}C-NMR chemical shift assignment of **49** are shown in Table-2.5

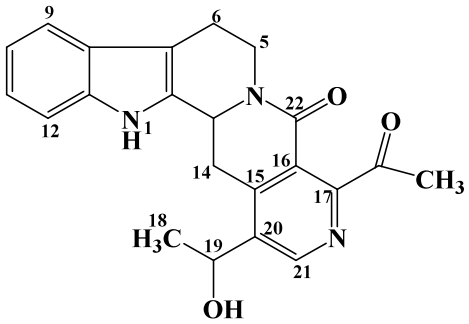
Table 2.5. Complete ^{1}H and ^{13}C-NMR chemical shift assignment as well as $^{1}H/^{13}C$ one-bond shift correlations of **49** as determined from HSQC spectrum

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	126.5 (C)
3.	5.05	51.2 (CH)
4.	-	-
5.	5.05	
	2.86	40.2 (CH_2)
6.	3.00	
	1.56	20.9 (CH_2)
7.	-	111.6 (C)
8.	-	152.2 (C)
9.	7.48, d, (7.6)	119.1 (CH)
10.	7.03, dt, (7.6, 7.0)	110.5 (CH)
11.	7.12, dt, (8.1, 7.0)	126.5 (CH)
12.	7.39, d, (8.1)	118.3 (CH)
13.	-	133.6 (-C-)
14.	3.80	31.1 (CH_2)
	2.98	
15.	-	133.6 (C)
16.	-	110.5 (C)
17.	8.90	152.2 (CH)
18.	2.0	25.30 (CH_3)
19.	-	110.5 (CH)
20.	8.56	143.9 (-CH-)
21.	-	163.4 (C)

[a]Multiplicity was determined by DEPT experiment.

Based on these spectral studies, structure **(49)** was established for this new alkaloid.

2.3.6 Latifoliamide E (50)



50

Latifoliamide E (**50**) was purified as a yellow colored gum. Its IR spectrum displayed intense absorption at 3283 (OH), 2922 (CH), and 1615 (amide carbonyl) cm^{-1}. The UV spectrum showed absorption maxima at 223 and 290 nm, indicating the presence of indole functionality. Its molecular formula, $C_{22}H_{23}N_3O_3$ (calcd. 377.1739), was established by HRIES that showed molecular ion peak at m/z 377.1742.

The ^{1}H-NMR spectrum (DMSO, 400 MHz, **Fig**. A-1) of **50** showed signals at δ 7.38 (d, *J* = 7.6 Hz), 7.04 (dd, *J* = 8.0, 7.6 Hz), 6.99 (dd, *J* = 8.0, 8.0 Hz), 7.33 (d *J* = 8.6 Hz), due to the C-9, C-10, C-11, and C-12 protons of *ortho*-substituted phenyl moiety. These ^{1}H-NMR signals along with UV spectral data suggested the indol-like structure of **50**. The C-5 and C-6 methylene protons resonated at δ 2.95, 4.78 and 2.95, 3.80, respectively. The C-18 methyl and C-19 methine protons resonated at δ 1.46 and, 5.41 respectively. The COS-45^o spectrum of **50** (**Fig**. A-5) showed the presence of four isolated spin systems "a-d" in this new alkaloid. The first spin system "a" was due to the presence of an *ortho*-substituted phenyl group. H-9 (δ 7.38) of spin system "a" showed cross-peaks with H-10 (δ 7.04) which in turn exhibited vicinal coupling with H-11 (δ 6.99). The cross-peaks

between H-11 and H-12 (δ 7.33) were also observed in the COSY-45° spectrum. The second spin system "b" was comprised of H_2-5 (δ 2.95, 4.78) and H_2-6 (δ 2.95 and 3.80) as both of these methylene protons showed vicinal couplings. The spin system "c" started with H-3 (δ 5.29) that showed cross-peaks with H_2-14 (δ 2.96 and 3.31). The spin system "d" was observed in the COSY-45° spectrum as H-19 (δ 5.41) showed cross-peaks with each other.

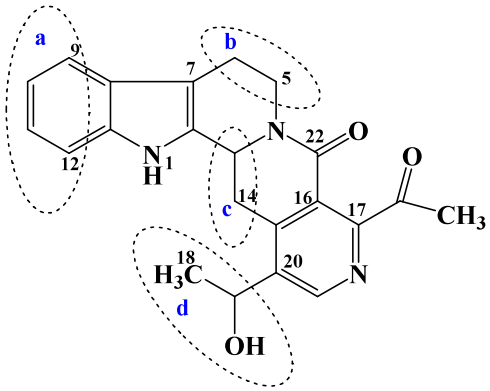


Figure 2.18 Spin systems "a-d" of compound (**50)** obtained from the COSY-45° spectrum.

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz) of **50** (**Fig**. A-2) showed the resonance of all twenty two carbons. The C-22 amide carbonyl resonated at δ 168.0. The C-19 resonated at δ 60.5. A combination of DEPT (**Figs**. A-3,4) and broad-band ^{13}C-NMR spectral data of **50**, revealed the presence of seven methine, three methylene, two methyl and ten quaternary carbons. The HSQC experiment (Fig. A-6) helped to establish ^{1}H/^{13}C one-bond shift correlations. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of **50** and ^{1}H/^{13}C one-bond shift correlations of **50** as determined from HSQC spectrum are shown in Table-2.6.

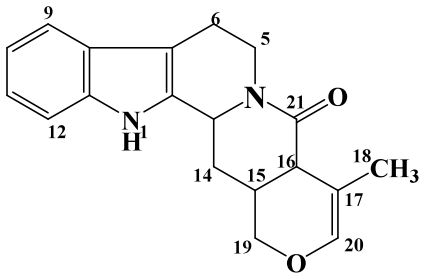
Table 2.6. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of **50** and $^{1}H/^{13}C$ one-bond shift correlations as determined from HSQC spectrum

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	126.9 (C)
3.	5.29	53.6 (CH)
4.	-	-
5.	2.95	
	4.78	42.3 (CH_2)
6.	2.95	
	3.80	30.6 (CH_2)
7.	-	111.2 (C)
8.	-	126.9 (C)
9.	7.38, d, (7.6)	124.5 (CH)
10.	7.04, dd, (8.0, 7.6)	117.5 (CH)
11.	6.99, dd, (8.0, 8.0)	118.5 (CH)
12.	7.33, d, (8.6)	114.2 (CH)
13.	-	134.8 (C)
14.	2.96	
	3.31	30.6 (CH_2)
15.	-	135.8 (C)
16.	-	119.4 (C)
17.	-	134.9 (C)
18.	1.46	11.5 (CH_3)
19.	5.41	60.5 (CH)
20.	-	136.4 (C)
21.	8.5	120.8 (CH)
22.	-	168.0 (C)
$CH_3C=O$	2.03	20.6
$CH_3C=O$	-	206.0 (C)

[a]Multiplicity was determined by DEPT spectrum.

A combination UV, MS, ^{I}H, ^{13}C, COSY HSQC and HMBC spectral data led us to assign structure (**50**) to this new alkaloid.

2.3.7 Latifoliamide F (51)



51

Latifoliamide F **(51)** was obtained as a yellow colored compound. Its IR spectrum displayed intense absorption at 3338 (NH), 2945 (CH) and 1654 (amide carbonyl) cm^{-1}. The UV spectrum showed absorption maxima at 221 and 289 nm, indicating the presence of indole nucleus. Its HREIMS displayed a molecular ion peak at m/z 308.1527 that provided molecular formula $C_{19}H_{20}N_2O_2$ (calcd. 308.1525).

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz, **Fig**. A-50) of **51** showed signals at δ 7.45 (d, *J* = 8.0 Hz), 7.26 (dt, *J* = 8.0, 7.96 Hz) 7.14 (dt, *J* = 8.0, 7.96 Hz) and 7.52 (d, J = 7.8 Hz) due to the C-9, C-10, C-11, and C-12 protons of the *ortho*-substituted phenyl moiety. These ^{1}H-NMR data along with UV spectral data suggested that compound (**51**) had indole-like structure. Signals at δ 5.06, 2.60 and 2.96, 2.46 were due to the C-5 and C-6 methylene protons respectively. The C-18 methyl protons resonated at δ 1.35. The COS-45^o spectrum of **51** (**Fig**. A-54) helped to establish partial structures "a-c". The first partial structure "a" was due to the presence of an *ortho*-substituted benzene group. H-9 (δ 7.45) of partial structure "a" showed cross-peaks with H-10 (δ 7.26) which in turn exhibited vicinal coupling with H-11 (δ 7.14). The later displayed ^{1}H-^{1}H spin correlation with H-12 (δ 7.52). The second partial structure "b" was consisted of H_2-5 (δ 5.06 and

2.60) and H_2-6 (δ 2.96 and 2.46). Both of these methylene protons exhibited COSY-45^o interaction with each other. The third partial structure "c" started with H-14 (δ 3.00) that showed cross-peaks with H-15 (δ 2.96). Partial structure "a-c" are shown in Fig. 2.7. The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz, **Fig**. A-51) showed the resonance of all nineteen carbons.

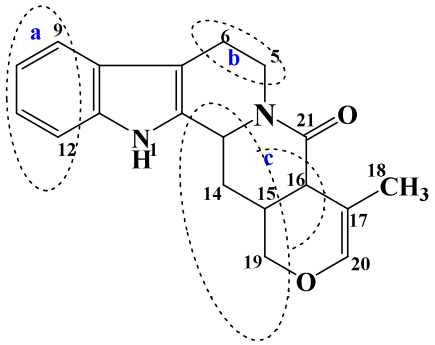


Figure 2.19 Spin system "a-d" of compound (**51**)**,** as deduced from the COSY 45^o spectrum.

The DEPT experiments (**Figs**. A-52, 53) were performed in order to establish the multiplicity of each carbon signal. The HSQC experiment (Fig. A-55) was performed in order to establish ^{1}H / ^{13} C one-bond correlations of all pronated carbons. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of **51** as determined from HSQC spectrum and ^{1}H /^{13}C one-bond shift correlations of all hydrogen bearing carbons as determined by HSQC spectrum are shown in Table 2.7.

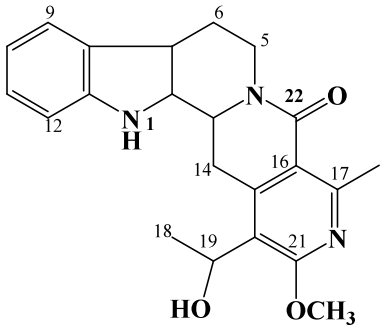
Table 2.7. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of **51** and $^{1}H/^{13}C$ one-bond shift correlations as determined from HSQC spectrum

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	127.6 (C)
3.	5.05	53.6 (CH)
4.	-	-
5.	5.06	
	2.60	43.3 (CH_2)
6.	2.96	
	2.46	21.1 (CH_2)
7.	-	111.4 (C)
8.	-	122.2 (C)
9.	7.45, d, (8.0)	118.3 (CH)
10.	7.26, dt, (8.0, 7.96)	120.0 (CH)
11.	7.14, dt, (8.0, 7.96)	123.4 (CH)
12.	7.52, d, (7.8)	135.9 (CH)
13.	-	164.3 (CH)
14.	3.00	
	3.00	43.3 (CH_2)
15.	2.96	53.7 (CH)
16.	3.03	56.0 (CH)
17.	-	111.4 (CH_3)
18.	1.35	14.1
19	4.56	80.0 (CH_2)
	3.96	
20.	8.10	148.8 (C)
21.	-	164.3 (C)

[a]Multiplicity was determined by HSQC spectrum.

These spectroscopic studies led us to characterize compound **(51)** as latifoliamide D.

2.3.8 Latifoliamide G (52)



52

Latifoliamide G (**52)** was isolated as a yellow colored solid. Its IR spectrum displayed intense absorption at 3703 (OH) and 1236 (amide carbonyl) cm^{-1}. Its UV spectrum showed absorption maxima at 225 and 293 nm, indicating the presence of indole moiety. The HREIMS of **52**, provided its molecular formula $C_{22}H_{23}N_3O_3$ (calcd. 377.1739), as it exhibited the molecular ion peak at m/z 377.1742

The ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz, **Fig**. A-45), of **52** showed signals at δ 7.49 (d, *J* = 7.8 Hz), 7.13 (dt, *J* = 7.2, 6.9 Hz), 7.20 (dt, *J* = 8.0, 6.9 Hz) and 7.40 (d, *J* = 8.0 Hz) due to the C-9, C-10, C-11, and C-12 protons respectively. This multiplicity pattern is characteristic of *ortho*-substituted phenyl group. These ^{1}H data along with UV spectral data as discussed previously suggested that compound (**52)** was a member of indole alkaloid. Resonances at 5.00, 3.00 and 3.95, 1.96 were due to the C-5 and C-6 methylene protons respectively. A three proton doublet at δ 1.48 was due to the C-18 methyl proton. *O*-methyl protons appeared at δ 3.50. A careful interpretation of the COSY spectrum (**Fig**. A-47) of compound (**52**) revealed the presence of four isolated spin systems "a-d". The first spin system "a" was due to the *ortho*-phenyl moiety present in **52**. H-9 (δ 7.49)

showed cross-peaks with H-10 (δ 7.13) that in turn showed COSY-45° interactions with H-11 (δ 7.20). The later exhibited vicinal couplings with H-12 (δ 7.40). The second spin system "b" was due to the C-5 (δ 5.00, 3.00) and C-6 (δ 3.95, 1.96) methylene protons. The third spin system "c" started with H-3 (δ 4.99) that showed cross-peaks with H_2-14 δ 3.00, and 1.99. The fourth spin system "d" was due to 2-hydroxy ethyl group in which H-19 (δ 3.98) showed cross-peaks with H_3-18 (δ 1.48).

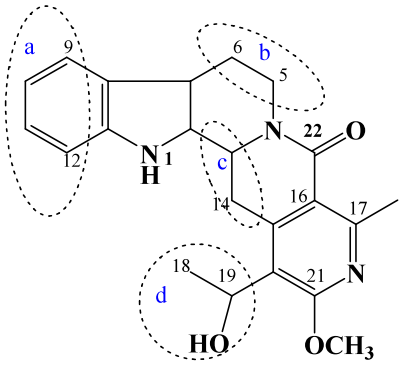


Figure 2.20 Spin systems "a-d" of compound **(52)** obtained from the COSY-45° spectrum

The ^{13}C-NMR spectrum ($CDCl_3$, 100 MHz, **Fig**. A-46) showed the resonance of all twenty-two carbons. The C-22 resonated at δ 164.6. A downfield aliphatic C-19 appeared at δ 72.0. Its downfield chemical shift value was indicative of the presence of a geminal hydroxyl group. A combination of DEPT and broad-band ^{13}C-NMR spectral data of **52** revealed the presence of seven methine, three methylene, two methyl, and ten quaternary carbons in **52**. The HSQC experiment (F**ig**. A-48) was performed to establish ^{1}H/^{13}C one-bond correlations, and this data is presented in Table 2.8. Complete^{1}H and ^{13}C-NMR chemical shift assignments are listed in Table 2.8.

Table 2.8. Complete ^{1}H and ^{13}C-NMR chemical shift assignment of compound (**52)** as well as HSQC spectral data

Carbon No.	**^{1}H**	**^{13}C**
	δ (J in Hz)	δ (multiplicity[a])
1.	-	-
2.	-	127.7 (C)
3.	4.99	53.3 (CH)
4.	-	-
5.	5.00	
	3.00	43.3 (CH_2)
6.	3.95	
	1.96	19.4 (CH_2)
7.	-	111.5 (C)
8.	-	122.3 (C)
9.	7.49, d, (7.8)	118.3 (CH)
10.	7.13, dt, (7.2, 6.9)	120.6 (CH)
11.	7.20, dt, (8.0, 6.9)	120.0 (CH)
12.	7.40, d, (8.0)	132.5 (CH)
13.	-	135.9 (C)
14.	3.00	
	1.99	56.4 (CH_2)
15.	-	150.6 (C)
16.	-	127.5 (C)
17.	9.80	132.5 (CH)
18.	1.48, d, (6.3)	19.4 (CH_3)
19.	3.98	72.0 (CH)
20.	-	111.5 (C)
21.	3.5	150.6 (C)
22.	-	164.5 (C)

[a]DEPT spectrum was used to establish multiplicity at carbon signals.

Based on these spectral studies, structure (**52**) was assigned to this new alkaloid.

Angustoline (**45**); greenish-yellow colored gum, 8.0 mg; UV (MeOH): λmax 225, 293 nm; IR (MeOH): νmax 3350 (OH), 2950 (CH), 1631 (amide carbonyl) cm^{-1}; HREIMS *m/z* 333.1475 (calcd. for $C_{20}H_{19}N_3O_2$, 333.1477); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.1; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.1

Latifoliamide A (**46**); yellow colored solid, 8.8 mg; UV (MeOH): λmax 221, 288 nm; IR (MeOH): νmax 3529 (NH) and 3120 (CH) cm^{-1}; HREIMS *m/z* 332.1678 (calcd. for $C_{20}H_{22}N_2O_2$, 332.1681); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.2; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.2

Latifoliamide B (**47**); yellow colored gum, 3.8 mg; UV (MeOH): λmax 221, 293 nm; IR (MeOH): νmax 3276 (NH), 2925 (CH), and 1628 (amide carbonyl) cm^{-1}; HREIMS *m/z* 315.1376 (calcd. for $C_{20}H_{17}N_3O$, 315.1372); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.3; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.3

Latifoliamide C (**48**); greenish-yellow colored solid, 15 mg; UV (MeOH): λmax 224, 295 nm; IR (MeOH): νmax 3339 (OH), and 2944 (CH) $cm^{-}1$; HREIMS *m/z* 352.1783 (calcd. for $C_{21}H_{24}N_2O_3$, 352.1787); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.4; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.4

Latifoliamide D (**49**); yellow colored gum, 6.4 mg; UV (MeOH): λmax 220, 289 nm; IR (MeOH): νmax 3319 (NH), 2941(CH), and 1670 (amide carbonyl) cm^{-1}; HREIMS *m/z* 303.1377 (calcd. for $C_{19}H_{17}N_3O$, 303.1374); ^{1}H-NMR (DMS, 400M Hz) = see Table 2.5; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.5

Latifoliamide E (**50**); yellow colored solid, 14.6 mg; UV (MeOH): λmax 223, 290 nm; IR (MeOH): νmax 3283 (OH), 2922 (CH), and 1615 (amide carbonyl) cm^{-1}; HREIMS *m/z* 377.1742 (calcd. for $C_{22}H_{23}N_3O_3$, 377.1739); ^{1}H-NMR (DMSO, 400M Hz) = see Table 2.6; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.6

Latifoliamide F (**51**); yellow colored gum, 3.2 mg; UV (MeOH): λmax 221, 289 nm; IR (MeOH): νmax 3338 (NH), 2945 (CH) and 1654 (amide carbonyl) cm^{-1}; HREIMS *m/z* 308.1527 (calcd. for $C_{19}H_{20}N_2O_2$, 308.1525); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.7; ^{13}C-NMR ($CDCl^3$, 100 MHz) = see Table 2.7

Latifoliamide G (**52**); yellow colored solid, 2.1 mg; UV (MeOH): λmax 225, 293 nm; IR (MeOH): νmax 3703 (OH) and 1236 (amide carbonyl) cm^{-1}; HREIM m*/z* 377.1742 (calcd. for $C_{22}H_{23}N_3O_3$, 377.1739); ^{1}H-NMR ($CDCl_3$, 400M Hz) = see Table 2.8; ^{13}C-NMR ($CDCl_3$, 100 MHz) = see Table 2.8

2.4 REFERENCES

1 Akubue PI, Mittal GC. Clinical evaluation of a traditional herbal practice in Nigeria: a preliminary report. J Ethnopharmacol 1982 ;6(3):355-359.

2 Igoli JO, Ogaji OG, Tor-Anyiin NP, Igoli NP. Traditional medicine practice amongst the Igede people of Nigeria. Part II. Afr J Trad CAM 2005;2(2):134-152.

3 Elujoba A. Female infertility in the lands of traditional birth attendants in south-western Nigeria. Fitoterapia 1995;66:239-248.

4 Deeni YY, Hussain HS. Screening for antimicrobial activity and for alkaloids of Nauclea latifolia. J Ethnopharmacol 1991;35(1):91-96.

5 Benoit-Vical F, Valentin A, Cournac V, Pélissier Y, Mallié M, Bastide JM. In vitro antiplasmodial activity of stem and root extracts of Nauclea latifolia S.M. (Rubiaceae). J Ethnopharmacol 1998;61(3):173-178.

6 Di Giorgio C, Lamidi M, Delmas F, Balansard G, Ollivier E. Antileishmanial activity of quinovic acid glycosides and cadambine acid isolated from Nauclea diderrichii. Planta Med 2006 ;72(15):1396-1402.

7 Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, et al. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. Phytomedicine 2000 ;7(1):31-38.

8 Gidado A, Ameh DA, Atawodi SE. Effects of Nauclea latifolia leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr J Biotecnol 2005;4(1):91-93.

9 Nworgu ZAM, Onwukaeme DN, Afolayan AJ, Amaechina FC, Ayinde BA. Preliminary studies of blood pressure lowering effect of Nauclea latifolia in rats. Afr J Pharm Pharmacol 2008;2(2):37-41.

10 Musa AD, Yusuf GO, Ojogbane EB, Nwodo OFC. Screening of eight plants used in folk medicine for the treatment of typhoid fever. J, Chem Pharm Res 2010;2(4):7-15.

11 Ogbonna DN, Sokari TG, Agomuoh AA. Antimalarial activities of some selected traditional herbs from south eastern Nigeria against Plasmodium species. Res J Parasitol 2008;3(1):25-31.

12 Abbah J, Amos S, Chindo B, Ngazal L, Vongtau HO, Adzu B, et al. Pharmacological evidence favouring the use of *Nauclea latifolia* in malaria ethnopharmacy: Effects against nociception, inflammation, and pyrexia in rats and mice. J Ethnopharmacol 2010;127(1):85-90.

13 Shigemori H, Kagata T, Ishiyama H, Morah F, Ohsaki A, Kobayashi J. Naucleamides A—E, New Monoterpene Indo Alkaloids from *Nauclea latifolia.* Chem Pharm Bull 2003;51(1):58-61.

14 Ngnokam D, Ayafor JF, Conolly JD, Nuzillard JM. Nuclefolinin: a new alkaloid from the roots of Nauclea latifolia. Bull Chem Ethiop 2003;17(2):173-176.

15 Ameh S, Obodozie O, Gamaniel S, Abubakar M, Garba M. Physicochemical variables and real time stability of the herbal substance of Niprd-AM1®- an antimalarial developed from the root of *Nauclea latifolia* S.M. (Rubiaceae). Int J Phytomedicine 2010;2(3):332-340.

16 Moundipa PF, Melanie KGF, Bilong-Bilong CF, Bruchhaus I. *In vitro* Amoebicidal Activity of some Medicinal Plants of the Bamun Region (Cameroon). Afr J Trad CAM 2005;2(2):113-121.

17 Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, et al. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother Res 2000 ;14(8):630-634.

18 Ogueke CC, Chikwendu CI, Iwouno JO, Ogbulie JN. Effect Of Crude Ethanol Extract Of Nauclea Latifolia On Some Clinical Isolates Of Food Importance And Its Toxicological Potentials. New York Science Journal 2010;3(9):97-105.

19 Rotimi VO, Mosadomi HA. The effect of crude extracts of nine African chewing sticks on oral anaerobes. J Med Microbiol 1987;23(1):55-60.

20 Falodun A, Igwe A, Obasuyi O. Anti-Microbial Evaluation of a Herbal Dental Remedy Stem Bark of Nuclea latifolia-Family Rubiaceae. J Appl Sci 2007;7(18):2696-2700.

21 Okwori AEJ, Okeke CI, Uzoechina A, Etukudoh NS, Amali MN, Adetunji JA, et al. The antibacterial potentials of *Nauclea latifolia.* Afr J Biotecnol 2008;7(10):1394-1399.

22 Agyare C, Mensah AY, Osei-Asante S. Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. BLACPMA 2006;5(6):113-117.

23 Musa AD, Nwodo OC, Yusuf GO. A comparative study of the antibacterial activity of aqueous ethanol and chloroform extracts of some selected medicinal plants used in Igalaland of Nigeria. Der Pharmacia Sinica 2011;2(1):222-227.

24 Musa AD, Yusuf GO, Ojogbane EB, Nwodo OFC. Screening of Eight Plants Used In Folkloric Medicine for the Treatment of Typhoid Fever. J Chem Pharm Res 2010;2(4):7-15.

25 Gidado A, Ameh DA, Atawodi SE. Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. Afr J Biotechnol 2005;4(1):91-93.

26 Nworgu ZAM, Eferakeya A, Onwukaeme DN, Afolayan AJ, Ameachina FC, Ayinde BA. The effect of active fractions of the roots of Nauclea latifolia Smith (Rubiaceae) on blood pressure of normotensive rabbits. J Appl Sci Res 2009;5(12):2208-2212.

27 Adedapo AA, Sofidiya MO, Afolayan AJ. Anti-inflammatory and analgesic activities of the aqueous extracts of Margaritaria discoidea (Euphorbiaceae) stem bark in experimental animal models. Rev Biol Trop 2009 ;57(4):1193-1200.

28 Taiwe GS, Ngo Bum E, Dimo T, Tella E, Weiss N, Dawe A, et al. Antidepressant, myorelaxant and anti-anxiety-like effects of Nauclea latifolia smith (Rubiaceae) roots extract in Murine models. International Journal of Pharnmacology 2010;6(4):364-371.

29 Takayama H, Tsutsumi S, Kitajima M, Santiarworn D, Liawruangrath B, Aimi N. Gluco-indole Alkaloids from Nauclea cadamba in Thailand and Transformation of 3a-Dihydrocadambine into the Indolopyridine Alkaloid, 16-Carbomethoxynaufoline. Chem Pharm Bull 2003;51(2):232-233.

30 McLean S, Murray DG. Isolation of indole (P-carboline), pyridine, and indole-pyridine alkaloids from Nauclea diderrichii. Can J Chem 1970;48:867-868.

31 Ata A, Udenigwe CC, Matochko W, Holloway P, Eze MO, Uzoegwu PN. Chemical constituents of *Nauclea latifolia* and their anti-GST and anti-fungal activities. Nat Prod Comm 2009;4(9):1185-1188.

32 Erdelmeier CO, Regenass U, Sticher O, Rali T. Indole alkaloids with in vitro antiproliferative activity from the ammoniacal extract of Nauclea orientalis. Planta Medica 1992; 58(1): 43-48.

33 Abreu P, Pereira A. New indole alkaloids from Sarcocephalus latifolius. Natural Product Letters 2001; 15(1): 43-48.

CHAPTER 3

Renin Inhibitory Assay

3.1 INTRODUCTION

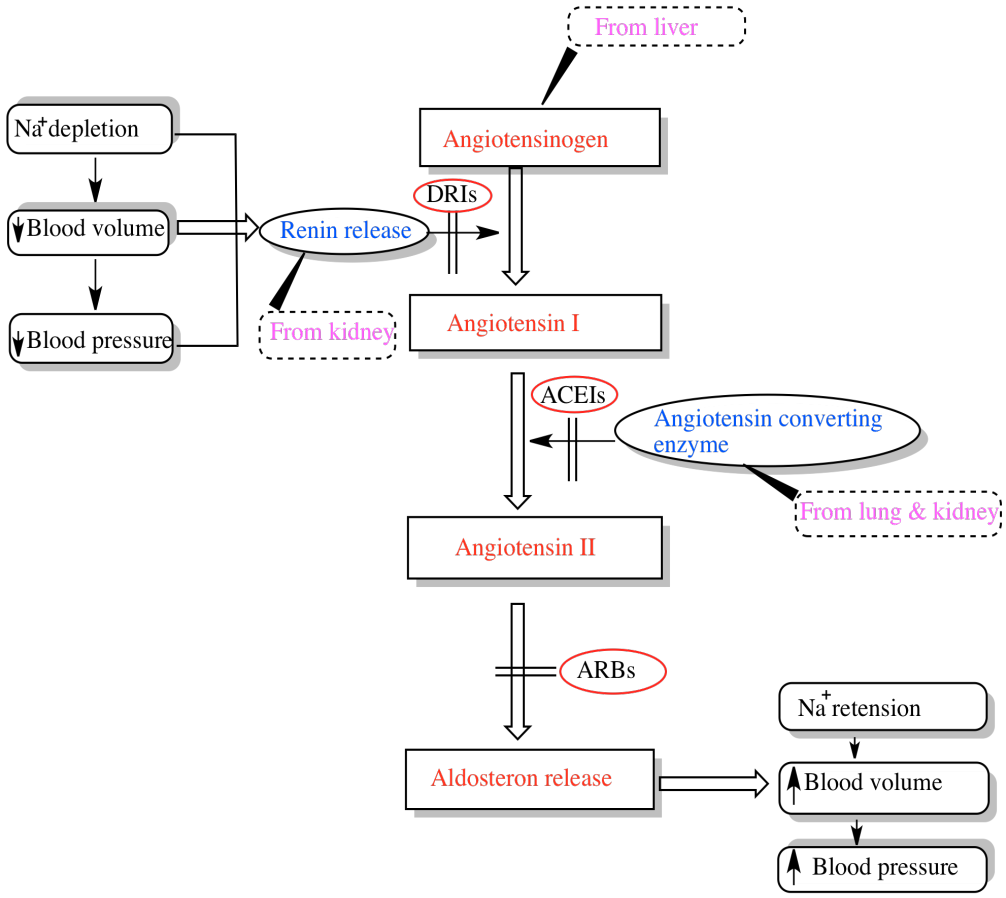
Hypertension is an abnormal and sustained elevation in blood pressure, beyond what is normal for the age of an individual. The incidence of hypertension is increasing day by day and has now become a global burden. In 2000, 26% of the adult population globally, were diagnosed with hypertension. The number of adults with hypertension in 2025 are projected to increase by about 60% to a total of 1.56 billion (1.54-1.58 billion)[1,2]. Hypertension has been identified as the third major factor for disability adjusted life years (DALY)[3]. It can primarily affect the heart leading to many heart complications including heart failure, or has secondary effect on other body organs and systems like brain (stroke), kidneys (kidney failure), etc.

3.1.1 The Renin Angiotensin System and its role in blood pressure regulation

The renin-angiotensin system (RAS) plays a pivotal role in controlling and maintaining arterial blood pressure, tissue perfusion and body water[4]. Attempts to find effective ways to treat hypertension led researchers to discover the methods by which the body regulates blood pressure. The RAS was discovered in 1898 by Tigerstedt and Bergmann by showing the existence of heat labile substance in crude extracts of rabbit kidney. This extracts caused a sustained increase in arterial blood pressure[5].

Skeggs and his colleagues in 1957 postulated three possible approaches to pharmacologically inhibit the RAS pathway. (i) Inhibition of angiotensin converting enzyme (ACE); (ii) Inhibition of renin, and (iii) interfering with angiotensin-II binding to its receptors. Since the step of this cascade, catalyzed by renin is the rate limiting step,

these researchers noted that this step is the most critical in the entire pathway[6,7]. The pharmacological inhibition of the enzymes, and receptors involved in the RAS pathway (Scheme 3.1), has thus offered a very important target for the discovery of drugs for treating not only heart and blood vessel diseases, but also other conditions like kidney and liver disease[8].



DRIs – Direct renin inhibitors

ACEIs – Angiotensin converting enzyme inhibitors

ARBs – Angiotensin receptor blockers

Scheme 3.1. RAS cascade and blood pressure regulation

3.1.2 The Enzyme Renin

Renin belongs to the aspartic proteases family of enzymes which also includes the enzymes pepsin, cathepsin, and chymosin[9]. It is made up of 340 amino acid residues and it is highly specific for its substrate. It is made up of 2 homologous lobes, with the active sites residing between the two lobes. The catalytic activity of the enzyme is due to the 2 aspartic acid residues (32 and 215) provided by each lobe. The active site can accommodate 7 amino acid units of the substrate angiotensinogen, and cleaves the Leu10-val11 peptide bond within angiotensinogen to generate angiotensin I (A-I). The reaction catalyzed by renin is the rate-limiting step in A-II formation. Both of these forms (A-I & A-II) cannot be synthesized in the absence of rennin[6].

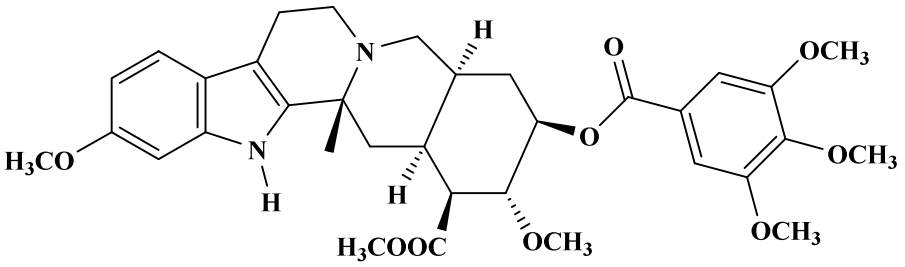
Renin is produced by the epithelial cells of the kidney. It is released when the kidney senses decrease in blood volume, extracellular volume, and arterial blood pressure. Renin acts on angiotensinogen, an alpha-2 globulin, with 452 amino acids, converting it to angiotensin I (10 amino acids). In the next step, angiotensin converting enzyme, produced from the lung and the kidney, converts angiotensin I to angiotensin II (8 amino acids). Angiotensin II causes the constriction of blood vessels and has a stimulatory effect on many organ systems of the body. The net effect is salt and water retention in the body and that causes increase in blood pressure.

3.1.3 Blood pressure medications: Historical background

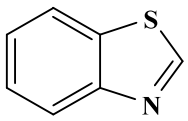
Over the years, scientists have made spirited efforts in discovering effective ways of treating hypertension. However, early attempts for the treatment of hypertension using drugs started after 1945. This began with the introduction of reserpine (**53)** in 1953. Reserpine was derived from the snakeroot plant *Rauwolfia serpentine*, used by Indians in

folk medicine. The active ingredient was then isolated and named reserpine, and was synthesized in 1956 by Robert Burns Woodward. Reserpine was the first antihypertensive drug to achieve wide clinical use because of its nearly universal effectiveness[10]. The thiazide diuretic (benzothiazide) (**54)**, which acts by increasing water excretion, was introduced in 1957, and the beta blockers (propranolol) (**55)**, in 1964. The calcium channel blockers (e.g. verapamil) (**56)**, which blocks the channels that carry calcium to the heart was introduced in the 1980s.

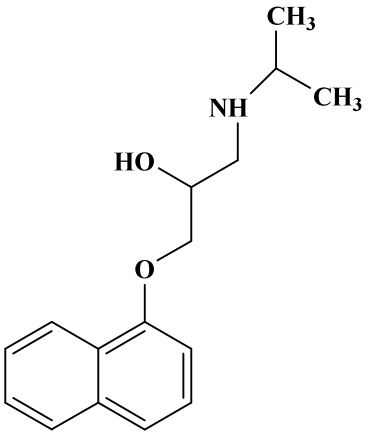
Studies on the discovery of drugs targeting the RAS pathway started with the development of angiotensin converting enzyme inhibitor captopril (**57)** by Cushman and his colleagues in 1977[11]. This was followed by the discovery of ramipril and enalapril which are pro drugs. The first angiotensin receptor blocker losartan (**58)** was discovered in 1986, and is the prototype from which all the other ARBs were derived[12]. It was approved by FDA in 1995, as the first non-peptide angiotensin II receptor blocker anti-hypertensive.



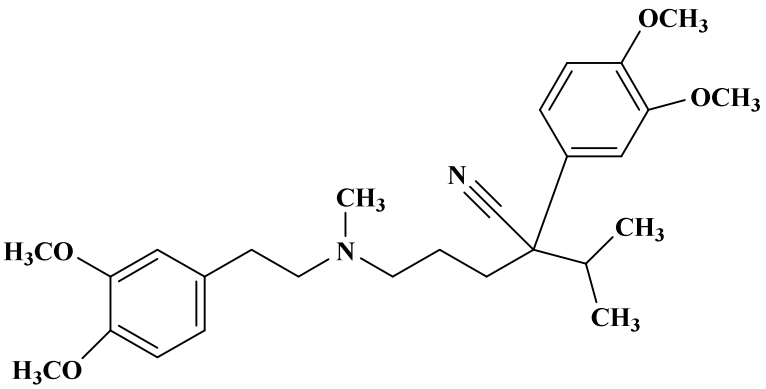
53



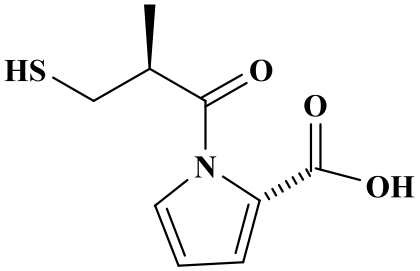
54



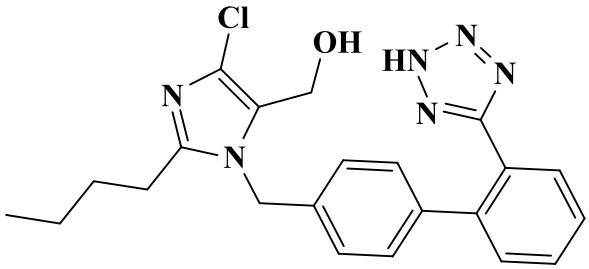
55



56



57



58

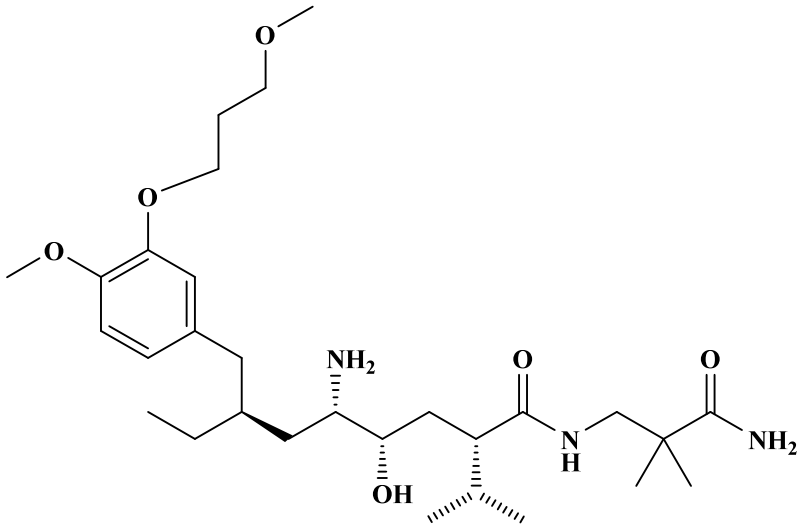
Despite the promise of ACE inhibitors and Angiotensin receptor blockers (ARBs,) nearly 70% of patients still fail to achieve adequate BP control, and the desired improvements in cardiovascular morbidity and mortality have also failed to materialize. Since both ACE inhibitors and ARBs interrupt the negative feedback loop of the renin system, renin can still be released. Therefore, these compounds do not completely block the RAS, which impedes a full effect in reducing BP in hypertension[13].

Direct inhibition of renin has been proposed as a therapeutic option for the treatment of arterial hypertension. Direct renin inhibitors target the rate-limiting step in the RAS

cascade, preventing renin release, and before ACE inhibitors and ARBs in the cascade[14]. Studies in both animal and human models have equally enumerated the superiority of direct renin inhibitors (DRIs) over other agents targeting other points in the RAS pathway[15,16].

An agent that inhibits renin enzyme will prevent its cleavage of angiotensinogen. Being the first enzymatic reaction in this pathway, this will block subsequent steps in the reaction pathway, and such agent would tend to exert a better control of hypertension[17]. Considerable research[18,19] over three decades has not yielded the much needed breakthrough in the quest for direct renin inhibitor antihypertensive drugs. This is partly because of difficulties in development, identifying suitable agents with the required combination of high affinity for renin's active site and sufficient bioavailability to permit chronic oral administration[20-23]. To date, only one synthetic drug aliskiren (**59)**, approved by FDA in 2007 is in use as a direct renin inhibitor[24].

However, the enzyme renin remains a very important drug target since the enzyme is specific for only one substrate-angiotensinogen. These inhibitors would also tend to produce fewer side effects[12].

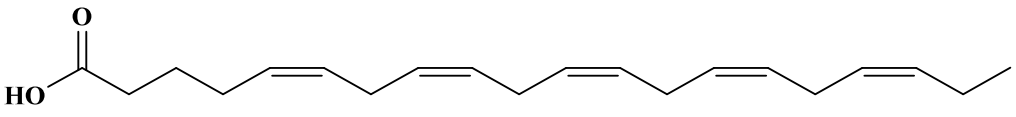


59

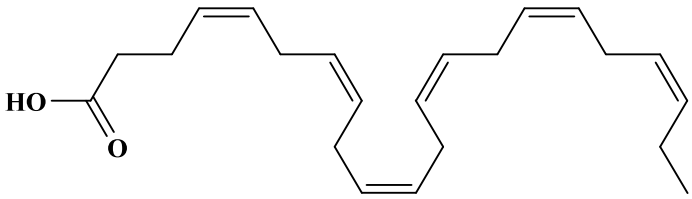
3.1.4 Role of Natural Products in the Treatment of Hypertension

Like other areas of human health, natural products have contributed greatly in the management of hypertension. Some food-derived peptides[25,26], have shown promising results in lowering high blood pressure. The omega-3 fatty acids (eicosanpentoic acid (**60)** and docosahexanoic acid (**61)** obtained from fish oils have been found to be possibly effective in hypertension when orally administered[27]. Garlic, has been shown to lower blood pressure, when eaten, due to its ability to relax smooth muscles, and cause the dilatation of blood vessels[27]. Hosseini and his colleagues discovered that aqueous garlic extract significantly lowered the serum level of ACE in diabetic and non diabetic rats[28]. Canola meal subjected to enzymatic proteolysis yielded inhibitory peptides that inhibited ACE with an IC_{50} values ranging from 18.1 to 82.5 μg protein ml^{-1}[29]. Enzymatic hydroxylates from flaxseed protein when investigated for *in vitro* inhibition of ACE and renin showed a concentration-dependent ACE inhibition with an IC_{50} values ranging

from 0.0275-0.151 mg ml^{-1}[30]. Milk derived bioactive peptides have also been shown to inhibit ACE *in vitro*[31-33].



60



61

Studies on different extracts from plants including ethanolic extract of *Rabdosia coetsa*, have shown promising inhibitory activities of the RAS pathway, particularly ACE[34-36]. A review of natural products inhibitors of angiotensin converting enzyme (ACE) between 1980 to 2000 shows that 321 species of plants and 158 natural substances inhibited the enzyme[37]. Screening of 20 plants used by traditional healers in South Africa also indicated some of the plants inhibited ACE by up to 97%[38].

It is important to note that despite the strategic importance of renin in the RAS cascade, and the huge amount of studies aimed at discovering DRI drugs, little success has been achieved, particularly in the area of natural product. This has made this current study interesting.

3.2 EXPERIMENTAL

3.2.1 General conditions

Renin inhibitory activities were measured spectrophotometrically using JASCO FP-6300 spectrofluorimeter (Japan Spectroscopic Company, Tokyo, Japan). Human recombinant Renin Inhibitor Screening Assay Kit was purchased from Cayman Chemicals (Ann Arbor, MI, USA). Other analytical grade reagents used in the renin bioassay were purchased from Fischer Scientific (Oakville, ON, Canada).

3.2.2 Bioassay Procedure

The eight compounds from *N. latifolia* were weighed in mg using Sartorius CP224S weighing balance, and each dissolved with equivalent volume of methanol to make 1mg/ml dilution (Scheme 3.2) . Further dilutions were made with the tris-buffer, to give the respective concentrations for the assay. Screening for inhibition was initially done with 50 and 5 micrograms concentrations. This was followed by the determination of the IC_{50} values using 5, 25, 50, and 100 microgram concentrations respectively. All the four concentrations of the compounds in the bioassay study were duplicated.

In vitro inhibition of the activity of human recombinant renin was conducted by fluorescence spectrometry using the Renin Inhibitor Screening Assay Kit as reported by Udenigwe, *et al*[30]. Briefly, the total assay volume of 190µl contained 10 µM Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dabcyl)-Arg(renin substrate dissolved in dimethyl sulphoxide), human recombinant renin and the compounds in 50 mM Tris-HCl buffer, pH 8.0, containing 100 mM NaCl. The Tris-HCl buffer was used instead of the compounds in the blank experiment. The renin substrate and compounds were mixed and pre-warmed to 37°C for 10 min to attain thermal equilibrium. Thereafter,

the reaction was initiated by adding human recombinant renin to the mixture; the increase in fluorescence intensity was monitored for 10 min in a JASCO FP-6300 spectrofluorimeter (Japan Spectroscopic Company, Tokyo, Japan) equipped with a thermostated cell compartment that was maintained at 37°C using a circulatory water bath. The spectrofluorimeter parameters were set as follows: excitation wavelength, 340 nm; emission wavelength, 490 nm; excitation bandwidth, 5 nm; emission bandwidth, 10 nm. The enzyme activity was expressed as reaction rate, arbitrary fluorescence intensity unit per min ($FIU.min^{-1}$). Renin inhibitory activity of each compound was calculated using the Equation:

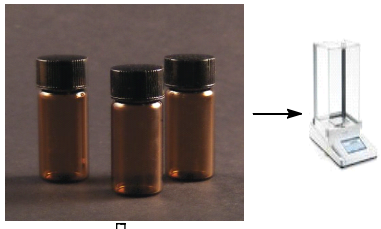
Renin inhibition (%) = $[(FIU.min^{-1}_{(control)} - FIU.min^{-1}_{(sample)}) / FIU.min^{-1}_{(control)}] \times 100$

Where:

$(FIU.min^{-1})_{(control)}$ = Fluorescence intensity unit per min for blank = Renin reaction rate in the absence of compounds.

$(FIU.min^{-1})_{(sample)}$ = Fluorescence intensity unit per min for samples = Renin reaction rate in the presence of compounds.

The IC_{50} values were determined using linear regression (**Figs** A-58-63). These IC50 values of the duplicated experiments were used to calculate the mean and standard deviation.



1:1 Mass/Vol dissolution in Methanol

Further dilution in Tris-buffer: 5 25, 50, 100 uM concs., using the CW, and FW

The Tocris molarity calculator:

Mass = Concentration x Volume x Molecular Weight

JASCO FP-6300 spectrofluorimeter

Spec. Parameters
- Maintained at 37 ^{0}C
-Excitation wavelength, 340nm
-Emission wavelength, 490nm
1. Blank experiment
2. Sub. + Comp. + warm(10 mins.)
3. Mixture + renin = Rxn iniciation
4. Change in Fluorescence intensity monitoring (10 mins)

Determination of Enzyme activity (Rxn rate)= Fluorescence Intensity Unit (FIU)

Total assay volume of 190µl contained:

- 20 µl of 10 µM Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dabcyl)-Arg(renin substrate dissolved in dimethyl sulphoxide).

- 10 µl of human recombinant renin

- 150 µl of 50 mM Tris-HCl buffer, pH 8.0, containing 100 mM NaCl.

- 10 µl of compounds

-Substrate is a synthetic peptide
-Peptide linked to a fluorophore(EDANS) at one end and to a non fluorescent chromophore(Dabcyl) at the other
-After cleavage by renin , the product (peptide-EDANS) is brightly fluorescent and can be easily analysed using a Fluorometer

Scheme 3.2. Flow chart of Renin Bioassay

3.3 RESULTS

The IC_{50} values of the renin inhibitory activity of the compounds are shown in Table 3.1. Compounds (**45-46**) and (**48-51**) displayed varying levels of renin inhibition, while compounds **47** and **52** did not exhibit any inhibition. The inhibitory activity of compounds (**49**), (**45**) and (**48**) were higher (11.26, 16.25 and 32.58 μM respectively), compared to the rest of the compounds in these bioassays.

Table 3.1. IC_{50} values of compounds (**45-52**) in anti-renin assay

Compounds	**IC_{50} (μM)***
45 (Angustoline)	16.3 ± 0.5
46 (Latifoliamide A)	72.0 ± 11.3
47 (Latifoliamide B)	-
48 (Latifoliamide C)	32.6 ± 2.0
49 (Latifoliamide D)	11.3 ± 1.1
50 (Latifoliamide E)	95.0 ± 6.9
51 (Latifoliamide F)	94.5 ± 3.0
52 (Latifoliamide G)	-

*IC_{50} value represents the concentration of compounds needed to inhibit 50% of the activity of Renin. ± represents the standard deviation.

3.4 DISCUSION

The result presented above provides an insight in the search for DRI agents, particularly from natural product origin. The RAS cascade plays a crucial role in the regulation of blood pressure[39,40]. The importance of renin, the rate limiting enzyme in the cascade has been variously enumerated[6,41-43]. It has also been shown that suppression of the RAS after treatment with either ACE inhibitors or ARB remains incomplete[44]. Development of renin inhibitors has faced different challenges including high cost of synthesis, lack of appropriate animal models, and inadequate bioavailability[41].

Currently, only alkisiren approved by FDA in 2007 is in use as the first and only direct renin inhibitor[45-47]. A handful of studies have been done on renin inhibitory activity of various foods[30,41-43,48-50]. Some studies have equally looked at the inhibitory activities of some synthetic peptides[51,52]. Screening for rennin inhibition using synthetic analogues of plant compounds has also been studied[53]. This study looks at the inhibitory activities of compounds isolated from *N. latifolia* against renin. Information available to us from published data indicated that this was the first time alkaloids from *N. latifolia* were tested for renin inhibitory activity. Six of the isolates (**49, 45, 48, 46, 51, 50)** displayed inhibitory activities, while two (**47, 52**) were inactive in this bioassay (Fig. 3.1). The inhibition of the compounds was concentration dependent, exhibiting higher inhibition at higher concentration of compounds. Compounds **49**, **45** and **48** displayed significant inhibition of renin with $1C_{50}$ values of 11.26, 16.25 and 32.58 µM respectively. Commercially purified soybean saponin inhibited human renin with an IC_{50} 59.9 µg ml^{-1}[48], and oleic and linoleic acids isolated from rice inhibited renin with inhibition kinetics of 15.8 and 19.8 µM respectively[41]. Saori *et al*, isolated soybean renin inhibitor from soy

bean with an IC_{50} value of 30 μg ml $^{-1}$[43], and extracts from two groups of minor legumes, inhibited renin with IC_{50} values of 0.27-0.42 and 0.67-1.75 mg ml^{-1} respectively. Chemical analogues of sodium houttuynin derived from the plant *Houttuynia cordata* inhibited renin with IC_{50} values of 273, 195 and 44 μM respectively. From the forgoing, the inhibition pattern displayed by the compounds isolated from *N. latifolia*, particularly compounds **49**, **45**, and **48**, exhibited anti-renin activity that is comparable to aforementioned natural products. Compounds **47** and **52** did not show any inhibition. However, this was an in vitro screening experiment. An in vivo study is warranted since these two environments are not identical. The spectrophotometer used in the experiment records fluorescence. The high level of inhibition exhibited by compounds **49** and **45** at a concentration of 50 μM resulted in the machine recording florescence at a negative value, which was interpreted as zero florescence. There was no standard in the experiment. Aliskiren, the only DRI so far licensed for use was not available when this study was done. Its IC_{50} is 0.6 nM. Further studies on structure-activity relationship are warranted to identify the active pharmacophore responsible for the inhibitory activity, and to possibly improve their bioactivity. Essential hypertension is a chronic medical condition which requires a prolonged period of drug administration. Major part of the setbacks in the successful production of direct renin inhibitor agents has been the identification of agents with good bioavailability after oral administration. *N. latifolia* is a common herb that has been in use in folk medicine by different people in different parts of the world. Most parts of this plant (leaves, stem, fruits, and roots) have been reported to be edible[54], and this could have pharmacokinetic benefit. Based on the

available bioactivity data, we assume that the antihypertensive activity of this plant might be due to the presence of alkaloids in the crude extract

3.5 REFERENCES

1 Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005;365(9455):217-223.

2 Chockalingam A, Campbell JR, Fodor JG. Worldwide epidemic of hypertension. Can J Cardiol 2006;22(7):553-555.

3 http://www.who.int/whr/2002/en/whr02_en.pdf

4 Atlas AS. The Renin-Angiotensin Aldosterone System:Pathophysiological Role and Pharmacologic Inhibition. J Manag Care Pharm 2007;13(8(suppl S-b)):S9-S20.

5 Piepho RW, Beal J. An overview of antihypertensive therapy in the 20th century. J Clin Pharmacol 2000;40(9):967-977.

6 Gradman AH, Kad R. Renin Inhibition in Hypertension. JACC 2008;51(5):519-528.

7 Skeggs LT, Kahn JR, Lentz K, Shumway NP. The preparation, purification, and amino acid sequence of a polypeptide renin substrate. J Exp Med 1957;106(3):439-453.

8 Harrison-Bernard LM. The renal renin-angiotensin system. Adv Physiol Educ 2009 ;33(4):270-274.

9 Wood JM, Stanton JL, Hofbauer KG. Inhibitors of renin as potential therapeutic agents. J Enzyme Inhib 1987;1(3):169-185.

10 Lopez-Munoz F, Bhatara VS, Alamo C, Cuenca E. Historical approach to reserpine discovery and its introduction in psychiatry. Actas Esp Psiquiatr 2004;32(6):387-395.

11 Erdos GE. The ACE and I: how ACE inhibitors came to be. FASEB 2006;20(8):1034-1038.

12 Siegl PK. Discovery of losartan, the first specific non-peptide angiotensin II receptor antagonist. J Hypertens 1993;11:S19-22.

13 Fisher NDL, Hollenberg NK. Renin Inhibition: What Are the Therapeutic Opportunities? J Am Soc Nephrol 2005;16:592-599.

14 Luis RM. Direct Renin Inhibitors in Hypertension - Outlook for End Organ Protection. European Cardiovascular Disease 2007 2007(2):57-60.

15 Fisher ND, Allan D, Kifor I, Gaboury CL, Williams GH, Moore TJ, et al. Responses to converting enzyme and renin inhibition. Role of angiotensin II in humans. Hypertension 1994 ;23(1):44-51.

16 Paassen VP, Zeeuw D, Navis G, De Jong PE. Renal and systemic effects of continued treatment with renin inhibitor remikiren in hypertensive patients with normal and impaired renal function. Nephrol Dial Transplant 2000;15(5):637-643.

17 O'Brien E, Barton J, Nussberger J, Mulcahy D, Jensen C, Dicker P, et al. Aliskiren reduces blood pressure and suppresses plasma renin activity in combination with a thiazide diuretic, an angiotensin-converting enzyme inhibitor, or an angiotensin receptor blocker. Hypertension 2007;49(2):276-284.

18 Galen FX, Devaux C, Atlas S, Guyenne T, Menard J, Corvol P, et al. New monoclonal antibodies directed against human renin. Powerful tools for the investigation of the renin system. J Clin Invest 1984;74(3):723-735.

19 Haber E. Renin inhibitors. Hypertension 1986;8(12):1093-1095.

20 Fisher ND, Hollenberg NK. Is there a future for renin inhibitors? Expert Opin Investig Drugs 2001;10(3):417-426.

21 Segall L, Covic A, Goldsmith DJA. Direct renin inhibitors: the dawn of a new era, or just a variation on a theme? Nephrol Dial Transplant 2007;22(9):2435-2439.

22 Nussberger J, Wuerzner G, Jensen C, Brunner HR. Angiotensin II suppression in humans by the orally active renin inhibitor Aliskiren (SPP100): comparison with enalapril. Hypertension 2002;39(1):E1-8.

(23) Campbell DJ. Renin Inhibitors - Mechanisms of Action. Aust Prescr 2009;32(5):132-135.

24 Gulec S. Renin inhibition in hypertension. Turk Kardiyol Dern Ars 2009; 37(Suppl 7): 15-22

25 Rainer H, Meisel H. Food-derived peptides with biological activity: from research to food applications. Current Opinion in Biotechnology 2007;18(169):163.

26 McCue P, Kwon YI, Shett K. Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. Asia Pac J Clin Nutr 2005;14:145-152.

27 Al-Qattan KK, Alnaqeeb MA, Ali M. The antihypertensive effect of garlic (*Allium sativum*) in the rat two-kidney–one-clip Goldblatt model. J Ethnopharmacol 1999; 66(2):217-222.

28 Hosseini M, Shafiee SM, Baluchnejadmojarad T. Garlic extract reduces serum angiotensin converting enzyme (ACE) activity in nondiabetic and streptozotocin-diabetic rats. Pathophysiology 2007 ;14(2):109-112.

29 Wu J, Aluko RE, Muir AD. Production of angiotensin I-converting enzyme inhibitory peptides from defatted canola meal. Bioresour Technol 2009;100(21):5283-5287.

30 Udenigwe CC, Lin Y, Hou W, Aluko RE. Kinetics of the inhibition of renin and angiotensin I-converting enzyme by flaxseed protein hydrolysate fractions. Journal of Functional Foods 2009;1(2):199-207.

31 Jakala P, Vapaatalo H. Antihypertensive peptides from milk proteins. Pharmaceuticals 2010; 3(1): 251-272.

32 FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. J. Nutr 2004; 134(4): 980S-988S.

33 Gobbetti M, Ferranti P, Smacchi E, Goffredi F, Addeo F. Production of Angiotensin-I-Converting-Enzyme-Inhibitory Peptides in Fermented Milks Started by *Lactobacillus delbrueckii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT4. Appl Environ Microbiol 200;66(9):3898-3904.

34 Li QL, Li BG, Zhang Y, Gao XP, Li CQ, Zhang GL. Three angiotensin-converting enzyme inhibitors from Rabdosia coetsa. Phytomedicine 2008;15(5):386-388.

35 Kang DG, Oh H, Chung HT, Lee HS. Inhibition of angiotensin converting enzyme by lithospermic acid B isolated from Radix Salviae miltiorrhiza Bunge. Phytother Res 2003;17(8):917-920.

36 Ahmed F, Siddesha JM, Urooj A, Vishwanath BS. Radical scavenging and angiotensin converting enzyme inhibitory activities of standardized extracts of *Ficus racemosa* stem bark. Phytother Res 2010;24(12):1839-1843.

37 Barbosa-Filho JM, Martins VKM, Rabelo AL, Moura MD, Silva MS, Cunha VLE, et al. Natural products inhibitors of the angiotensin converting enzyme (ACE). A review between 1980 - 2000. Rev Bras Farmacogn 2006;16(3):421-446.

38 Duncan AC, Jager AK, Vanstaden J$. Screening of Zulu medicinal plants for angiotensin converting enzyme (ACE) inhibitors. J Ethnopharmacol 1999;68:63-70.

39 Andersen K, Weinberger MH, Egan B, Constance CM, Ali MA, Jin J, et al. Comparative efficacy and safety of aliskiren, an oral direct renin inhibitor, and ramipril in hypertension: a 6-month, randomized, double-blind trial. J Hypertens 2008 ;26(3):589-599.

40 Novo S, Fazio G, Raccuglia E. Aliskiren: Just a New Drug for Few Selected Patients or an Innovative Molecule Predestinated to Replace Arbs and Ace-Inhibitors? Pharmaceuticals 2009;2(3):118-124.

41 Beaulieu PL, Gillard J, Bailey M, Beaulieu C, Duceppe J, Lavallee P, Wernic D. Practical synthesis of BILA 2157 BS, a potent and orally active renin inhibitor: use of an enzyme-catalysed hydrolysis for the preparation of homochiral succinic acid 1 derivatives. J Org Chem 1999; 64: 6622-6634.

42 Takahashi S, Hori K, Kumagai M, Wakabayashi S. Human Renin Inhibitory Activity in Legumes J Biol Macromol 2007;7(3):49-54.

43 Takahashi S, Hori K, Shinbo M, Hiwatashi K, Gotoh T, Yamada S. Isolation of human renin inhibitor from soybean: soyasaponin I is the novel human renin inhibitor in soybean. BBB 2008;72(12):3232-3236.

44 Azizi M, Menard J. Combined blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists. Circulation 2004;109(21):2492-2499.

45 Oh B, Mitchell J, Herron JR, Chung J, Khan M, Keefe DL. Aliskiren, an Oral Renin Inhibitor, Provides Dose-Dependent Efficacy and Sustained 24-Hour Blood Pressure Control in Patients With Hypertension. JACC ;49(11):1157-1163.

46 Riccioni G, Vitulano N, D'Orazio N, Bellocci F. Aliskiren, the first approved renin inhibitor: Clinical application and safety in the treatment of hypertension. Adv Ther 2009; 26(7):700-710.

47 Novo S, Fazio G, Raccuglia E. Aliskiren: Just a New Drug for Few Selected Patients or an Innovative Molecule Predestinated to Replace Arbs and Ace-Inhibitors? Pharmaceuticals 2009;2(3):118-124.

48 Hiwatashi K, Shirakawa H, Hori K, Yoshiki Y, Suzuki N, Hokari M, et al. Reduction of blood pressure by soybean saponins, renin inhibitors from soybean, in spontaneously hypertensive rats. BBB 2010;74(11):2310-2312.

49 Takahashi S, Hori K, Hokari M, Gotoh T, Sugiyama T. Inhibition of human renin activity by saponins. Biomed Res 2010;31(2):155-159.

50 Takahashi1 S, Tokiwano T, Suzuki1 N, Kodama I, Yoshizawa Y, Gotoh T. Renin Inhibitory Activity in Rice and Cereals. J Biol Macromol , 2010;10(3):83-91.

51 Kokubu T, Hiwada K, Murakami E, Muneta S, Morisawa Y, Yabe Y, et al. In vitro inhibition of human renin by statine-containing tripeptide renin inhibitor (ES-1005). J Cadiol Pharm 1987;10(Suppl7):S88-S90.

52 Cumin F, Evin G, Fehrentz JA, Seyer R, Castro B, Menard J, et al. Inhibition of human renin by synthetic peptides derived from its prosegment. J Biol Chem 1985 ;260(16):9154-9157.

53 Yuan L, Wu J, Aluko RE, Ye X. Kinetics of Renin Inhibition by Sodium Houttuyfonate Analogs BBB 2006;70(9):2275-2280.

54 Nworgu ZM, Owolabi OJ, Atomah JE. Effect of the ethanolic extract of Nauclea latifolia (Family: Rubiaceae) on the isolated uterus of non-pregnact rats. IJGP 2010; 4(1): 48-53.

CONCLUSIONS

In summary, phytochemical studies on the crude methanolic extract of *N. latifolia* resulted in the isolation of eight compounds **45-52**. The structures of these compounds were determined using spectroscopic methods. Compounds **46-52** were identified as new natural products, while compound **45** had been previously identified. Compounds **45, 46, 48-51**, showed a wide range of anti-renin activity. Compounds **49, 45** and **48** were found to be most potent, compared to the rest of the isolates. The bioactivity data compares favorably well with available studies on natural product renin inhibition. Careful examinations of structures (**45-52**) reveal that all of the compounds contain at least two nitrogen atoms in their structures. The renin inhibitory activities of these compounds might have resulted from this common functional group. Further pharmacokinetic and structure-activity relationship studies on these compounds would help determine their toxicity and bioavailability as well as the active pharmacophore required for renin inhibition. The various numbers of reactive groups in these compounds make them good candidates for possible reactions, whose product could be more active. Compounds **47** and **52** were inactive.

APPENDIX: SPECTRAL DATA OF COMPOUNDS

Figure A-21. NOESY spectrum of latifoliamde B (**47**) in $CDCl_3$

Figure A-22. ^{1}H-NMR spectrum of angustoline (**45**) in $CDCl_3$

Figure A-23. ^{13}C spectrum of angustoline (**45**) in $CDCl_3$

Figure A-24. DEPT 90 spectrum of angustoline (**45**) in $CDCl_3$

Figure A-25. DEPT 135 spectrum of angustoline (**45**) in $CDCl_3$

Figure A-26. COSY spectrum of angustoline (**45**) in $CDCl_3$

Figure A-27. HSQC spectrum of angustoline (**45**) in $CDCl_3$

Figure A-28. HMBC spectrum of angustoline (**45**) in $CDCl_3$

Figure A-29. NOESY spectrum of angustoline (**45**) in $CDCl_3$

Figure A-30. ^{1}H-NMR spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-31. ^{13}C spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-32. DEPT 90 spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-33. DEPT 135 spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-34. COSY spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-35. HSQC spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-36. HMBC spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-37. NOESY spectrum of latifoliamide A (**46**) in $CDCl_3$

Figure A-38. ^{1}H-NMR spectrum of latifoliamide C (**48**) in $CDCl_3$

Figure A-39. ^{13}C spectrum of latifoliamide C (**48**) in $CDCl_3$

Figure A-40. DEPT 90 spectrum of latifoliamide C (**48**) in $CDCl_3$

Figure A-41. DEPT 135 spectrum of latifoliamide C (**48**) in $CDCl_3$

Figure A-42. HSQC spectrum of latifoliamide C (**48**) in $CDCl_3$

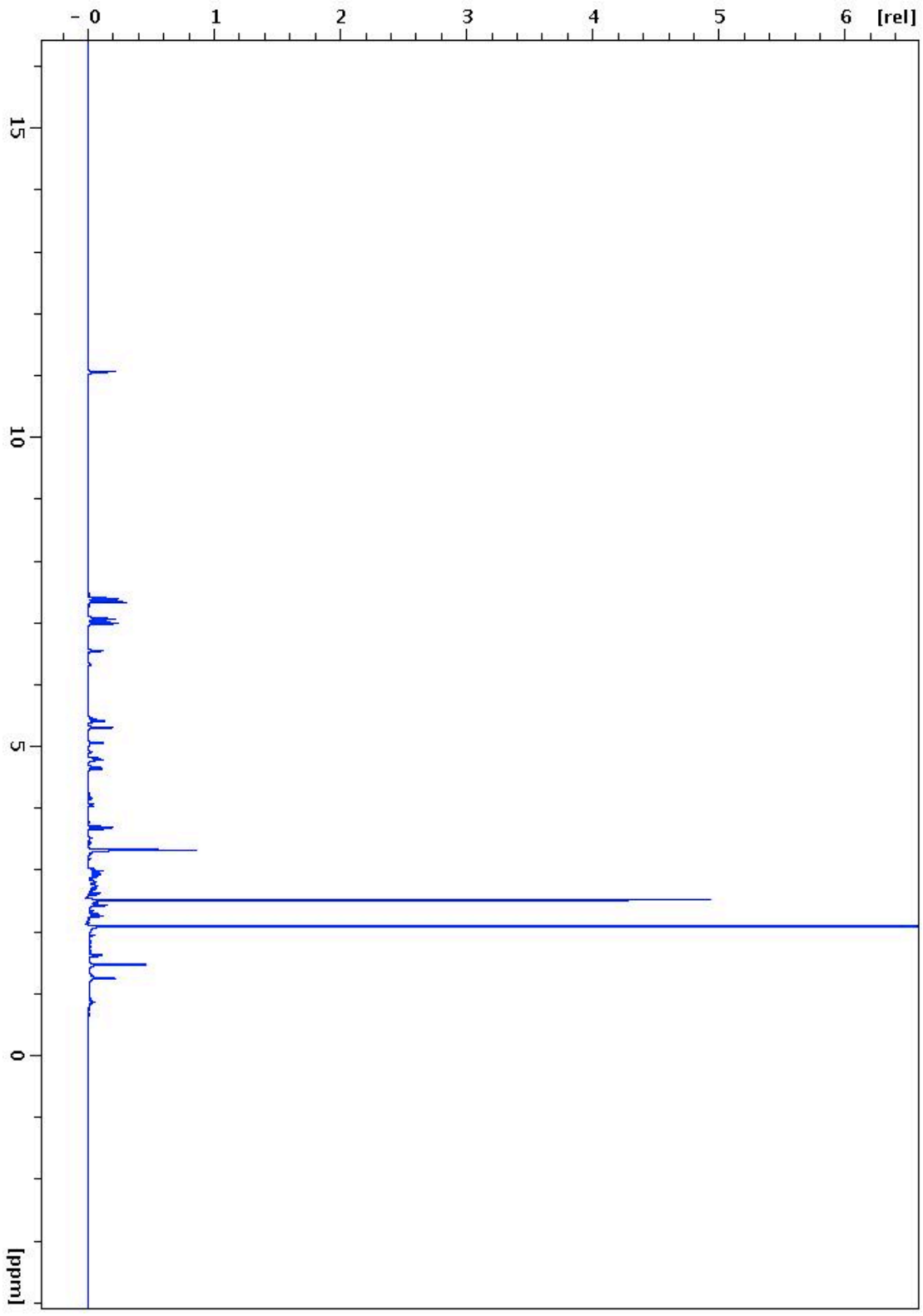


Figure A-1. ^{1}H-NMR spectrum of latifoliamide E (**50**) in DMSO

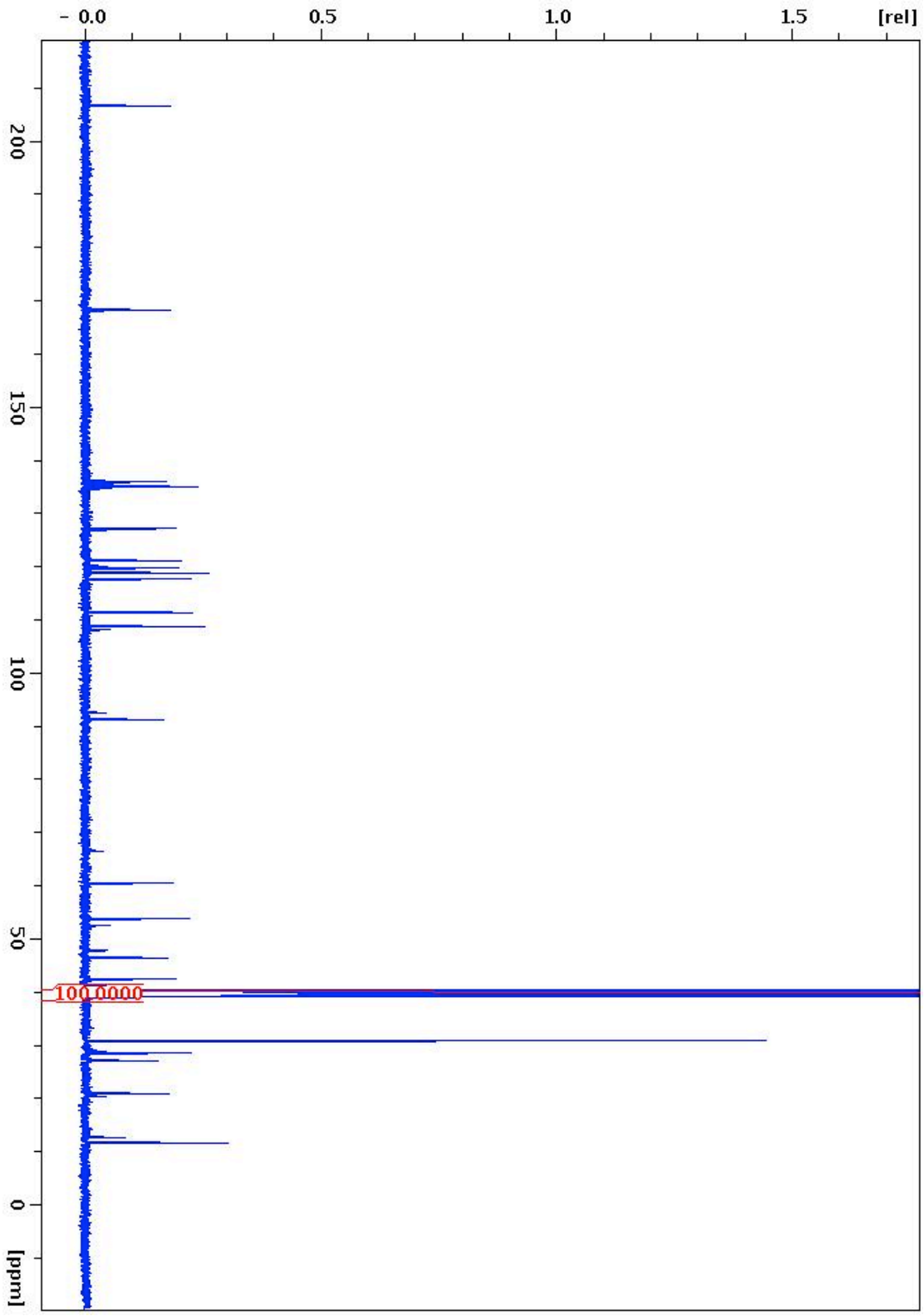


Figure A-2. ^{13}-C spectrum of latifoliamde E (**50**) in $CDCL_3$

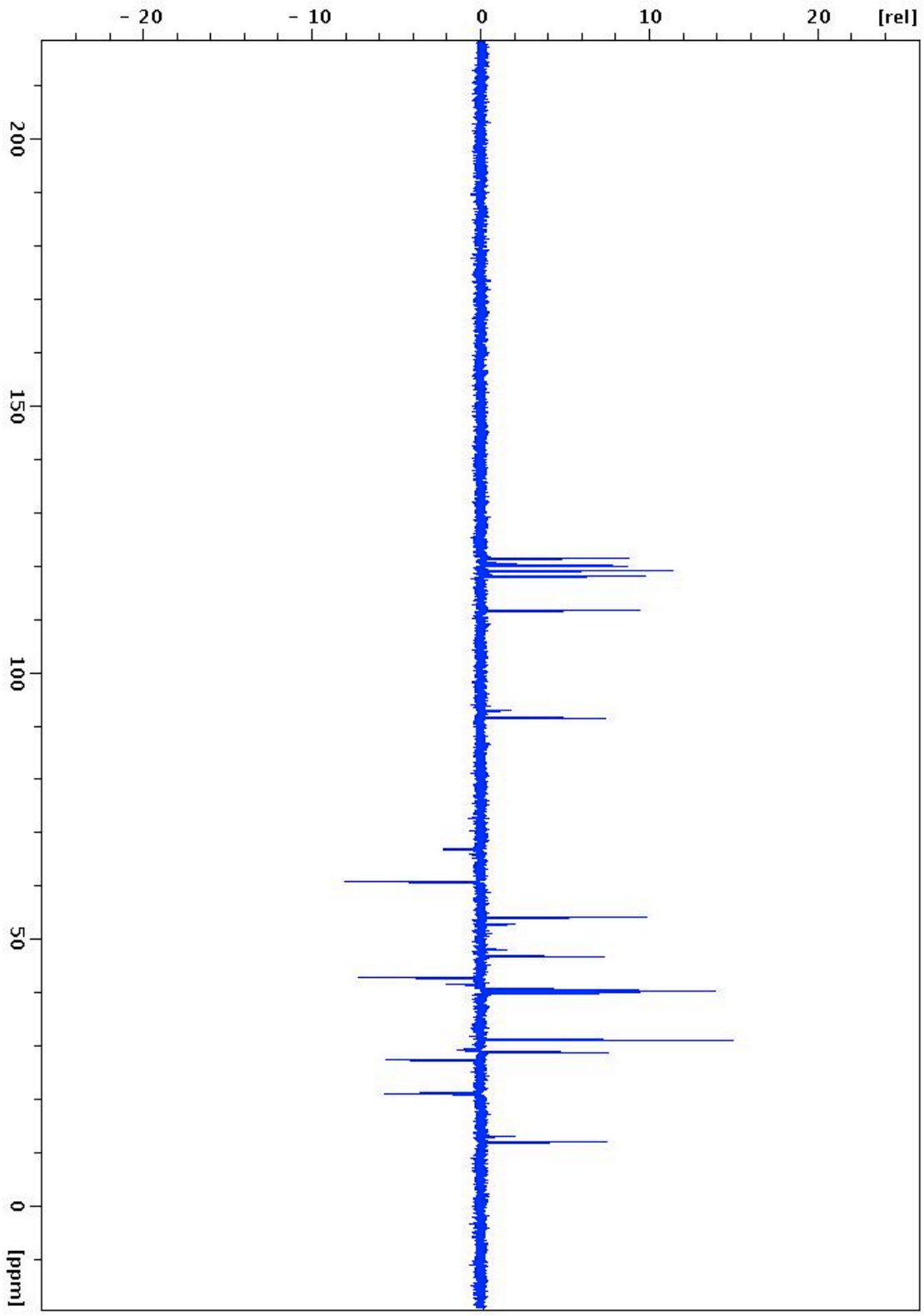


Figure A-3.DEPT 135 spectrum of latifoliamde E (**50**) in $CDCL_3$

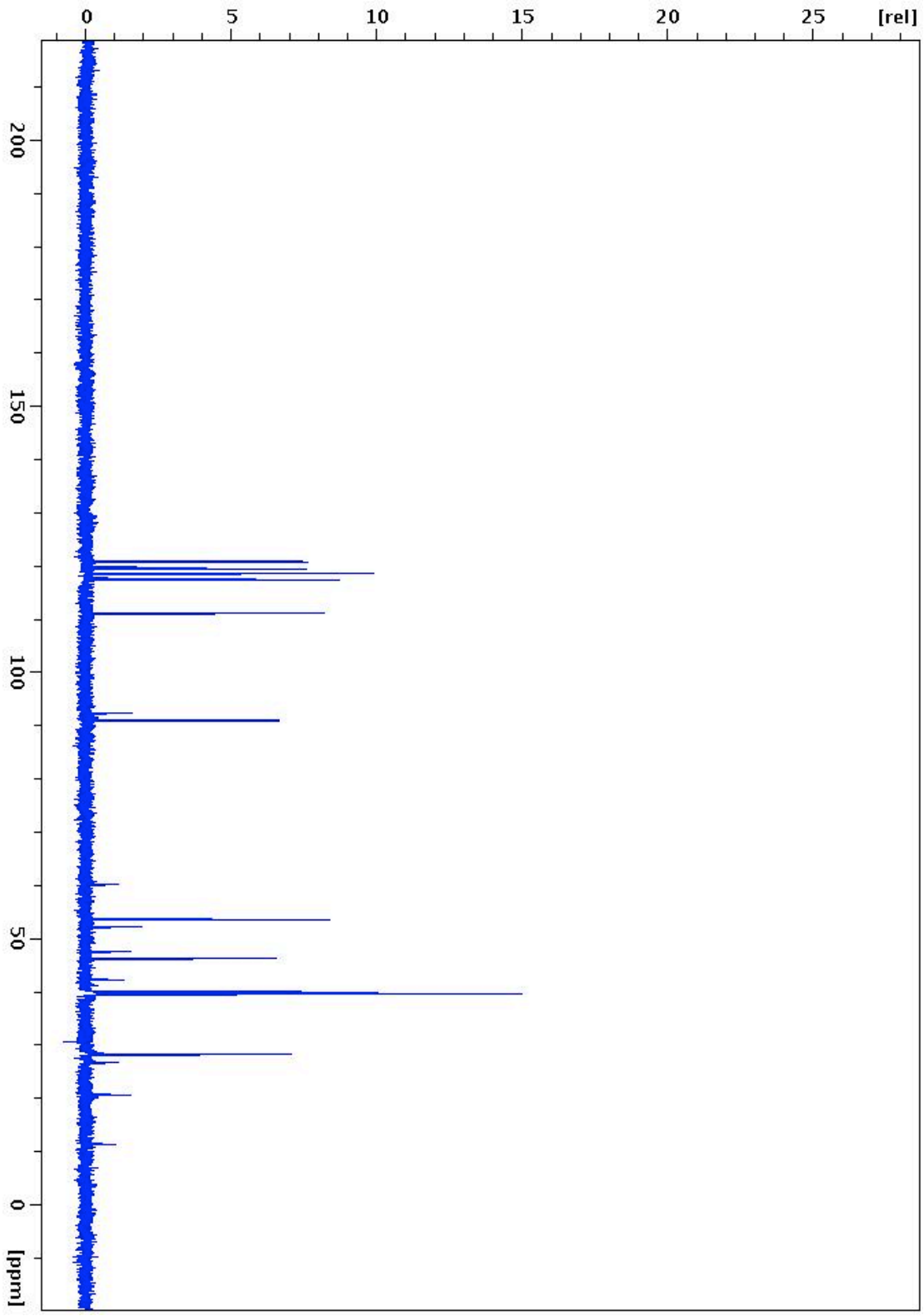


Figure A-4. DEPT 90 spectrum of latifoliamde E (**50**) in $CDCL_3$

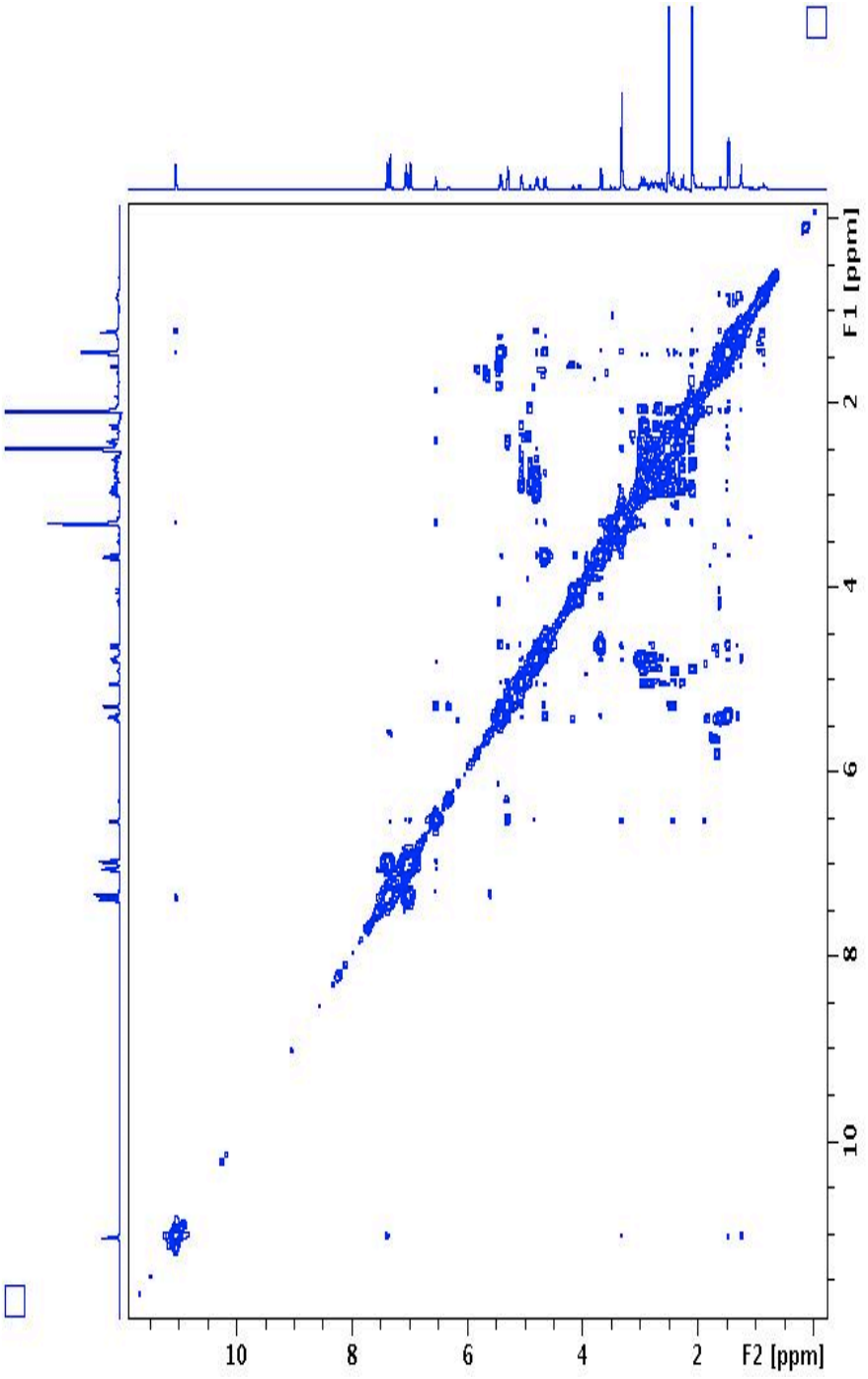


Figure A-5. COSY spectrum of latifoliamde E (**50**) in $CDCL_3$

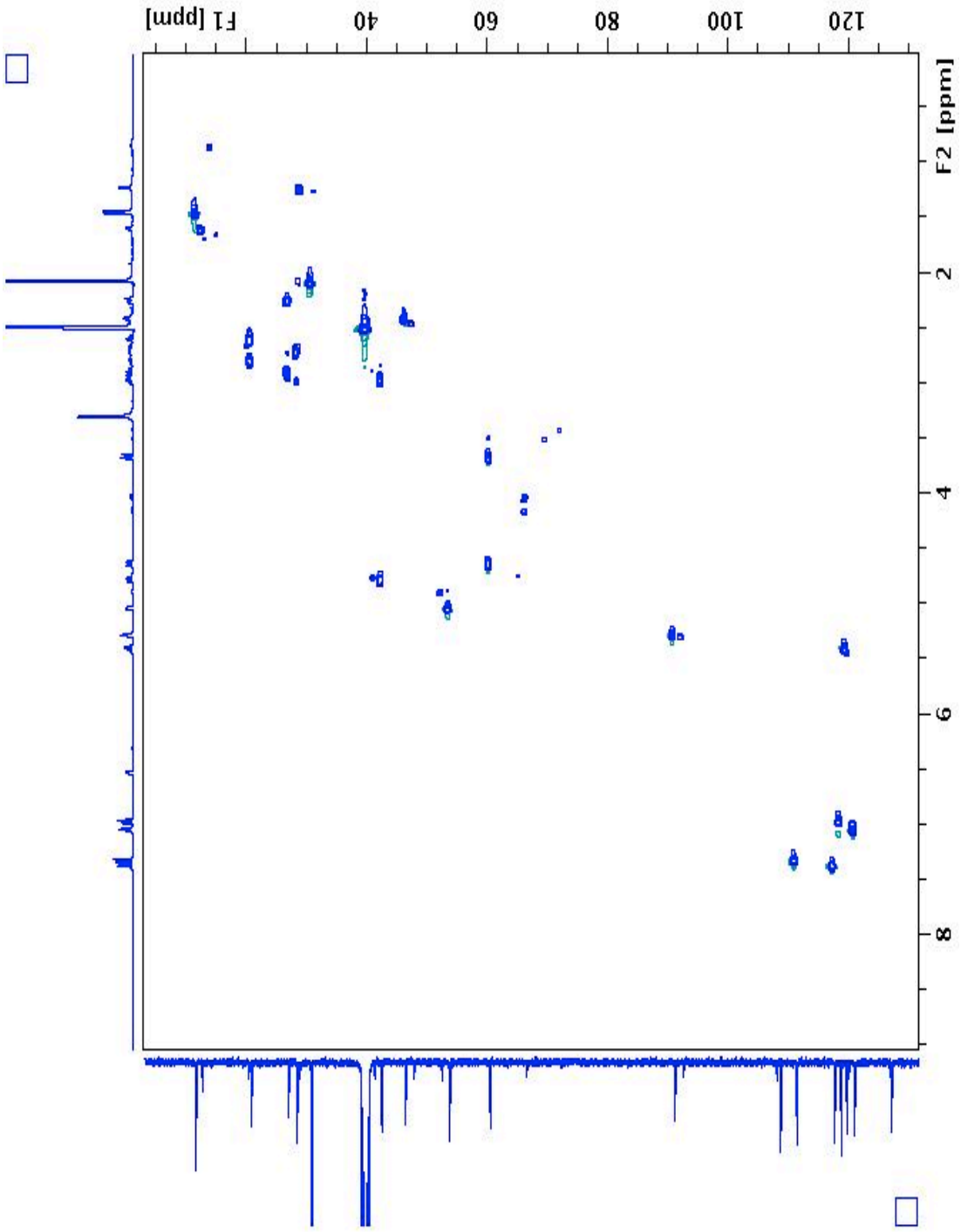


Figure A-6. HSQC spectrum of latifoliamde E (**50**) in $CDCl_3$

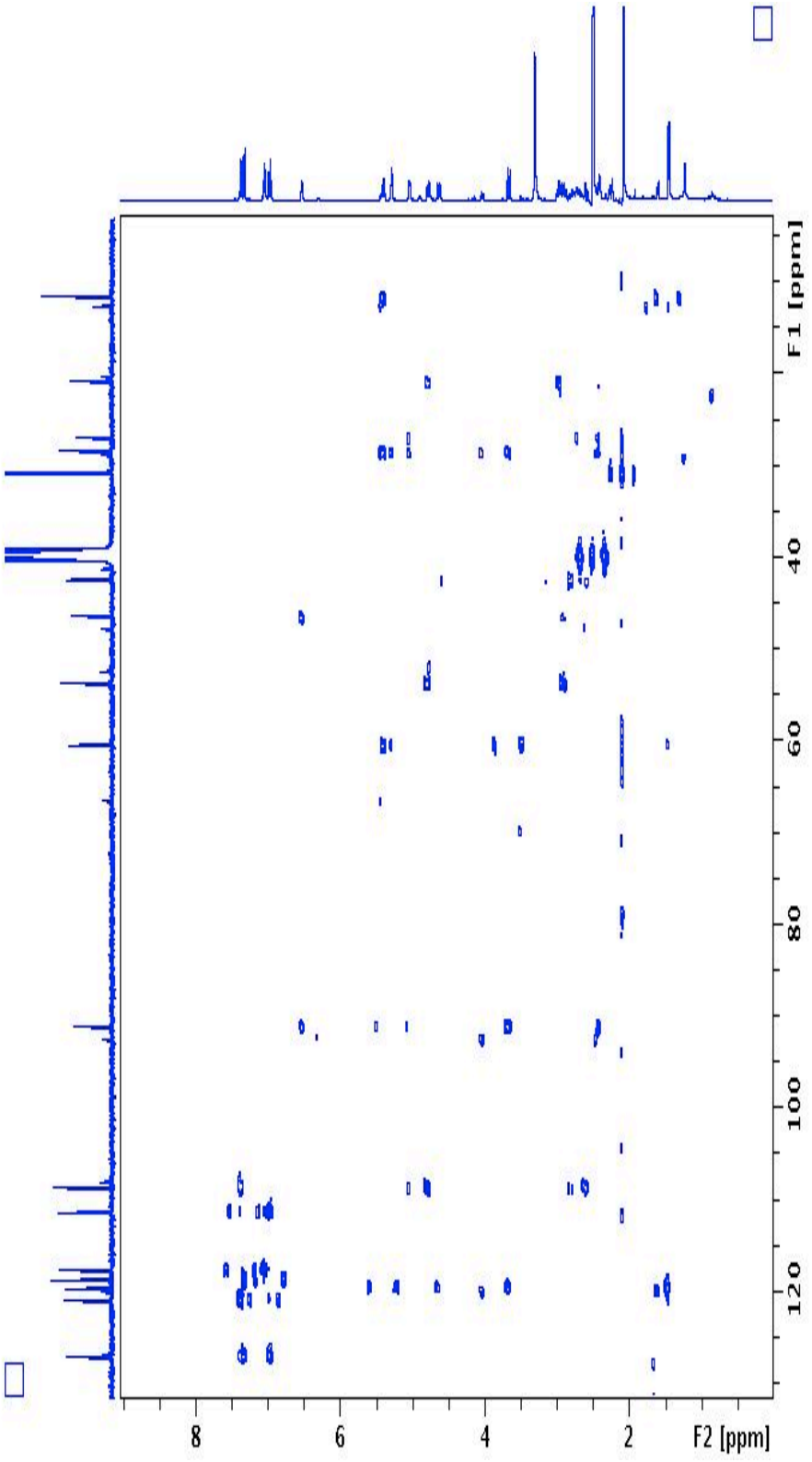


Figure A-7. HMBC spectrum of latifoliamde E (**50**) in $CDCl_3$

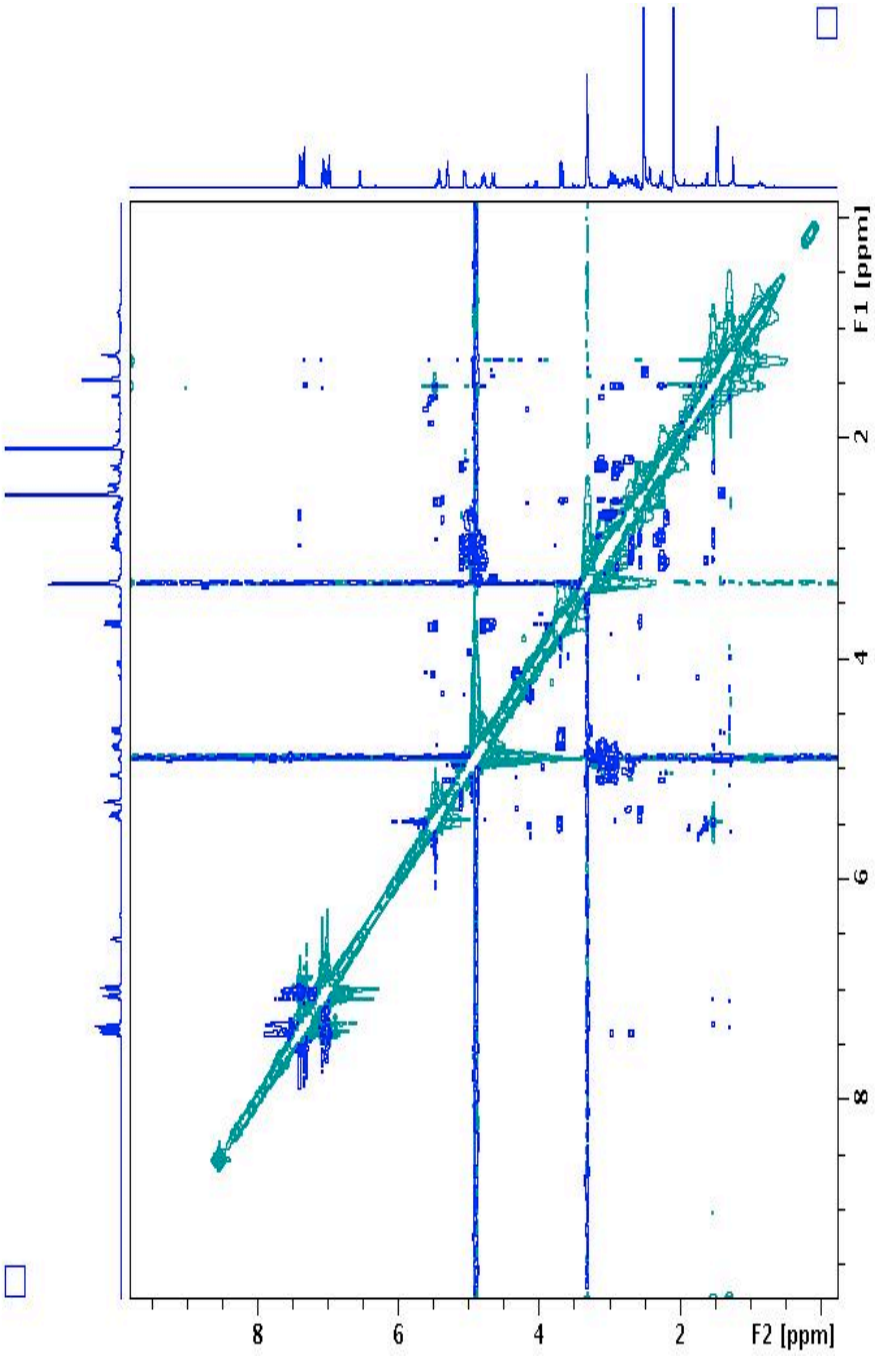


Figure A-8. NOESY spectrum of latifoliamde E (**50**) in $CDCl_3$

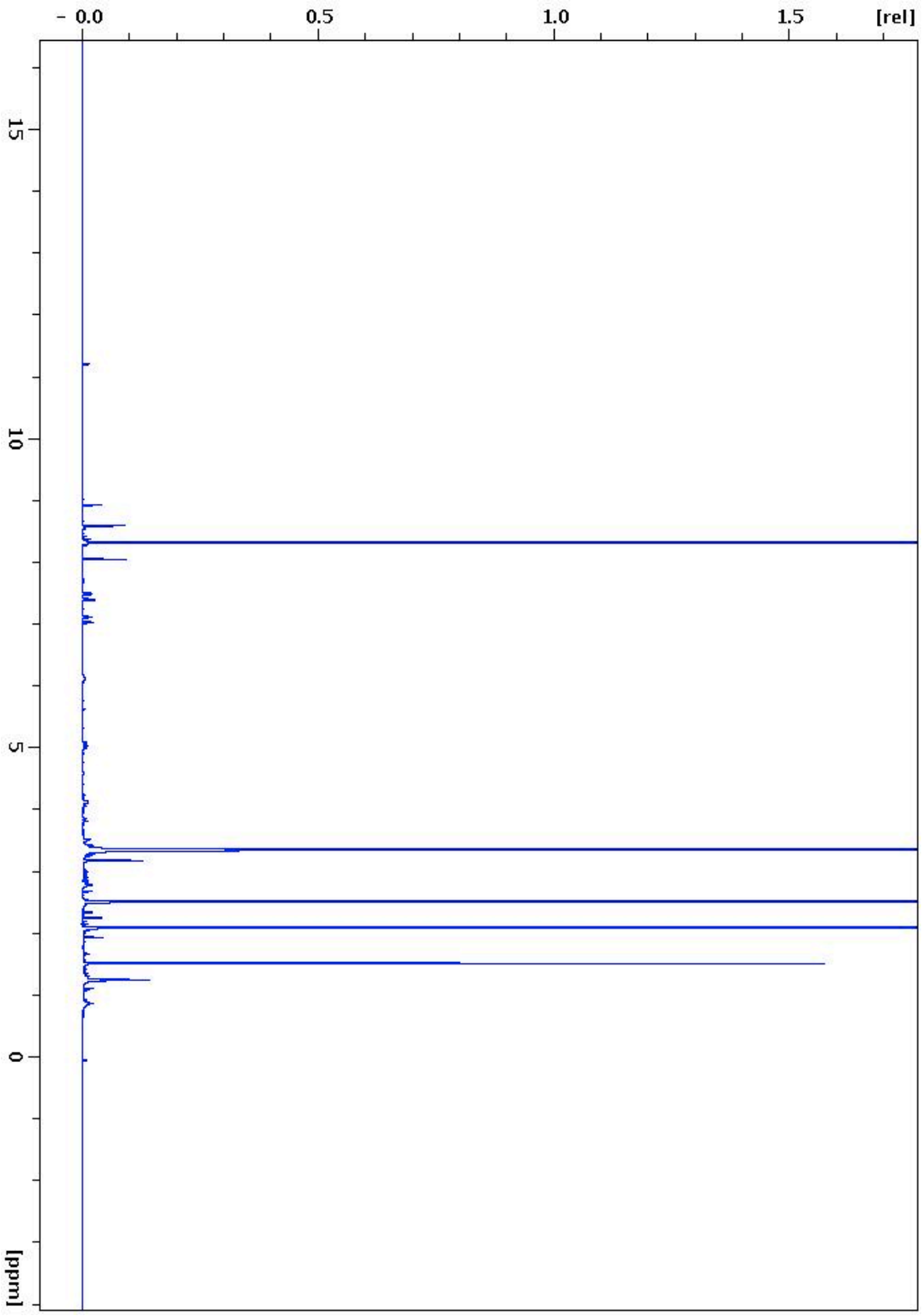


Figure A-9. ^{1}H-NMR spectrum of latifoliamide D (**49**) in DMSO

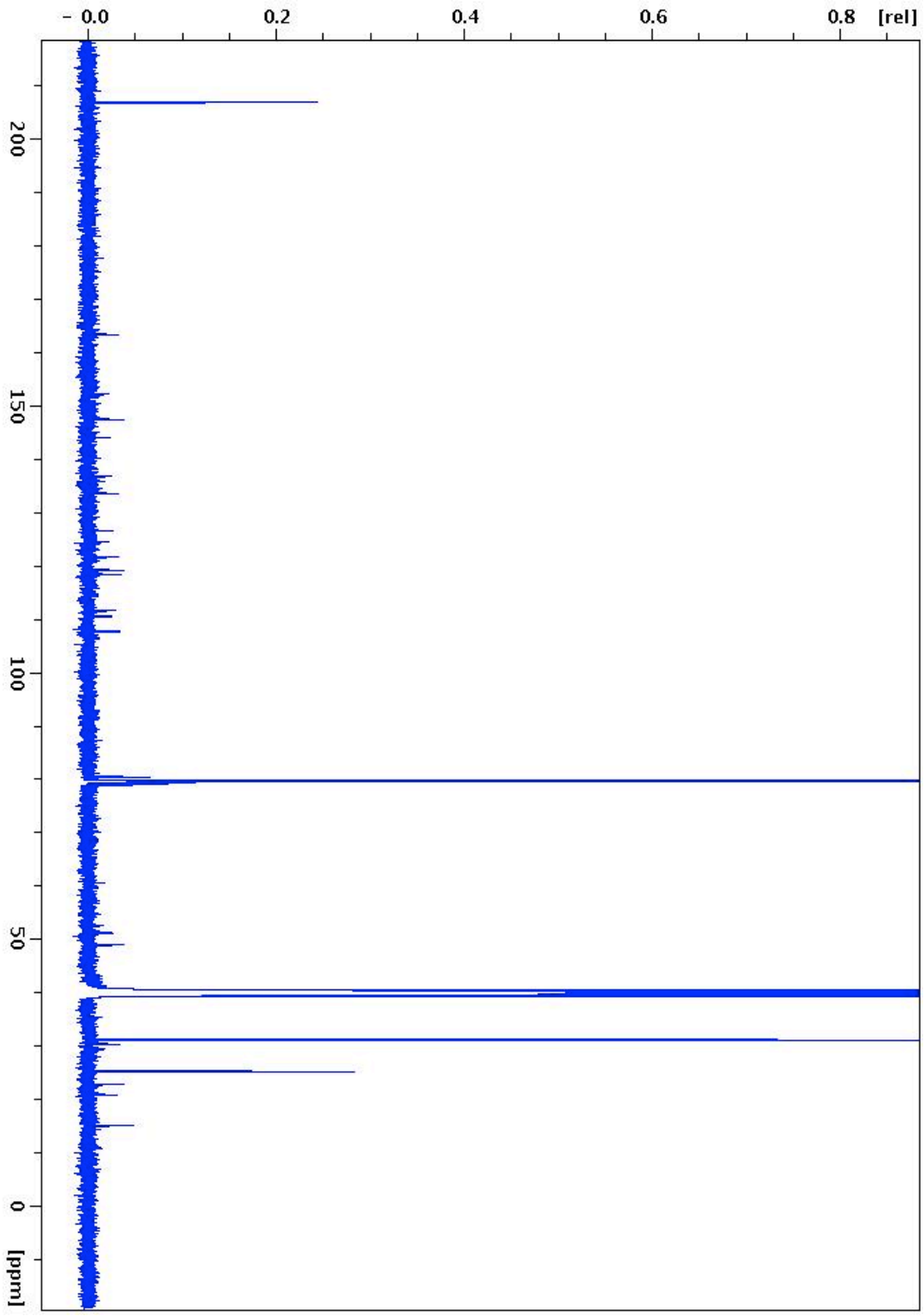


Figure A-10. ^{13}-C spectrum of latifoliamde D (**49**) in $CDCl_3$

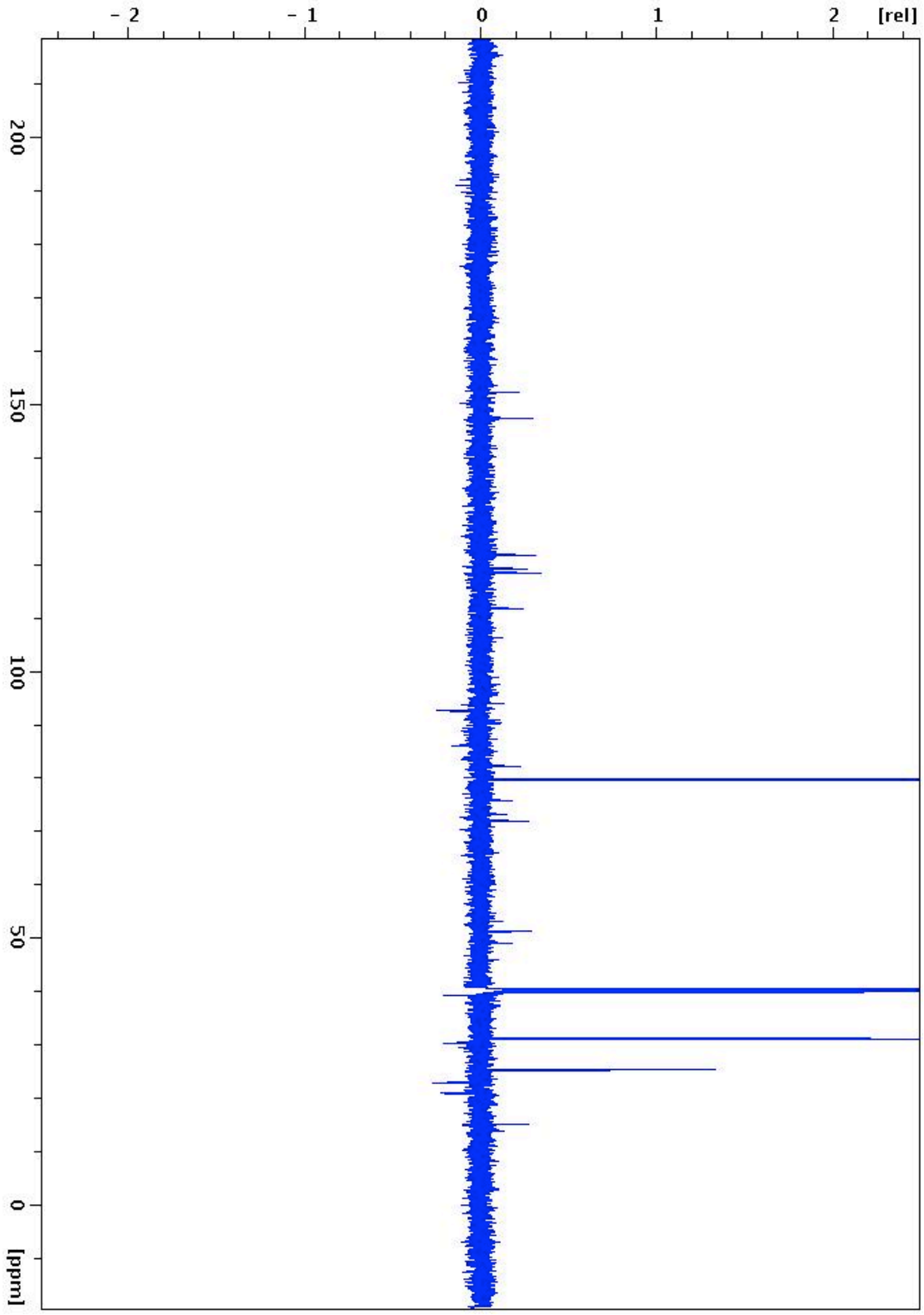


Figure A-11.DEPT 135 spectrum of latifoliamde D (**49**) in $CDCl_3$

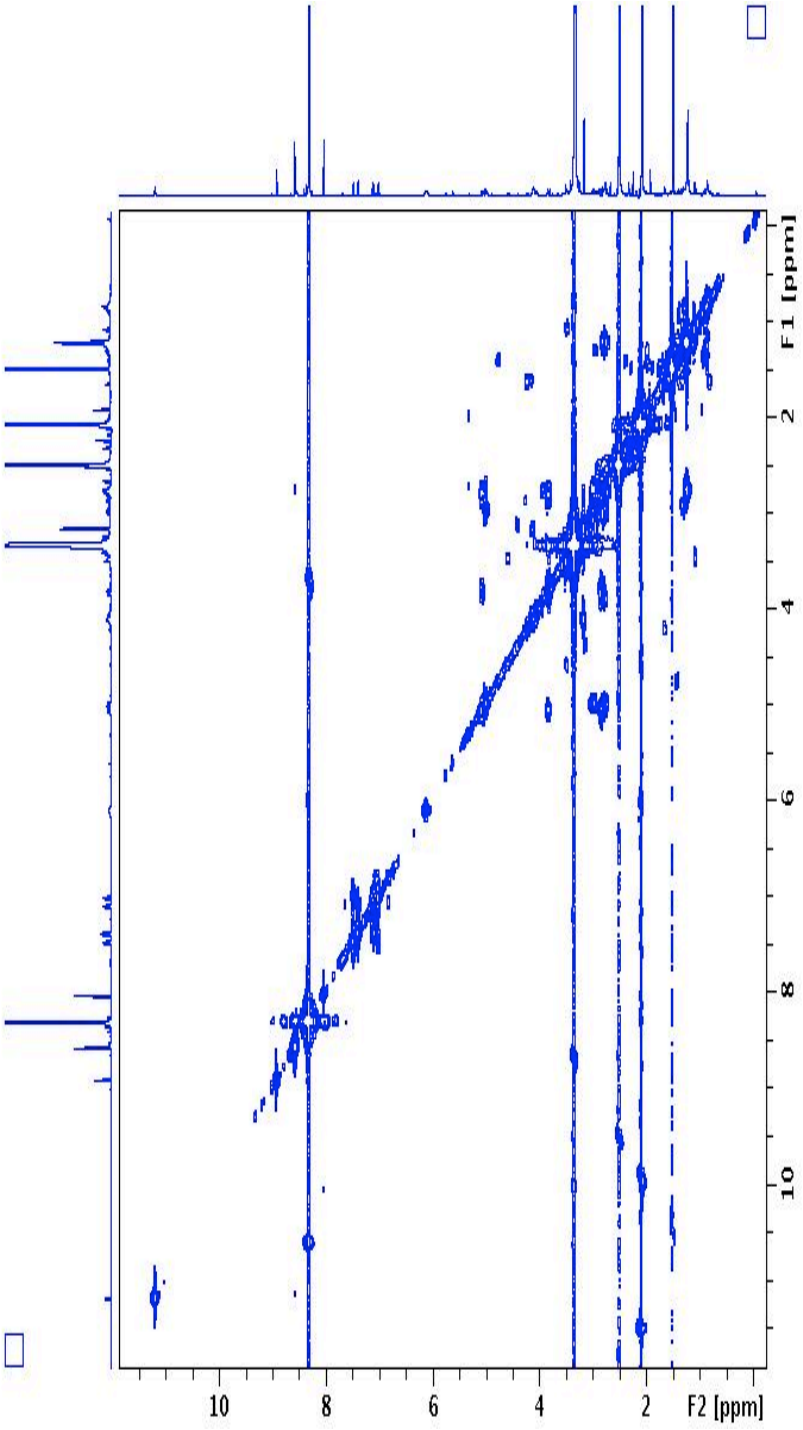


Figure A-12. COSY spectrum of latifoliamde D (**49**) in $CDCl_3$

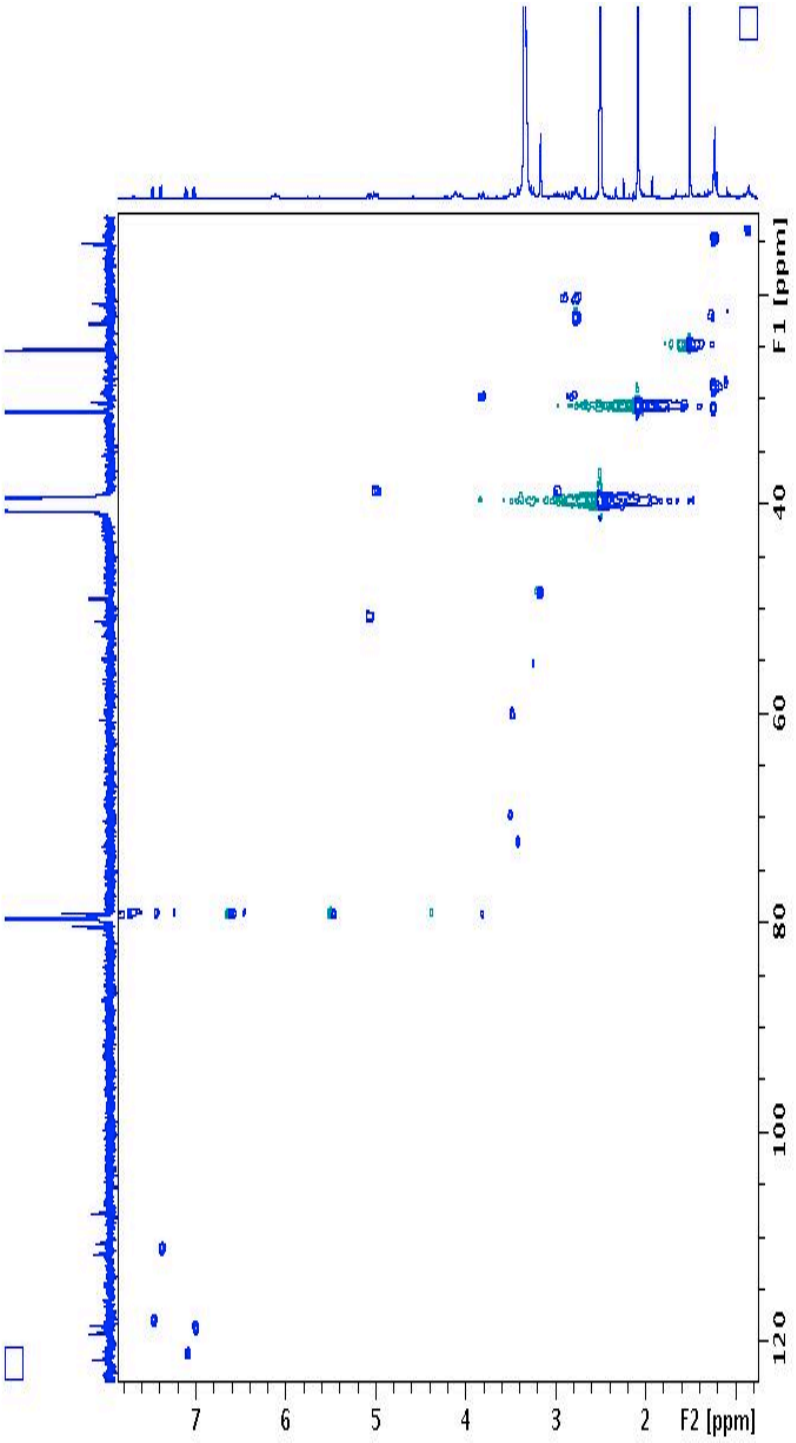


Figure A-13. HSQC spectrum of latifoliamde D (**49**) in $CDCl_3$

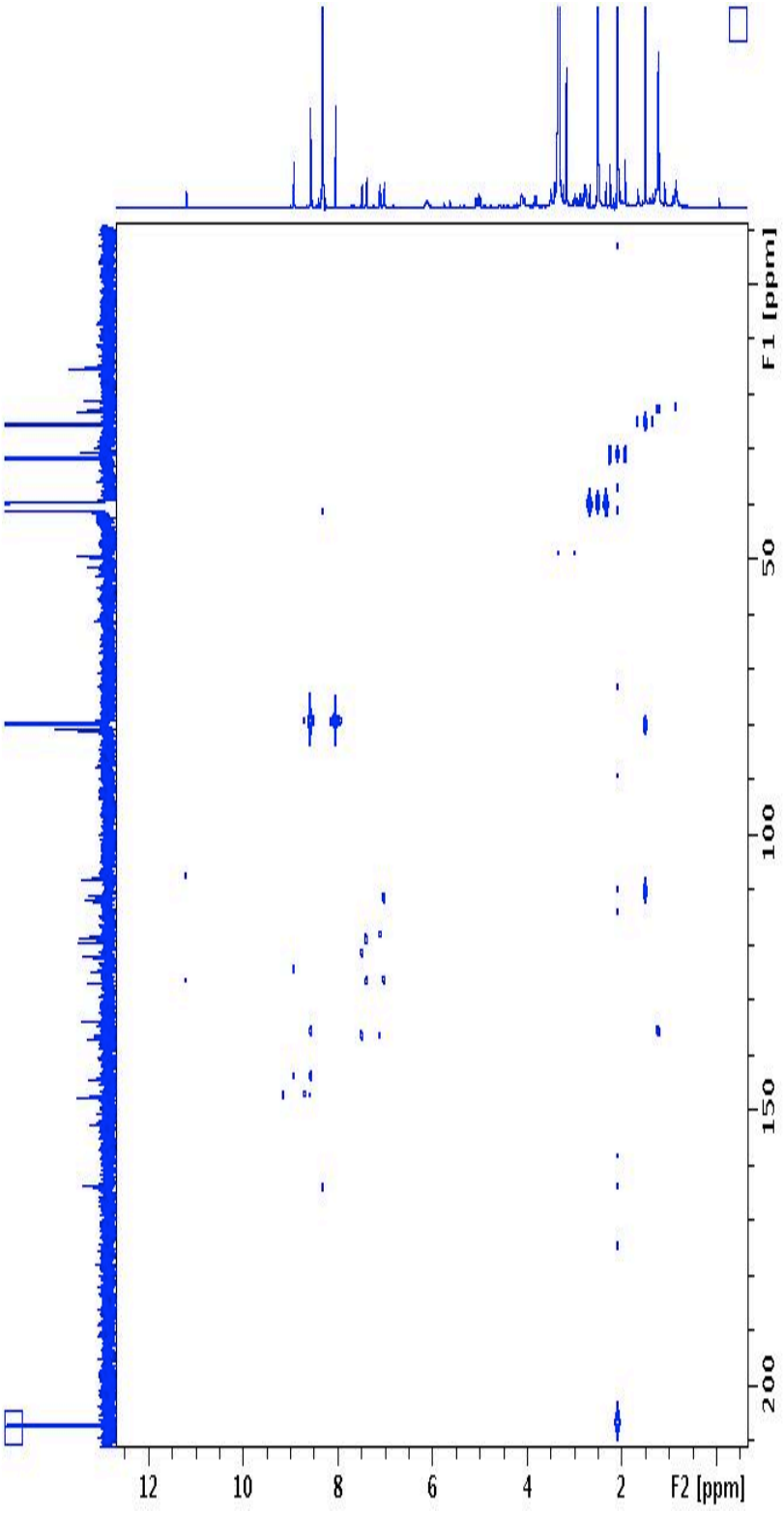


Figure A-14. HMBC spectrum of latifoliamde D (**49**) in $CDCl_3$

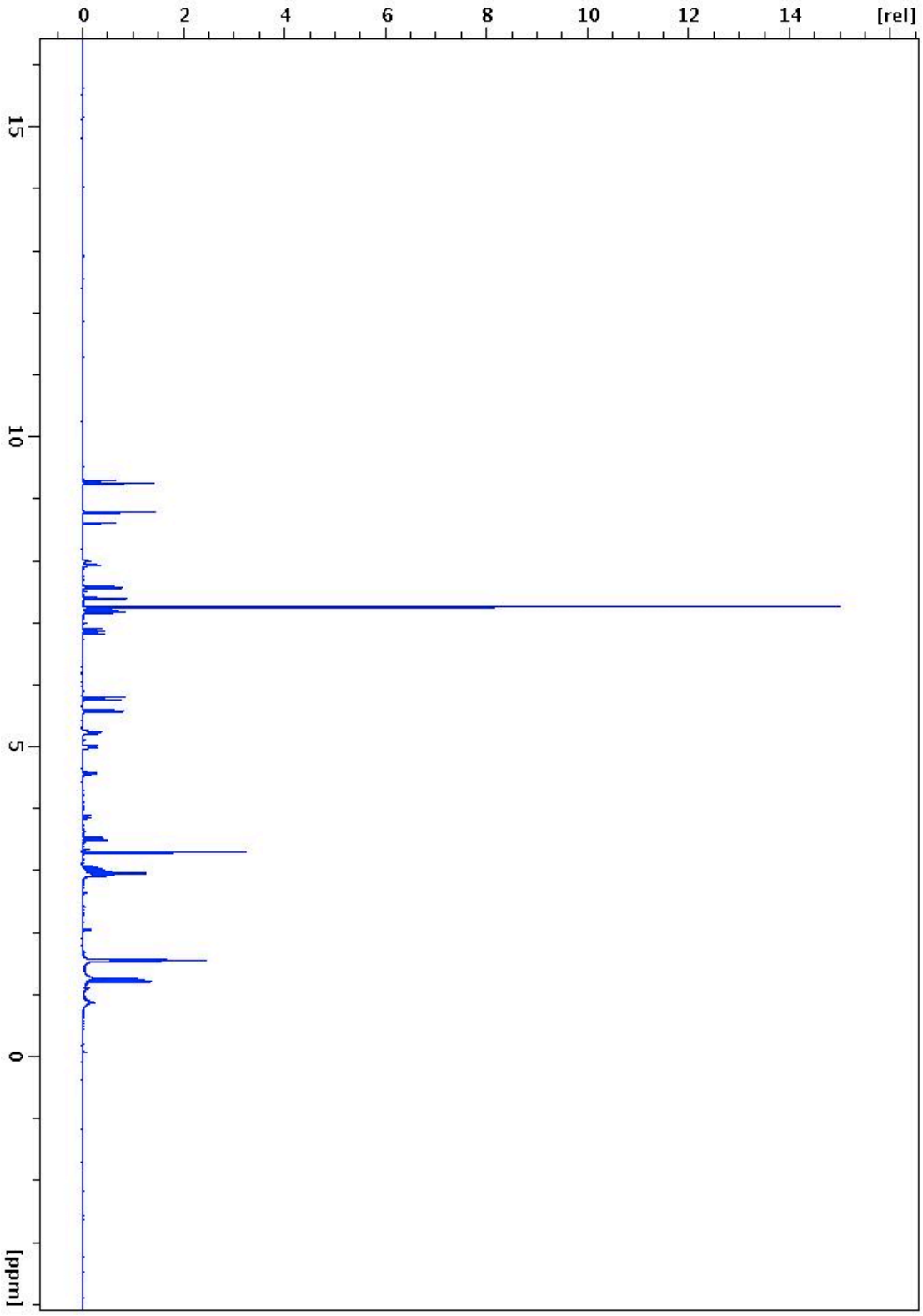


Figure A-15. ^{1}H-NMR spectrum of latifoliamide B (**47**) in $CDCl_3$

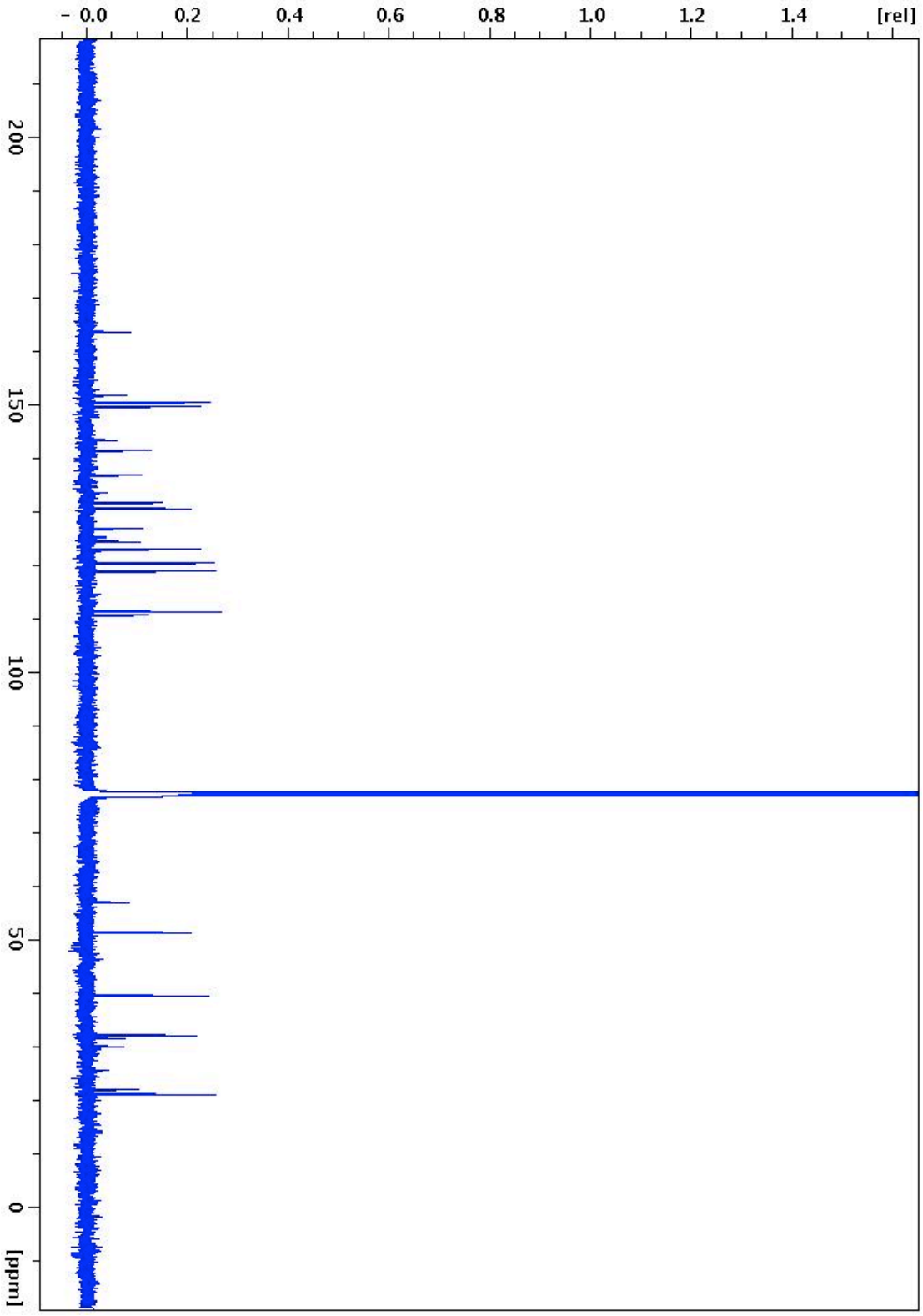


Figure A-16. ^{13}-C spectrum of latifoliamde B (**47**) in $CDCl_3$

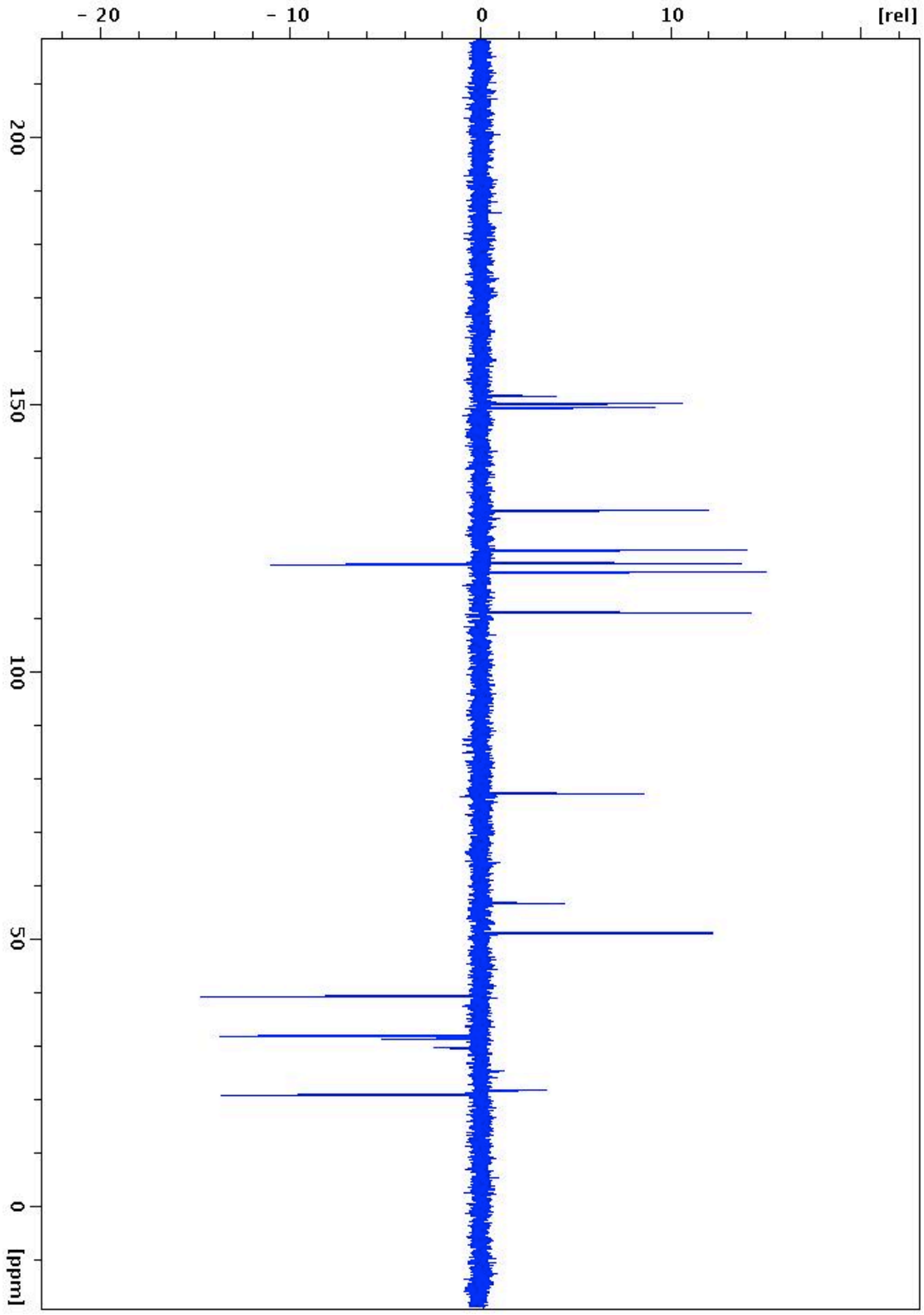


Figure A-17. DEPT 135 spectrum of latifoliamde B (**47**) in $CDCl_3$

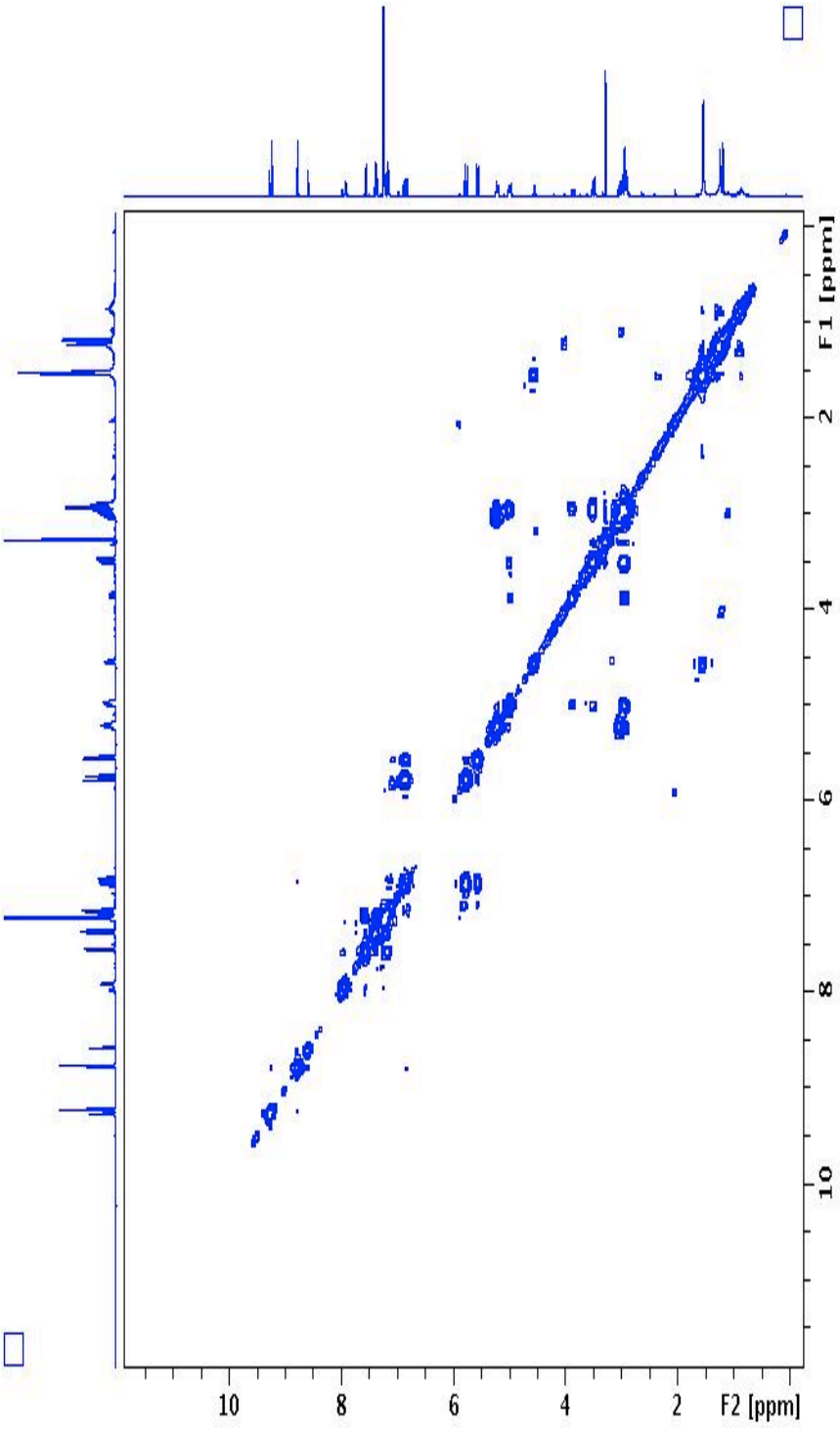


Figure A-18. COSY spectrum of latifoliamde B (**47**) in $CDCl_3$

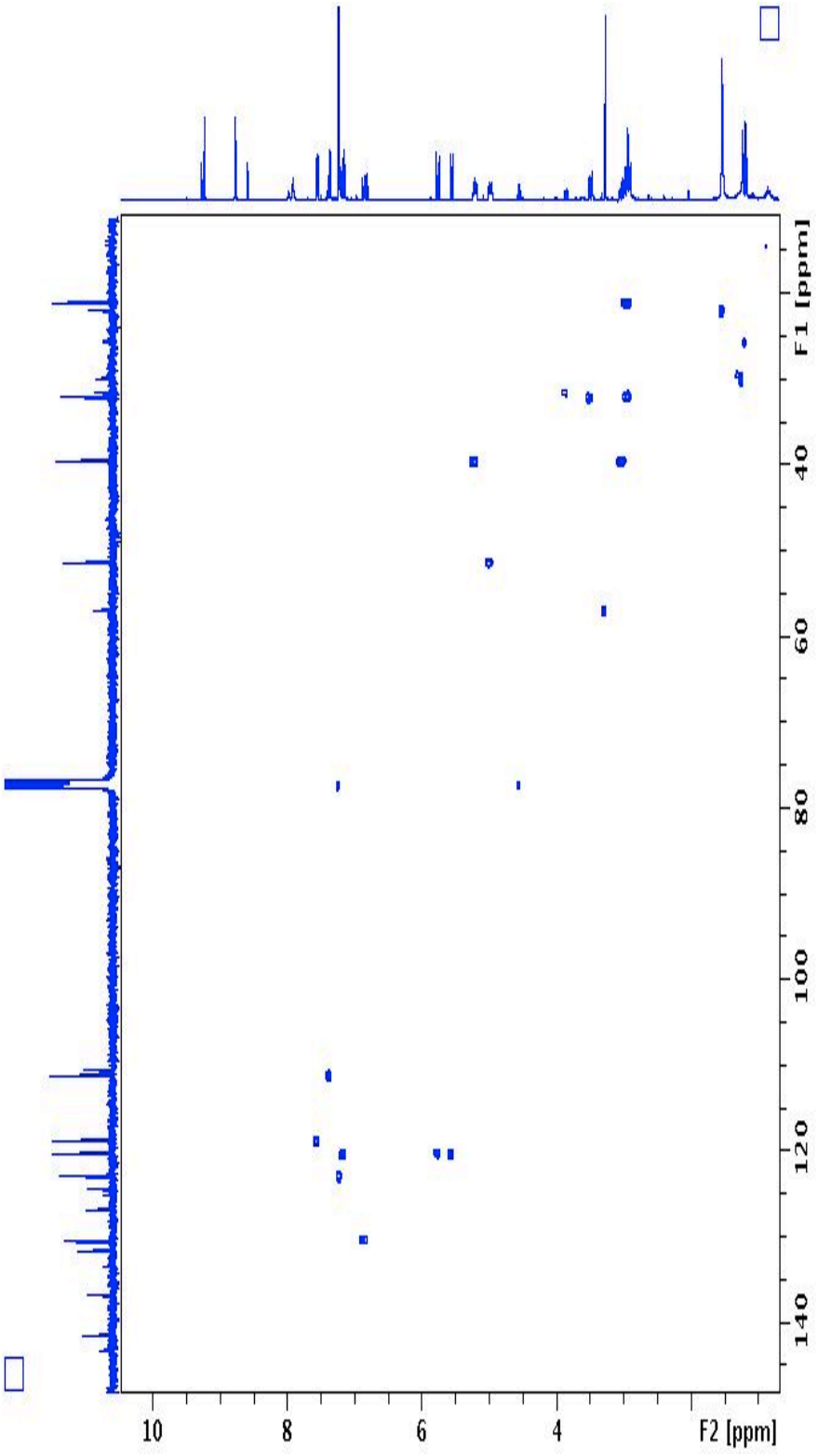


Figure A-19. HSQC spectrum of latifoliamde B (**47**) in $CDCl_3$

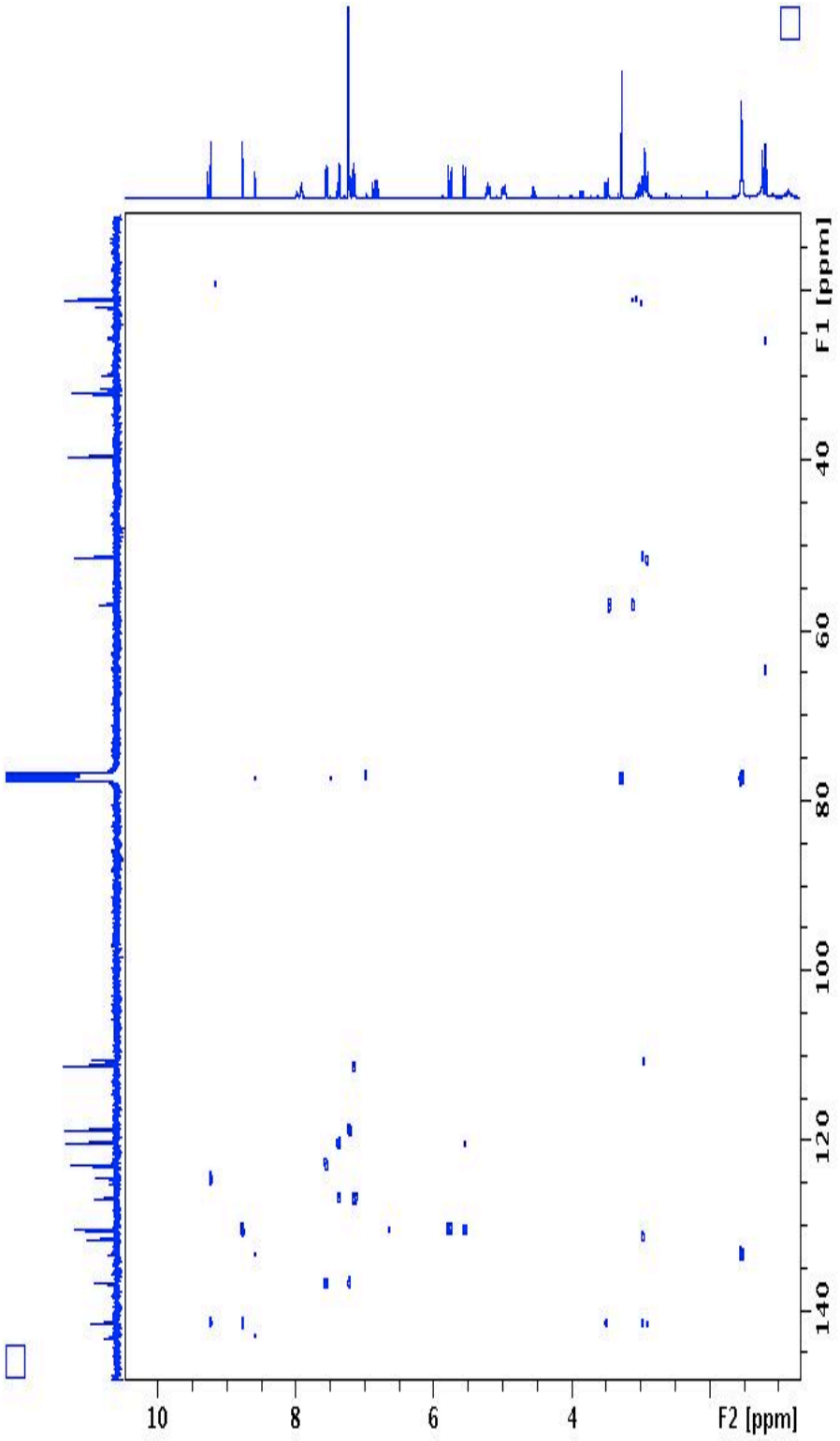


Figure A-20. HMBC spectrum of latifoliamde B (**47**) in $CDCl_3$

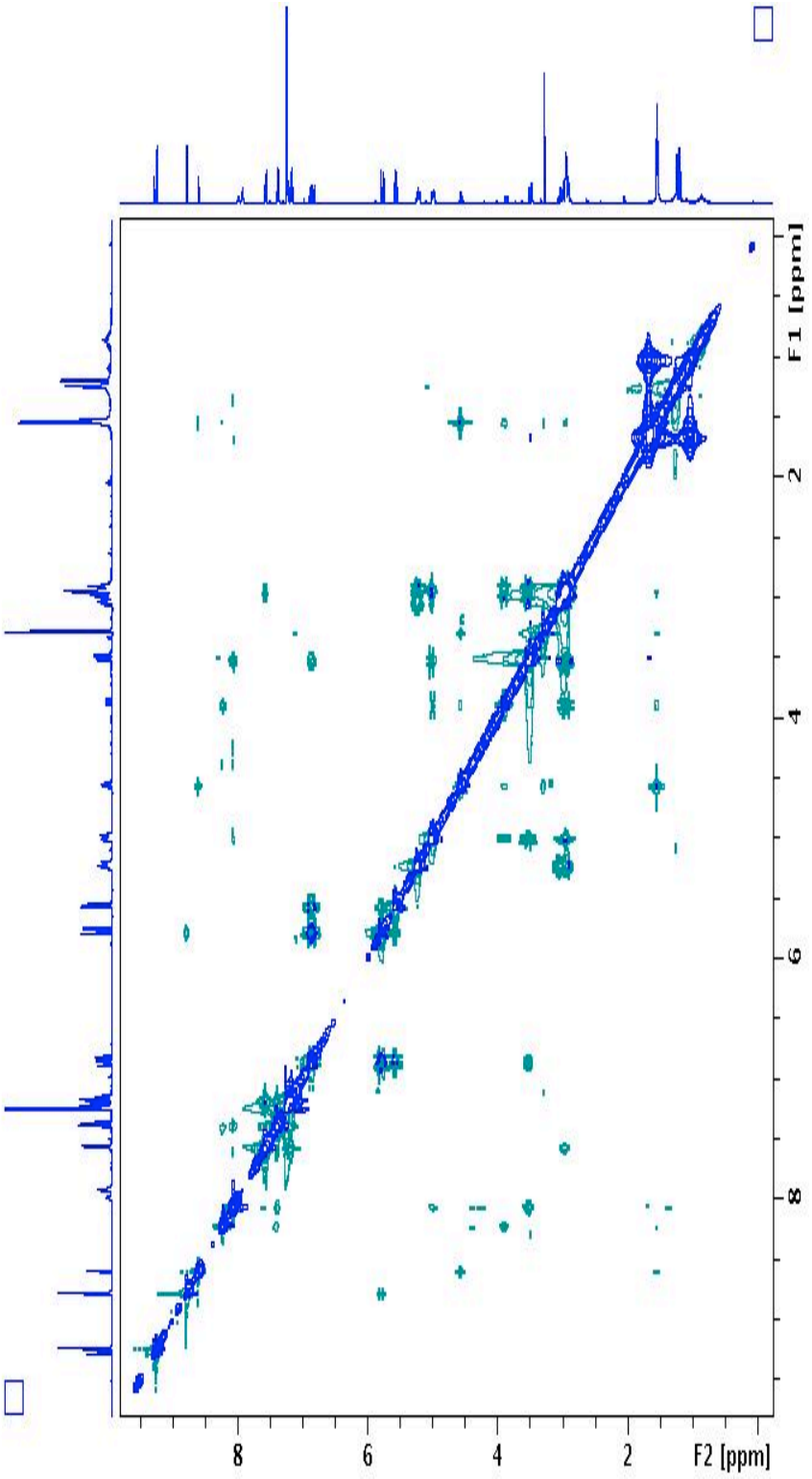


Figure A-21. NOESY spectrum of latifoliamde B (**47**) in $CDCl_3$

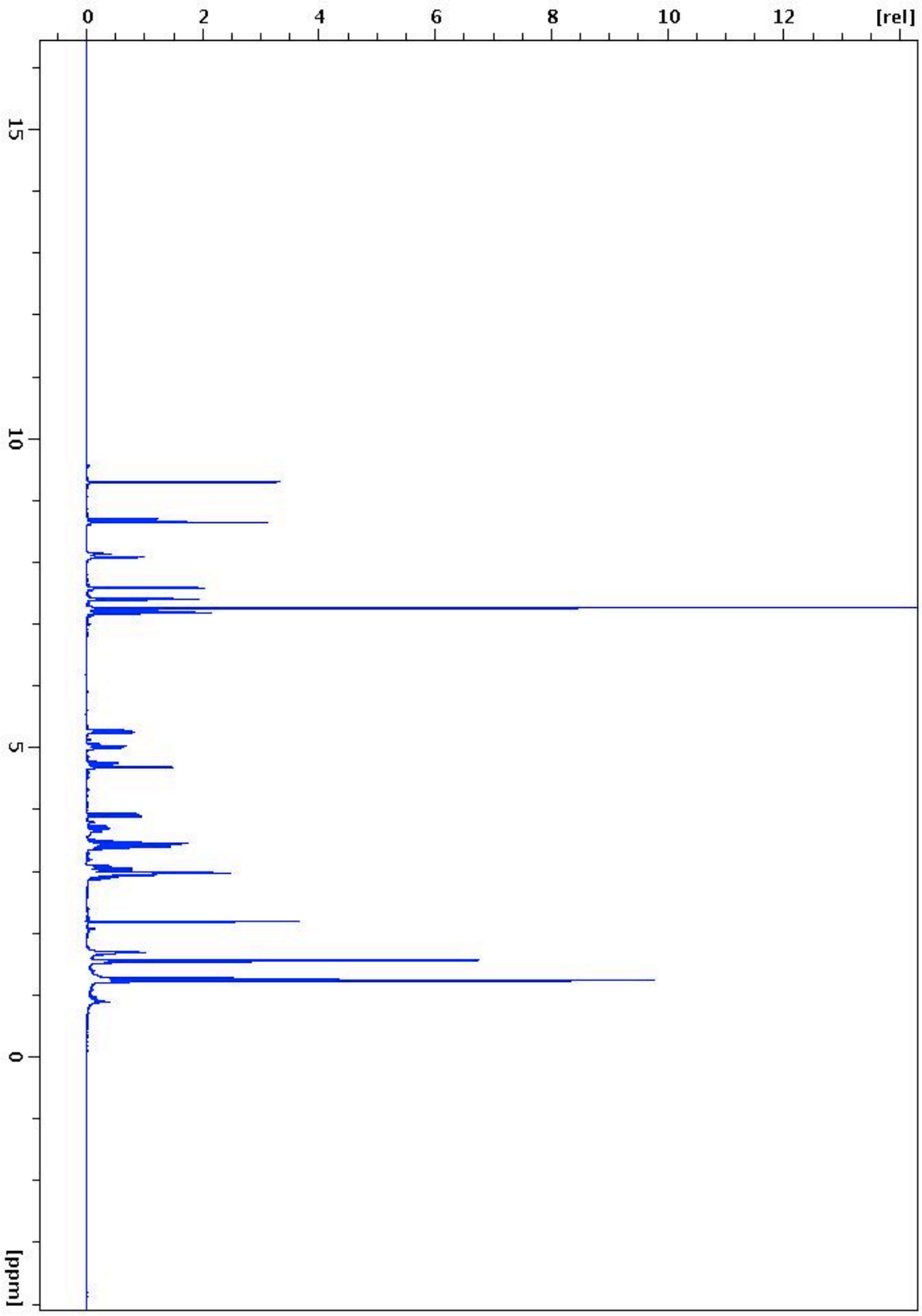


Figure A-22. ^{1}H-NMR spectrum of angustoline (**45**) in $CDCl_3$

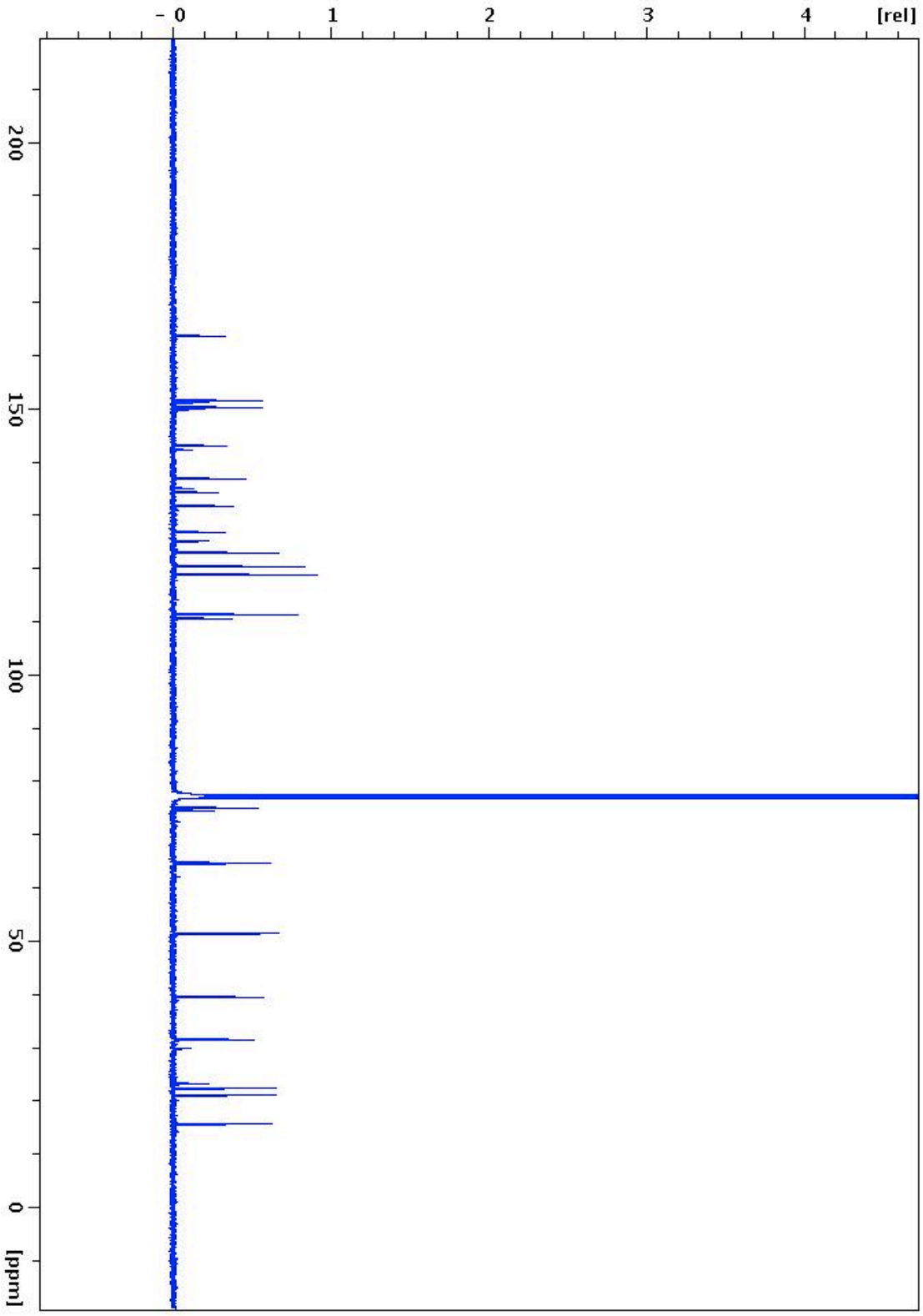


Figure A-23. ^{13}C spectrum of angustoline (**45**) in $CDCl_3$

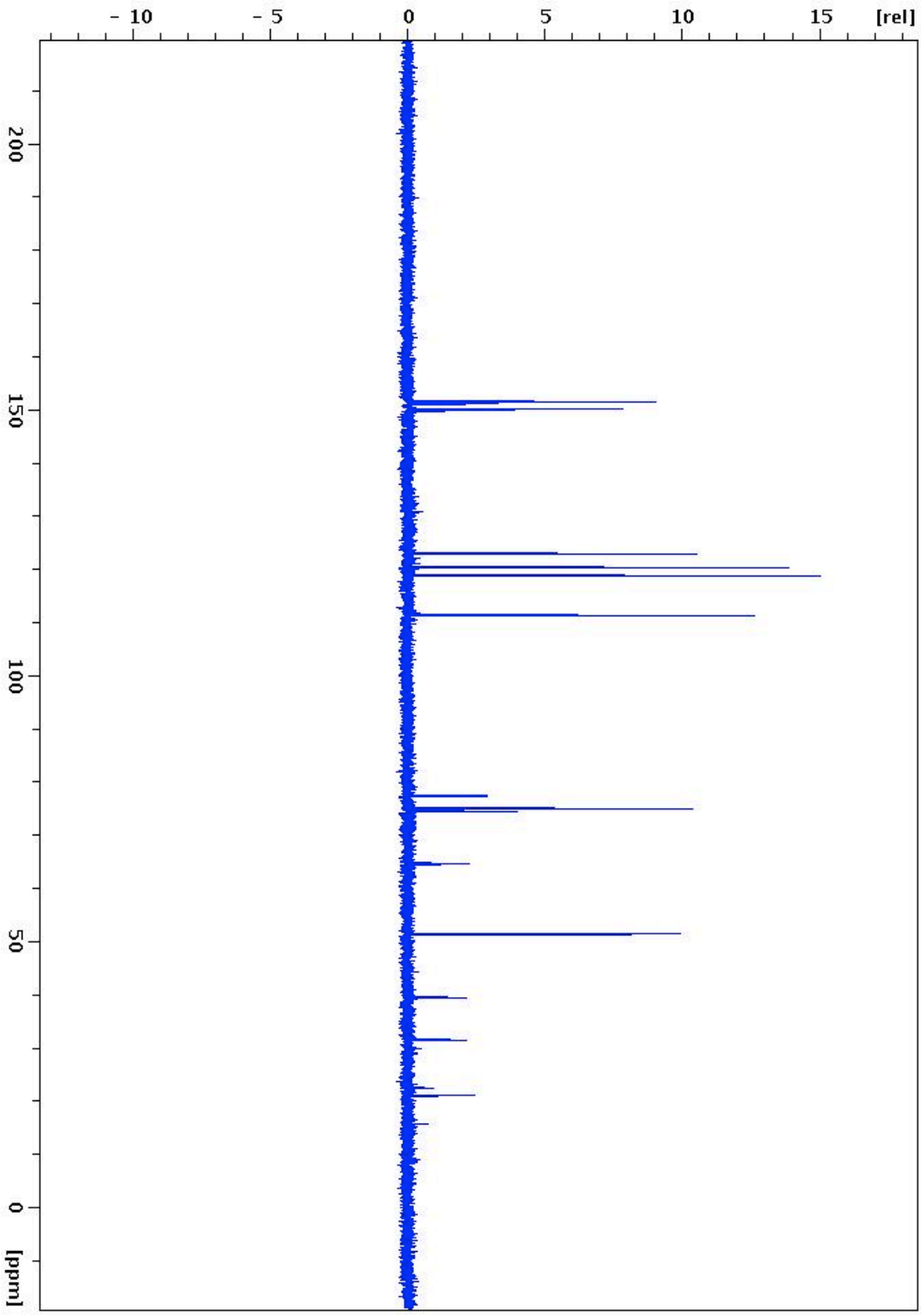


Figure A-24. DEPT 90 spectrum of angustoline (**45**) in $CDCl_3$

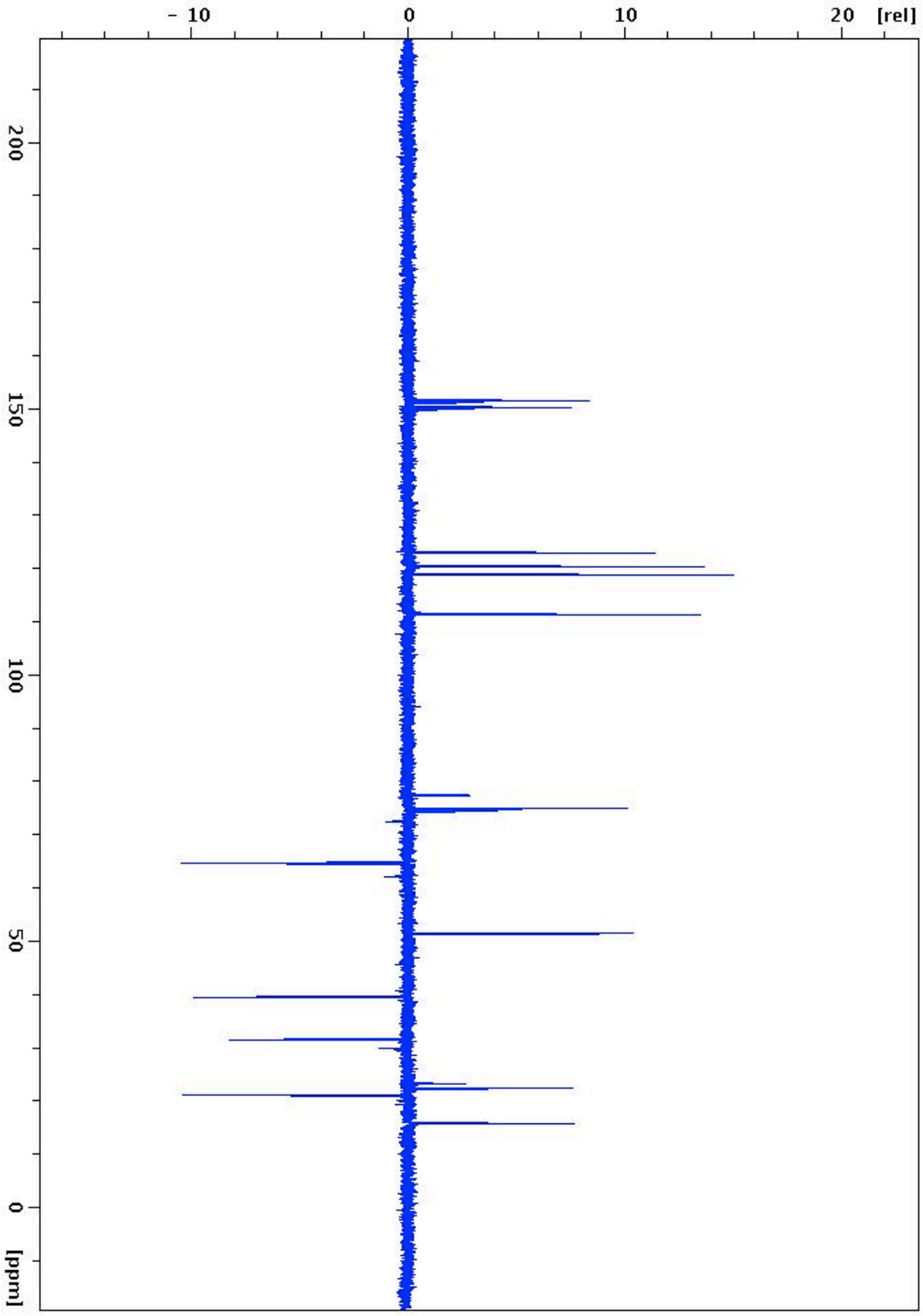


Figure A-25. DEPT 135 spectrum of angustoline (**45**) in $CDCl_3$

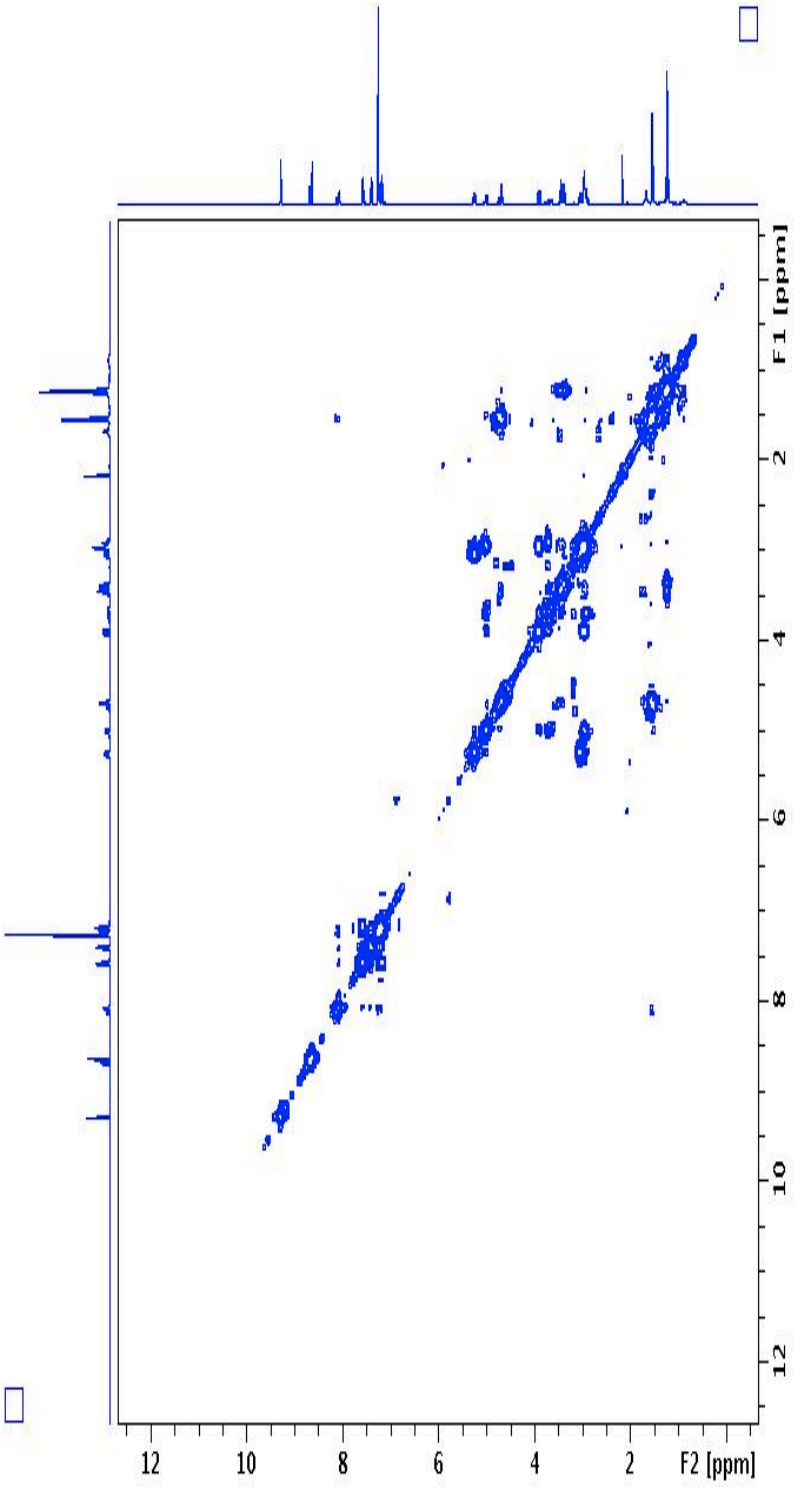


Figure A-26. COSY spectrum of angustoline (**45**) in $CDCl_3$

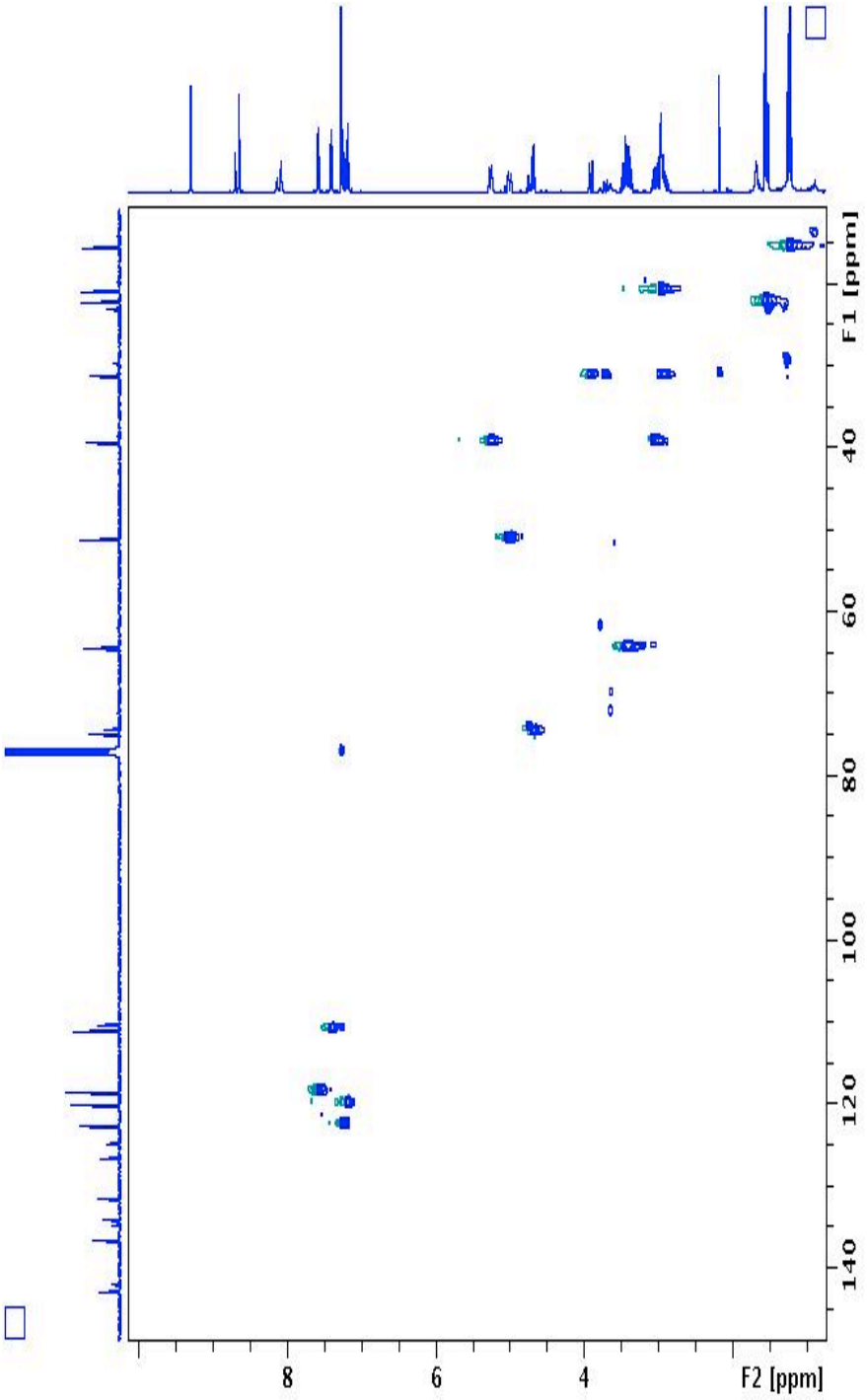


Figure A-27. HSQC spectrum of angustoline (**45**) in $CDCl_3$

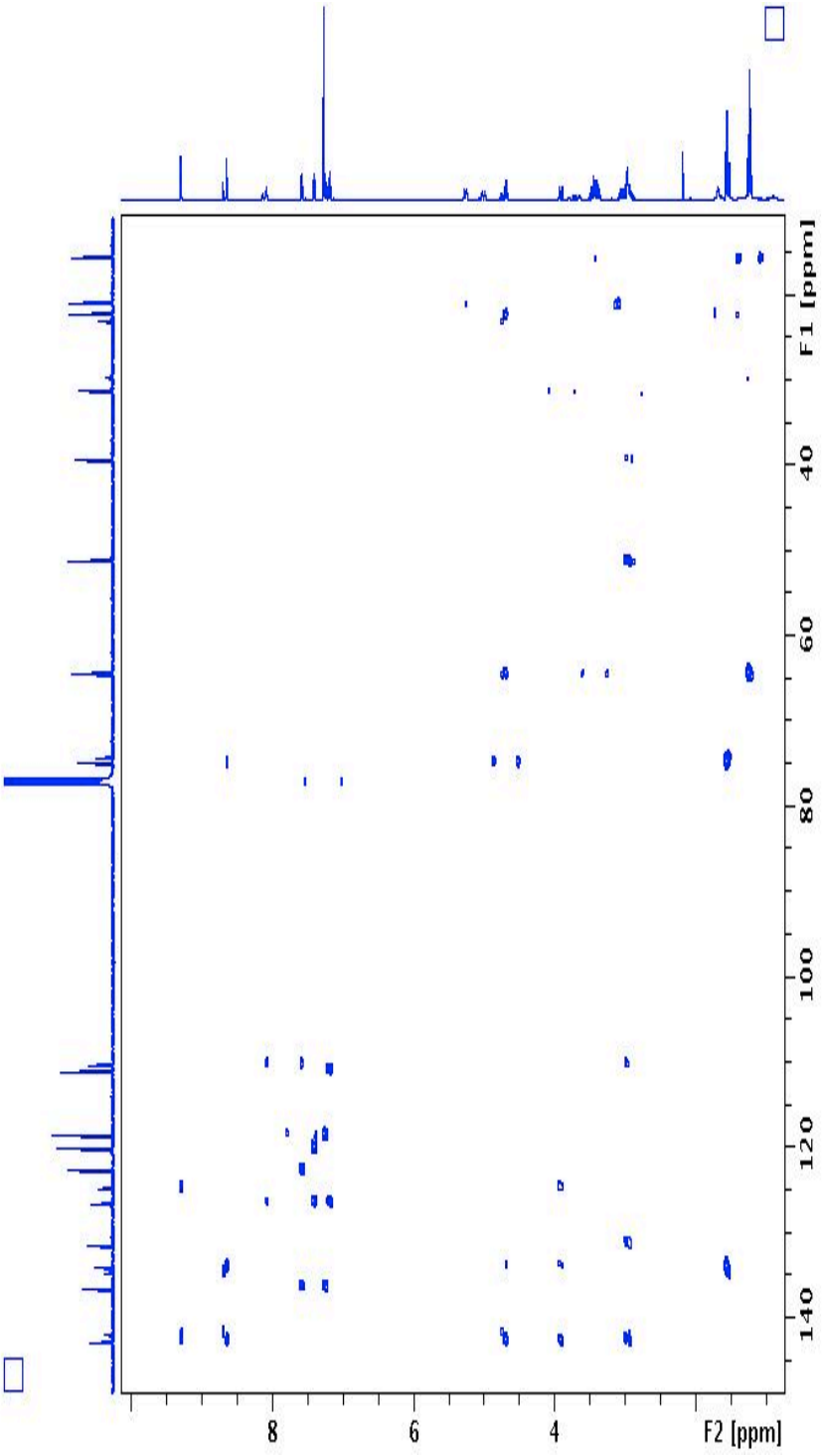


Figure A-28. HMBC spectrum of angustoline (**45**) in $CDCl_3$

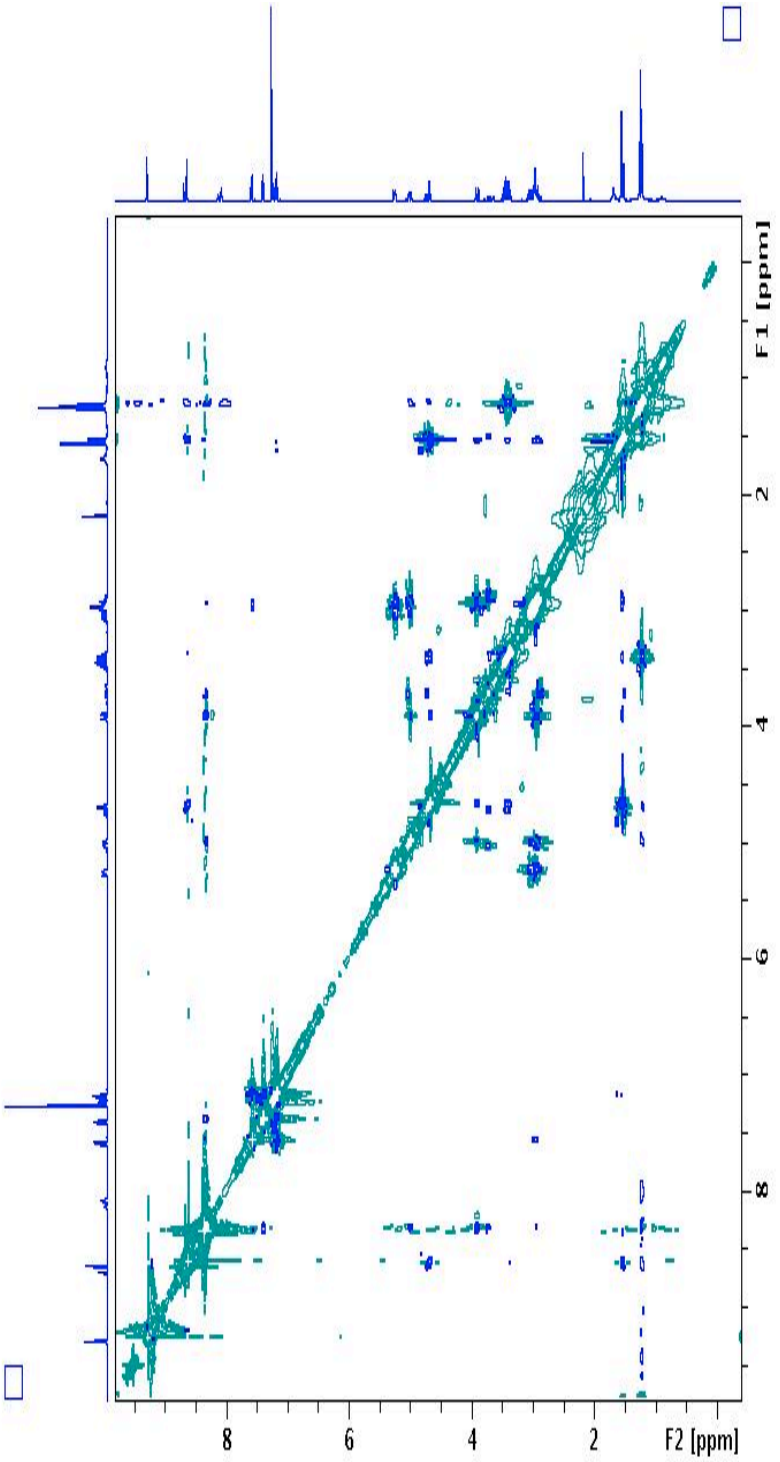


Figure A-29. NOESY spectrum of angustoline (**45**) in $CDCl_3$

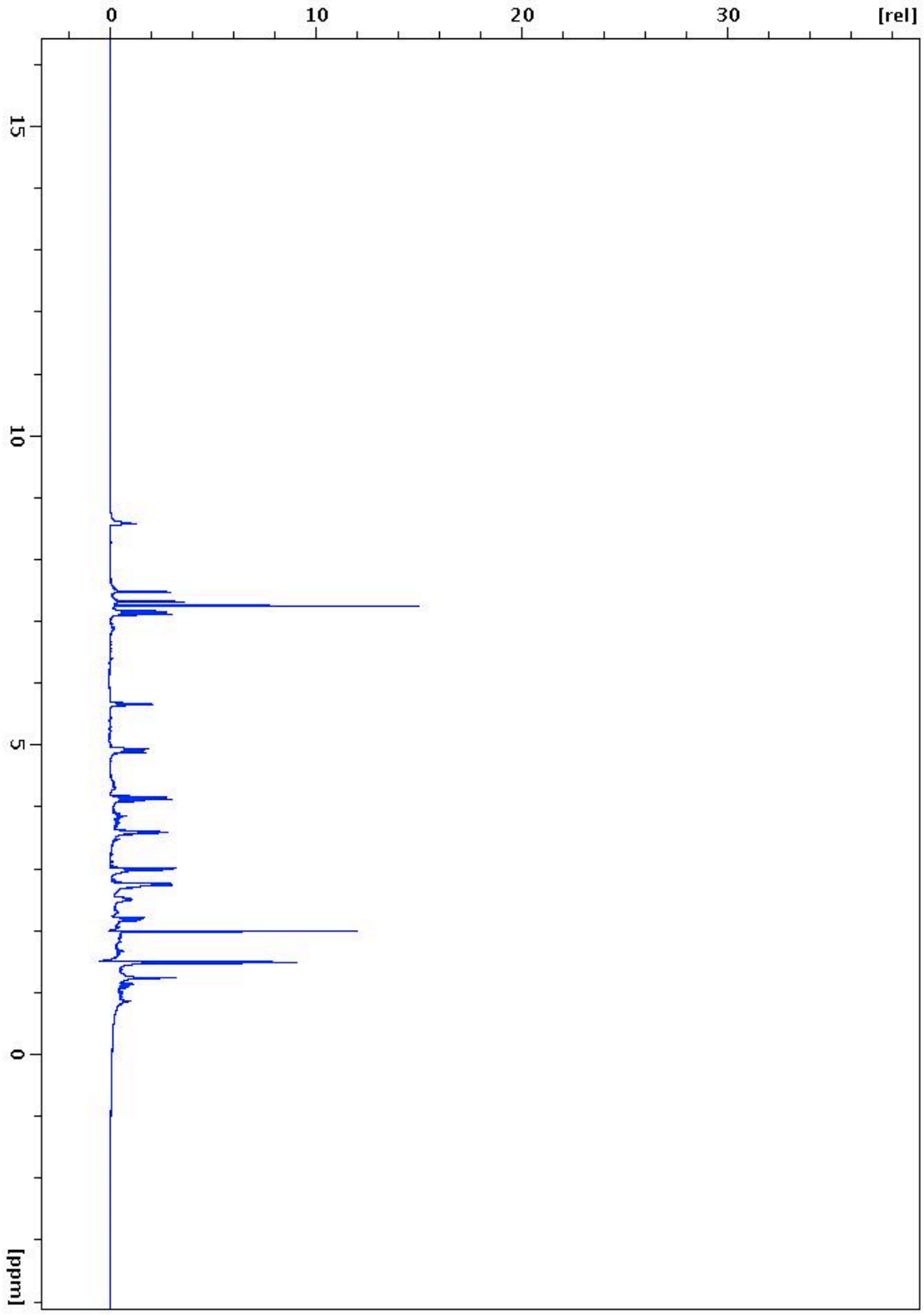


Figure A-30. ^{1}H-NMR spectrum of latifoliamide A (**46**) in $CDCl_3$

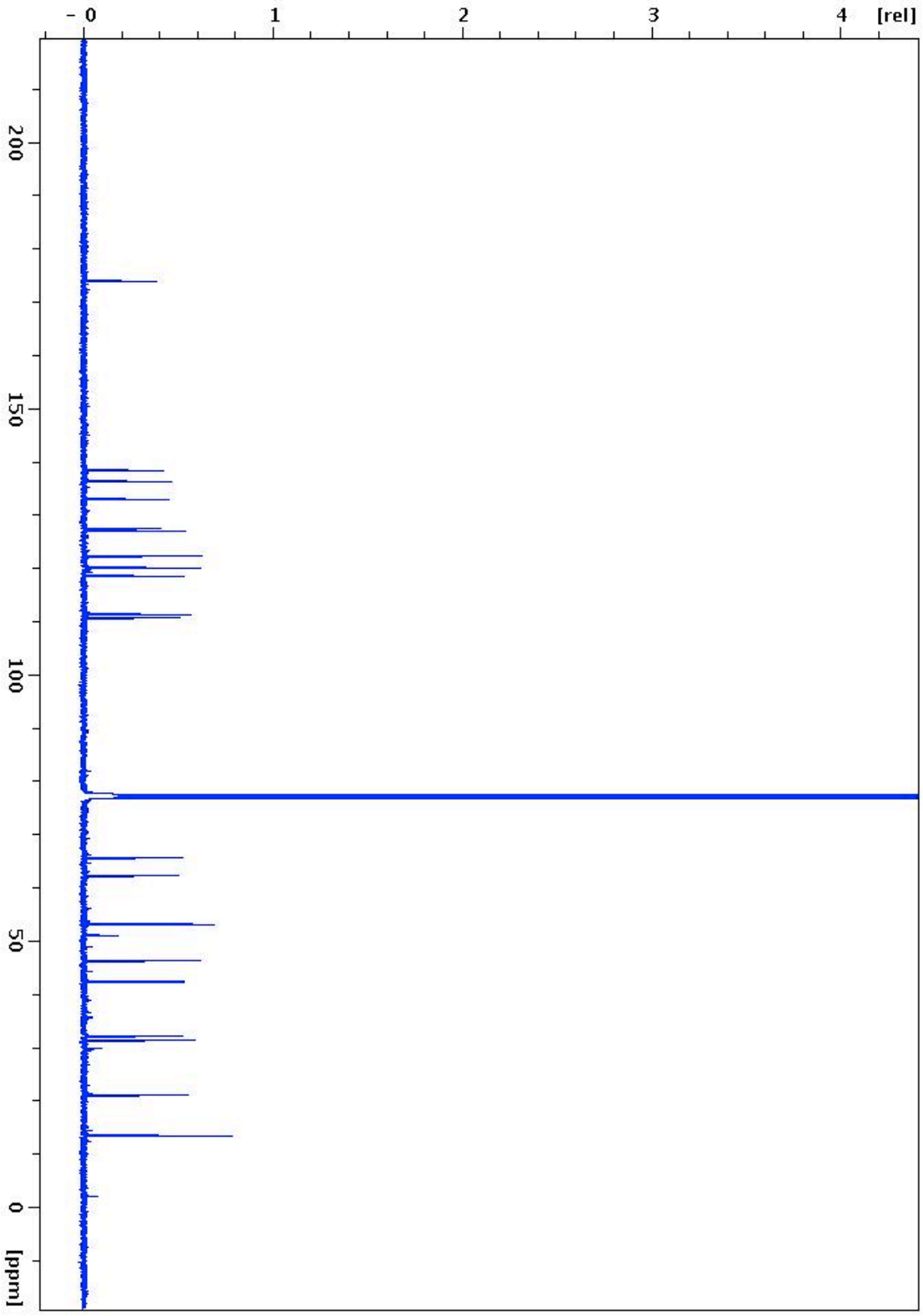


Figure A-31. ^{13}C spectrum of latifoliamide A (**46**) in $CDCl_3$

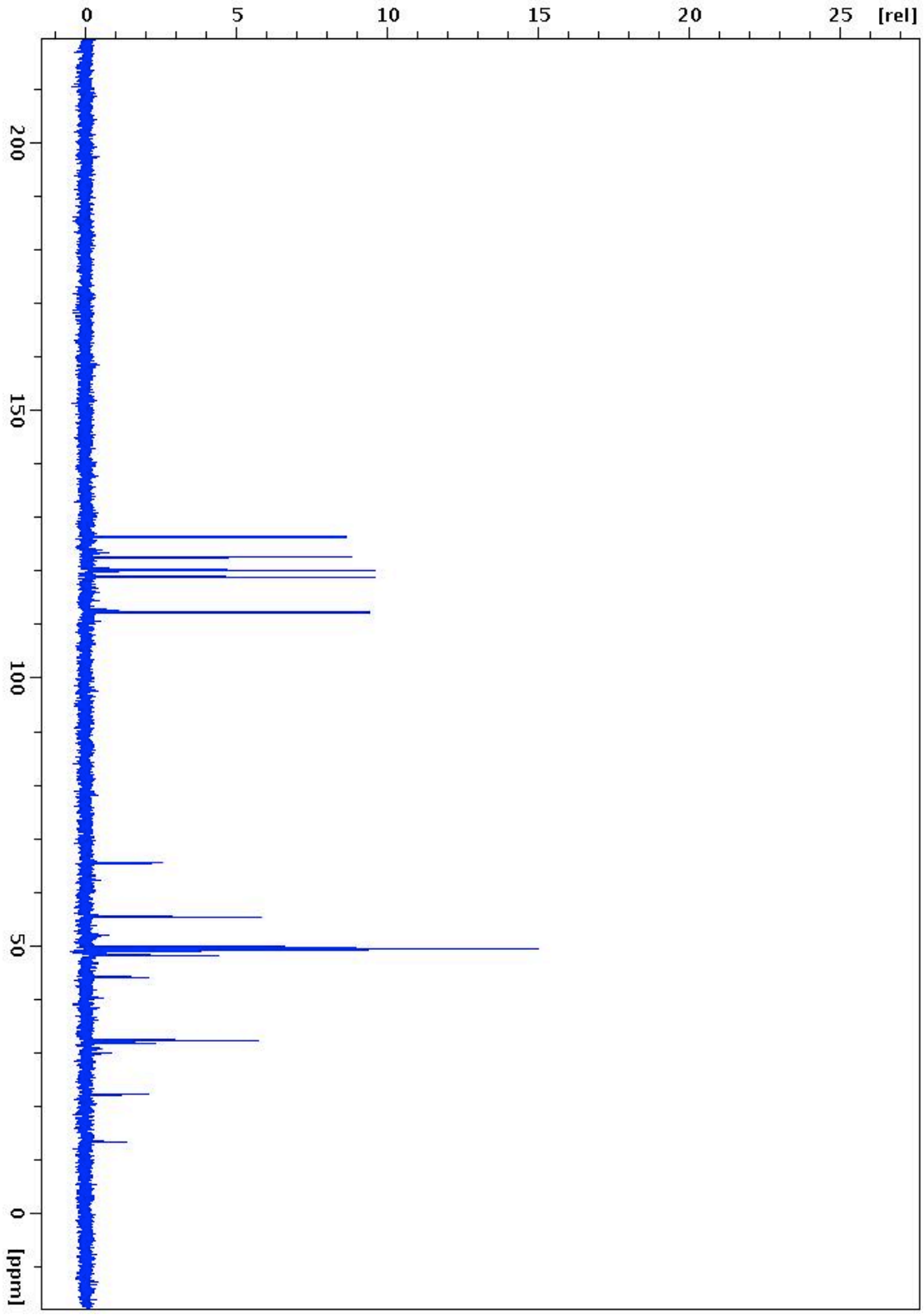


Figure A-32. DEPT 90 spectrum of latifoliamide A (**46**) in $CDCl_3$

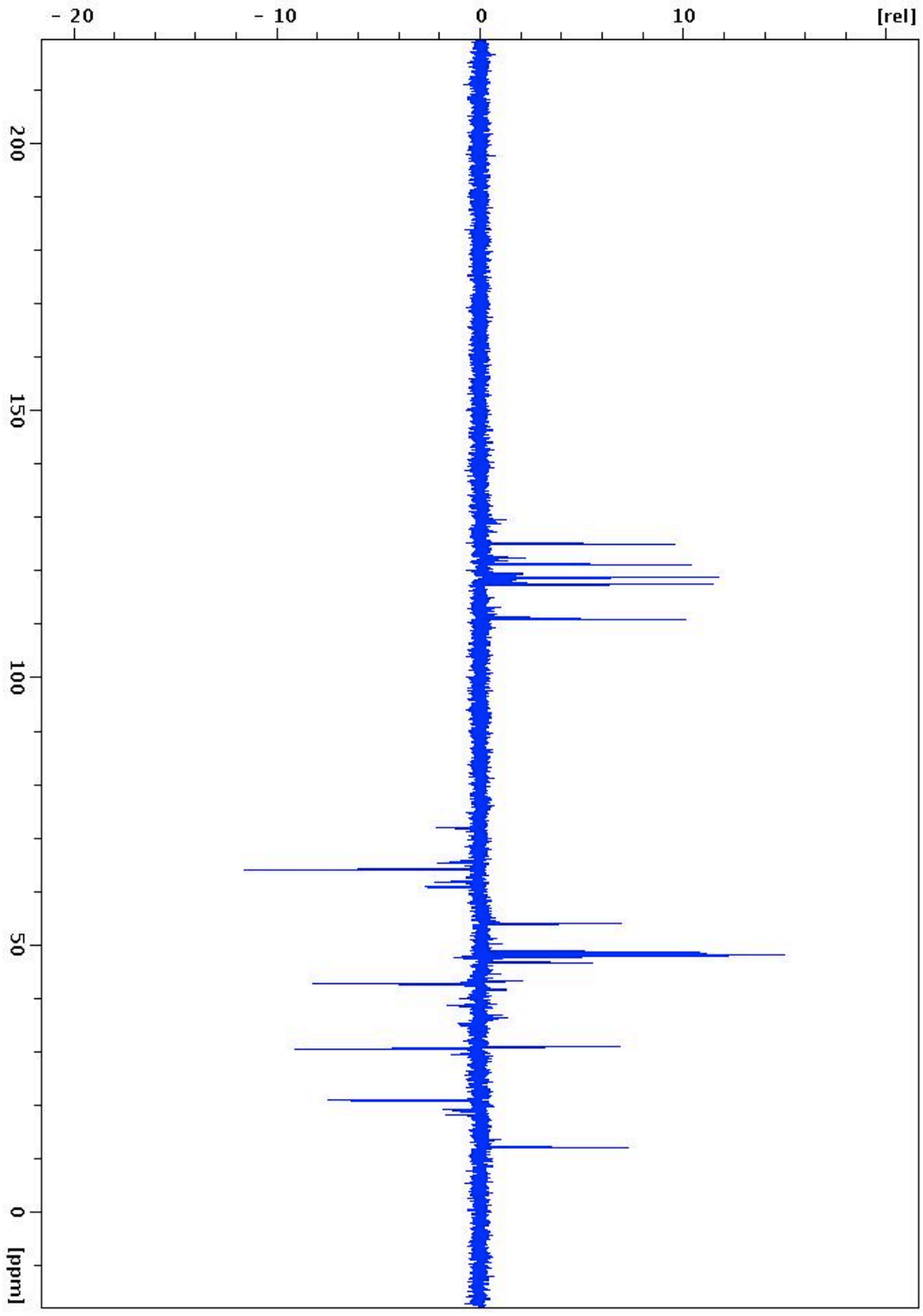


Figure A-33. DEPT 135 spectrum of latifoliamide A (**46**) in $CDCl_3$

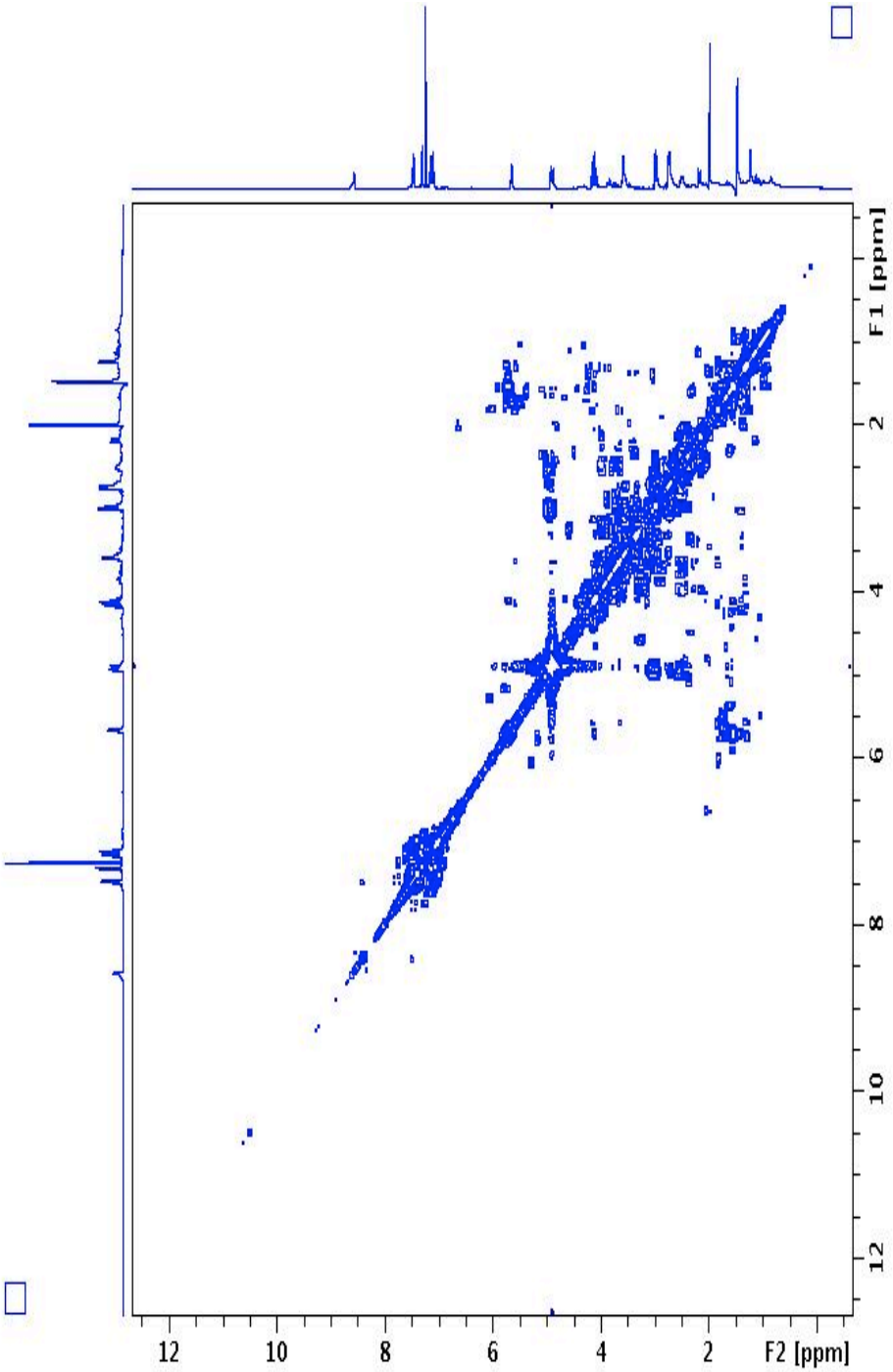


Figure A-34. COSY spectrum of latifoliamide A (**46**) in $CDCl_3$

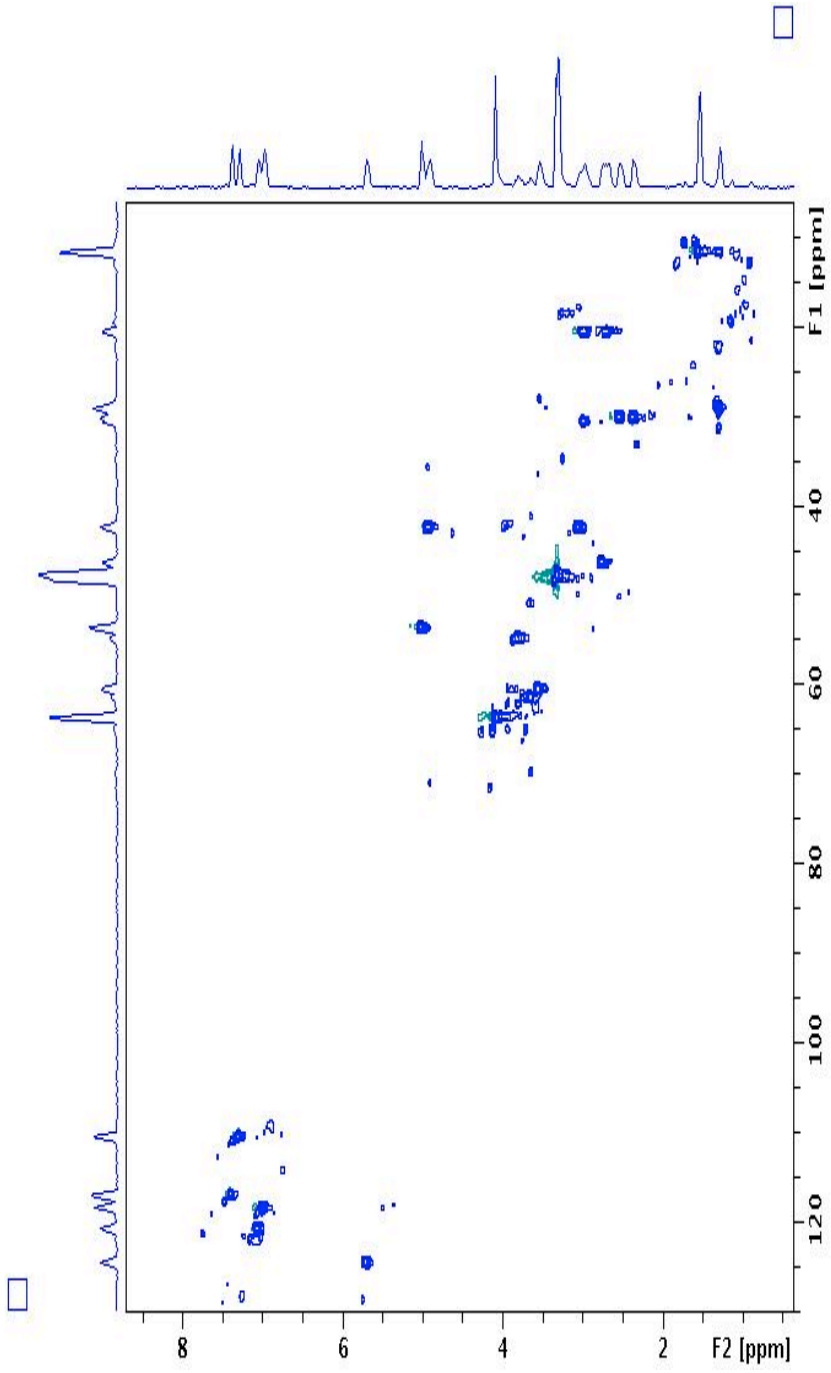


Figure A-35. HSQC spectrum of latifoliamide A (**46**) in $CDCl_3$

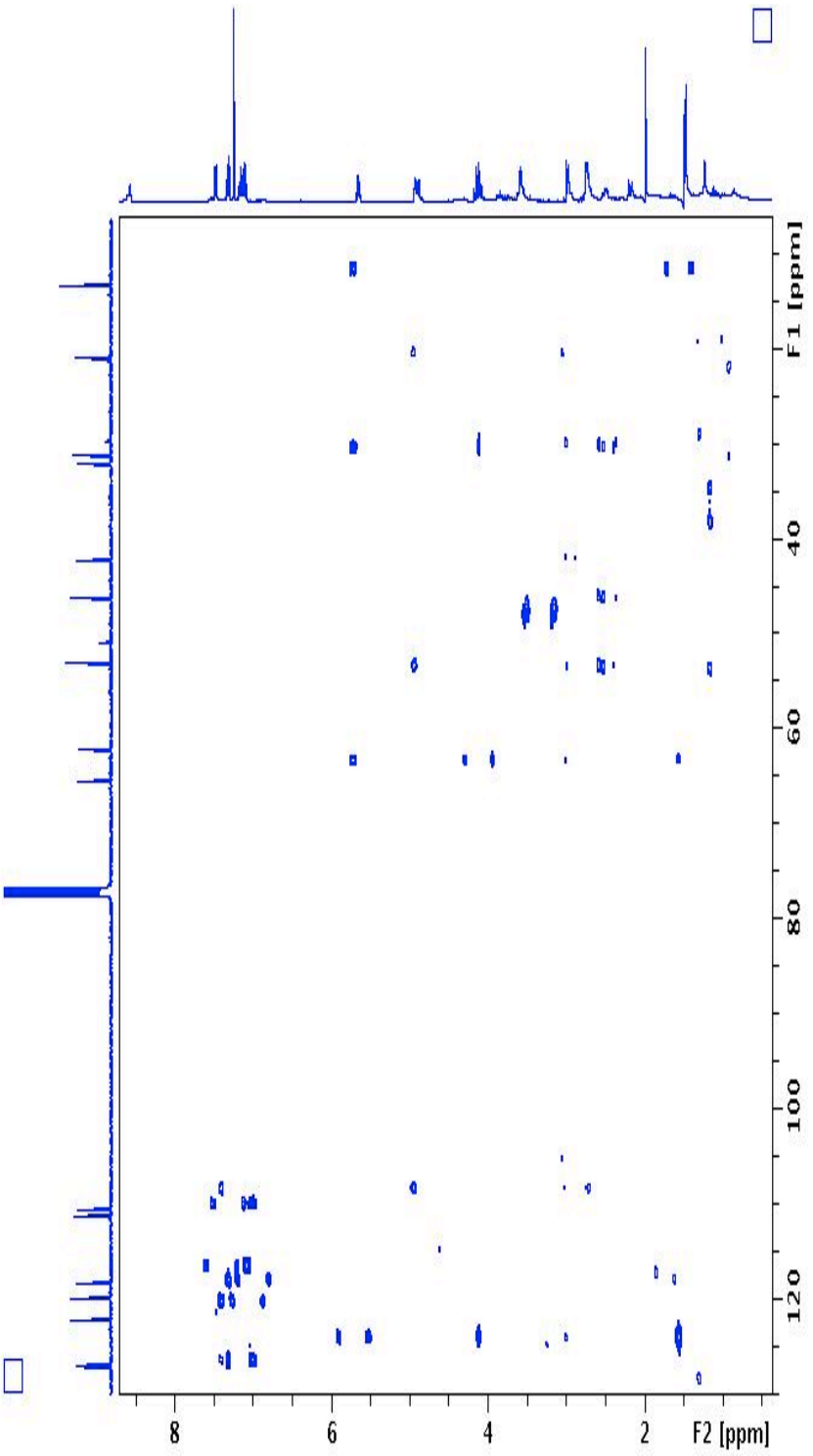


Figure A-36. HMBC spectrum of latifoliamide A (**46**) in $CDCl_3$

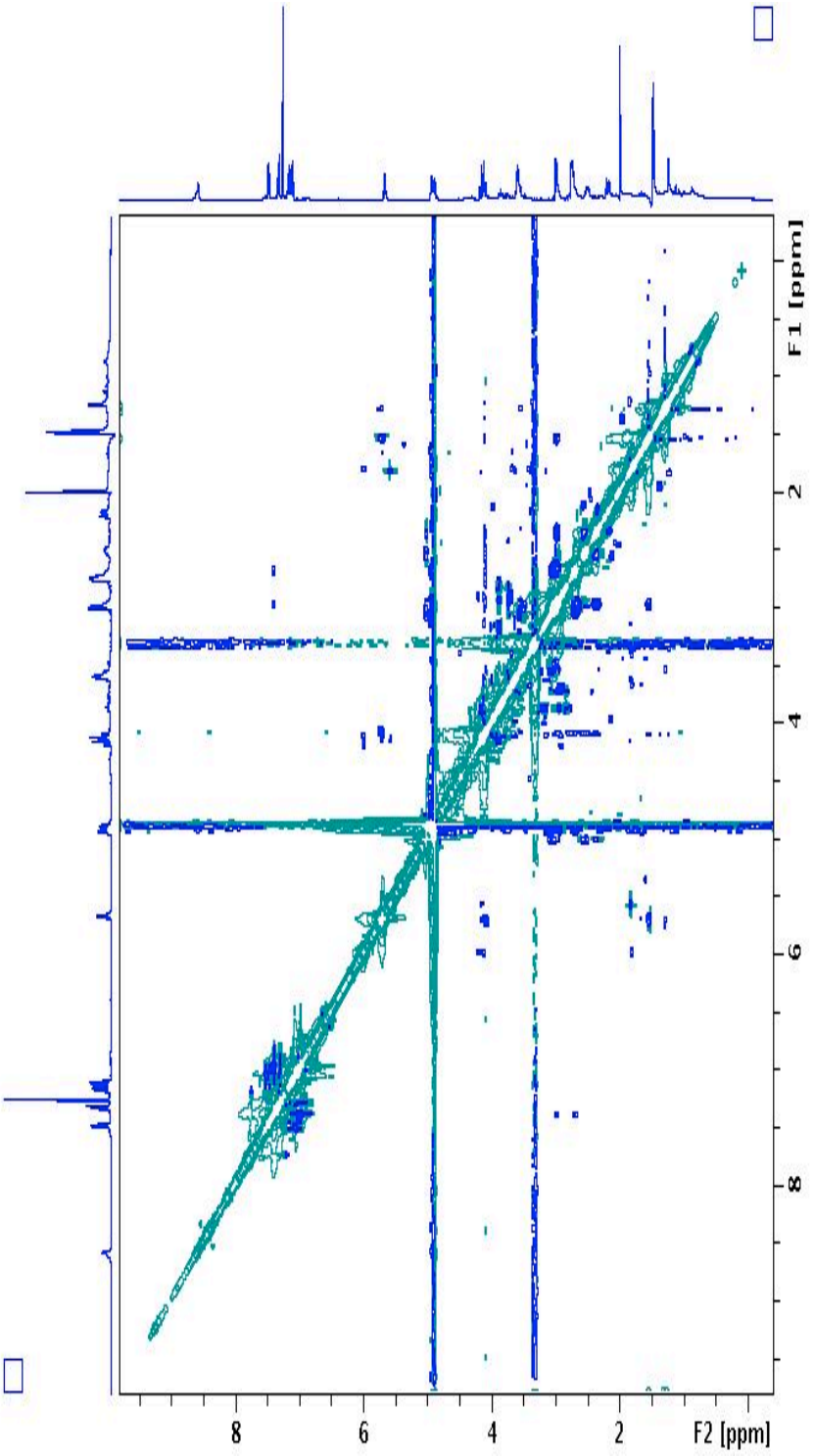


Figure A-37. NOESY spectrum of latifoliamide A (**46**) in $CDCl_3$

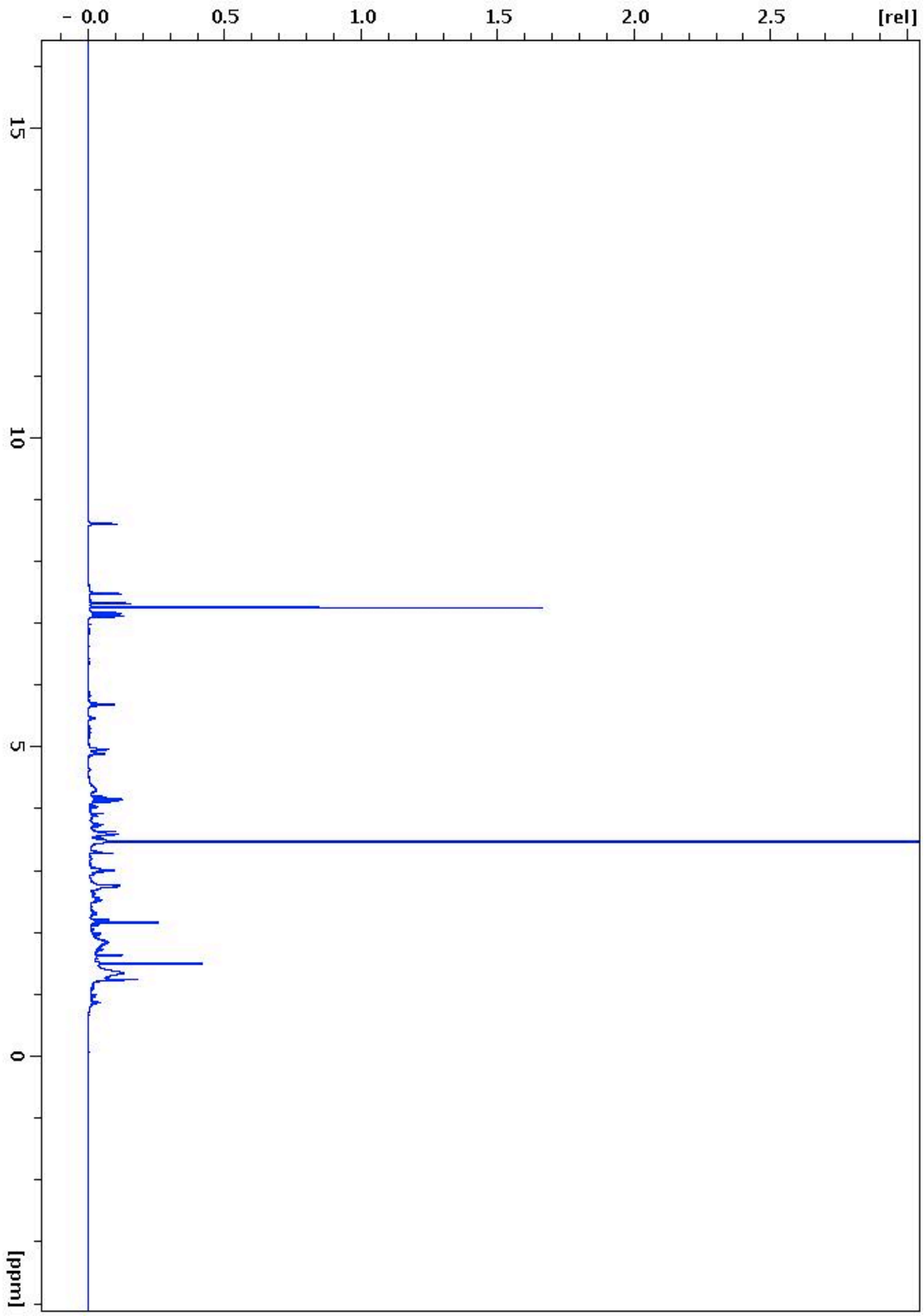


Figure A-38. ^{1}H-NMR spectrum of latifoliamide C (**48**) in $CDCl_3$

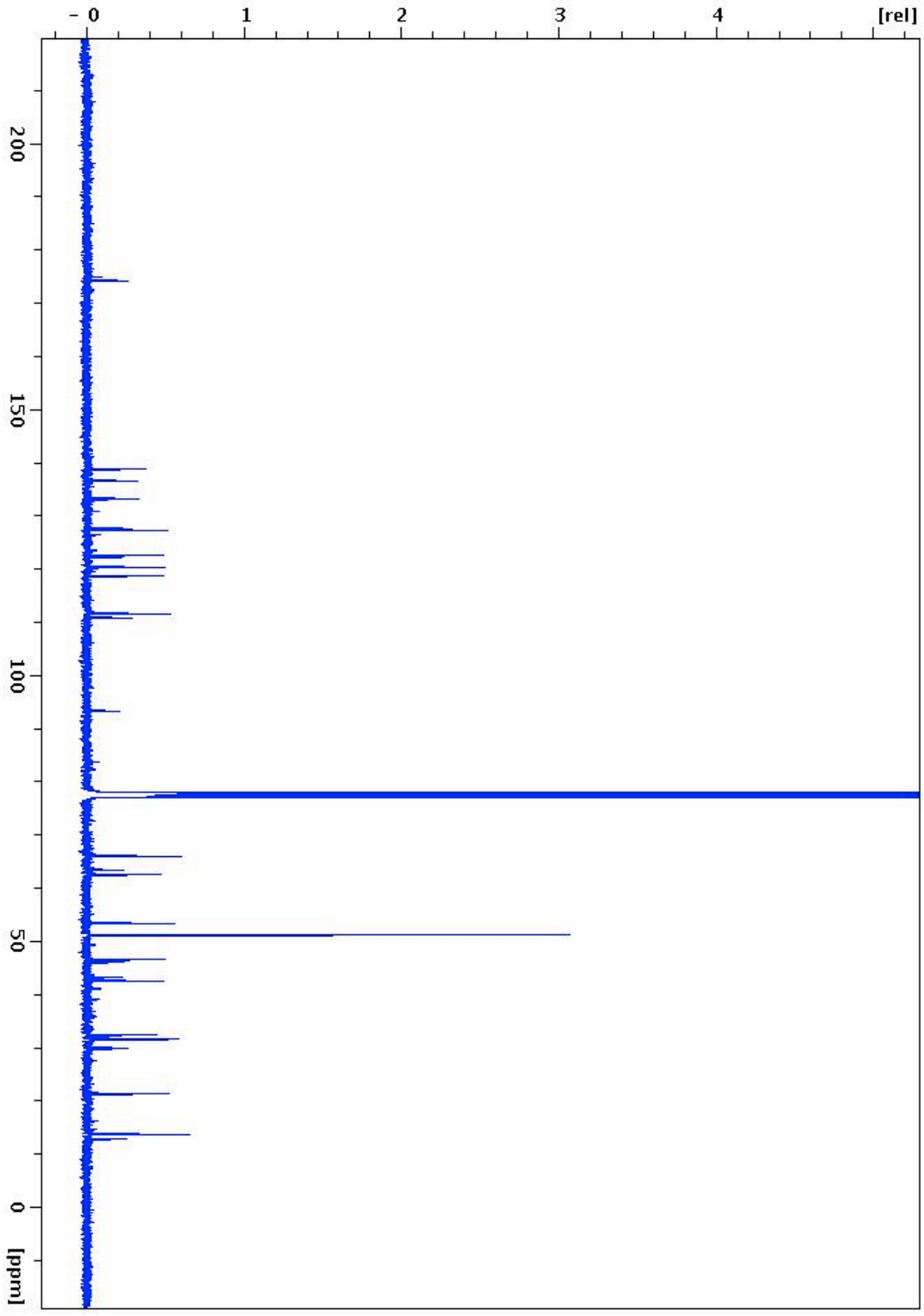


Figure A-39. ^{13}C spectrum of latifoliamide C (**48**) in $CDCl_3$

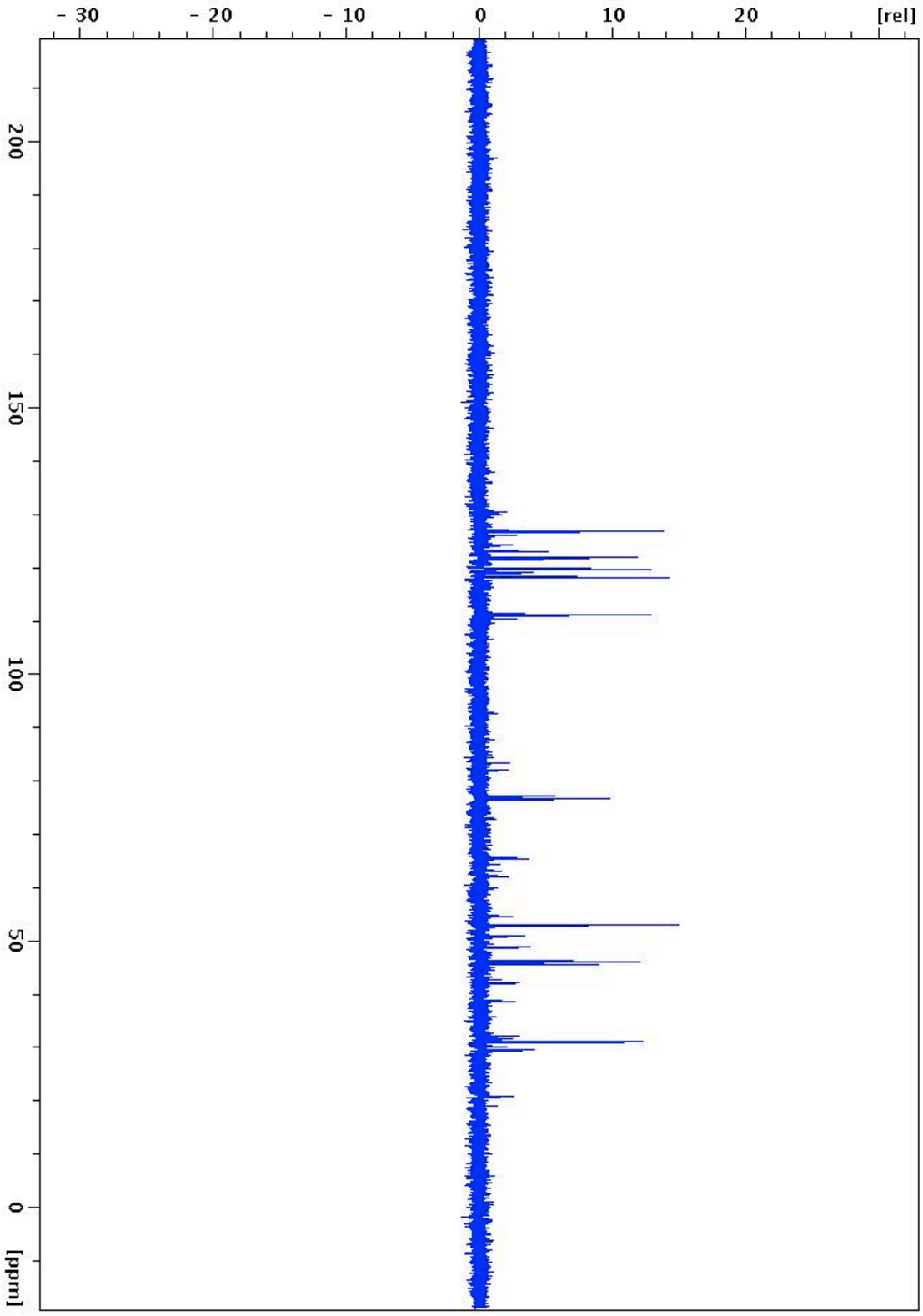


Figure A-40. DEPT 90 spectrum of latifoliamide C (**48**) in $CDCl_3$

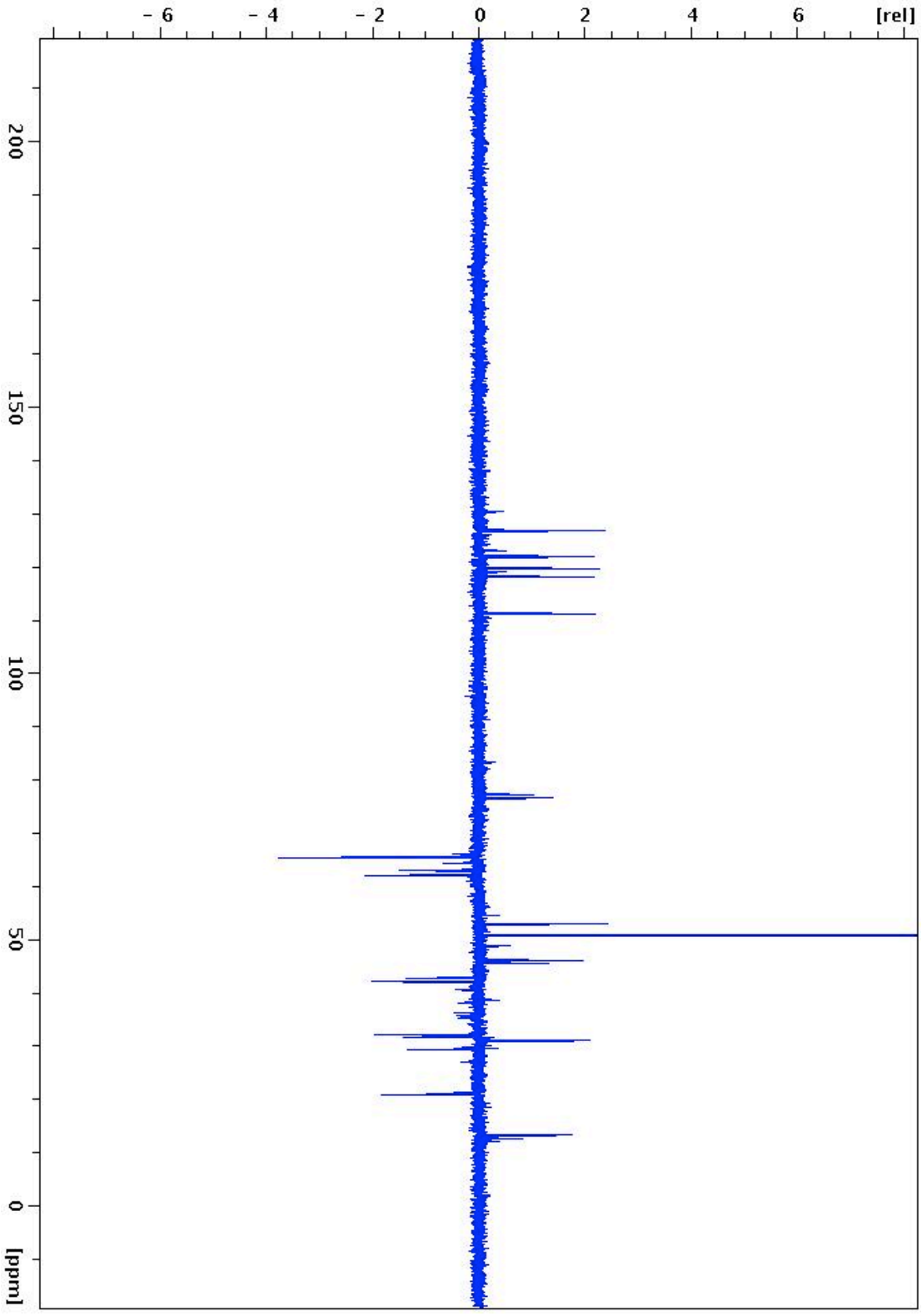


Figure A-41. DEPT 135 spectrum of latifoliamide C (**48**) in $CDCl_3$

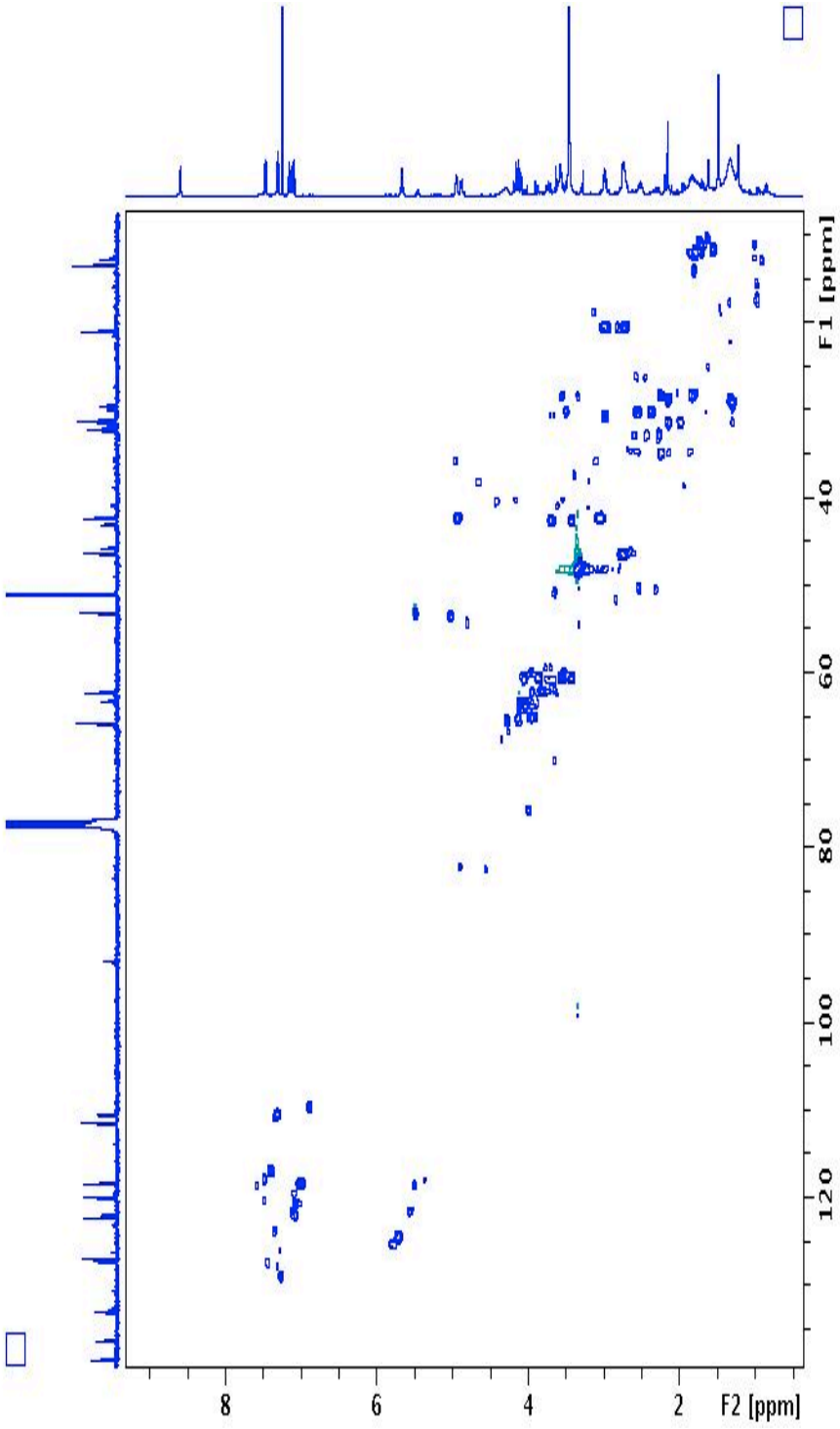


Figure A-42. HSQC spectrum of latifoliamide C (**48**) in $CDCl_3$

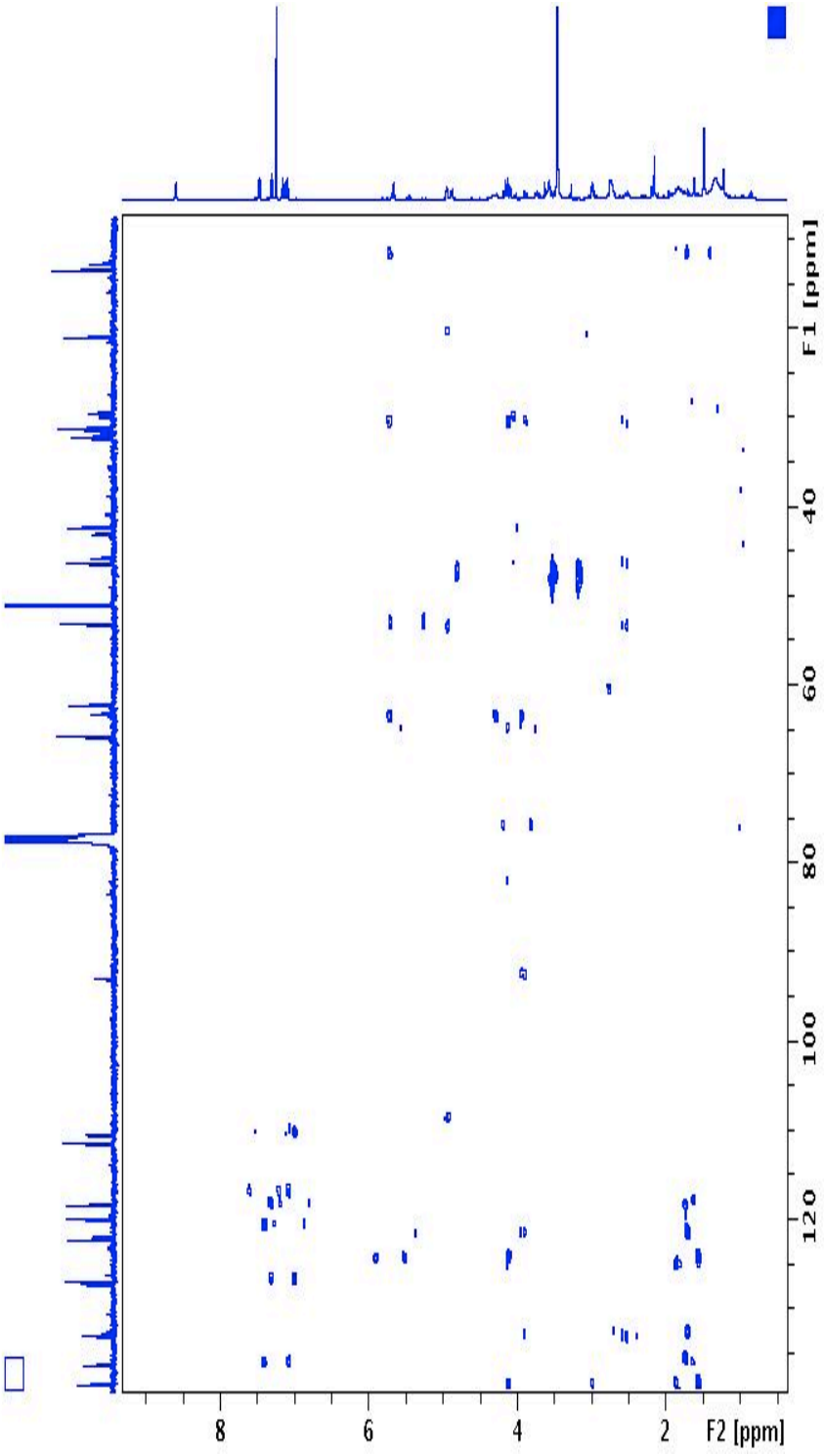


Figure A-43. HMBC spectrum of latifoliamide C (**48**) in $CDCl_3$

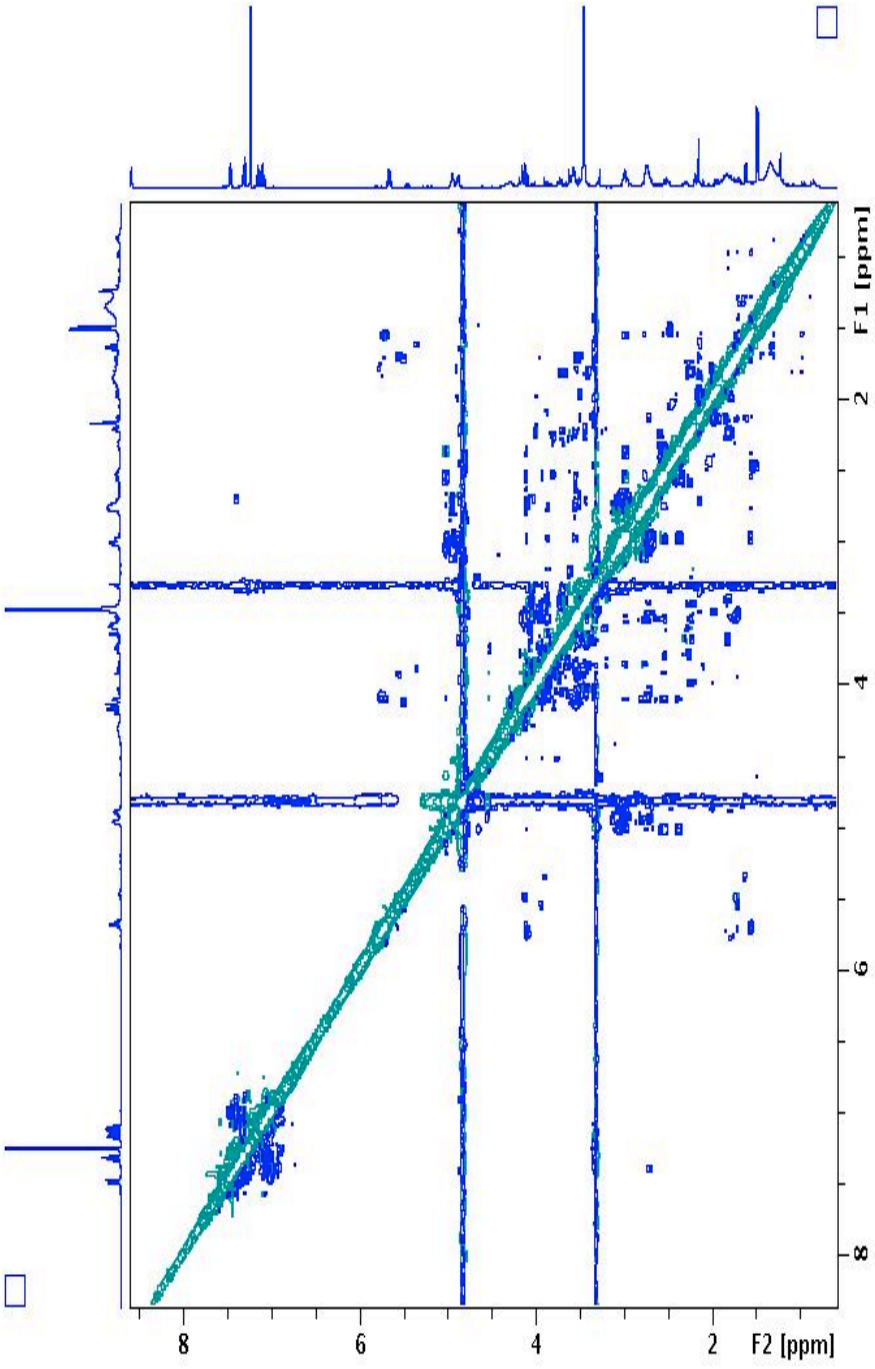


Figure A-44. NOESY spectrum of latifoliamide C (**48**) in $CDCl_3$

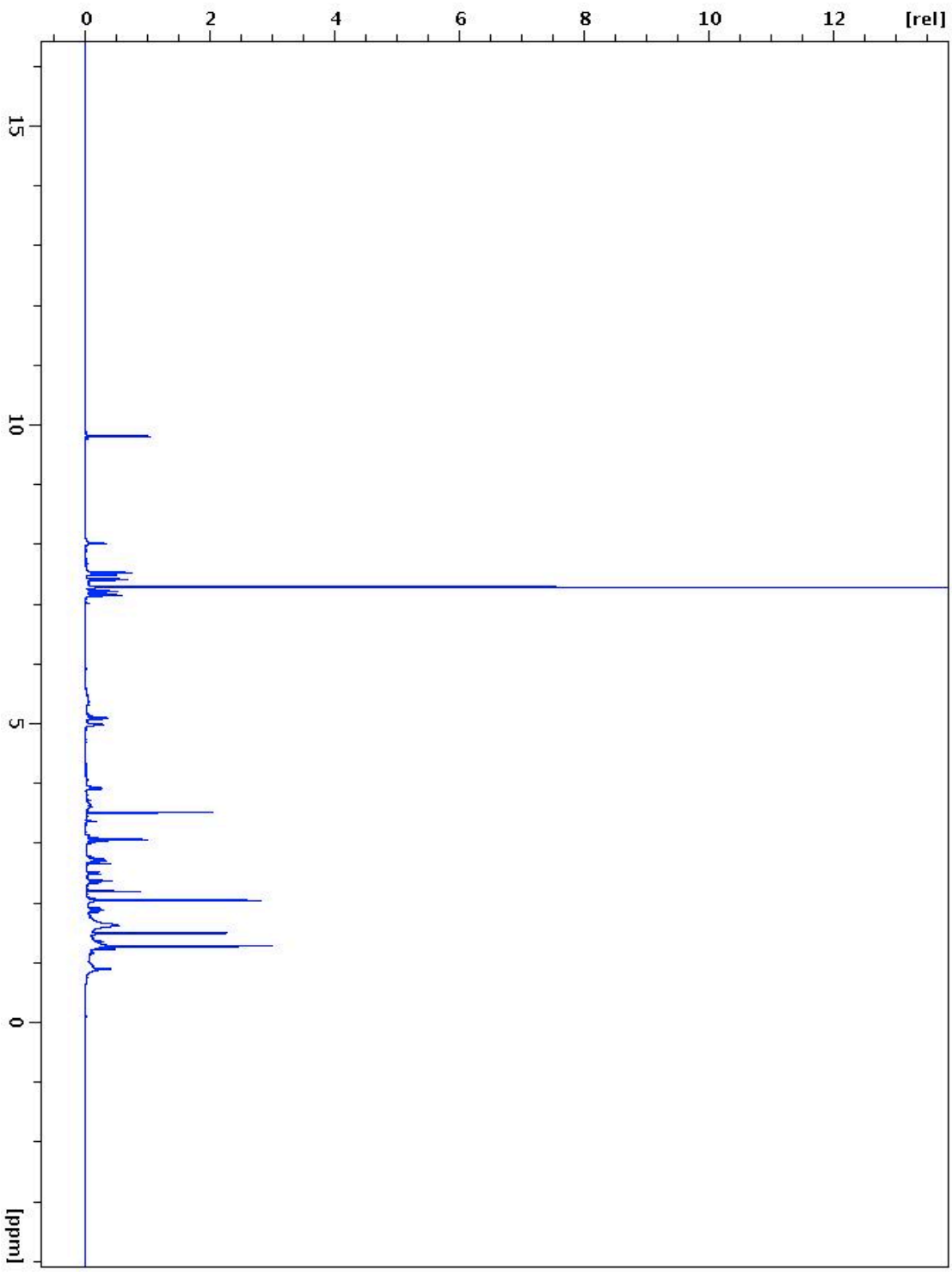


Figure A-45. ^{1}H-NMR spectrum of latifoliamide G (**52**) in $CDCl_3$

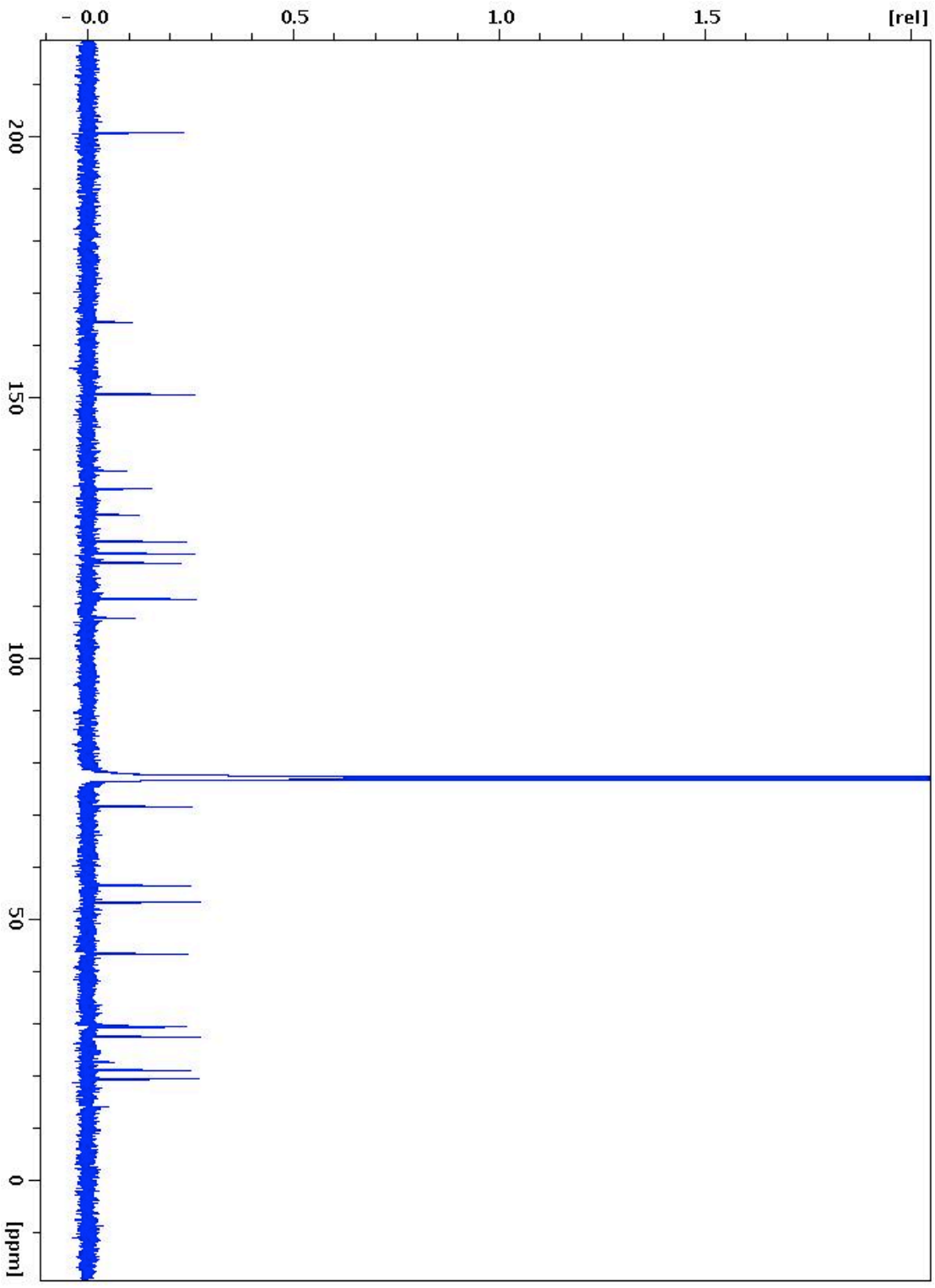


Figure A-46. ^{13}C spectrum of latifoliamide G (**52**) in $CDCl_3$

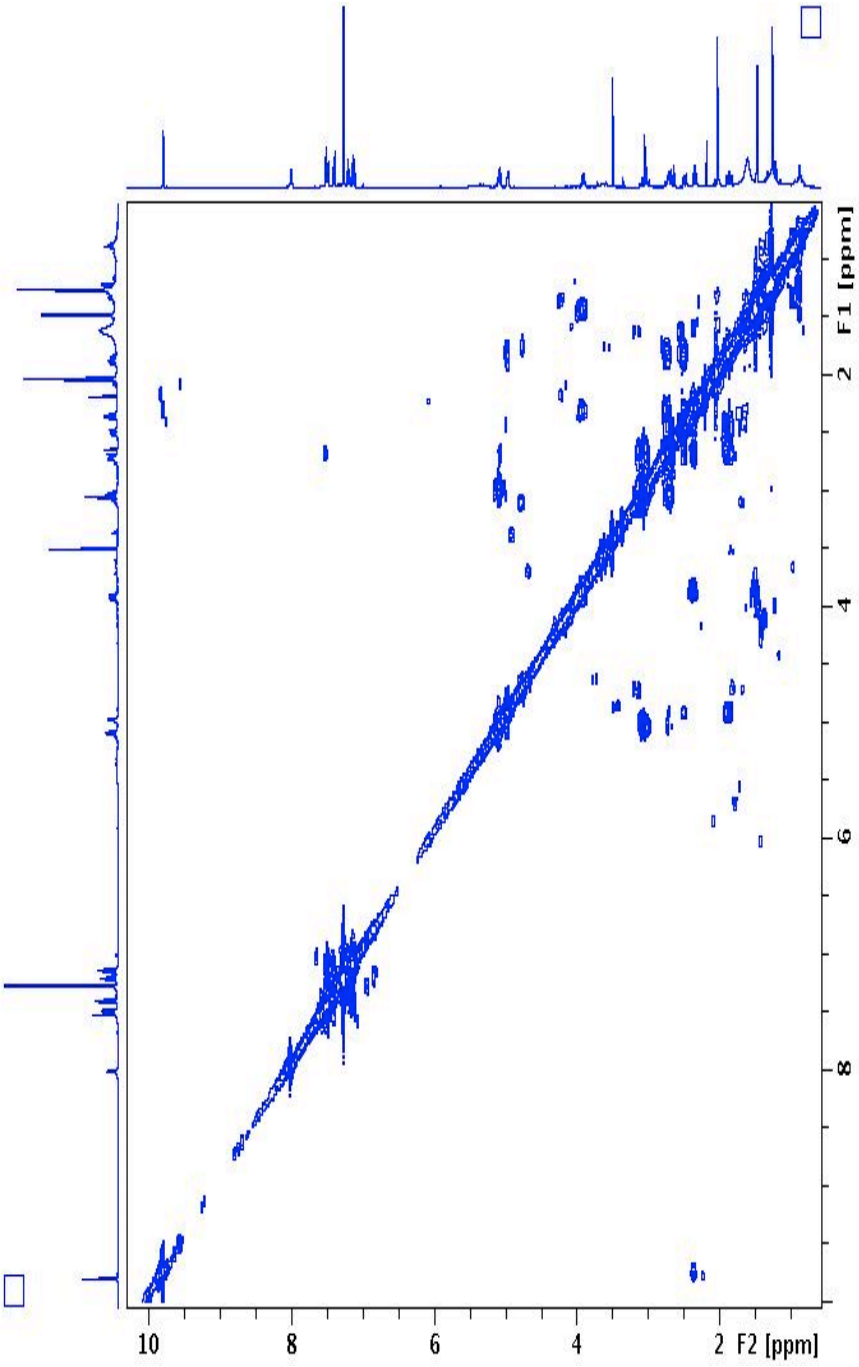


Figure A-47. COSY spectrum of latifoliamide G (**52**) in $CDCl_3$

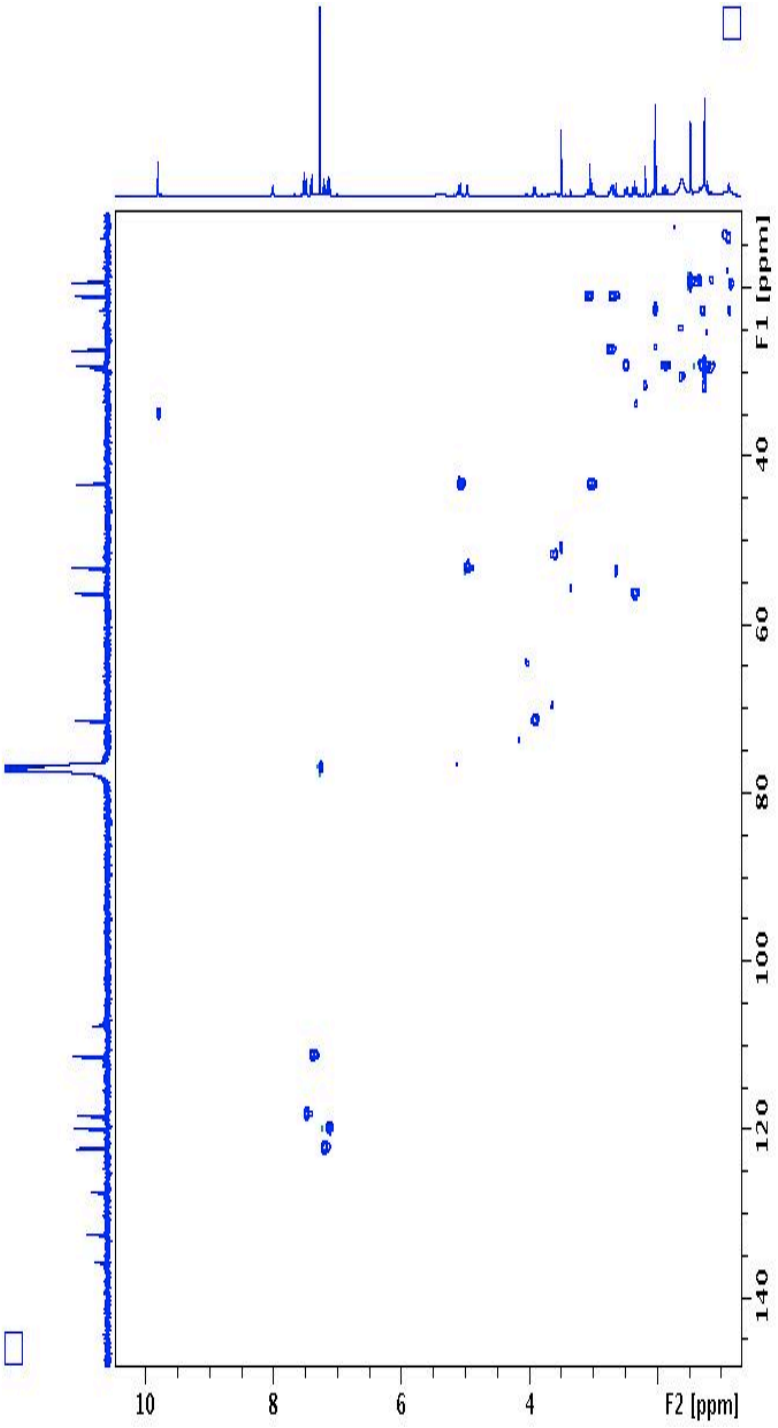


Figure A-48. HSQC spectrum of latifoliamide G (**52**) in $CDCl_3$

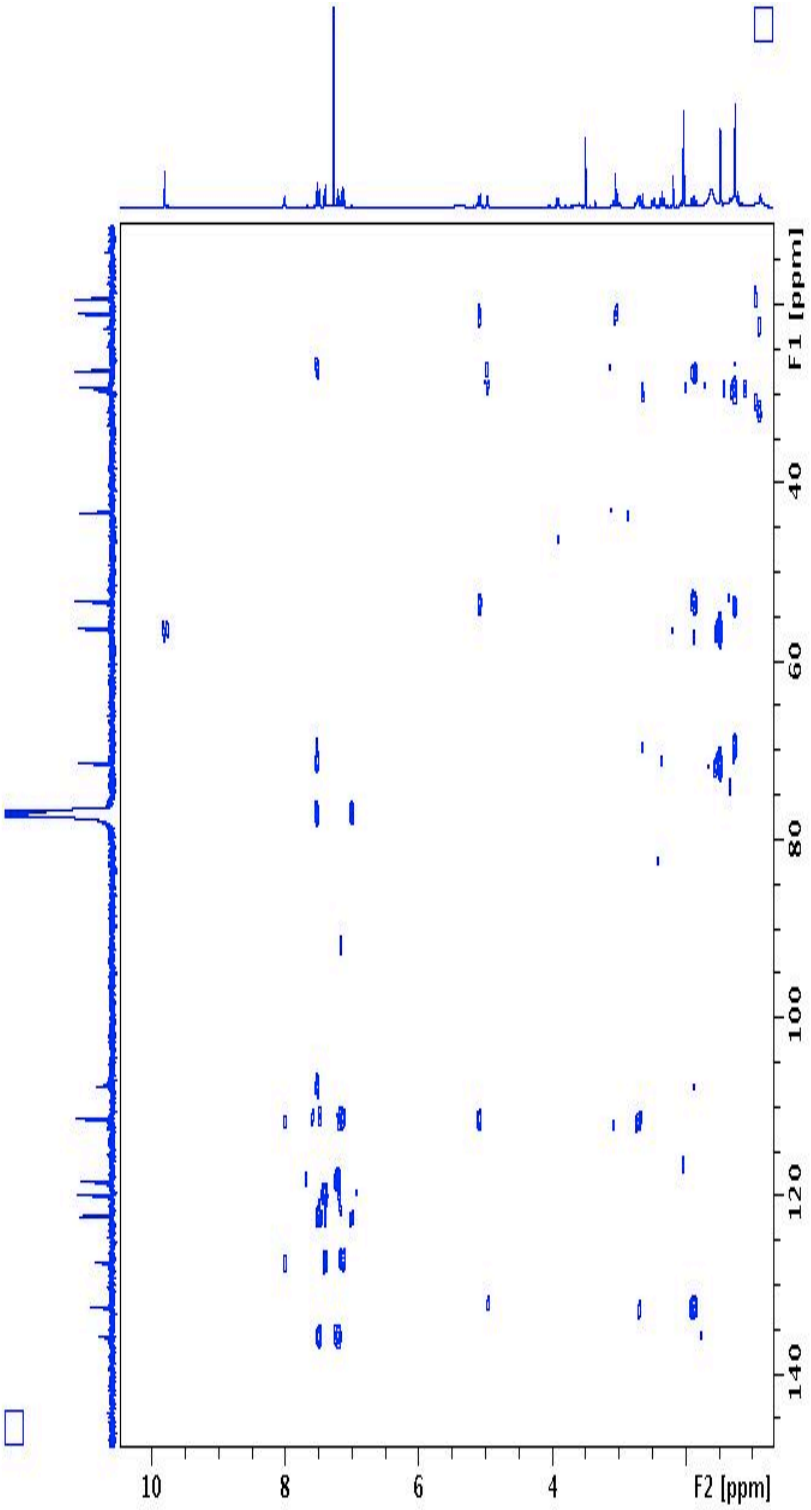


Figure A-49. HMBC spectrum of latifoliamide G (**52**) in $CDCl_3$

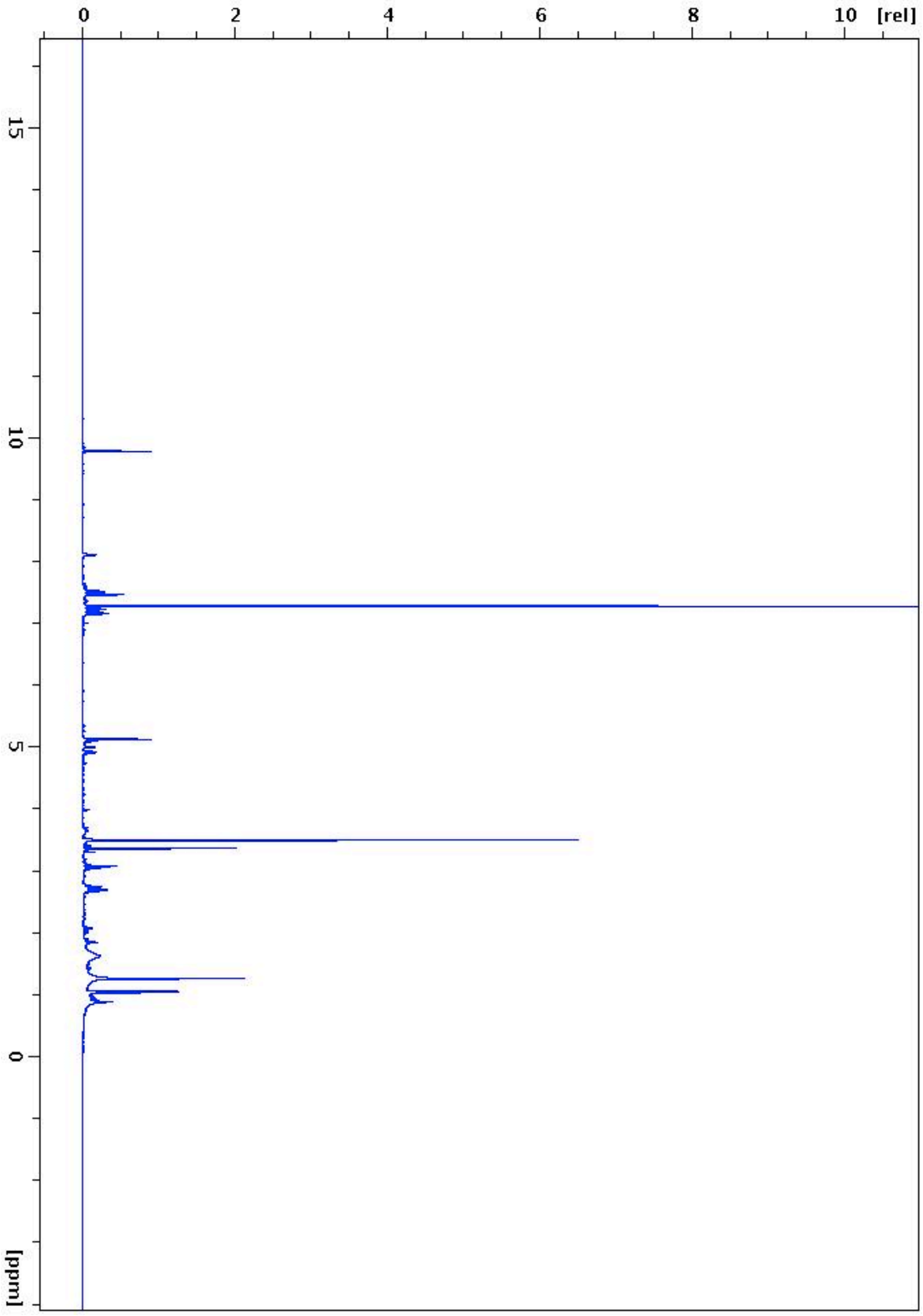


Figure A-50. ^{1}H-NMR spectrum of latifoliamide F (**51**) in $CDCl_3$

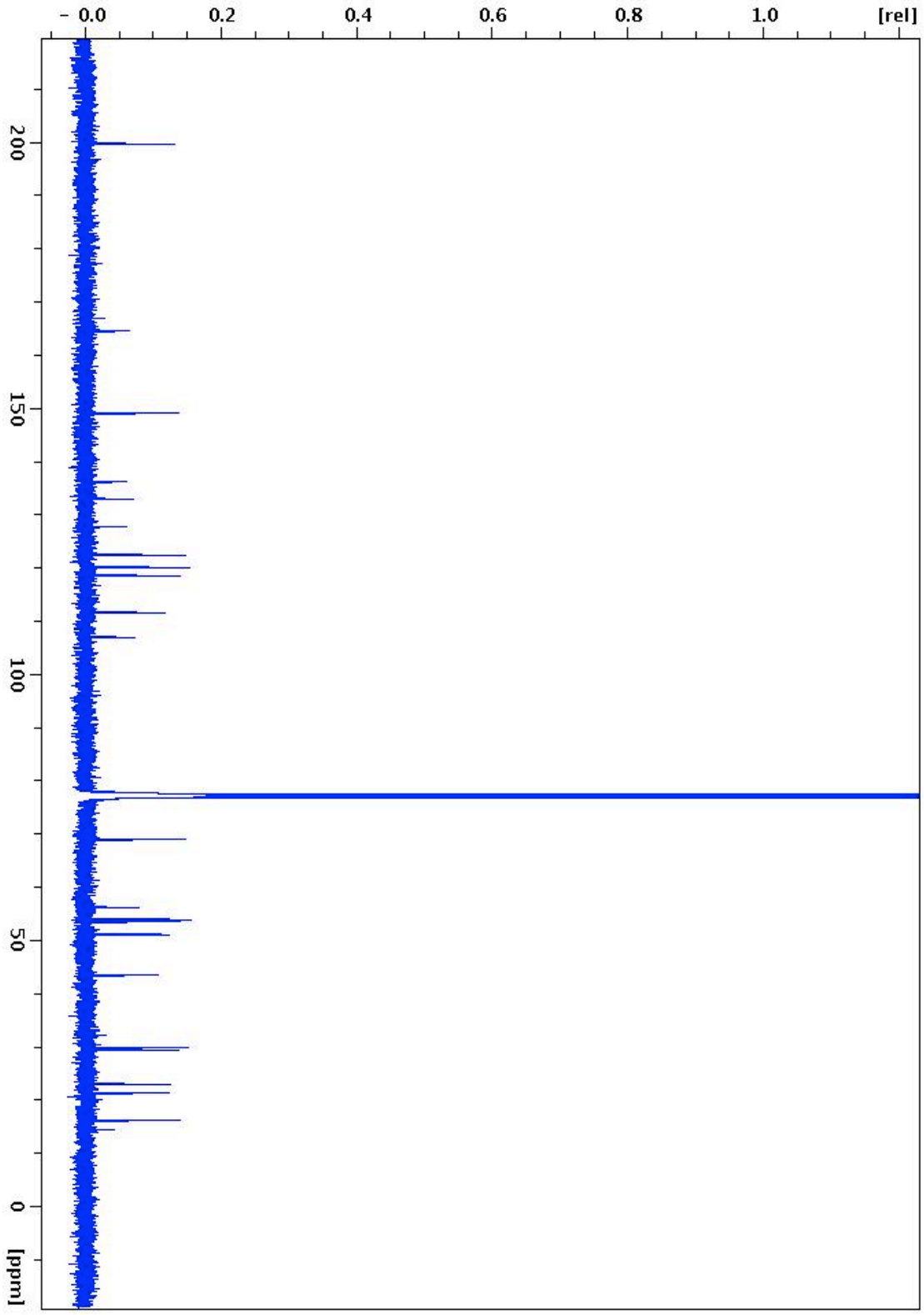


Figure A-51. ^{13}C spectrum of latifoliamide F (**51**) in $CDCl_3$

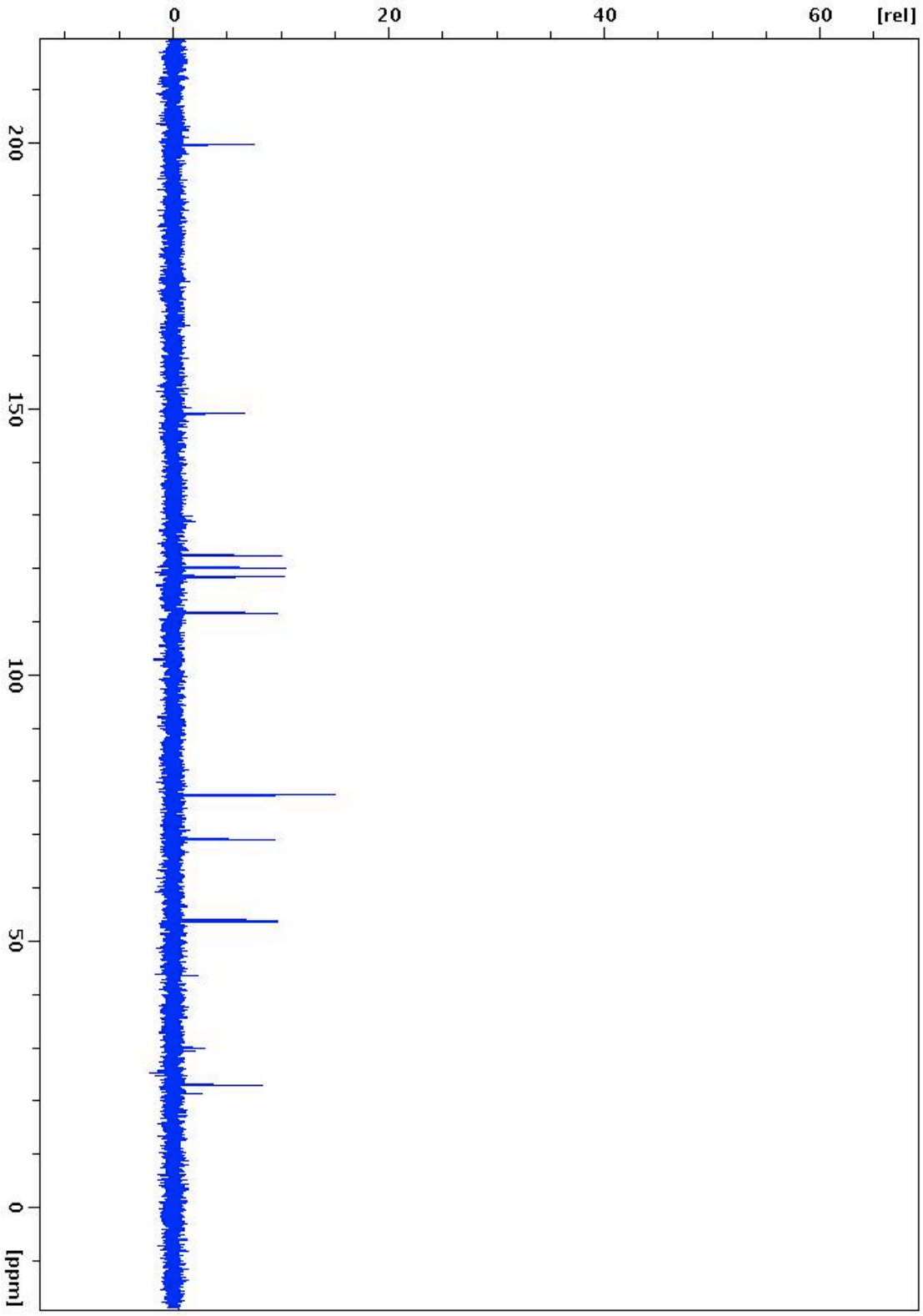


Figure A-52. DEPT 90 spectrum of latifoliamide F (**51**) in $CDCl_3$

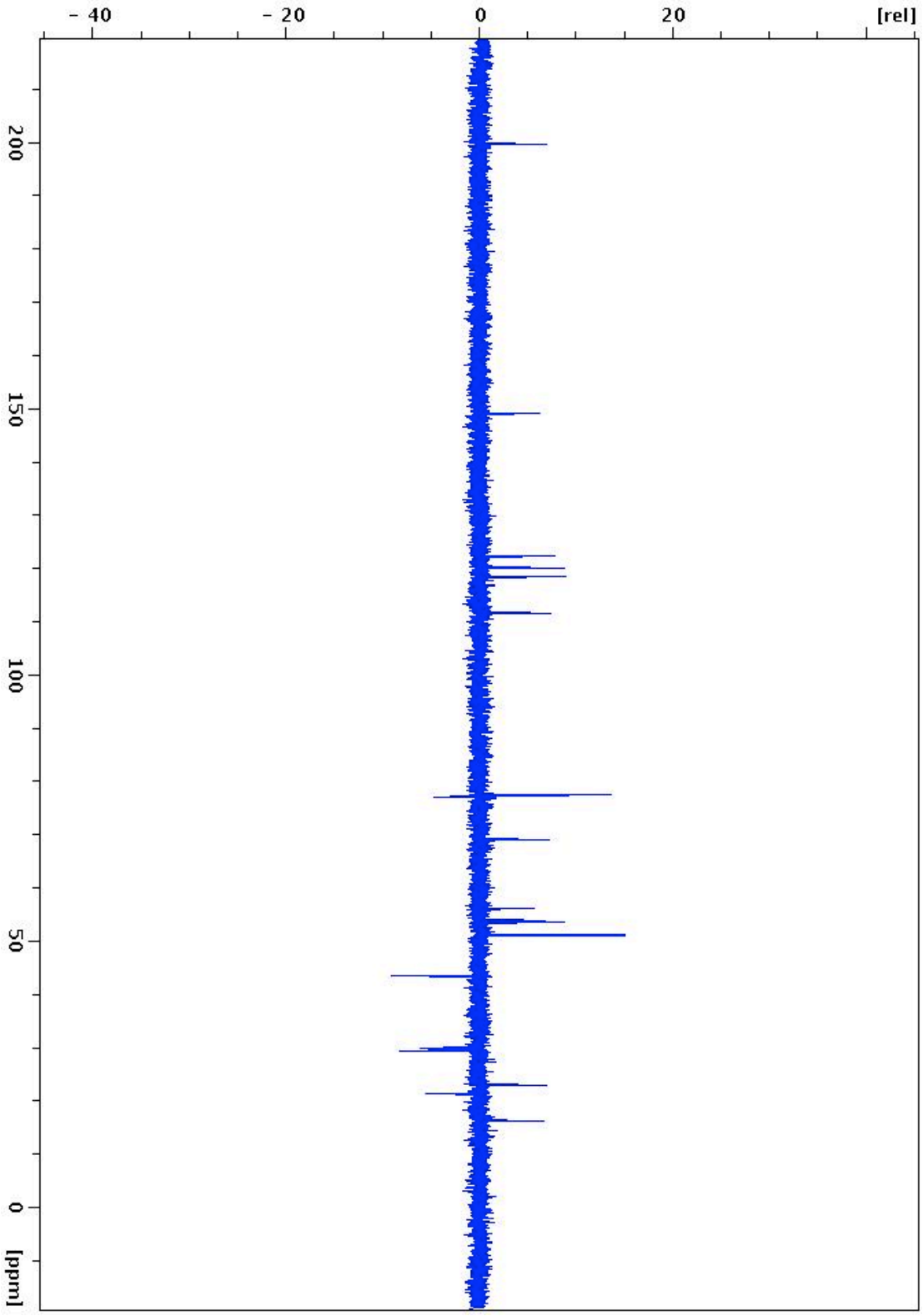


Figure A-53. DEPT 135 spectrum of latifoliamide F (**51**) in $CDCl_3$

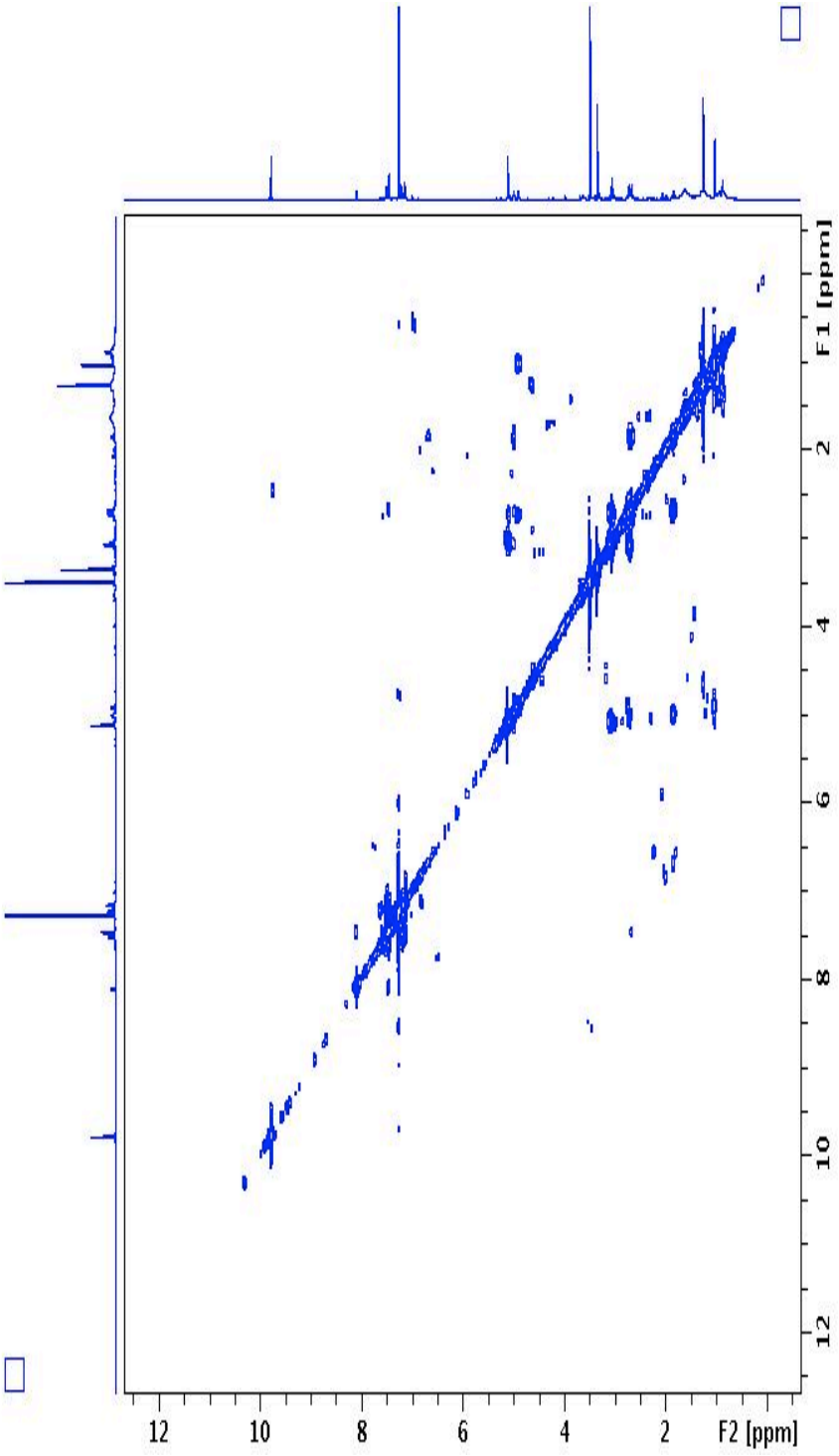


Figure A-54. COSY spectrum of latifoliamide F (**51**) in $CDCl_3$

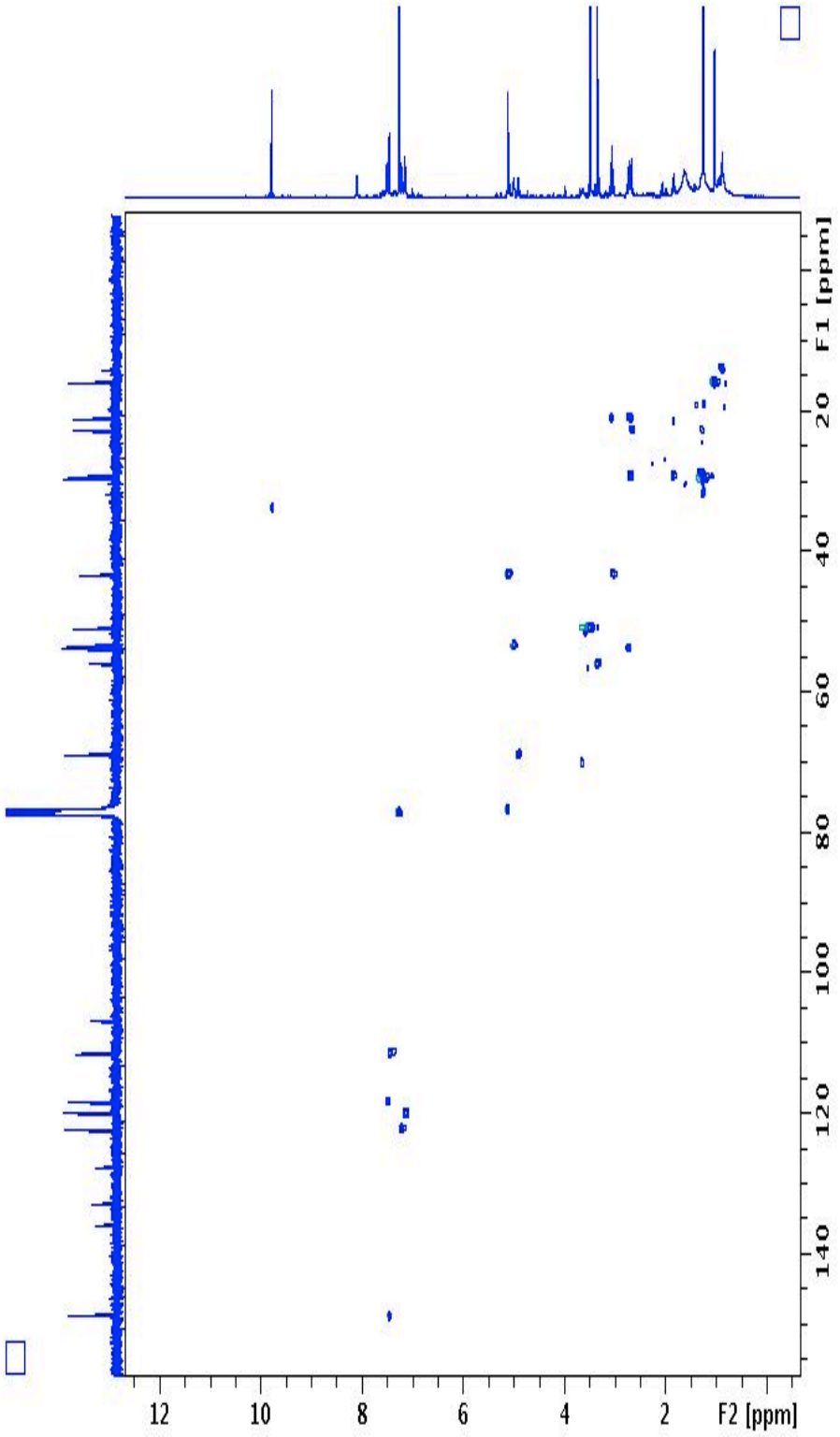


Figure A-55. HSQC spectrum of latifoliamide F (**51**) in $CDCl_3$

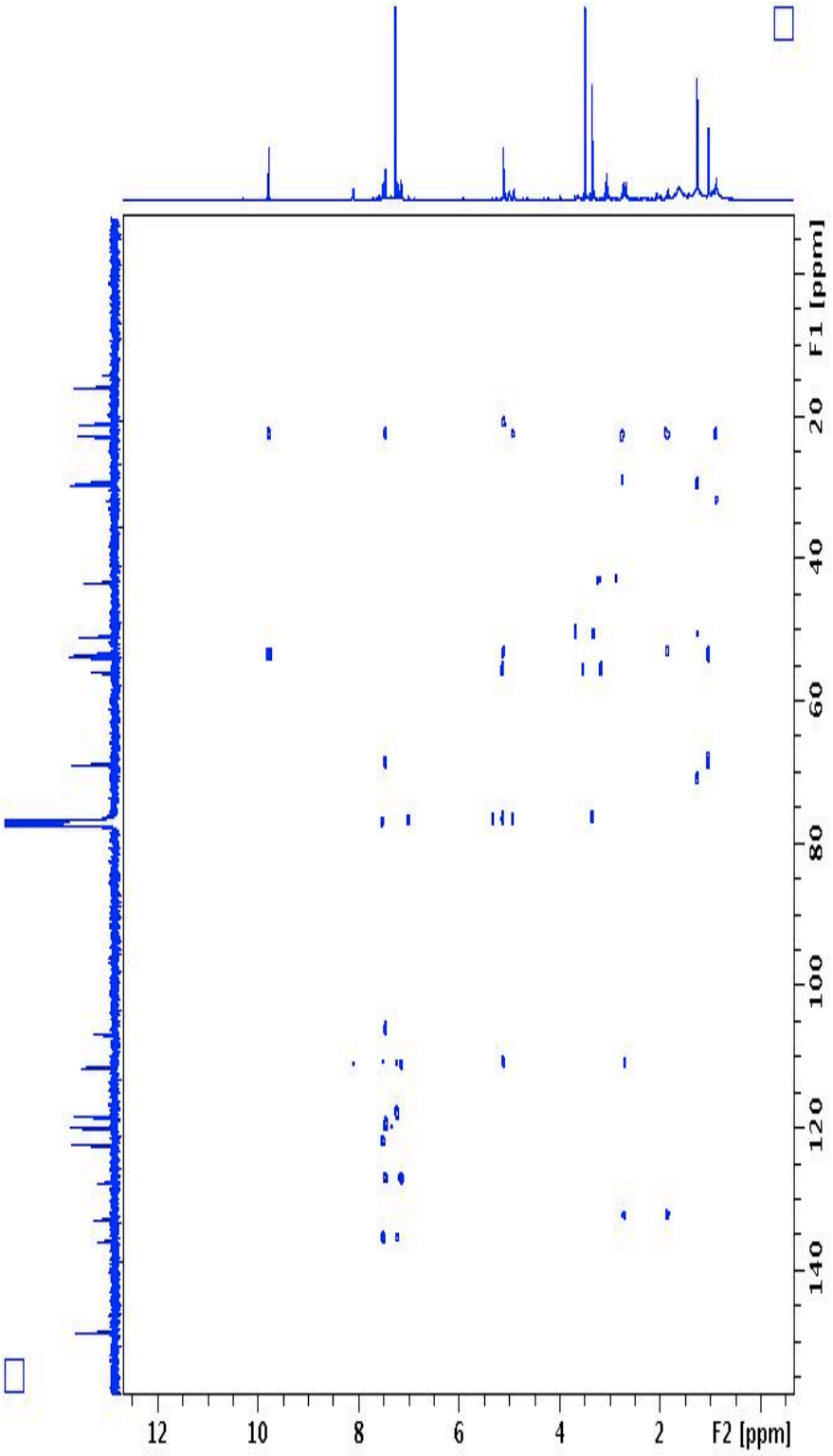


Figure A-56. HMBC spectrum of latifoliamide F (**51**) in $CDCl_3$

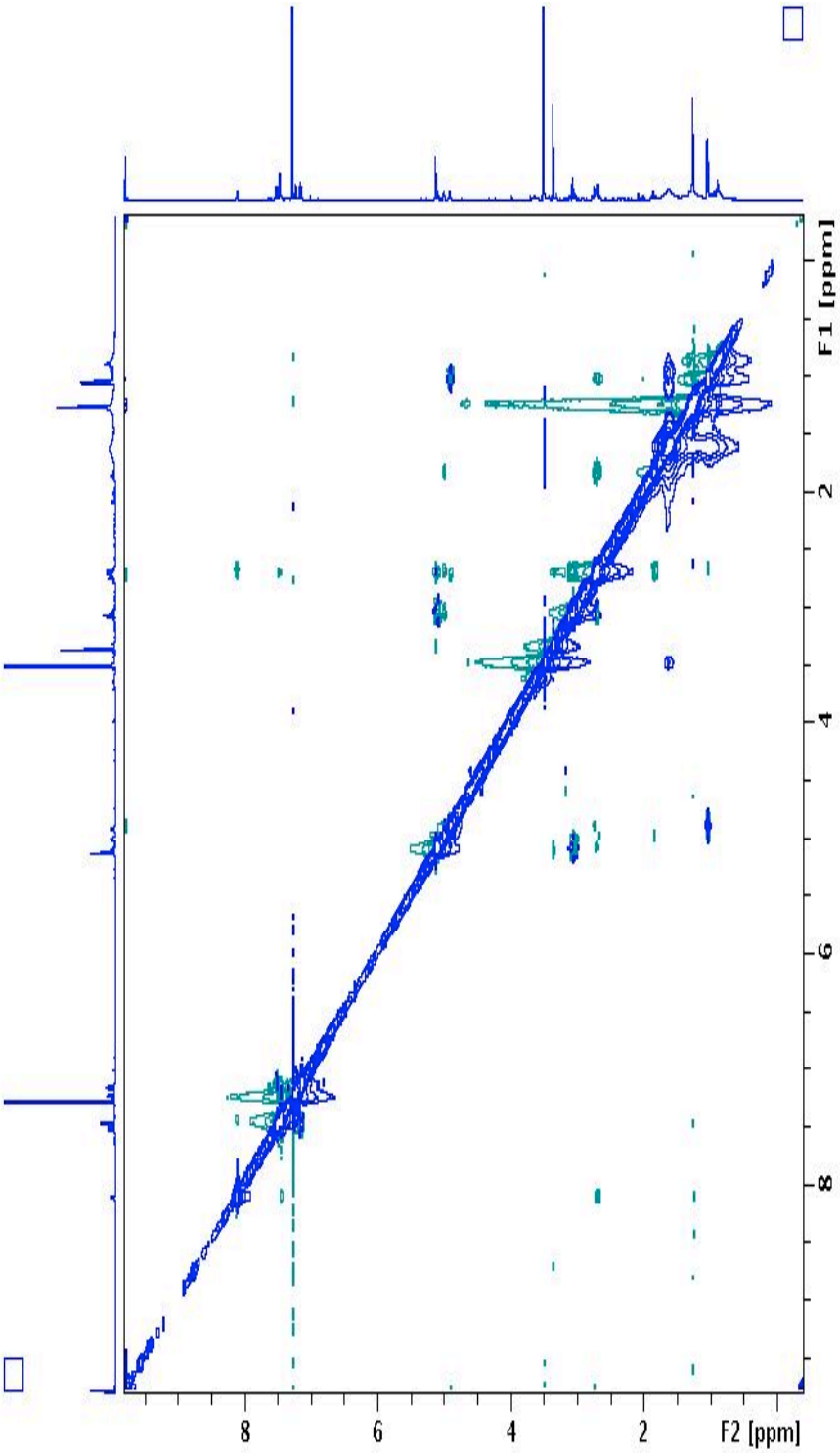


Figure A-57. N spectrum of latifoliamide F (**51**) in $CDCl_3$

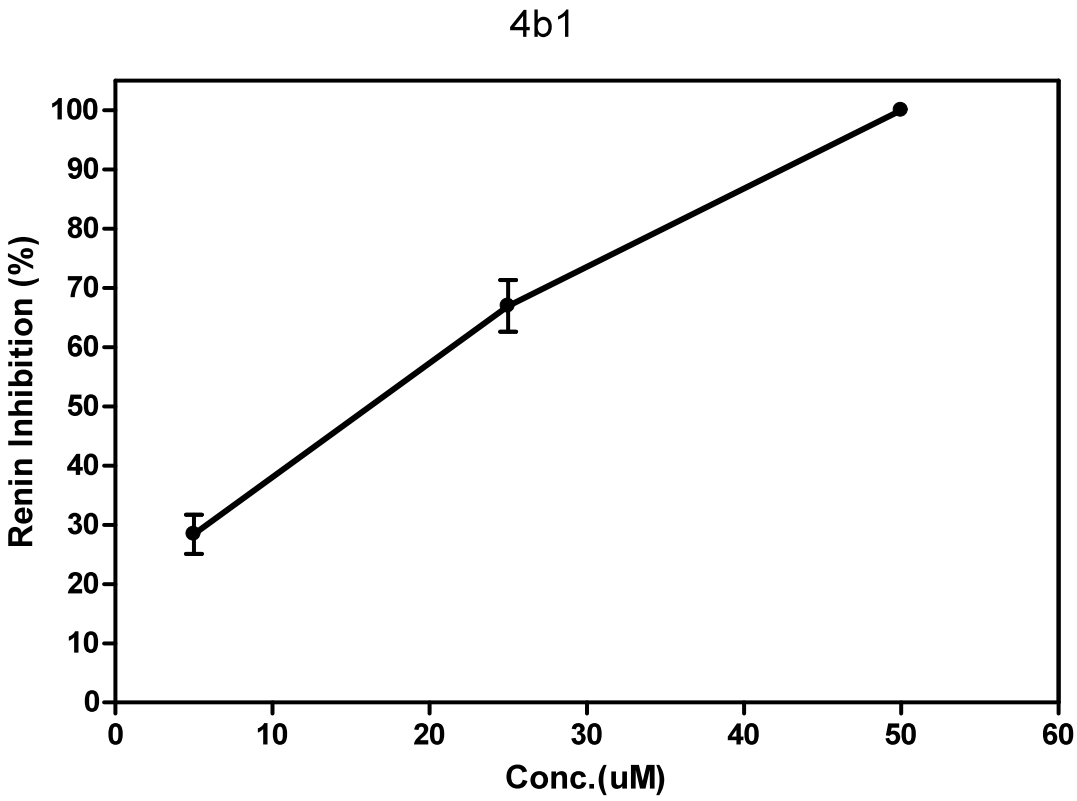


Figure A-58. A graph of Percentage inhibition vs. conc. of **45**

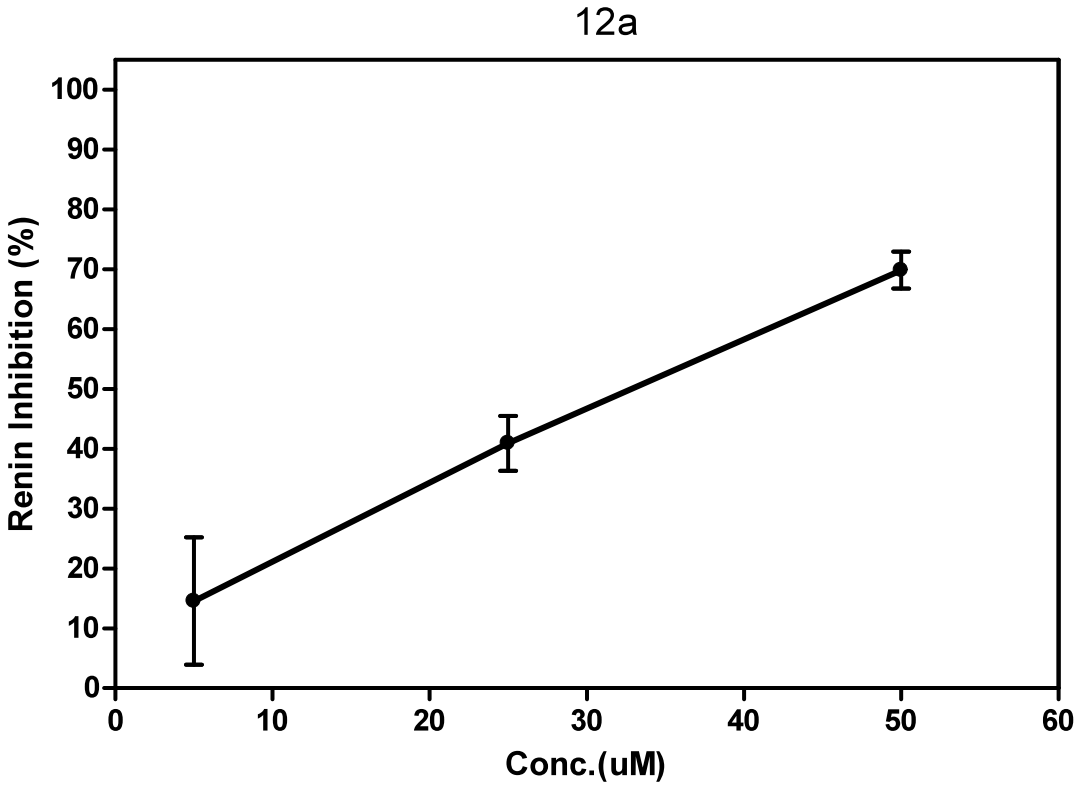


Figure A-59. A graph of Percentage inhibition vs. conc. of **48**

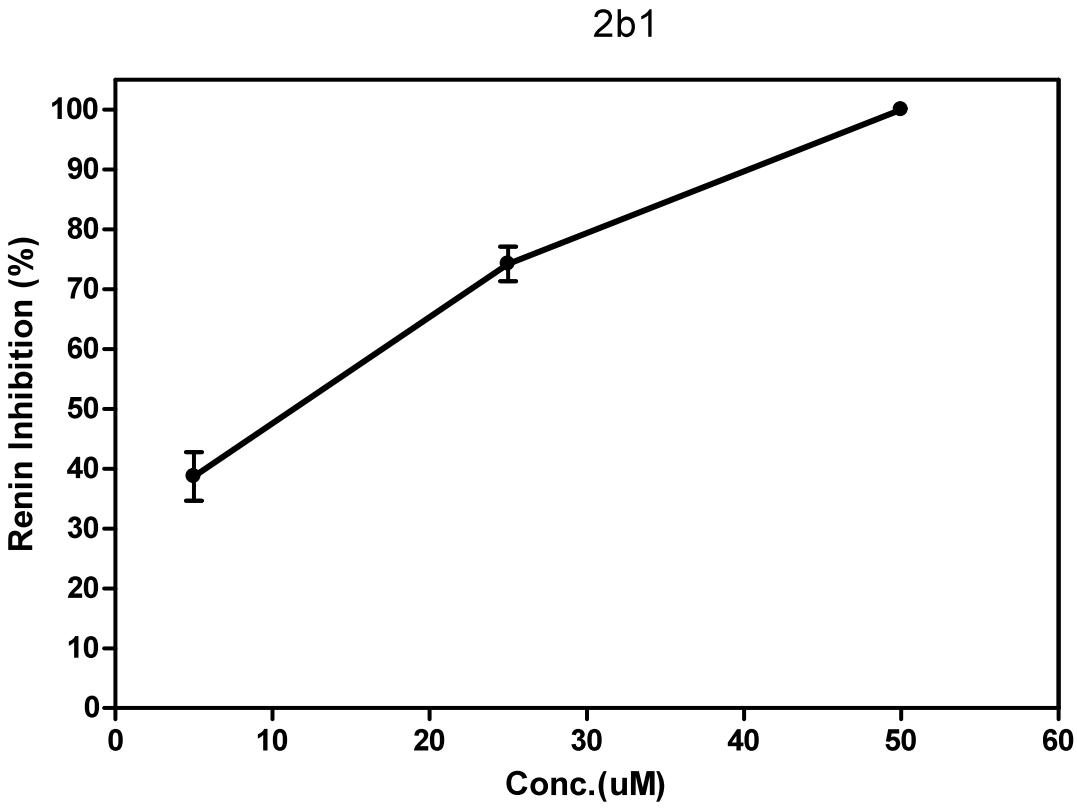


Figure A-60. A graph of Percentage inhibition vs. conc. of **49**

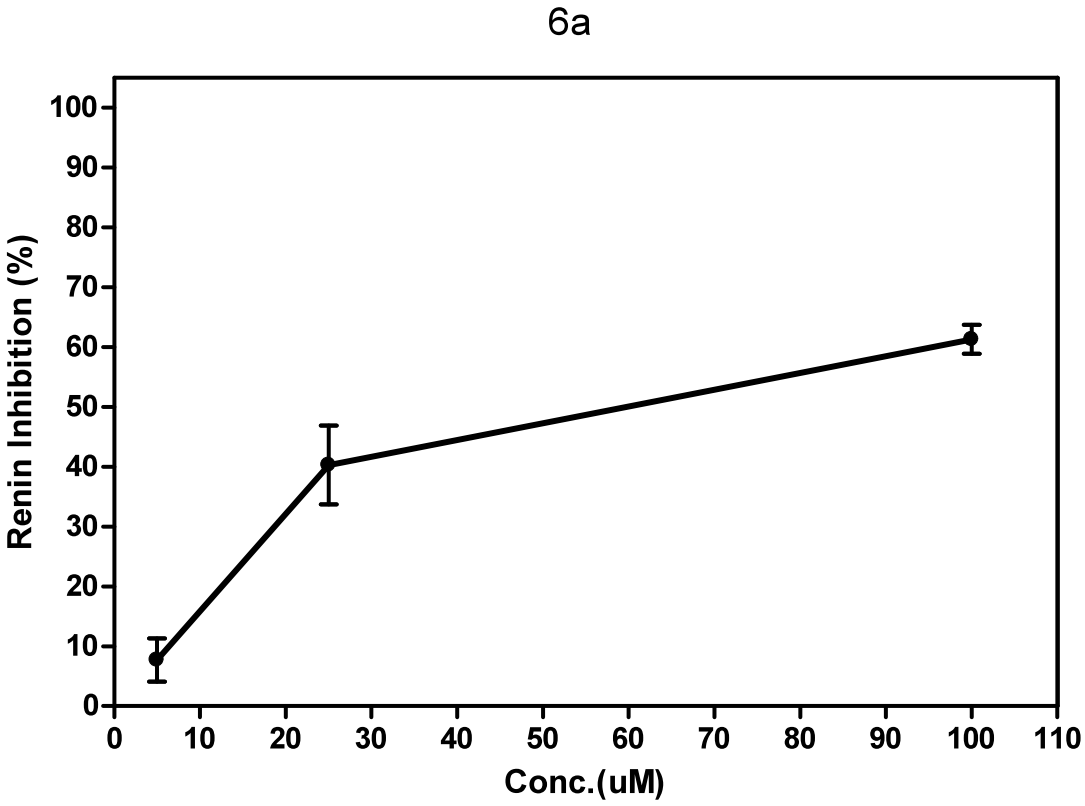


Figure A-61. A graph of Percentage inhibition vs. conc. of **46**

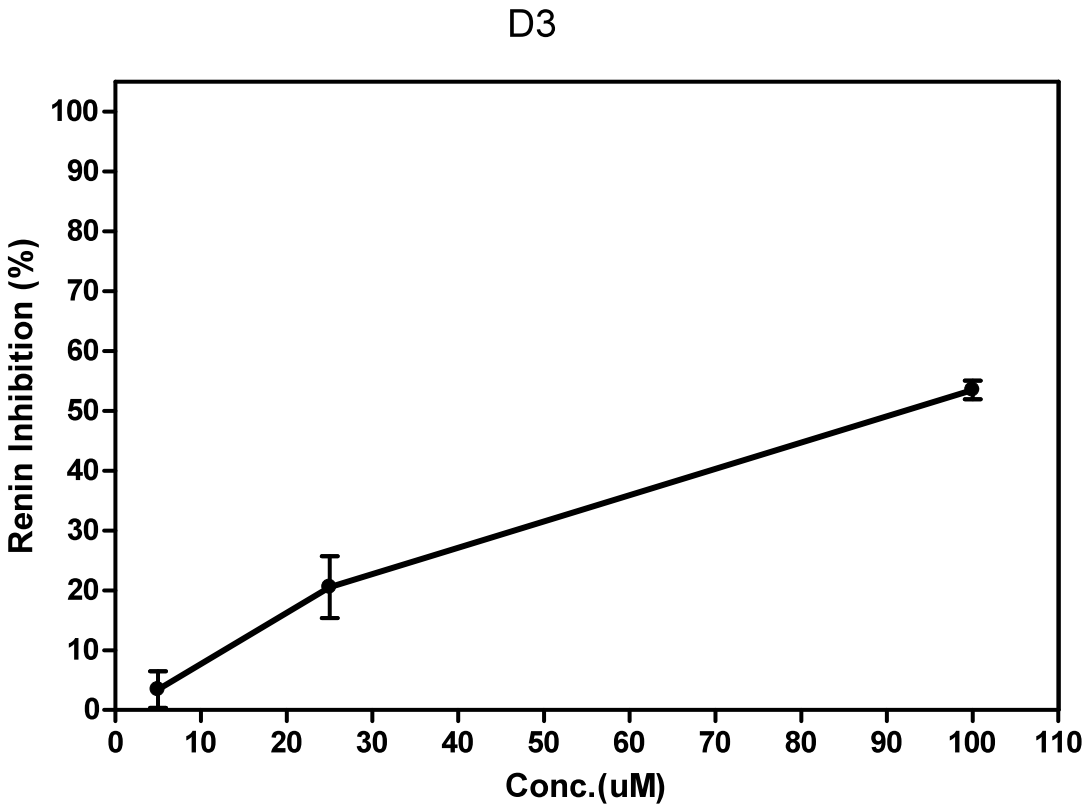


Figure A-62. A graph of Percentage inhibition vs. conc. of **50**

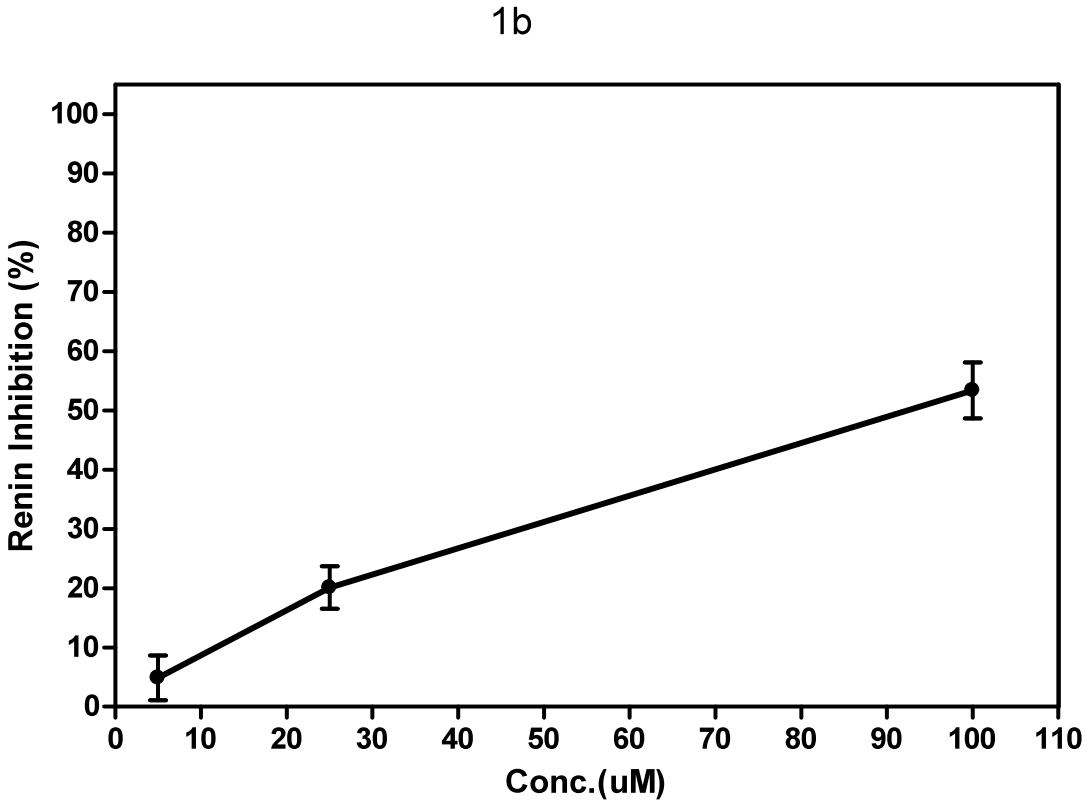


Figure A-63. A graph of Percentage inhibition vs. conc. of **51**