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Master's Degree Thesis

Phytochemical constituents of Akebiae caulis and their Aβ42 inhibitory activities

Graduate School of Chosun University

College of Pharmacy

Md. Anisuzzaman Chowdhury

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목통(Akebiae Caulis)의 화학성분과 Aβ42 저해활성

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Advisor: Prof. Woo Eun-Rhan

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This is to certify that the Master thesis
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List of Abbreviations

***A. quinata*:** *Akebia quinata*,

WHO: World Health Organization

AD: Alzheimer's disease

Fig.: Figure

APP: amyloid precursor protein

IL-6: Interleukin 6

MeOH: methanol

EtOAc: ethyl acetate

BuOH: n-butyl alcohol

H_2O: Water

CH_2Cl_2: methylene chloride

m/z: mass to charge ratio

MW: molecular weight

ppm: parts per million

RP: reverse phase

NP: normal phase

CC: column chromatography

UV: ultraviolet absorption

IR: infrared spectroscopy

NMR: nuclear magnetic resonance

MS: mass spectrum

EIMS: impact mass spectroscopy

HREIMS: high resolution electro impact mass spectroscopy

HPLC: high performance liquid chromatography

TLC: thin layer chromatography

***J*:** spin-spin coupling constant [Hz]

m: multiplet (in connection with NMR data)

s: singlet

t: triplet

br: broad

MHz: mega hertz

MPLC: medium pressure liquid chromatography

COSY: correlation spectroscopy

HSQC: Heteronuclear Single Quantum Coherence

HMBC: Heteronuclear Multiple Bond Correlation

PBS: phosphate-buffered saline;

FBS: Fetal bovine serum;

NaCl: Sodium Chloride

KCl: Potassium Chloride

CD spectra: Circular dichroism

KBSI: Korea Basic Science Institute

FD-MS: Field desorption mass spectrometry

MP: melting point

E.coli: Escherichia coli

NH_4OH: ammonium hydroxide

mg: milligram

ml: milliliter

nM: nanomolar

µM: micrometre

^{1}H NMR: Proton NMR

^{13}C NMR: Carbon NMR

CD_3OD: Deuterated methanol

Th-T assay: Thioflavin T

국문 초록

목통(Akebiae Caulis)의 화학성분과 Aβ42 저해활성

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목통(Akebiae Caulis) 은 으름덩굴(*Akebia quinata* Decaisne.) 또는 기타동속 식물의 줄기로 주피를 제거하고 가로로 자른 것으로 한방에서 소염, 이뇨, 통경에 사용된다. 목통의 약리활성으로는 이뇨, 항진균, 위액분비 억제, 지질저하 및 산후 유즙분비 촉진 작용 등이 알려져있다. 이전의 연구에 의하면 목통 (Akebiae Caulis) 으로부터 triterpenes, triterpene glycosides, phenylethanoid glycosides, megastigmane glycoside 등을 비롯한 다양한 성분이 분리, 보고되었다. 본 연구실의 예비실험결과 목통의 에틸아세테이트 분획이 Aβ-42에 대한 저해 뛰어난 활성을 나타내 활성성분의 분리를 시도하였다. 목통(Akebiae Caulis)의 에틸아세테이트분획에 대해 실리카겔, RP-18, MCI-gel, MPLC 컬럼크로마토그래피를 반복적으로 수행하여 **9**개의 화합물: Pulsatilla saponin A (**1**), Collinsonidin (**2**), Akebonic acid (**3**), Hederagenin (**4**), 1-(3′, 4′-dihydroxycinnamoyl) cyclopentane-2, 3-diol (**5**), Asperosaponin C (**6**), Leontoside A (**7**), Quinatic acid (**8**), and Quinatoside A (**9**)을 분리했다. 화합물들 (**1-9**)의 구조는^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}HCOSY, HSQC, HMBC 분광분석 등의 1D 및 2D NMR에 기초하여 확인하였다. 분리된 화합물 가운데 화합물 (**5**) 및 (**6**)은 목통에서 처음으로 분리된 화합물들이다. 분리된 화합물에 대해, Aβ-42 유도 fibrillogenesis의 저해활성을

조사하였다. 화합물 중, 화합물**2**, **3** 및 **6**은 Aβ-42 유도 fibrillogenesis 대해 상당한 억제효과를 나타냈다. 본 연구에서는 목통으로부터 분리된 화합물들에 대해Aβ-42 저해효과여부를 처음으로 확인하였다.

키워드: 목통 (Akebiae Caulis); 으름덩굴; Aβ42

Abstract

Phytochemical constituents of Akebiae Caulis and their Aβ42 inhibitory activities

Md. Anisuzzaman Chowdhury

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Akebia quinata DECAISENE (Lardizabalaceae) is a creeping woody vine that is widely distributed in East Asia, including Korea, China, and Japan. Traditionally, its dried stem is used mainly as a diuretic agent for the treatment of edema and rheumatic pain. Previous phytochemical investigations resulted in the isolation of triterpenes, triterpene glycosides, phenylethanoid glycosides and megastigmane glycoside. Amyloid β (Aβ), a main component of the senile plaque detected in Alzheimer's disease, induces cell death. However, a limited number of studies have addressed the biological and pharmacological effects of EtOAc fraction of Akebiae Caulis. In particular, the inhibitory activity against Aβ-42 induced fibrilogenesis of Akebiae Caulis remains unclear. In the present study, by means of repeated column chromatography using silicagel, LiChroprep RP-18, MCI-gel, and MPLC, nine compounds: Pulsatilla saponin A (**1**), Collinsonidin (**2**), Akebonic acid (**3**), Hederagenin (**4**), 1-(3′, 4′-dihydroxycinnamoyl) cyclopentane-2, 3-diol (**5**), Asperosaponin C (**6**), Leontoside A (**7**), Quinatic acid (**8**), and Quinatoside A (**9**) was isolated from the Akebiae Caulis. The chemical structures of compounds (**1-9**) were identified based on 1D and 2D NMR, including H-H COSY, HSQC, and HMBC spectroscopic analyses. Compound **5** and **6** were isolated from this plant for the first time. For these isolated compounds, the inhibitory activities of Aβ-42

induced fibrilogenesis were examined. Among these isolated compounds, compound **2**, **3**, and **6** have significant inhibitory effects against Aβ-42 induced fibrilogenesis. This is the first report on the Aβ-42 inhibitory effects of the components from Akebiae Caulis

Key Words: Akebiae Caulis; Lardizabalaceae, Aβ-42, fibrilogenesis,

1. Introduction

1.1. The β-Amyloid peptide and Alzheimer's disease

'Dementia'- the term is not a specific disease. It's a group of symptoms which affect mental tasks like loss of memory and other mental abilities. According to WHO (World Health Organization); approximately 47.5 million people around the world are living with dementia (**Fig. 1**) [**1**]. Alzheimer's disease (AD) is responsible for 60 to 70 percent of all cases of dementia [**2**]. AD characterized by progressive and insidious neurodegeneration of the central nervous system. The risk of developing Alzheimer's disease (AD) increase with the average age of 65 years or older [**3**].

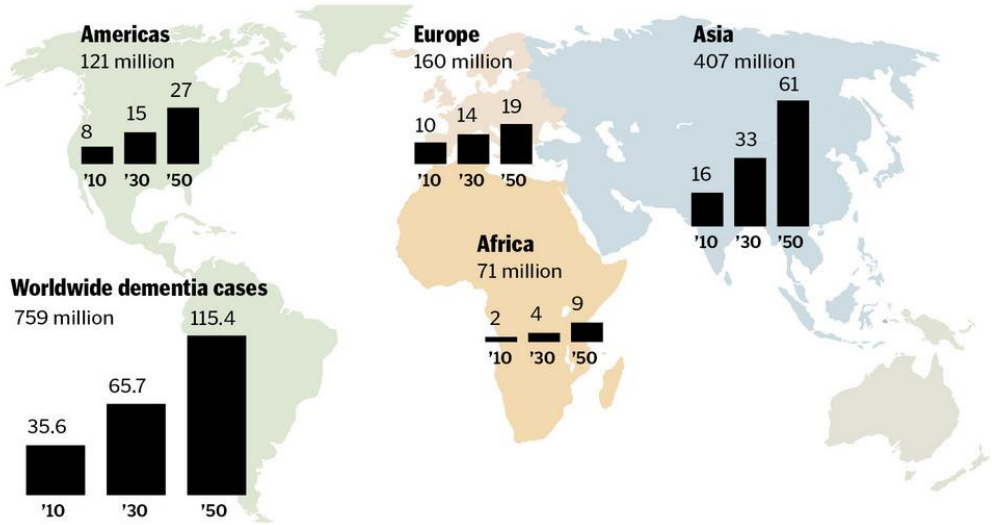


Fig.1. Worldwide prevalence of Alzheimer's Disease 2000-2030

The principal neuropathological features of AD are the presence of intracellular neurofibrillary tangles and the senile plaques (extracellular deposition of amyloid beta (Aβ) peptides). The third main feature of AD, which leads to diminished cell function and cell death by losing the connections between cells [**4**]. The peptide is derived from amyloid precursor protein (APP) (**Fig. 2**) by sequential cleavage of α

-, β -, and γ- secretases enzymes [**5**].

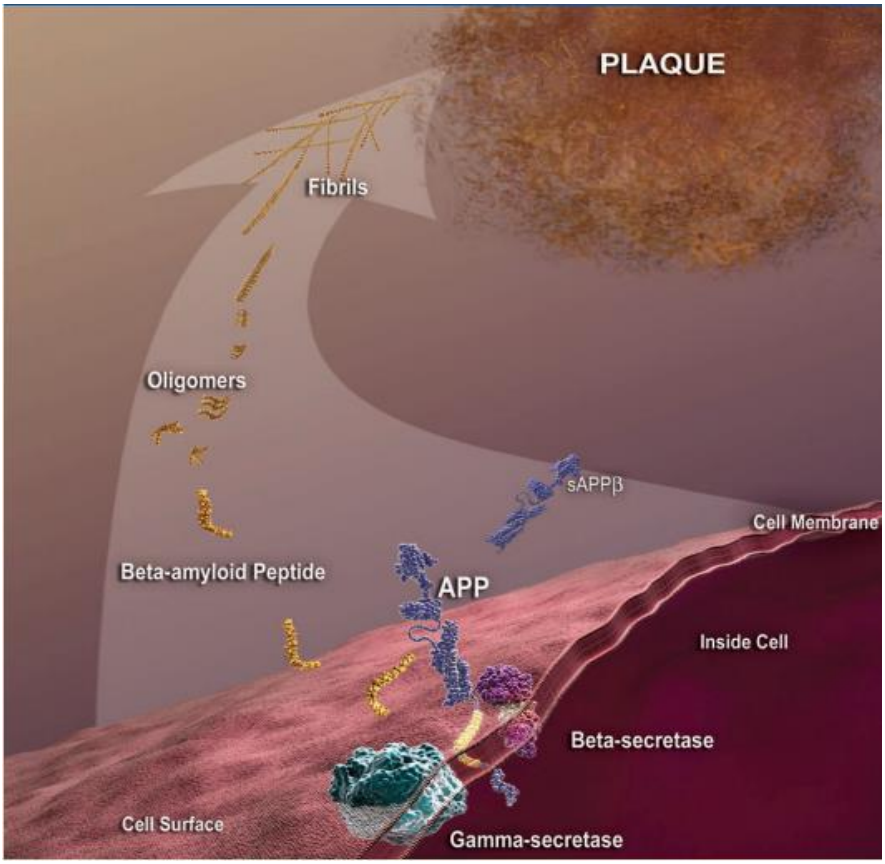


Fig.2: amyloid precursor protein (APP) to Amyloid plaques.

In the general pathway, alpha-secretase cleaves the APP molecule within the portion that has highly potential to become beta-amyloid. The cleavage releases from the neuron, which is beneficial to promote neuronal growth and survival. At the end of the beta-amyloid segment, gamma-secretase cleaves the remaining APP fragment. In the malignant pathway, beta-secretase first cleaves the APP molecule at one end of the beta-amyloid peptide, and the resulting APP fragment cleaved by gamma-secretase. After that beta-amyloid peptide is released and begins to stick to other beta-amyloid peptides (**Fig. 3**). These small and soluble aggregates of several beta-amyloid peptides are called oligomers. It is probably that, some oligomers are

cleared from the brain. Those cannot be cleared that clump together with more beta-amyloid peptides, which grow larger and form protofibrils and fibrils. These increasingly insoluble forms combine to become the well-known plaques that are features of AD [**4**].

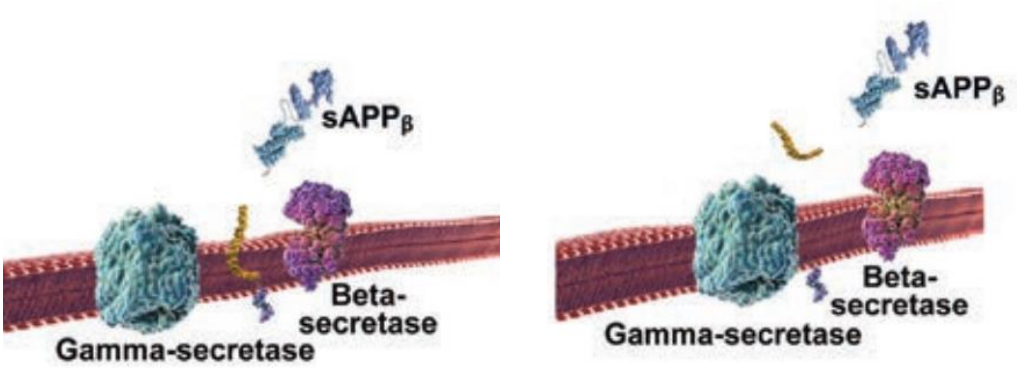


Fig. 3: Releasing sAPPβ from the cell and stick to other beta-amyloid peptides

Cleavage by γ-secretase is somewhat nonspecific, resulting in the formation of different C-terminal heterogeneous peptides. So, numerous different Aβ species exist, but those ending at position 40 (Aβ40) are the most abundant (~80-90%), followed by 42 (Aβ42, ~5-10%). The marginally longer forms of Aβ, specifically Aβ42, are more hydrophobic and fibrillogenic, and are the principal species deposited in the brain [**6, 7, 8**].

In the Alzheimer's brain; the **cortex shrivels up**, damaging areas involved in thinking, planning and remembering. Shrinkage is especially severe in the **hippocampus**, **ventricles** (fluid-filled spaces within the brain) grow larger (**Fig. 4**).

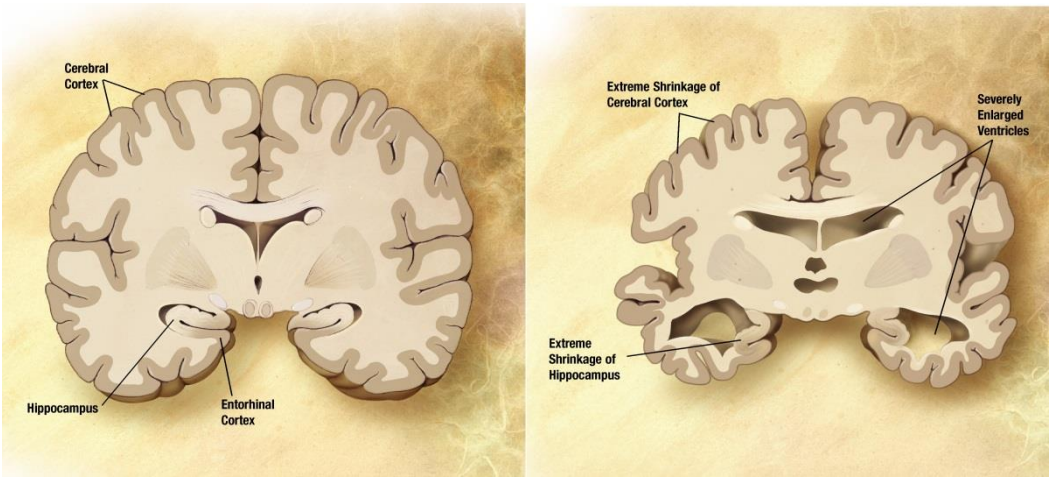


Fig.4: Comparison between normal brain and AD brain.

Because of its essential role in the Alzheimer's disease pathogenesis, the longer form of Aβ, (Aβ42) is considered a major target for the study of its underlying mechanism of fibrilogenesis. Therefore, it is the primary goal of a number of therapeutic strategies under development or in clinical trials to prevent the assembly of Aβ monomer into toxic oligomer or fibril. Hence, major experiments have involved the establishment of compounds, which are capable of inhibiting or reversing the Aβ aggregation process. Moreover, a number of diverse compounds including small molecules, antibodies, peptidic β-sheet breakers, and osmolytes, have been used to prevent or to reduce the aggregation of Aβ into oligomers or fibrils [**9**]. In this study we isolated several compounds from the A. Caulis and examined against Aβ42 induced fibrilogenesis.

1.2. Akebiae Caulis*:*



Fig. 5: *Akebia Quinata*; Leaves, Flowers, Fruits

Akebia quinata DECAISENE (Lardizabalaceae), commonly referred to as chocolate vine, grows naturally in Japan, China, and Korea. The dry ripe fruit and stem of *Akebia quinata* is used as an analgesic, an antiphlogistic, and a diuretic in traditional Chinese medicine [**10**]. And also used as ingredients of a traditional Korean weight-loss tea as a folk remedy [**13**]. Regarding the biological activity of *Akebia quinata* extract has been shown to have antioxidant activity and free radical scavenging capability [**11**] cytotoxicity against cancer cells [**12**] anti-obesity and hypolipidemic effects [**13**] antiatherogenic effects [**14**] Antinociceptive effect [**15**] and IL-6 inhibitory activity [**16**].

Previous phytochemical investigations resulted in the isolation of triterpenes, triterpene glycosides, and phenylethanoid glycosides [**17-19**], lignan glycoside [**16**], megastigmane glycoside [**20**].

Here the stem of *Akebia quinata* (known as Akebiae Caulis) was extracted and fractionated further into the nBuOH, CH_2Cl_2, Hexane and EtOAc fractions. This experiment was carried out to identify the chemical constituents from EtOAc fraction of Akebiae Caulis. And in this study, we investigated the effect of isolated compounds from Akebiae Caulis in Aβ42-induced fibrillogenesis. However, isolation and identification of constituents from EtOAc fraction of this plant and its

inhibitory activity has not reported yet. By activity oriented purification procedure, we therefore now report nine (9) compounds were isolated from the EtOAc fraction of Akebiae Caulis. For this isolated compound, the inhibitory activities of Aβ42-induced fibrillogenesis were examined. The chemical structures of compounds (**1 - 9**) were identified based on 1D and 2D NMR, including H-H COSY, HSQC, and HMBC spectroscopic analyses. The results are described in this thesis.

Fig. 6: *Akebia Quinata*; stem (known as Akebiae Caulis)

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The stem (Akebiae Caulis) of *A. quinata* were collected in Gyeongju, Gyeongbuk province, Korea, in August 2011 and identified by Dr. J. H. Lee, Professor of the department of Korean Medicine, Dongguk University. A voucher specimen (CSU-877-17) has been deposited in the Herbarium of the College of Pharmacy, Chosun University.

2.1.2. Chemicals, reagents and chromatography

TLC and column chromatography were performed on pre-coated Silica Gel F plates (Merck, art. 5715), RP-18F plates (Merck, art. 15389) and silica gel 60 (40-63 and 63-200 mm, Merck), MCI gel CHP20P (75-150m, Mitsubishi Chemical Co.), Sephadex LH-20 (25-100 μm, Sigma) as well as LiChroprep RP-18 (40-63 μm, Merck) and MPLC (Grace, USA, Reveleris flash Chromatography system, Part No. 5148513). Low pressure liquid chromatography was carried out over a Merck Lichroprep Lobar® -A RP-18 (240 9 10 mm) column with a FMI QSY-0 pump (ISCO).

Phosphate-buffered saline (PBS) [10 mM phosphate buffer (pH 7.4), 137 mM NaCl, and 2 mM KCl] was purchased from Amresco (Solon, OH). Fetal Bovine Serum (FBS) were purchased from Welgene (Daegu, Korea). All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise stated.

2.2. Methods

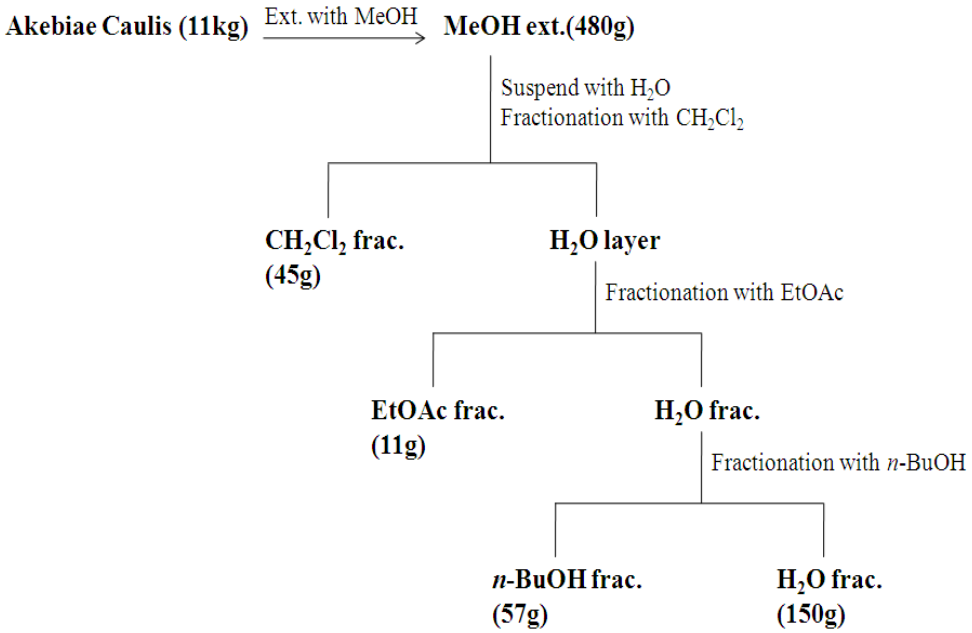
2.2.1. General experimental procedures

Optical rotations were measured using an Autopol-IV polarimeter. IR spectra were recorded on an IMS 85 (Bruker). CD spectra were recorded on a JASCO J-810 spectropolarimeter. HR-ESI–MS spectra were obtained on a Q- TOF (Synapt HDMS system, Waters, USA) mass spectrometer. NMR spectra, COSY, heteronuclear single quantum coherence (HSQC) and HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer (KBSI - Gwangju center) operating at 600 and 500 MHz for both ^{1}H and ^{13}C, with chemical shifts given in ppm (δ). Semi-preparative HPLC was performed using a Waters HPLC system equipped with Waters 600 Q-pumps, a 996 photodiode array detector, and an YMC-Pack ODS-A column (250×10 mm i.d., 5 μm), flow rate 4.0 mL/min.

2.2.2. Extraction and Isolation

2.2.2.1 Extraction

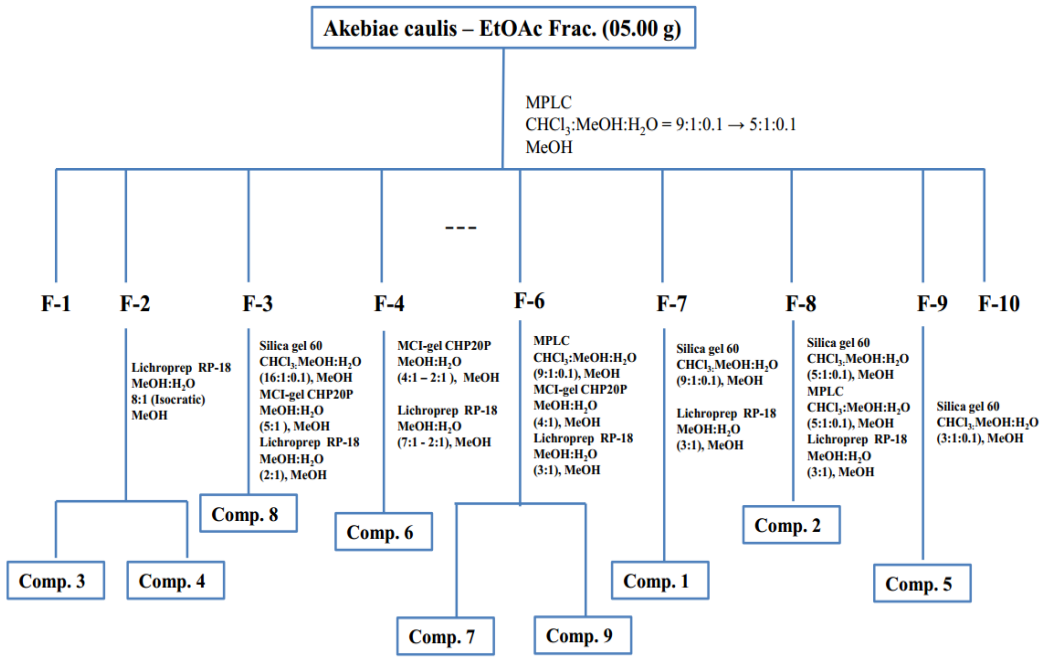
The air-dried stem of Akebiae Caulis (11 kg) were cut and extracted with MeOH three times for 4 h at 80℃. The resultant MeOH extract (480 g) was suspended in water (1.5 L×3) and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and *n*-butanol. Each fraction was evaporated *in vaccuo* to yield the residues of CH_2Cl_2 (45.2 g), EtOAc (11.0 g), *n*-BuOH (57.0 g), and water (150.3 g) extracts (**Scheme. 1**).



Scheme 1: Extraction and fraction of EtOAc fraction from Akebiae Caulis

2.2.2.2. Isolation

The EtOAc fraction (05.0 g) was chromatographed over a MPLC, using a gradient solvent system of $CHCl_3$: MeOH: H_2O = (9:1:0.1), to give ten subfractions (F1 ~ F10) (**Scheme. 2**).



Scheme 2: Isolation of compound 1-9 from EtOAc fraction of Akebiae Caulis

Compound 1:

The subfraction F-7 (682.33 mg) was subjected to a Silica-gel column chromatography (CC) eluting with an isocratic solvent system of $CHCl_3$: MeOH: H_2O (9:1:0.1) to yield six subfractions (F-7-1 ~ F-7-6). The subfraction F-7-3 (263.22mg) was subjected to Lichroprep RP-18 Column chromatography eluting with an isocratic solvent system of MeOH: H_2O (3: 1) to produce **compound 1** (14.23mg).

White amorphous powder

Molecular formula: $C_{41}H_{66}O_{12}$

Molecular weight: 750.96

Melting point: 334-335 ℃

ESI-MS: m/z 749.6 $[M-H]^-$,

IR (KBr) cm^{-1}: 3419 (OH), 1687 (−C = O), 1637 (−C=C−)

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.62 (1H, m, H-3), 5.24 (1H, br s, H-12), 2.84 (1H, dd, J = 4, 14.5, H-18), 3.52 (1H, m, H-23 a), 3.33 (1H, m, H-23 b), 0.97 (3H, s, H-24), 0.70 (3H, s, H-25), 0.82 (3H, s, H-26), 1.17 (3H, s, H-27), 0.91 (3H, s, H-29), 0.94 (3H, s, H-30), 4.55 (1H, d, J = 6 Hz, Ara-H-1), 5.16 (1H, br, s, Rha-H-1), 1.24 (3H, s, Rha-H-6)

^{13}C NMR (500 MHz, CD_3OD) δ:

35.08 (C-1), 26.67 (C-2), 82.40 (C-3), 44.08 (C-4), 49.99 (C-5), 18.96 (C-6), 39.80 (C-7), 40.66 (C-8), 48.27 (C-9), 37.78 (C-10), 24.24 (C-11), 123.69 (C-12), 145.46 (C-13), 43.13 (C-14), 29.00 (C-15), 24.68 (C-16), 47.85 (C-17), 42.93 (C-18), 47.44 (C-19), 31.77 (C-20), 34.00 (C-21), 33.56 (C-22), 64.72 (C-23), 16.54 (C-24), 14.02 (C-25), 17.91 (C-26), 26.61 (C-27), 179.41 (C-28), 33.77 (C-29), 24.15 (C-

30), Ara: 104.49 (C-1), 76.78 (C-2), 74.07 (C-3), 69.34 (C-4), 72.28 (C-5), Rha: 102.04 (C-1), 70.31 (C-2), 72.16 (C-3), 73.83 (C-4), 64.94 (C-5), 18.11 (C-6).

Compound 2:

The subfraction F-8 (1.07 g) was subjected to MPLC eluting with an isocratic solvent system of $CHCl_3$: MeOH: H_2O (5:1:0.1) to yield six subfractions (F-8-1 ~ F-8-6). The subfraction F-8-4 (137.81mg) was subjected to Lichroprep RP-18 Column chromatography eluting with an isocratic solvent system of MeOH: H_2O (3: 1) to produce **compound 2** (3.38mg).

Colorless needle

Molecular formula: $C_{41}H_{66}O_{13}$

Molecular weight: 766.95

Melting point: 250-252 ℃

FAB-MS: m/z 789 [M + Na] +

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.61 (1H, m, H-3), 5.24 (1H, br s, H-12), 2.85 (1H, dd, J = 4, 14.5, H-18), 4.34 (1H, m, H-23 a), 3.35 (1H, m, H-23 b), 0.98 (3H, s, H-24), 0.72 (3H, s, H-25), 0.82 (3H, s, H-26), 1.17 (3H, s, H-27), 0.91 (3H, s, H-29), 0.94 (3H, s, H-30), 4.54 (1H, d, J = 6 Hz, Ara-H-1), 5.16 (1H, d, J = 8.1 Hz, Gluc-H-1), 3.69 (1H, m, Gluc-H-6a), 3.67 (1H, m, Gluc-H-6b).

^{13}C NMR (500 MHz, CD_3OD) δ:

35.10 (C-1), 26.62 (C-2), 83.64 (C-3), 44.00 (C-4), 48.44 (C-5), 19.03 (C-6), 39.62 (C-7), 40.67 (C-8), 48.25 (C-9), 37.86 (C-10), 24.25 (C-11), 123.59 (C-12), 145.55

(C-13), 43.12 (C-14), 29.02 (C-15), 24.70 (C-16), 47.91 (C-17), 42.96 (C-18), 47.47 (C-19), 31.78 (C-20), 34.02 (C-21), 33.62 (C-22), 65.26 (C-23), 16.58 (C-24), 13.50 (C-25), 17.98 (C-26), 26.47 (C-27), 182.22 (C-28), 33.77 (C-29), 24.17 (C-30), Ara: 105.64 (C-1), 71.25 (C-2), 84.39 (C-3), 69.70 (C-4), 67.01 (C-5), Gluc: 106.27 (C-1), 77.78 (C-2), 78.03 (C-3), 72.23 (C-4), 75.43 (C-5), 62.43 (C-6).

Compound 3 and 4:

The subfraction F-2 (205.60 mg) was subjected to Lichroprep RP-18 Column chromatography eluting with an isocratic solvent system of MeOH: H2O (8:1) to produce **compound 3** (2.96mg) and **compound 4** (11.77 mg).

Compound 3:

White amorphous powder

Molecular formula: $C_{29}H_{44}O_3$

IR (KBr): 3424, 2935, 1691, 1653, 1463, 1384, 1297, 1212, 1102, 996, 886.

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.33 (1H, br, s, H-3), 5.31 (1H, br s, H-12), 2.72 (1H, dd, J = 4, 14.5, H-18), 2.14 (1H, m, H-21 a), 2.08 (1H, m, H-21 b), 0.96 (3H, s, H-23), 0.84 (3H, s, H-24), 0.93 (3H, s, H-25), 0.82 (3H, s, H-26), 1.22 (3H, s, H-27), 4.61 (2H, br, s, H-29).

^{13}C NMR (500 MHz, CD_3OD) δ:

34.32 (C-1), 26.65 (C-2), 77.01 (C-3), 43.13 (C-4), 52.11 (C-5), 19.49 (C-6), 39.31 (C-7), 40.87 (C-8), 50.16 (C-9), 38.33 (C-10), 24.43 (C-11), 124.27 (C-12), 144.81 (C-13), 42.90 (C-14), 29.18 (C-15), 24.58 (C-16), 31.15 (C-17), 23.04 (C-18), 41.95 (C-19), 149.98 (C-20), 38.46 (C-21), 34.32 (C-22), 29.00 (C-23), 15.90 (C-24), 14.58 (C-25), 17.87 (C-26), 26.35 (C-27), 181.37 (C-28), 107.28 (C-29).

Compound 4:

White amorphous powder

Molecular formula: $C_{30}H_{48}O_4$

Molecular weight: 472.70

Melting point: 325-328 ℃

ESI-MS: m/z 472 $[M]^+$ (18), 248 (l00), 203 (66)

^{1}H-NMR (500 MHz, Pyridine-d_5) δ:

3.65 (1H, m, H-3), 5.53 (1H, br s, H-12), 3.35 (1H, dd, J = 4, 14.5, H-18), 3.65 (1H, m, H-23 a), 3.47 (1H, m, H-23 b), 0.96 (3H, s, H-23), 1.27 (3H, s, H-24), 0.92 (3H, s, H-25), 0.97 (3H, s, H-26), 1.31 (3H, s, H-27), 1.03 (3H, s, H-29), 1.05 (3H, s, H-30).

^{13}C NMR (500 MHz, Pyridine-d_5) δ:

35.49 (C-1), 29.60 (C-2), 79.33 (C-3), 43.45 (C-4), 49.40 (C-5), 20.08 (C-6), 34.48 (C-7), 40.67 (C-8), 47.76 (C-9), 38.65 (C-10), 25.11 (C-11), 123.83 (C-12), 146.11 (C-13), 43.29 (C-14), 30.07 (C-15), 27.45 (C-16), 47.95 (C-17), 41.02 (C-18), 40.20 (C-19), 30.90 (C-20), 34.56 (C-21), 31.26 (C-22), 57.08 (C-23), 17.85 (C-24), 16.83 (C-25), 18.72 (C-26), 29.39 (C-27), 181.50 (C-28), 32.25 (C-29), 25.04 (C-30).

Compound 5:

The subfraction F-9 (404.11 mg) was subjected to a Silica-gel column chromatography (CC) eluting with an isocratic solvent system of $CHCl_3$: MeOH: H2O (3:1:0.1) to yield seven subfractions (F-9-1 ~ F-9-7). The subfraction F-9-6

(56.34mg) was subjected to Silica-gel column chromatography (CC) again to elute with an isocratic solvent system of $CHCl_3$: MeOH: H2O (3:1:0.1) to produce **compound 5** (19.50 mg).

Brown Powder

Molecular formula: $C_{14}H_{16}O_6$

UV (MeOH) λ_{max} (log ε) 205 (3.95), 215 (3.94), 243 (3.76), 299 (3.81), 327 (3.90) nm

IR (KBr) ν_{max} 3414 (OH), 1694 (C=O) cm^{-1}

EIMS *m/z* 281 (10), $[M + H]^+$ 180 (20), 163 (82)

^{1}H-NMR (300 MHz, CD_3OD) δ:

7.04 (1H, d, J = 2 Hz, H-2), 6.77 (1H, d, J = 8.2 Hz, H-5), 6.91 (1H, dd, J = 8.2, 2 Hz, H-6), 7.56 (1H, d, J = 15.8 Hz, H-7), 6.28 (1H, d, J = 15.8 Hz, H-8), 5.38 (1H, m, H-1′), 3.69 (1H, dd, J = 8.3, 3 Hz, H-2′), 4.18 (1H, s, H-3′), 2.02-2.17 (2H, m, H-4′), 2.02-2.17 (2H, m, H-5′).

^{13}C NMR (75 MHz, CD_3OD) δ:

127.92 (C-1′), 115.25 (C-2′), 146.92 (C-3′), 149.64 (C-4′), 115.62 (C-5′), 123.09 (C-6′), 147.05 (C-7′), 116.63 (C-8′), 169.25 (C-9′), 74.86 (C-1), 73.16 (C-2), 72.51 (C-3), 38.70 (C-4), 40.55 (C-5),

Compound 6:

The subfraction F-4 (405.47 mg) was subjected to a MCI-gel column chromatography (CC) eluting with a gradient solvent system of MeOH: H2O (4:1 ~ 2:1) to yield seven subfractions (F-4-1 ~ F-4-7). The subfraction F-4-7 (35.23 mg)

was subjected to Lichroprep RP-18 Column chromatography to elute with a gradient solvent system of MeOH: H2O (7:1 ~ 2:1) to produce **compound 6** (3.96 mg).

White amorphous powder

Molecular formula: $C_{35}H_{56}O_7$

Molecular weight: 588.81

Melting point: 214-216 ℃

IR (KBr) γ_{max} 3440, 2944, 1694, 1462, 1388, 1087 cm^{-1}

Negative ESI-MS m/z 587 $[M–H]^-$

Negative TOF-MS m/z 587.3996 $[M–H]^-$

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.13 (1H, dd, J = 5, 12 Hz, H-3), 5.24 (1H, br s, H-12), 2.85 (1H, dd, J = 4, 14.5 Hz, H-18), 1.04 (3H, s, H-23), 0.84 (3H, s, H-24), 0.95 (3H, s, H-25), 0.82 (3H, s, H-26), 1.16 (3H, s, H-27), 0.91 (3H, s, H-29), 0.94 (3H, s, H-30), 4.27 (1H, d, J = 6 Hz, Ara-H-1).

^{13}C NMR (500 MHz, CD_3OD) δ:

35.08 (C-1), 27.16 (C-2), 90.82 (C-3), 40.33 (C-4), 57.15 (C-5), 19.49 (C-6), 39.90 (C-7), 40.70 (C-8), 30.95 (C-9), 38.05 (C-10), 24.23 (C-11), 123.69 (C-12), 145.42 (C-13), 43.03 (C-14), 29.00 (C-15), 24.67 (C-16), 47.86 (C-17), 42.92 (C-18), 47.44 (C-19), 31.78 (C-20), 33.99 (C-21), 34.14 (C-22), 28.71 (C-23), 17.12 (C-24), 16.09 (C-25), 17.90 (C-26), 26.56 (C-27), 182.29 (C-28), 33.75 (C-29), 24.15 (C-30), Ara: 107.33 (C-1), 74.46 (C-2), 72.95 (C-3), 69.69 (C-4), 66.58 (C-5).

Compound 7:

The subfraction F-6 (928.28 mg) was subjected to MPLC eluting with an isocratic solvent system of $CHCl_3$: MeOH: H2O (9:1:0.1) to yield five subfractions (F-6-1 ~ F-6-5). The subfraction F-6-2 (195.81 mg) was subjected to MCI-gel column chromatography (CC) eluting with an isocratic solvent system of MeOH: H2O (4:1) to yield five subfractions (F-6-2-1 ~ F-6-2-5). Therefore, subfraction F-6-2-2 (26.56 mg) was subjected to Lichroprep RP-18 Column chromatography to elute with an isocratic solvent system of MeOH: H2O (3:1) to produce **compound 7** (12.17 mg).

White amorphous powder

Molecular formula: $C_{35}H_{56}O_8$

Molecular weight: 604.81

Melting point: 276-278 ℃

FD-MS: m/z 627 $[M + Na]^+$

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.60 (1H, m, H-3), 5.24 (1H, br s, H-12), 2.85 (1H, dd, J = 4, 14.5 Hz, H-18), 3.59 (1H, m, H-23 a), 3.31 (1H, m, H-23 b), 0.98 (3H, s, H-24), 0.71 (3H, s, H-25), 0.82 (3H, s, H-26), 1.18 (3H, s, H-27), 0.91 (3H, s, H-29), 0.94 (3H, s, H-30), 4.31 (1H, d, J = 6 Hz, Ara-H-1).

^{13}C NMR (500 MHz, CD_3OD) δ:

35.08 (C-1), 26.62 (C-2), 83.45 (C-3), 44.03 (C-4), 52.09 (C-5), 19.00 (C-6), 39.62 (C-7), 40.68 (C-8), 48.28 (C-9), 37.83 (C-10), 24.22 (C-11), 123.70 (C-12), 145.44 (C-13), 43.13 (C-14), 28.99 (C-15), 24.70 (C-16), 47.82 (C-17), 42.91 (C-18), 47.41 (C-19), 31.76 (C-20), 33.90 (C-21), 33.60 (C-22), 64.96 (C-23), 16.54 (C-24),

13.55 (C-25), 17.91 (C-26), 26.47 (C-27), 182.15 (C-28), 33.73 (C-29), 24.14 (C-30), Ara: 106.45 (C-1), 74.67 (C-2), 73.10 (C-3), 69.91 (C-4), 66.98 (C-5).

Compound 8:

The subfraction F-3 (479.88 mg) was subjected to a Silica-gel column chromatography (CC) eluting with an isocratic solvent system of $CHCl_3$: MeOH: H2O (16:1:0.1) to yield five subfractions (F-3-1 ~ F-3-5). The subfraction F-3-3 (241.54 mg) was subjected to MCI-gel column chromatography (CC) eluting with an isocratic solvent system of MeOH: H2O (5:1) to yield six subfractions (F-3-3-1 ~ F-3-3-6). Therefore, subfraction F-3-3-3 (52.60 mg) was subjected to Lichroprep RP-18 Column chromatography to elute with an isocratic solvent system of MeOH: H2O (2:1) to produce **compound 8** (5.33 mg).

Colorless Powder

MP: 247-249 ℃

Molecular formula: $C_{29}H_{44}O_4$

IR ($CHCl_3$) γ_{max}: 3450, 1690, 1630

ESI-MS: (+) m/z 479 $[M+Na]^+$; (-) m/z 455 $[M-H]^+$

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.77 (1H, br, s, H-3), 5.30 (1H, br s, H-12), 3.67 (1H, m, H-23a), 3.39 (1H, m, H-23b), 0.92 (3H, s, H-24), 0.81 (3H, s, H-25), 1.04 (3H, s, H-26), 1.22 (3H, s, H-27), 4.59 (2H, br, s, H-29),

^{13}C NMR (500 MHz, CD_3OD) δ:

34.45 (C-1), 26.22 (C-2), 71.44 (C-3), 44.12 (C-4), 50.78 (C-5), 19.62 (C-6), 39.43

(C-7), 40.83 (C-8), 48.10 (C-9), 38.16 (C-10), 24.51 (C-11), 123.97 (C-12), 145.06 (C-13), 43.10 (C-14), 29.08 (C-15), 24.76 (C-16), 47.00 (C-17), 47.90 (C-18), 43.07 (C-19), 150.25 (C-20), 31.27 (C-21), 34.34 (C-22), 66.40 (C-23), 16.32 (C-24), 17.84 (C-25), 22.98 (C-26), 26.62 (C-27), 182.19 (C-28), 107.08 (C-29).

Compound 9:

The subfraction F-6 (928.28 mg) was subjected to MPLC eluting with an isocratic solvent system of $CHCl_3$: MeOH: H2O (9:1:0.1) to yield five subfractions (F-6-1 ~ F-6-5). The subfraction F-6-2 (195.81 mg) was subjected to MCI-gel column chromatography (CC) eluting with an isocratic solvent system of MeOH: H2O (4:1) to yield five subfractions (F-6-2-1 ~ F-6-2-5). Therefore, subfraction F-6-2-2 (26.56 mg) was subjected to Lichroprep RP-18 Column chromatography to elute with an isocratic solvent system of MeOH: H2O (3:1) to produce **compound 9** 2.56 mg).

Colourless Powder

MP: 256-260 ℃

Molecular formula: $C_{34}H_{52}O_8$

EIMS: m/z 456 $[M- 142]^+$ (3), 410 (3), 232, 207 (23), 187 (l00), 176 (25)

^{1}H-NMR (500 MHz, CD_3OD) δ:

3.61 (1H, m, H-3), 5.30 (1H, br s, H-12), 3.59 (1H, m, H-23a), 3.29 (1H, m, H-23b), 0.71 (3H, s, H-24), 0.98 (3H, s, H-25), 0.84 (3H, s, H-26), 1.21 (3H, s, H-27), 4.58 (2H, br, s, H-29), 4.32 (1H, d, J = 6 Hz, Ara-H-1)

^{13}C NMR (500 MHz, CD_3OD) δ:

37.84 (C-1), 26.48 (C-2), 83.46 (C-3), 44.03 (C-4), 48.28 (C-5), 18.99 (C-6), 39.63

(C-7), 40.69 (C-8), 47.90 (C-9), 39.43 (C-10), 24.52 (C-11), 123.91 (C-12), 145.17 (C-13), 43.12 (C-14), 29.12 (C-15), 24.68 (C-16), 47.34 (C-17), 48.13 (C-18), 43.05 (C-19), 150.26 (C-20), 31.27 (C-21), 33.59 (C-22), 64.95 (C-23), 13.55 (C-24), 16.55 (C-25), 18.02 (C-26), 26.64 (C-27), 182.25 (C-28), 107.08 (C-29).

2.2.3. Preparation of amyloid beta solution

Amyloid beta peptides were expressed in *E. coli* as fusion proteins and purified as described before [**21**]. The purified peptides were solubilized in 100% 1, 1, 1, 3, 3, 3, - hexafluoro-2-propanol, and dried under nitrogen flow and subsequently, under a vacuum for 30 min. The peptide aliquots were stored at −20°C until use. Immediately before use, the peptides were dissolved in 0.1% NH_4OH at a concentration of 2 mg/ml followed by bath sonication for 10 min at 4℃. The solution was diluted at the desired concentration with PBS.

2.2.4. Thioflavin T Binding Assay for the Study of Aβ42 Fibrillogenesis.

For the polymerization assay, Aβ42 (20 μM) was incubated in PBS at 37°C in the presence or absence of **compound (1-8)** in a final volume of 30 μl without shaking. Twenty μl from each reaction was mixed with 80 μl of 5 μM Th-T in PBS solution. Fluorescence was measured on a microplate spectrofluorometer Gemini-XS (Molecular Devices CA, USA) using excitation at 440 nm and emission at 490 nm [**22**].

3. Results and Discussion

3.1. Structure determination of isolated compounds from Akebiae Caulis

The structures of compounds (1 - 9) were identified based on 1D and 2D NMR, including H - H COSY, HSQC, HMBC spectroscopic analyses.

3.1.1. Compound 1

Compound 1 was obtained white amorphous powder and molecular formula was determined as $C_{41}H_{66}O_{12}$ by ESI-MS: m/z 749.6 $[M-H]^-$, IR (KBr) cm^{-1}: 3419 (OH), 1687 (−C = O), 1637 (−C=C−). The ^{13}C NMR and HSQC data compound 1 showed 41 carbon and expected triterpenoid typed compound. From the ^{1}H-NMR data **compound 1** showed seven methyl group at δ_H 0.97 (3H, s, H-24), δ_H 0.70 (3H, s, H-25), δ_H 0.82 (3H, s, H-26), δ_H 1.17 (3H, s, H-27), δ_H 0.91 (3H, s, H-29), δ_H 0.94 (3H, s, H-30), and sugar methyl at δ_H 1.24 (3H, s, Rha-H-6). At the same time, in the spectra of compound **2** we found with the aid of COSY two doublet signals of protons, having a spin-spin coupling with one another, from the chemical shifts, which corresponded to αβ protons in a CH_2OH group at the C-23 atom. There is a carboxyl group at δ_C 179.41 (C-28). From the carbon NMR data the spectrum showed two chemical shifts at 104.49 and 102.04, which referred two sugars moiety. Based on the above results, the structure of **compound 1** was identified as **Pulsatilla saponin A** or 3-O-α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**23**] **[24]**.

3.1.2. Compound 2

Compound 2 was obtained Colorless needle and molecular formula was determined as $C_{41}H_{66}O_{13}$ by EI-MS: m/z 767 $[M+H]^+$, 605 $[M+H\text{-glc }(C_6H_{11}O_5)]^+$,

472 [M+H-glc-ara ($C_{11}H_{19}O_9$)] $^+$, 471 [M-glc-ara] $^+$, 455 [M+ H-glc-ara-OH] $^+$. IR (Nujol) ν_{max} 3660, 3500, 3420, 3300, 1690, 1620, 1460, 1375, 1300, 1265, 1240, 1210, 1200, 1170, 1130, 1090, 1060, 1030, 1000, and 785 cm^{-1}. The 1H and the 13C nmr spectra showed signals for six quaternary methyls: ^{1}H-nmr signals at δ 0.72, 0.82, 0.91, 0.94, 0.98, 1.17 (each 3H, s) and ^{13}C-nmr signals at 33.77, 26.47, 24.17, 17.98, 16.58, and 13.50 ppm. The spectra also exhibited signals due to the following groups at positions similar to those of hederagenin: a carboxylic acid group (δ 182.22), a trisubstituted double bond **(**δ 123.59 and 145.55, a proton signal at δ 5.24 br, s), a primary alcoholic group (δ 65.26, proton signals at δ 3.35 and 4.34 each (1H, m) and a secondary alcoholic hydroxyl group (δ 83.64, proton signal at δ 3.61, m). The anomeric carbons at δ 106.27 and 105.64 in collinsonidin indicated the presence of two sugar units. The arabinosyl unit was shown to be directly attached at C-3 of the aglycon by a heteronuclear multiple bond correlation between the signals of the anomeric proton of the arabinosyl unit at δ 4.54 and C-3 of the aglycon at d 83.64. Based on the above results, the structure of **compound 2** was identified as **Collinsonidin** or Hederagenin 3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**25**].

3.1.3. Compound 3

Compound 3 was obtained white amorphous powder and molecular formula was determined as $C_{29}H_{44}O_3$ by EI-MS (70 eV): 440 (40, M^+), 422 (10, [M-H_2O] $^+$), 394 (29, [M-HCOOH] $^+$), 248 (58), 232 (84), 219 (34), 207 (65), 187 (78), 175 (53), 145 (44), 131 (55), 107 (54), 91 (64), 69 (77). The 1H and the 13C nmr spectra showed signals for five quaternary methyls: ^{1}H-nmr signals ***at*** δ 0.82, 0.84, 0.93, 0.96, 1.22 (each 3H, **s)** and ^{13}C-nmr signals at 29.00, 26.35, 24.17, 17.87, 15.90, and 14.58 ppm. The spectra also exhibited signals due to the following groups a

carboxylic acid group (δ 181.37), a trisubstituted double bond **(**δ 124.27 and 144.81, a proton signal at δ 5.31 br, s), and a secondary alcoholic hydroxyl group (δ 77.01, proton signal at δ 3.33, br, s). Further, the low field carbon signals at δ 149.98 ppm and 107.28 ppm were assigned to the exomethylene C-20 and C-29 respectively. Based on the above results, the structure of **compound 3** was identified as **Akebonic acid** or 30-Noroleana-12,20(29)-dien-28-oic acid, 3-hydroxy-, (3β)- by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**26**].

3.1.4. Compound 4

Compound 4 was obtained white amorphous powder and molecular formula was determined as $C_{30}H_{48}O_4$ by ESI-MS: m/z 472 $[M]^+$ (18), 248 (l00), 203 (66). The chemical shifts and DEPT experiment suggested that it was a CH_2OH group. In the HMBC experiment, H-23 showed long-range correlation with C-3, C-4, C-5 and C-24 and thus structural unit could be proposed. In the COSY experiment, H-3 showed a *J* correlation with H-2, thus, from the HMBC spectra H-2 showed long-range correlation with C-4 and C-10. Based on the above results, the structure of **compound 4** was identified as **Hederagenin** or Olean-12-en-28-oic acid, 3,23-dihydroxy-, (3β,4α)- by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**27**] **[28]**. Hederagenin is the well-known structure.

3.1.5. Compound 5

Compound 5 was obtained brown powder and molecular formula was determined as $C_{14}H_{16}O_6$ by EIMS *m/z* 281 (10), $[M + H]^+$ 180 (20), 163 (82). Signals at *δ* 149.64, 146.92, 127.92, 123.09, 115.62, and 115.25 in the ^{13}C NMR of **compound 5** were typical of a 3′, 4′-dihydroxylcinnamoyl group. The signals at *δ* 74.86, 73.16, and 72.51 were assigned to the hydroxymethine carbons C-1, C-2, and C-3. The ^{1}H

NMR of compound **5** revealed two aromatic protons appearing as doublets at δ 7.56 (J = 15.8 Hz, H-7′) and 6.28 (J = 15.8 Hz, H-8′), the coupling constant of which suggested that they were *trans*-oriented. The presence of a caffeic acid moiety in **compound 5** was confirmed from peaks at *m*/*z* 180 and 163 in the EIMS, while those at δ 146.8 and δ 115.3, together with correlations indicated by the gHMBC spectrum between H-7′ and H-8′ with the carbonyl group at δ 168.7. The multiple signals at δ 5.38 (H-1) and 4.18 (H-3) together with the doublet of doublets at δ 3.69 (H-2) were attributed to three hydroxymethine hydrogens. Based on the above results, the structure of **compound 5** was identified as **1-(3′, 4′-dihydroxycinnamoyl) cyclopentane-2, 3-diol** by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**29**].

3.1.6. Compound 6

Compound 6 was obtained white amorphous powder and molecular formula was determined as $C_{35}H_{56}O_7$ by Negative ESI-MS m/z 587 $[M–H]^-$; Negative TOF-MS m/z 587.3996 $[M–H]^-$. The downfield chemical shift of C-3 (δ C 90.82) in the ^{13}C NMR spectra implied that compound 6 is a monodesmosidic glycoside with sugar moiety at C-3. The coupling constants (6.0 Hz) of the anomeric protons suggested that arabinose has an α-anomeric orientation. The proton and carbon signals were assigned unambiguously using ^{1}H, ^{13}C, HSQC, HMBC, NOESY NMR experiments. Thus, the structure of compound 6 was determined as 3-O- α-L-arabinopyranosyl oleanolic acid. The aglycon of **compound 6** was identified as oleanolic acid and sugar moiety was an arabinose named **Asperosaponin C** or 3-O-α-L-arabinopyranosyl oleanolic acid by comparison of the ^{1}H and ^{13}C NMR data with literature values [**30**].

3.1.7. Compound 7

Compound 7 was obtained white amorphous powder and molecular formula was determined as $C_{35}H_{56}O_8$ by FD-MS: m/z 627 $[M + Na]^+$. The ^{1}H-NMR spectrum of compound **7** exhibited anomeric signal at δ 4.31 (1H, d, J = 6.00 Hz H-1') indicating the presence of one sugar moiety. The ^{13}C-NMR spectrum suggested that the aglycone of compound **7** is an oleanane-type triterpene. The olefinic resonances at δ 145.44 and 123.70 corresponding to quaternary and methine behavior revealed the presence of unsaturation at C-12 in an oleanane skeleton. The ^{13}C-NMR spectrum further showed anomeric carbon signals at δ 106.45 which indicated the presence of one sugar moiety. This suggested that compound 7 should be a hederagenin glycoside containing only one arabinosyl. C-23 was assigned to δ 64.96 as this was close to the assignment in hederagenin. From the ^{13}C NMR and ^{1}H NMR spectra, it can be concluded that the structure of **compound 7** was hederagenin 3-0-α-L-arabinopyranoside, which was identical to **Leontoside A** or 3-O-[α-L-Arabinopyranosyl] hederagenin, previously isolated from literature [**31**] **[32]**.

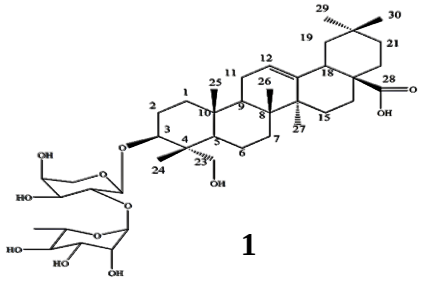
3.1.8. Compound 8

Compound 8 was obtained colorless powder and molecular formula was determined as $C_{29}H_{44}O_4$ by ESI-MS: (+) m/z 479 $[M+Na]^+$; (-) m/z 455 $[M-H]^+$. The ^{1}H and the ^{13}C nmr spectra showed signals for four quaternary methyls: ^{1}H-nmr signals at δ 0.81, 0.92, 1.04, 1.22 (each 3H, s**)** and ^{13}C-nmr signals at 26.62, 22.98, 17.84, and 16.32 ppm. The spectra also exhibited signals due to the following groups a carboxylic acid group (δ 182.19), a trisubstituted double bond **(**δ 123.97 and 145.06, a proton signal at δ 5.30 br, s), and a secondary alcoholic hydroxyl group (δ 71.44, proton signal at δ 3.77, br, s). Further, the low field carbon signals at δ 150.25 ppm and 107.08 ppm were assigned to the exomethylene

C-20 and C-29 respectively. Based on the above results, the structure of **compound 8** was identified as **Quinatic acid** or 30-Noroleana-12,20(29)-dien-28-oic acid, 3, 23-dihydroxy-, (3α,4β) by comparing ^{1}H-NMR and ^{13}C-NMR data with those reported in the literature [**27**].

3.1.9. Compound 9

Compound 9 was obtained colorless powder and molecular formula was determined as $C_{34}H_{52}O_8$ by EIMS: m/z 456 [M- 142] $^{+}$ (3), 410 (3), 232, 207 (23), 187 (l00), 176 (25). 13CNMR spectrum of compound 9 showed the presence of two olefinic bonds. One of them was located at C- 12(δ 123.91) and C-13 (δ 145.17) and the other signals appeared at δ 150.26 and δ 107.08 indicating the presence of an exomethylene group. On the other hand, the ^{13}C NMR spectrum of 1 showed the presence of only one anomeric carbon signal at δ 106.49 and the five carbon signals of a sugar moiety, which were identical with those reported for an α-L-arabinosyl moiety named as **Quinatoside A** or 3-O-α-L-arabinopyranosyl-3-norhederagenin [**33**].



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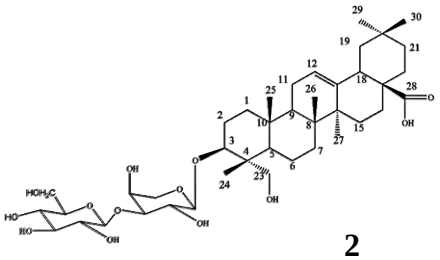

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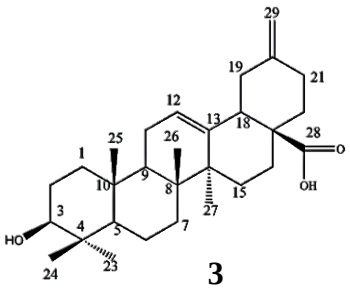

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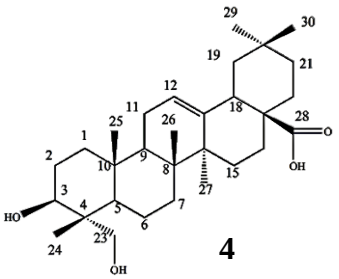

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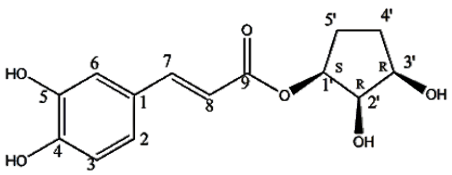

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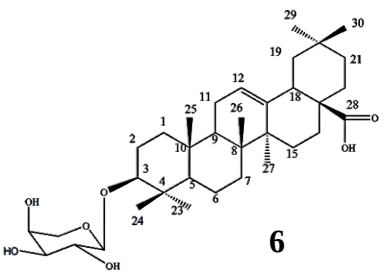

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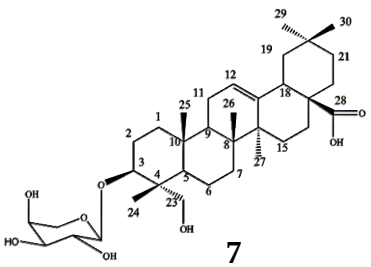

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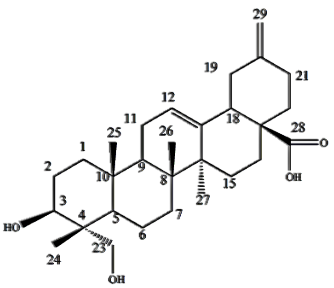

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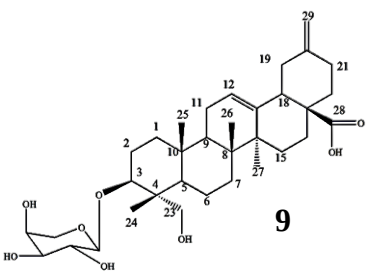

9

Fig. 7: Structures of compound 1-9 from Akebia Caulis.

[27]

3.2. Inhibition of Aβ42 induced fibrilogenesis

To examine the effects of **compound 1-8** on fibril formation of Aβ42, 20 μM of peptide was incubated in the presence of 40 μM concentrations of **compound 1-8** and Th-T assay was performed for measurement of fibrillogenesis activity. Before testing the isolated compound 1-8 from EtOAc fraction of Akebiae Caulis, the crude fractions CH_2Cl_2 and EtOAc of Akebiae Caulis and MeOH fraction of *Angelica keiskei* was experimented on **Aβ42 induced fibrilogenesis (Fig.8.)** and EtOAc fraction showed significant activity. The fluorescence profiles of Aβ42 aggregation with 40 μM concentration of **compound 1-8** are shown in **Fig. 9 & 10**. Aβ42 alone showed a characteristic sigmoidal curve when incubated at 37° C for 6h [**34**]. In the presence of 40 μM of compound 1-8, the final fluorescence level indicative of the amount of mature fibrils formed. The data suggested that, compound 2, 3, 4 and 6 showed significant activity and compound 1, 5 and 7 have moderate activity.

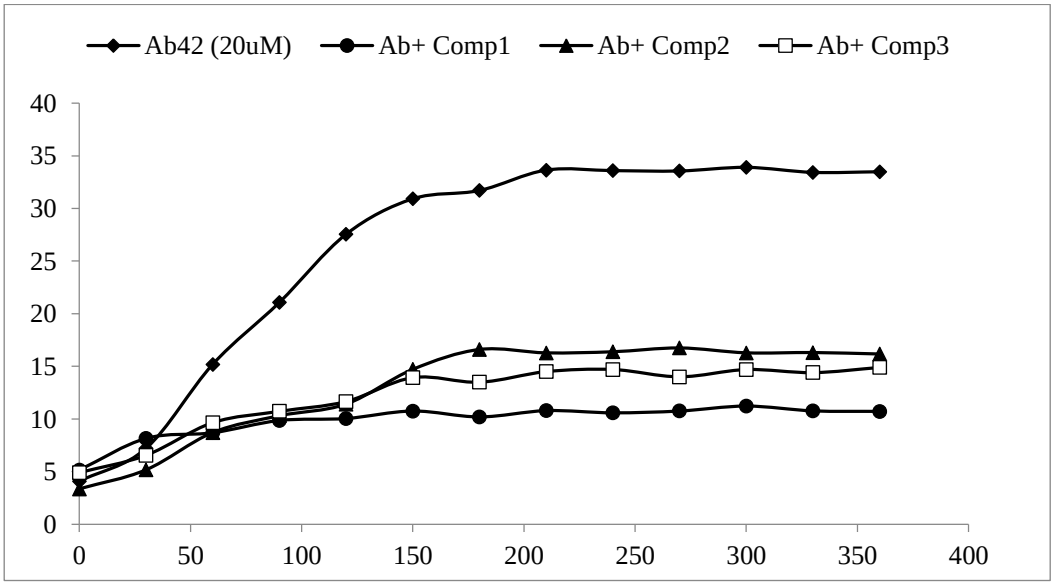

Fig. 8. Inhibitory effect of CH_2Cl_2 (Chem 2) & EtOAc (Chem 3) fractions of Akebiae Caulis and *Angelica keiskei* (Chem 1) on Aβ42 induced fibrilogenesis

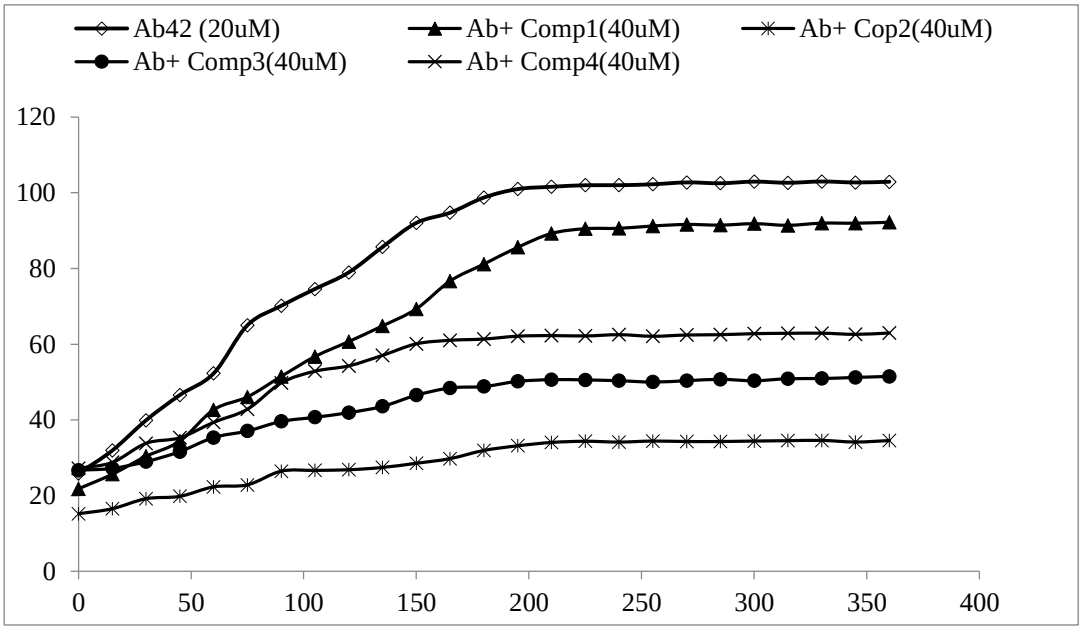

Fig. 9. Inhibitory effect of compound 1 -4 on Aβ42 induced fibrilogenesis

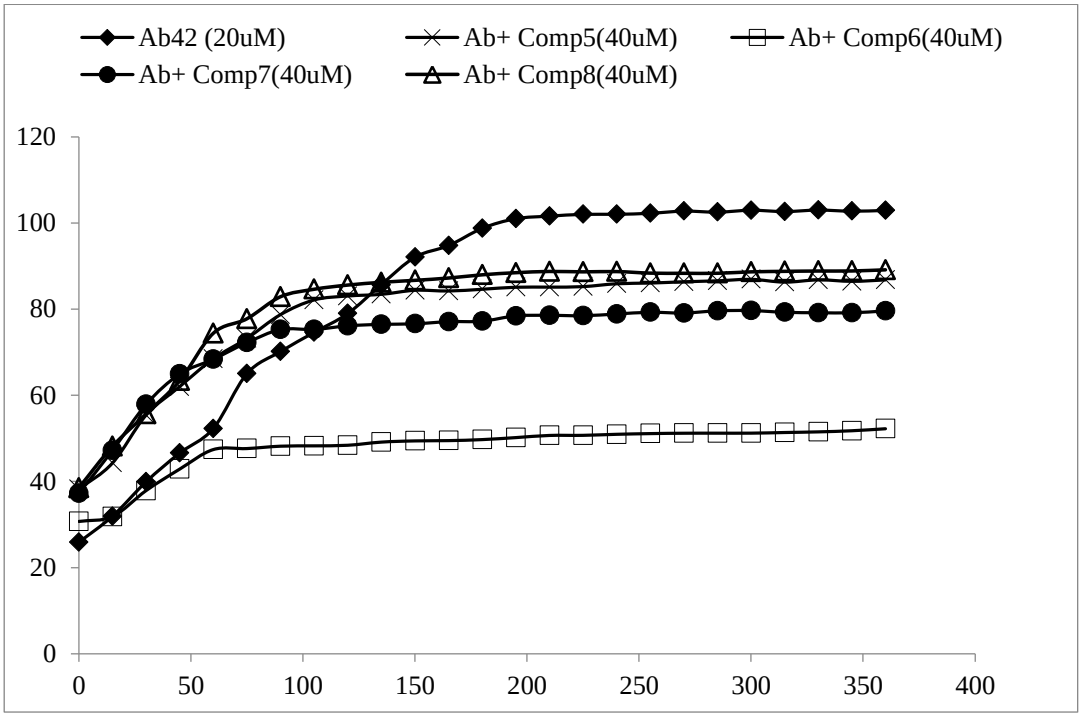

Fig. 10. Inhibitory effect of compound 5 -8 on Aβ42 induced fibrilogenesis

4. Conclusion

Beta-amyloid peptides, specifically Aβ42, are the principal species deposited in the brain and more hydrophobic and fibrillogenic. And cause protofibrils and fibrils to form the well-known plaque, which are the basic features of Alzheimer's disease (AD). In this study, to inhibit the Aβ42 induced fibrilogenesis, the isolated active compounds from EtOAc fraction of Akebiae Caulis were examined. As the active constituents, eight triterpenoid saponins, named Pulsatilla saponin A (**1**), Collinsonidin (**2**), Akebonic acid (**3**), Hederagenin (**4**), Asperosaponin C (**6**), Leontoside A (**7**), Quinatic acid (**8**), and Quinatoside A (**9**) and one quinic acid derivatives 1-(3′, 4′-dihydroxycinnamoyl) cyclopentane-2, 3-diol (**5**), were isolated from this plant through activity-guided isolation. From the study, Collinsonidin (**2**), Akebonic acid (**3**), Hederagenin (**4**), and Asperosaponin C (**6**), showed potent inhibitory effect on Aβ42 induced fibrilogenesis and considering Pulsatilla saponin A (**1**), 1-(3′, 4′-dihydroxycinnamoyl) cyclopentane-2, 3-diol (**5**) and Leontoside A (**7**), pointed the effect moderately. This is the first report on the inhibitory effect of the isolated components from Akebiae Caulis against fibrilogenesis The observation of this study suggest that the isolated compounds, especially compound 2, 3, 4, and 6 might be suppress the plaque formation by Aβ42. And that the active constituents of Akebiae Caulis could be considered as nontoxic source for development of agents against Alzheimer's disease (AD).

5. References:

1. Dementia Fact sheet N°362. *World Health Organization*. April 2012. Retrieved **28**, November 2014.

2. Burns. A., Iliffe. S., Alzheimer's disease. *BMJ*, 2009, **338**: b158.

3. Mendez. M.F., Early-onset Alzheimer's disease: nonamnestic subtypes and type 2 AD. *Archives of Medical Research*, 2012, **43**(8): 677–85.

4. Alzheimer's disease: Unraveling the Mystery. *NIH-National Institute on Aging*, 2008.

5. Olsson. F., Schmidt. S., Althoff. V., Munter. L.M., Jin. S., Rosqvist. S., Lendahl. U., Multhaup. G., Lundkvist. J., Characterization of intermediate steps in amyloid beta (Aβ) production under near-native conditions. *J. Biol. Chem.* 2014, **289** (3): 1540–50.

6. Selkoe. D.J., Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev*, 2001, **81**(2): 741 -66.

7. Hardy. J., Selkoe. D.J., The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, 2002, **297**(5580): 353-6.

8. Hardy. J.A., Higgins. G.A., Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 1992, **256**(5054): 184-5.

9. Sharoar. M.G., Thapa. A., Shahnawaz. M., Ramasamy. V.S., Woo. E.R., Shin. S.Y., Park. I.S., Kaempferol-3-O-rhamnoside abrogates amyloid beta toxicity by modulating monomers and remodeling oligomers and fibrils to non-toxic aggregates. *J Biomed Sci*, 2012, **19**:104.

10. Tang. W., Eisenbrand. G., *Akebia quinata* (Thunb.) Decne. In: Chinese

Drugs of Plant Origin. *Springer Berlin Heidelberg*, Heidelberg, 1992, 59–67.

11. Rim. A.R., Kim. S.J., Jeon. K.I., Park. E., Park. H.R., Lee. S.C., Antioxidant activity of extracts from *Akebia quinata* Decne. *J. Food Sci. Nutr.*, 2006, **11**: 84–87.

12. Jung. H.J., Lee. C.O., Lee. K.T., Choi. J., Park. H. J., Structure-activity relationship of oleanane disaccharides isolated from *Akebia quinata* versus cytotoxicity against cancer cells and NO inhibition. *Biological & Pharmaceutical Bulletin*, 2004, **27**: 744–747.

13. Sung. Y.Y., Kim. D.S., Kim. H.K., *Akebia quinata* extract exerts anti-obesity and hypolipidemic effects in high-fat diet-fed mice and 3T3-L1 adipocytes. *Journal of Ethnopharmacology*, 2015, **168**: 17–24.

14. Koo. H.J., Sung. Y.Y., Kim. H.K., Inhibitory effects of *Akebia quinata* ethanol extract on TNF-α-mediated vascular inflammation in human aortic smooth muscle cells. *Molecular Medicine Reports*, 2103, **7**: 379-383.

15. Choi. J., Jung. H.J., Lee. K.T., Park. H.J., Antinociceptive and Anti-Inflammatory Effects of the Saponin and Sapogenins Obtained from the Stem of *Akebia quinata. J Med Food*, 2005, **8**(1): 78–85.

16. Jin. H.G., Kim. A.R., Ko. H.J., Lee. S.K., Woo. E.R., Three New Lignan Glycosides with IL-6 Inhibitory Activity from *Akebia quinata*. *Chem. Pharm. Bull.*, 2014, **62**(3): 288–293.

17. Gao. H., Wang. Z., Triterpenoid saponins and phenylethanoid glycosides from stem of *Akebia trifoliata* var. australis. *Phytochemistry*, 2006, **67**: 2697–2705.

18. Mimaki. Y., Kuroda. M., Yokosuka. A., Hiroshi. H., Fukushima. M.,

Sashida. Y., Triterpenes and triterpene saponins from the stems of Akebia trifoliata. Chemical & Pharmaceutical Bulletin, 2003, **51**: 960–965.

19. Mimaki. Y., Doi. S., Kuroda. M., Yokosuka. A., Triterpene glycosides from the stems of *Akebia quinata*. *Chemical & Pharmaceutical Bulletin*, 2007, **55**: 1319–1324.

20. Jin. H.G., Kim. A.R., Ko. H.J., Woo. E.R., A new megastigmane glycoside from *Akebia quinata*. *Arch. Pharm. Res.*, 2015, **38**:591–597.

21. Shahnawaz. M., Thapa. A., Park I.S., Stable activity of a deubiquitylating enzyme (Usp2-cc) in the presence of high concentrations of urea and its application to purify aggregation-prone peptides. *Biochem Biophys Res Commun*, 2007, **359**(3): 801 –805.

22. Thapa. A., Woo. E.R., Chi. E.Y., Sharoar. M.G., Jin. H.G., Shin. S.Y., Park. I.S., Biflavonoids Are Superior to Monoflavonoids in Inhibiting Amyloid-beta Toxicity and Fibrillogenesis via Accumulation of Nontoxic Oligomer-like Structures. *Biochemistry*, 2011, **50**(13): 2445–2455.

23. Li. T., Pan. H., Feng. Y., Li. H., Zhao. Y., Bioactivity-guided isolation of anticancer constituents from *Hedera nepalensis* K. Koch. *South African Journal of Botany*, 2015, 100: 87 –93.

24. Shi. B.J., Li. Q., Zhang. X.Q., et al., Triterpene glycosides from the aerial parts of *Pulsatilla chinensis*. *Acta Pharmaceutica Sinica*, 2007, **42**: 862.

25. Joshi. B. S., Moore. K. M., Pelletier. S. W., Saponins From *Collinsonia canadensis.* *J. Nat. Prod.*, 1992, **55**: 1468-1476.

26. Shao-Hua Wu, Shu-Ming Yang, Da-Gang Wu, You-Wei Cheng and Qian Peng. Three Novel 24, 30-Dinortriterpenoids, Paeonenoides A–C, from *Paeonia veitchii*. Helvetica Chimica Acta, Volume 88(2), pages 259–

265.

27. Ikuta. A., Itokawa. H., Triterpenoids of *Paeonia Japonica* callus tissue. *Phytochemistry*, 1988, **27**(9): 2813-2815.

28. Joshi. B.S., Singh. K.L., Roy R., Complete assignments of ^{1}H and ^{13}C NMR spectra of the pentacyclic triterpene hederagenin from *Nigella sativa* Linn. *Magn. Reson. Chem*., 1999, **37**: 295-298.

29. Cardoso. C.L., Bolzani. V.D.S., Silva. D.H.S., Ishii. H., Berova. N., Nakanishi. K., The Absolute Configuration of 1-(3′, 4′-Dihydroxycinnamoyl) cyclopentane-2, 3-diol from the Amazonian Tree *Chimarrhis turbinate. J. Nat. Prod.* 2006, **69:** 1046-1050.

30. Ji. D., Wu. Y., Zhang. B., Zhang. C.F., Yang. Z.L., Triterpene saponins from the roots of *Dipsacus asper* and their protective effects against the Aβ25–35 induced cytotoxicity in PC12 cells. *Fitoterapia*, 2012, **83**: 843–848.

31. Li. X.C., Wang. D.Z., Su-Gong Wu. S.G., Yang. C.R., Triterpenoid saponins from *Pulsatilla Campanella*. *Phytochemistry*, 1990, **29**(2): 595-599.

32. Aftab. K., Shaheen. F., Mohammad. F.V., Noorwala. M., Ahmad. V.U., Phyto-Pharmacology of Saponins from *Symphytum officinale* L. *Saponins Used in Traditional and Modern Medicine*, 1996, 404: 429-442.

33. Ikuta. A., Itokawa. H., 30-Noroleanane saponins from callus tissues of *Akebia Quinata*. *Phytochemstry*, 1989, **28**(10): 2663-2665.

34. Ono. K., Yoshiike. Y., Takashima. A., Hasegawa. K., Naiki. H., Yamada. M., Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *J Neurochem*, 2003, **87**(1):172–181.

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showed me the path to success and I am walking on that path. In brief, I deeply appreciate all of the professors, relatives, colleagues, and friends who have helped me a lot. I sincerely wish them all the best in their lives.

APPENDIX

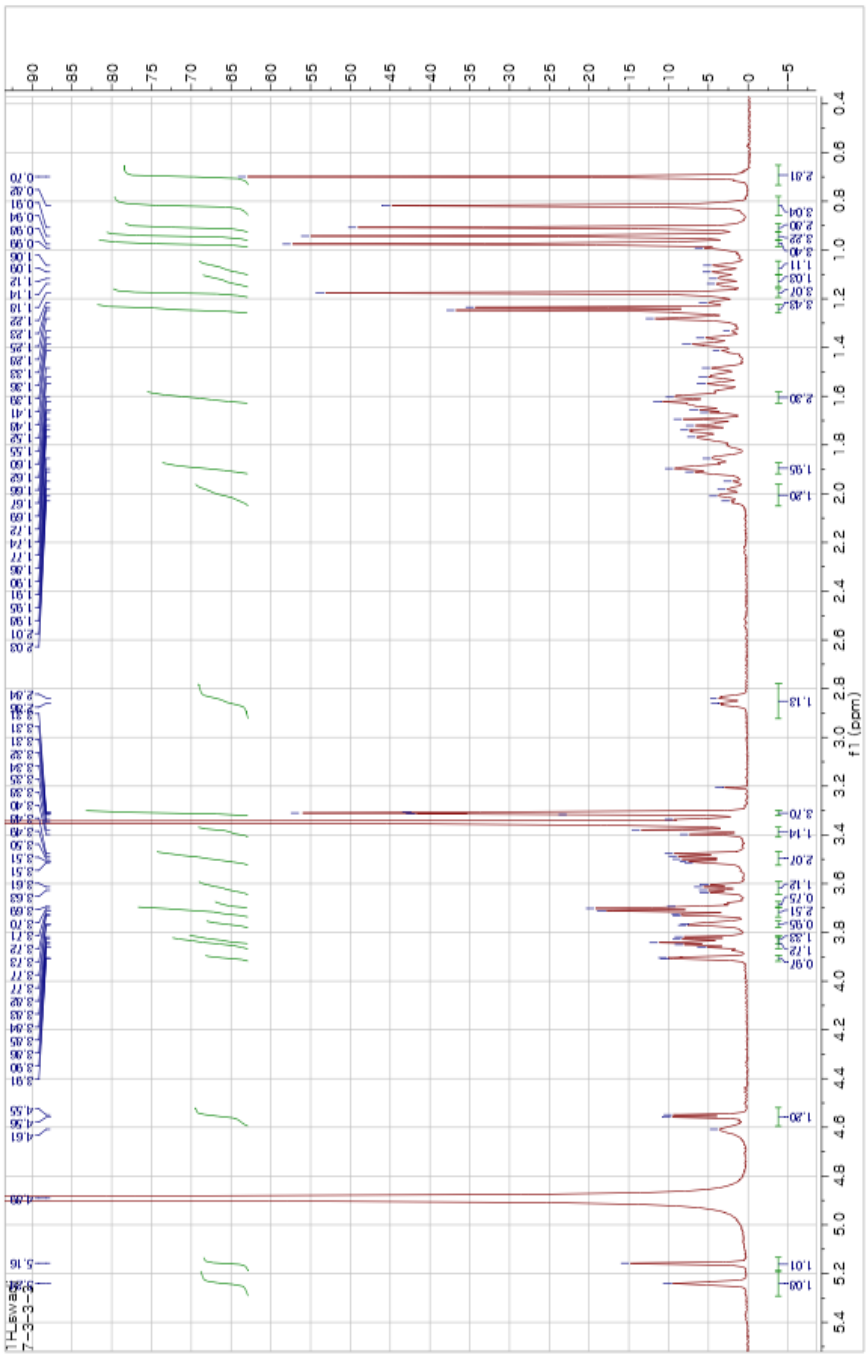

Fig.11: ^{1}H-NMR spectrum of compound 1 (500 MHz, CD_3OD)

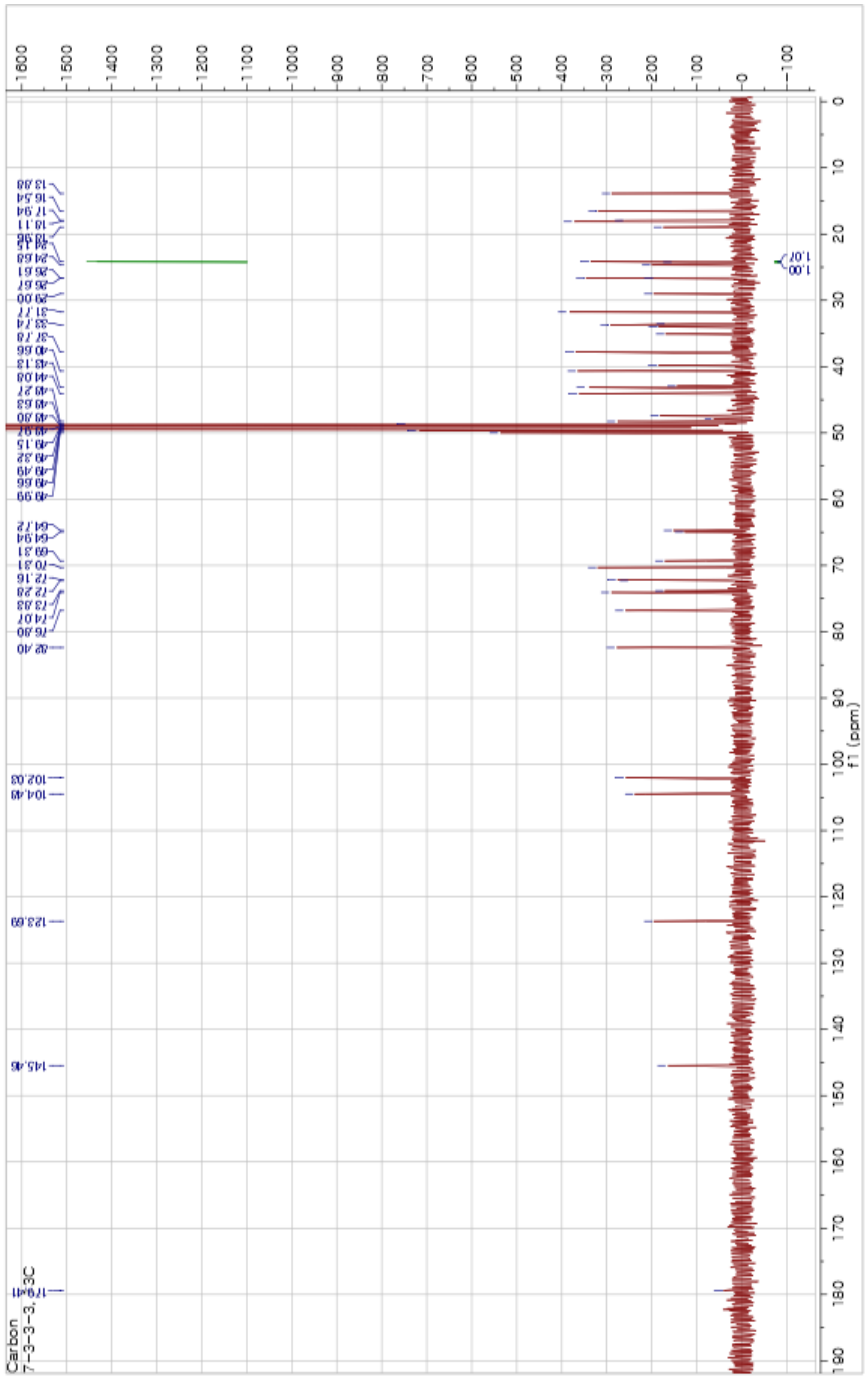

Fig. 12: ^{13}C-NMR spectrum of compound 1 (500 MHz, CD_3OD)

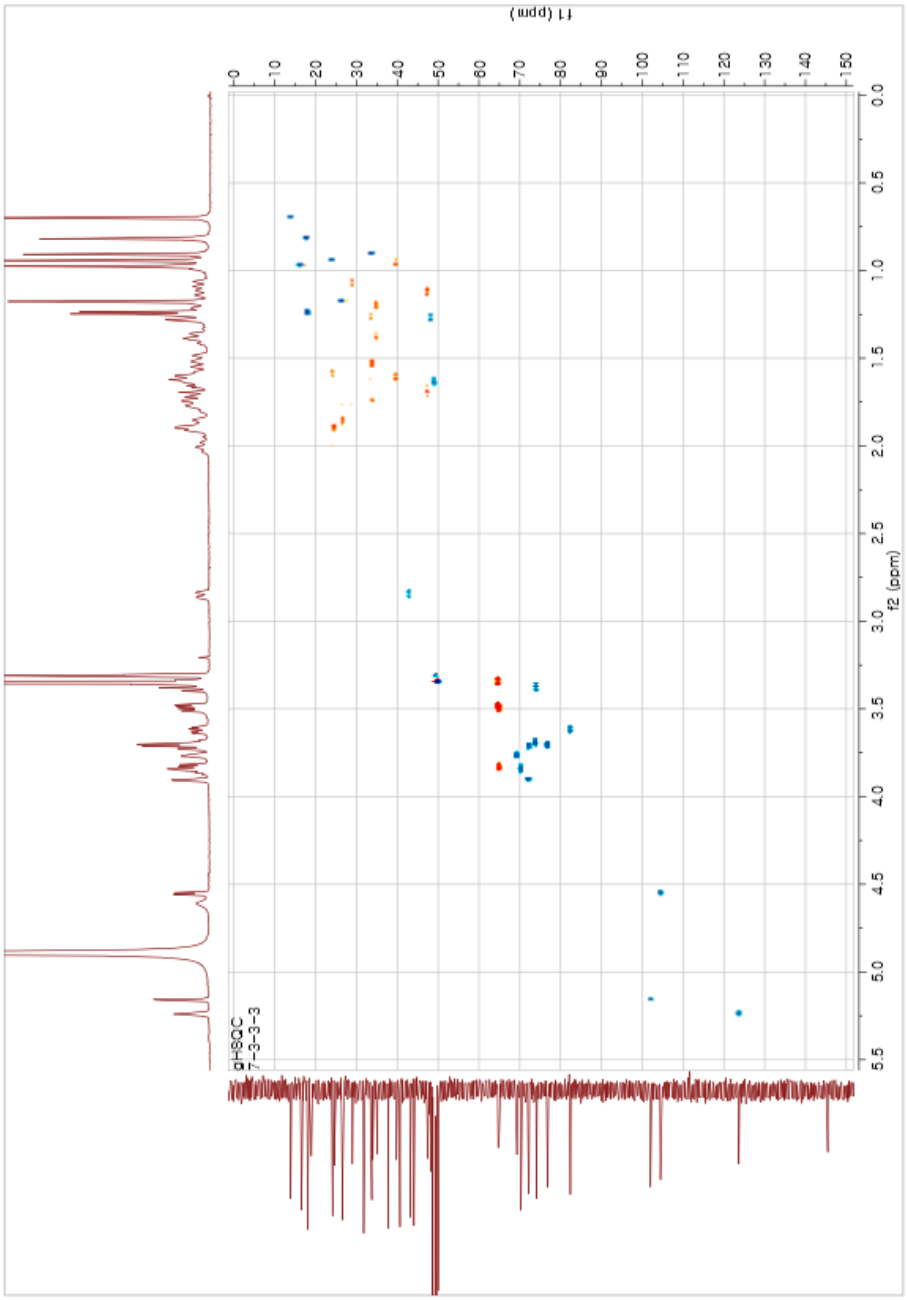

Fig.13: HSQC spectrum of compound 1 (500 MHz, CD_3OD)

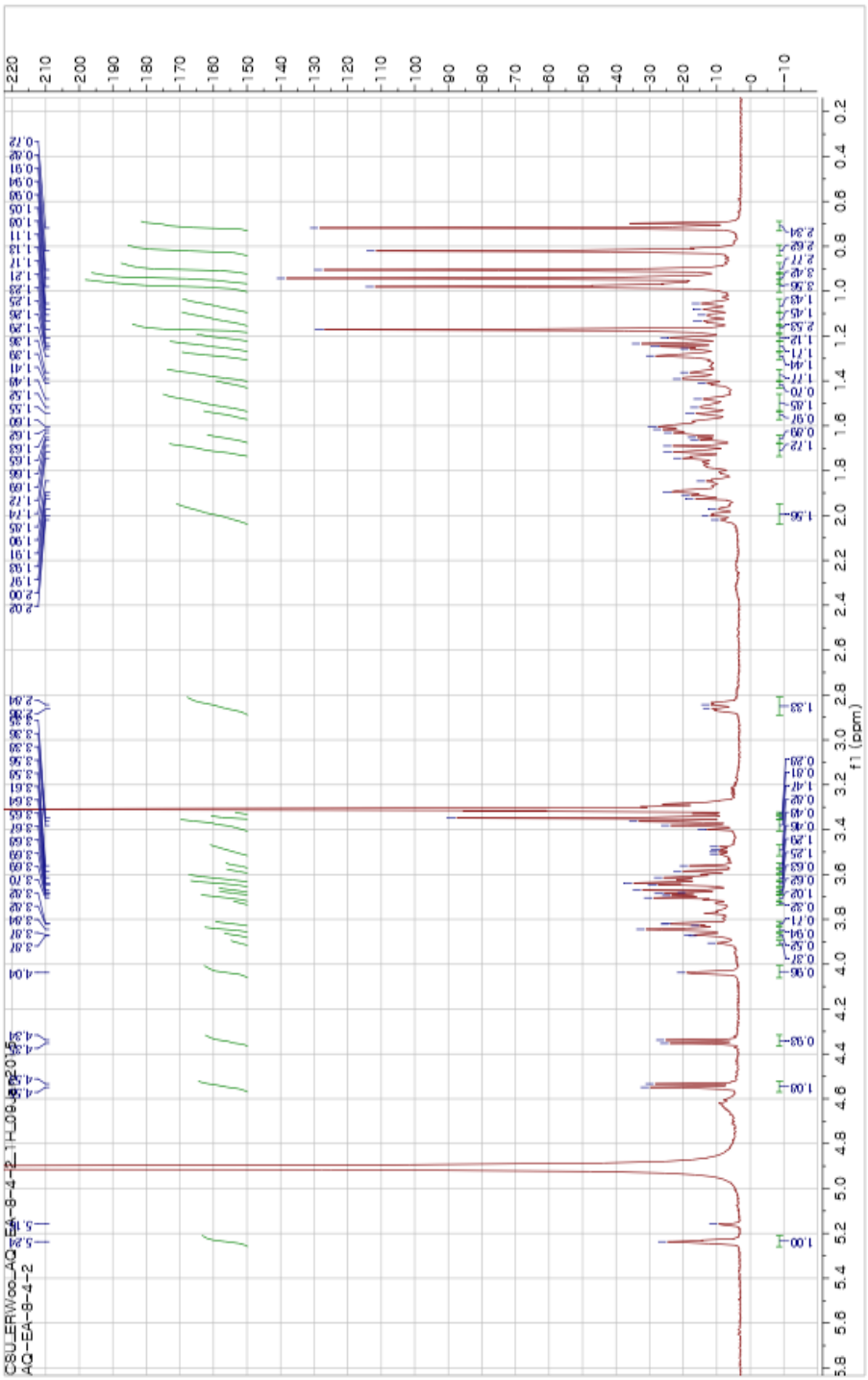

Fig.14: ^{1}H-NMR spectrum of compound 2 (500 MHz, CD_3OD)

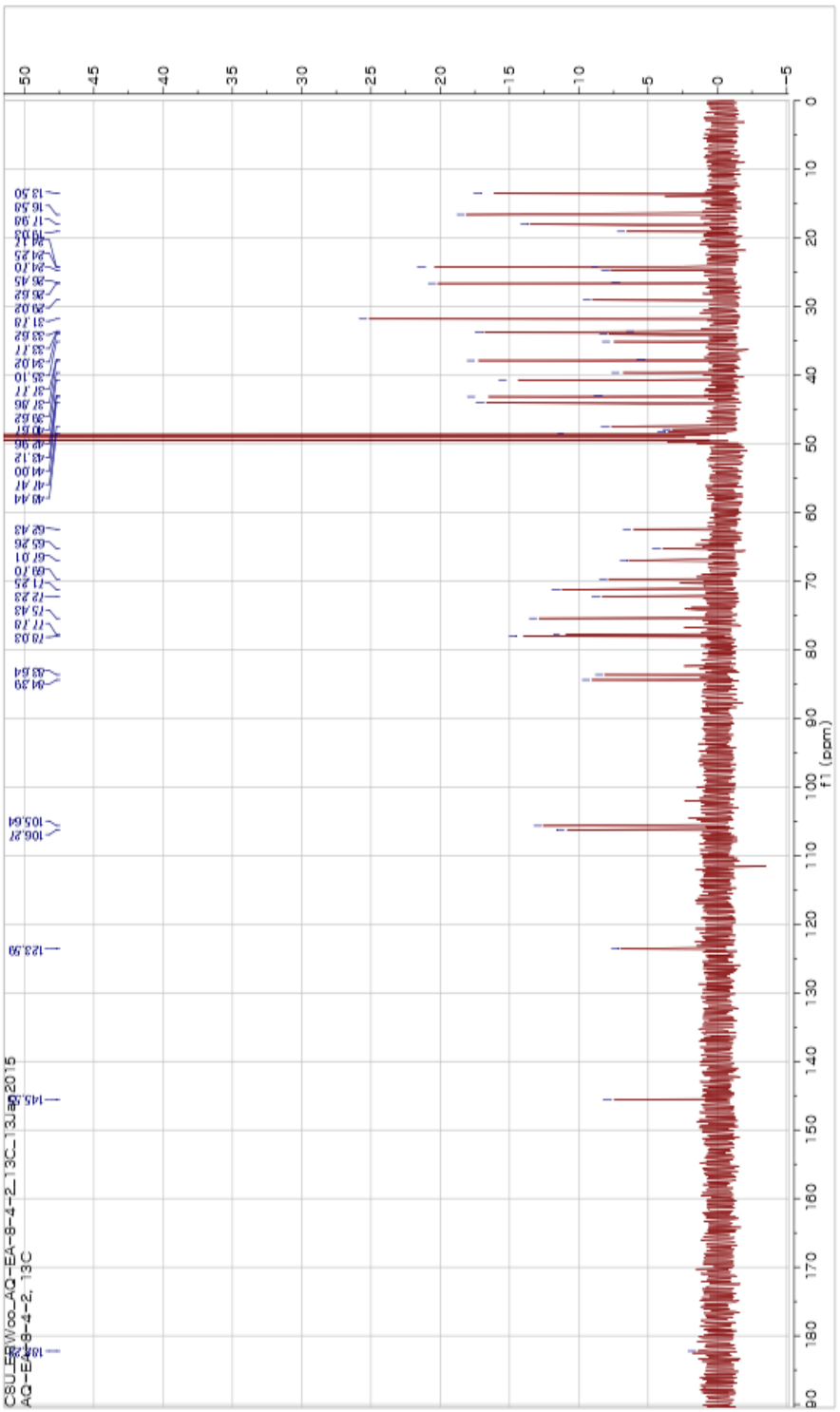

Fig. 15: ^{13}C-NMR spectrum of compound 2 (500 MHz, CD_3OD)

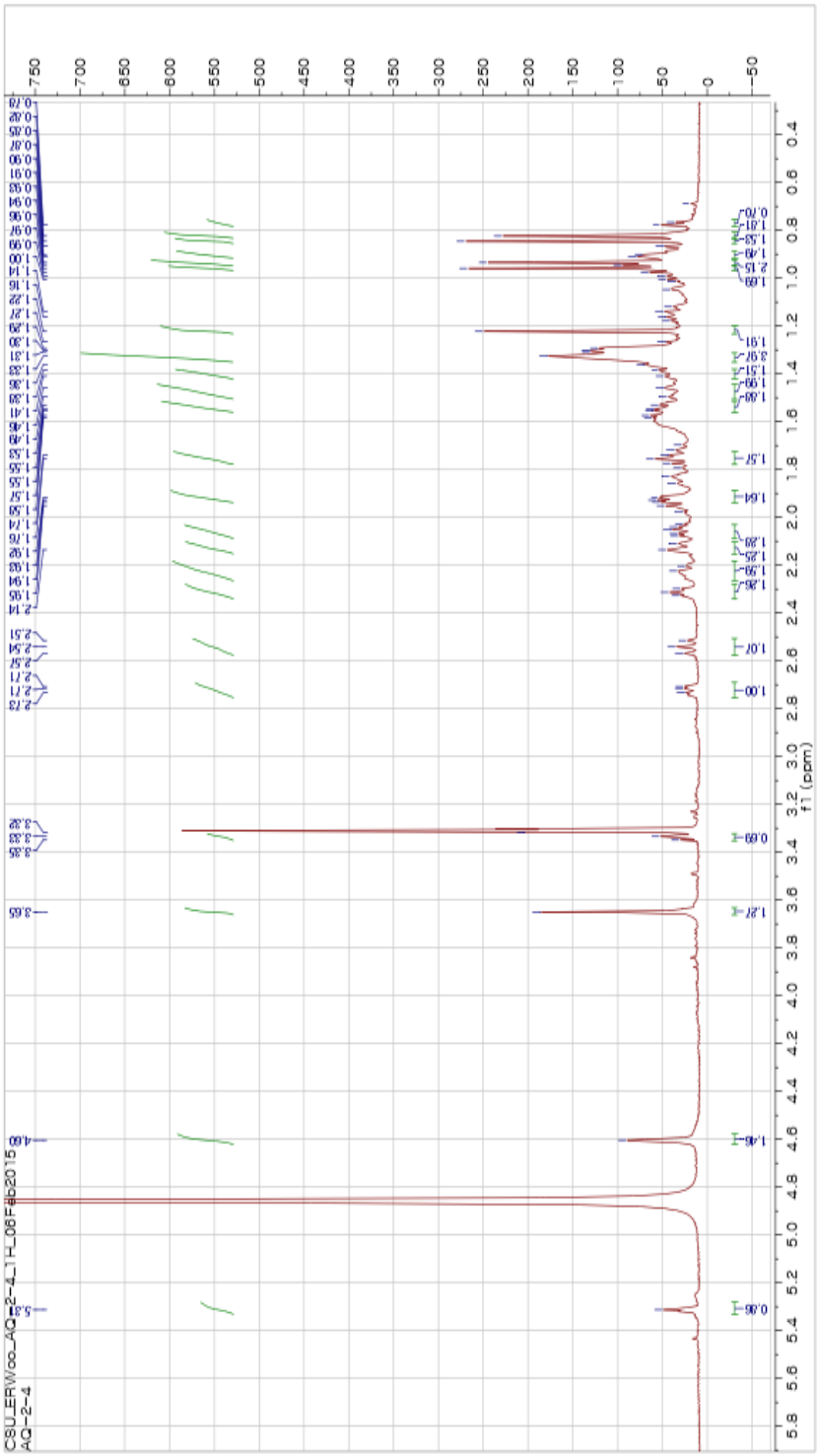

Fig.16: ^{1}H-NMR spectrum of compound 3 (500 MHz, CD_3OD)

Fig. 17: ^{13}C-NMR spectrum of compound 3 (500 MHz, CD_3OD)

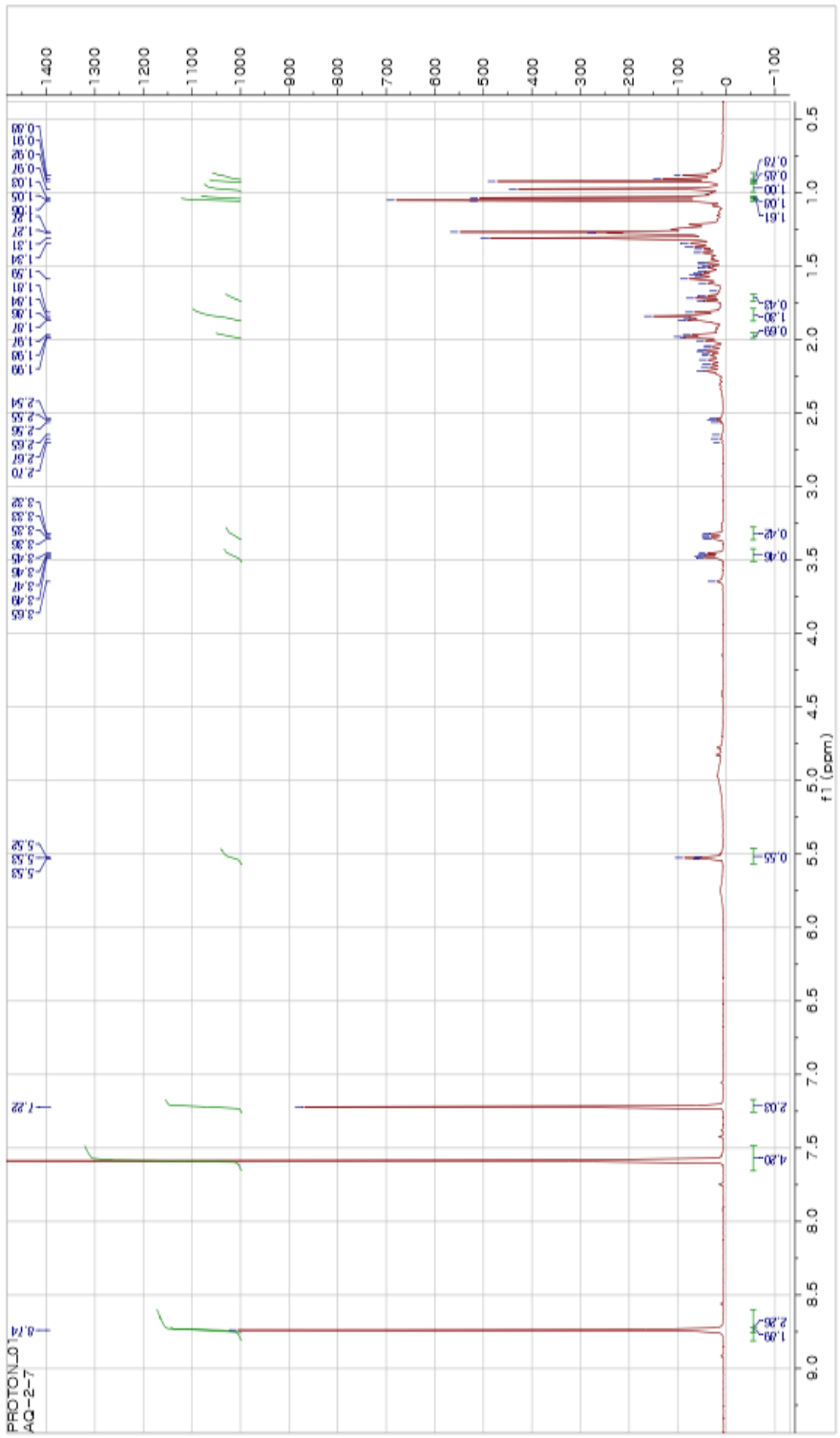

Fig.18: ^{1}H-NMR spectrum of compound 4 (500 MHz, Pyridine-D_5)

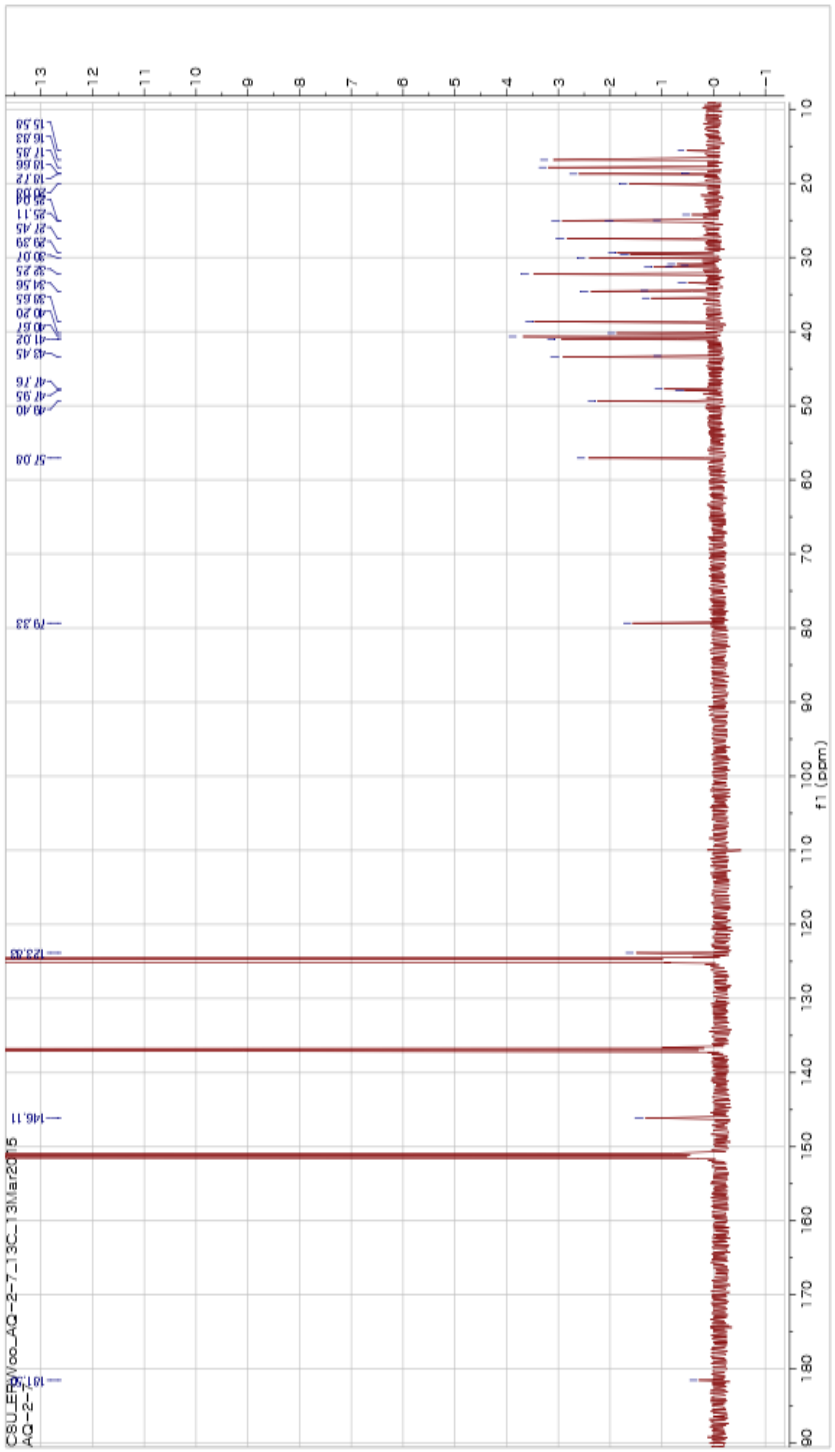

Fig. 19: ^{13}C-NMR spectrum of compound 4 (500 MHz, Pyridine-D_5)

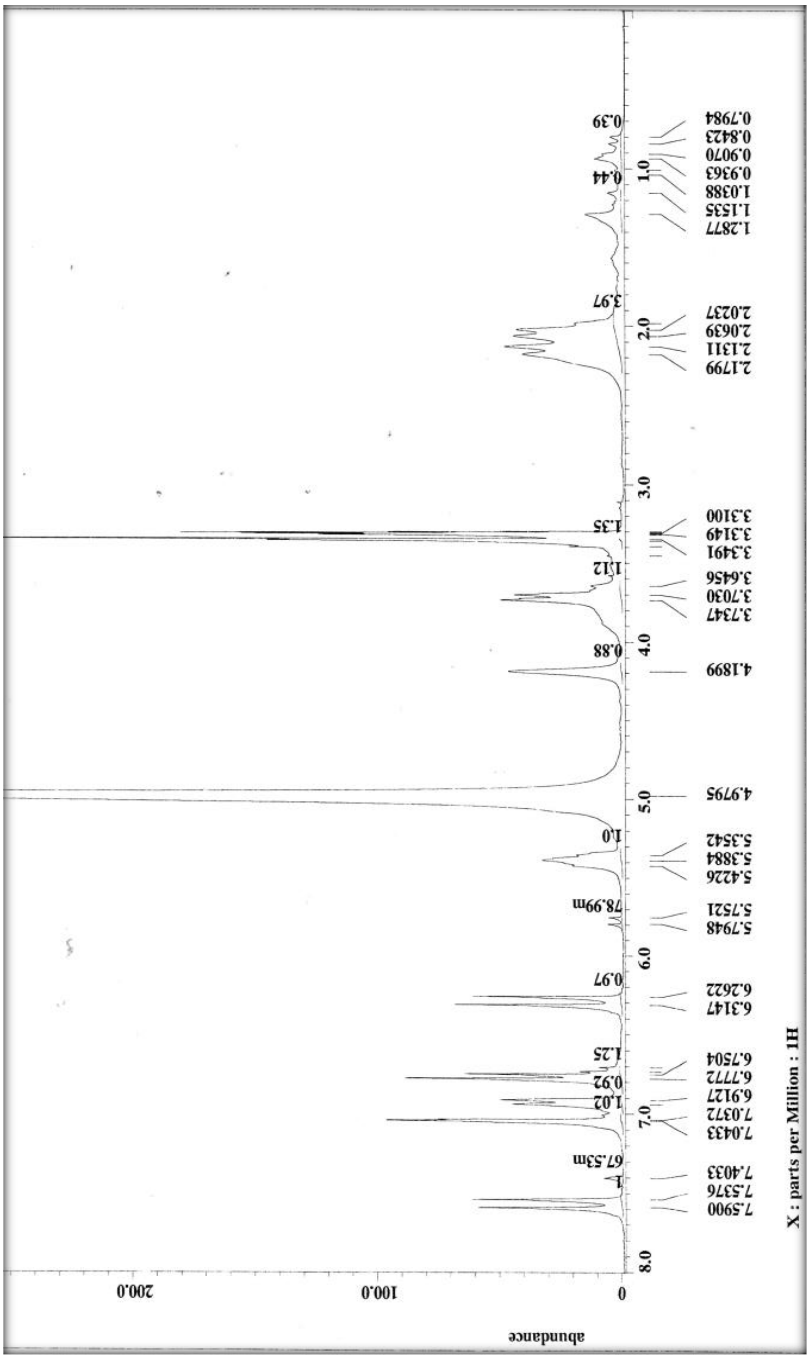

Fig.20: ^{1}H-NMR spectrum of compound 5 (300 MHz, CD_3OD)

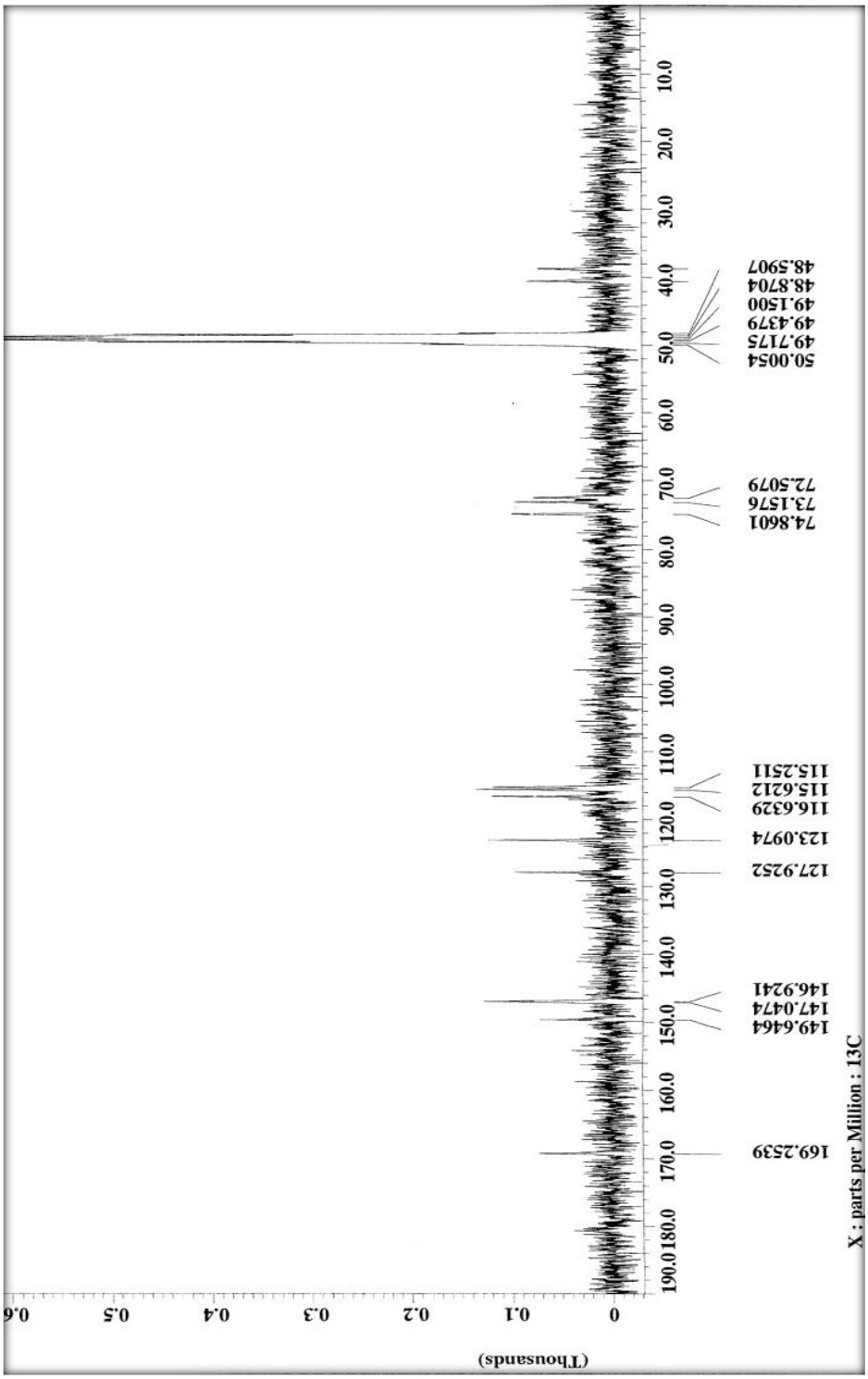

Fig. 21: ^{13}C-NMR spectrum of compound 5 (75 MHz, CD_3OD)

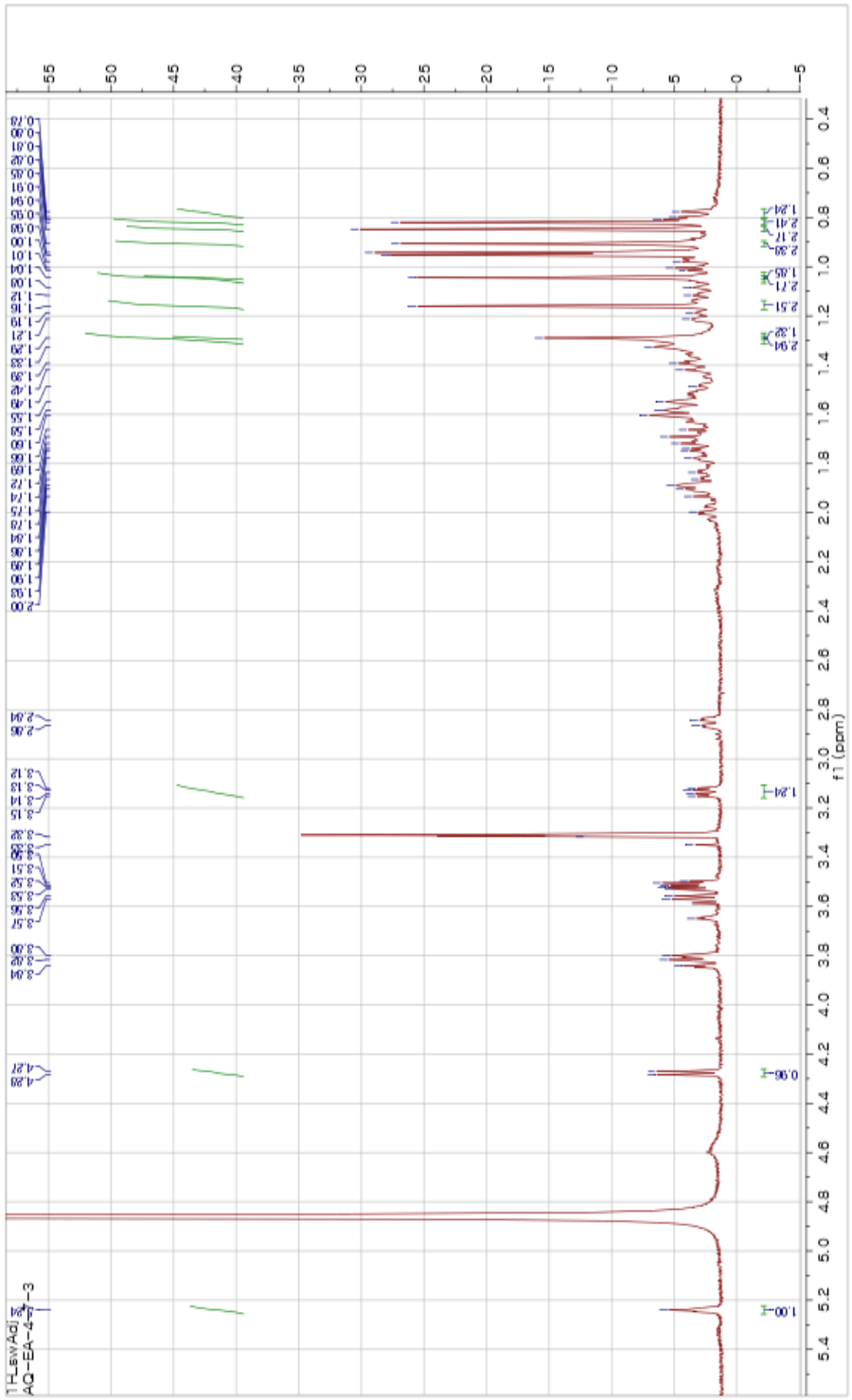

Fig.22: ^{1}H-NMR spectrum of compound 6 (500 MHz, CD_3OD)

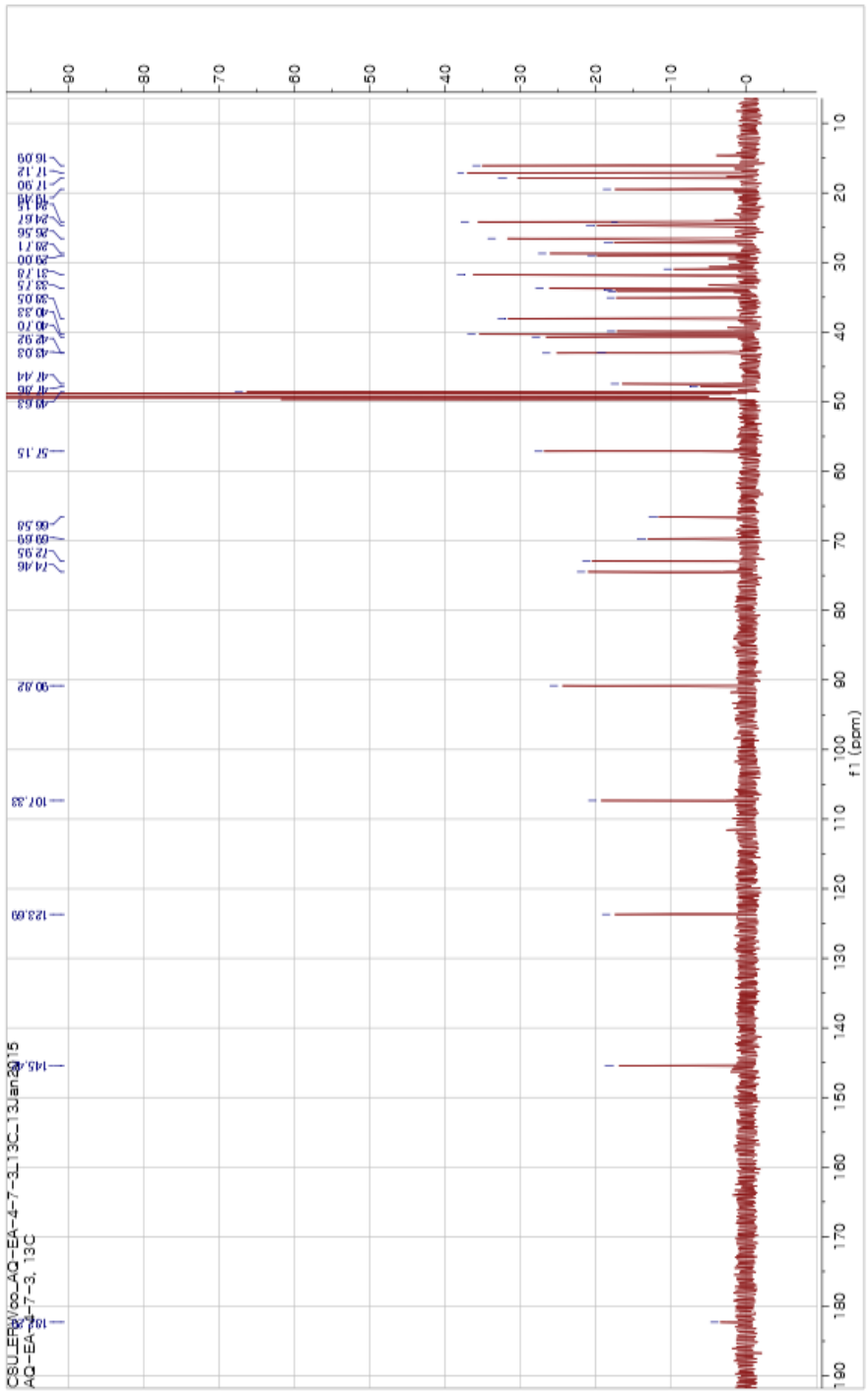

Fig. 23: ^{13}C-NMR spectrum of compound 6 (500 MHz, CD_3OD)

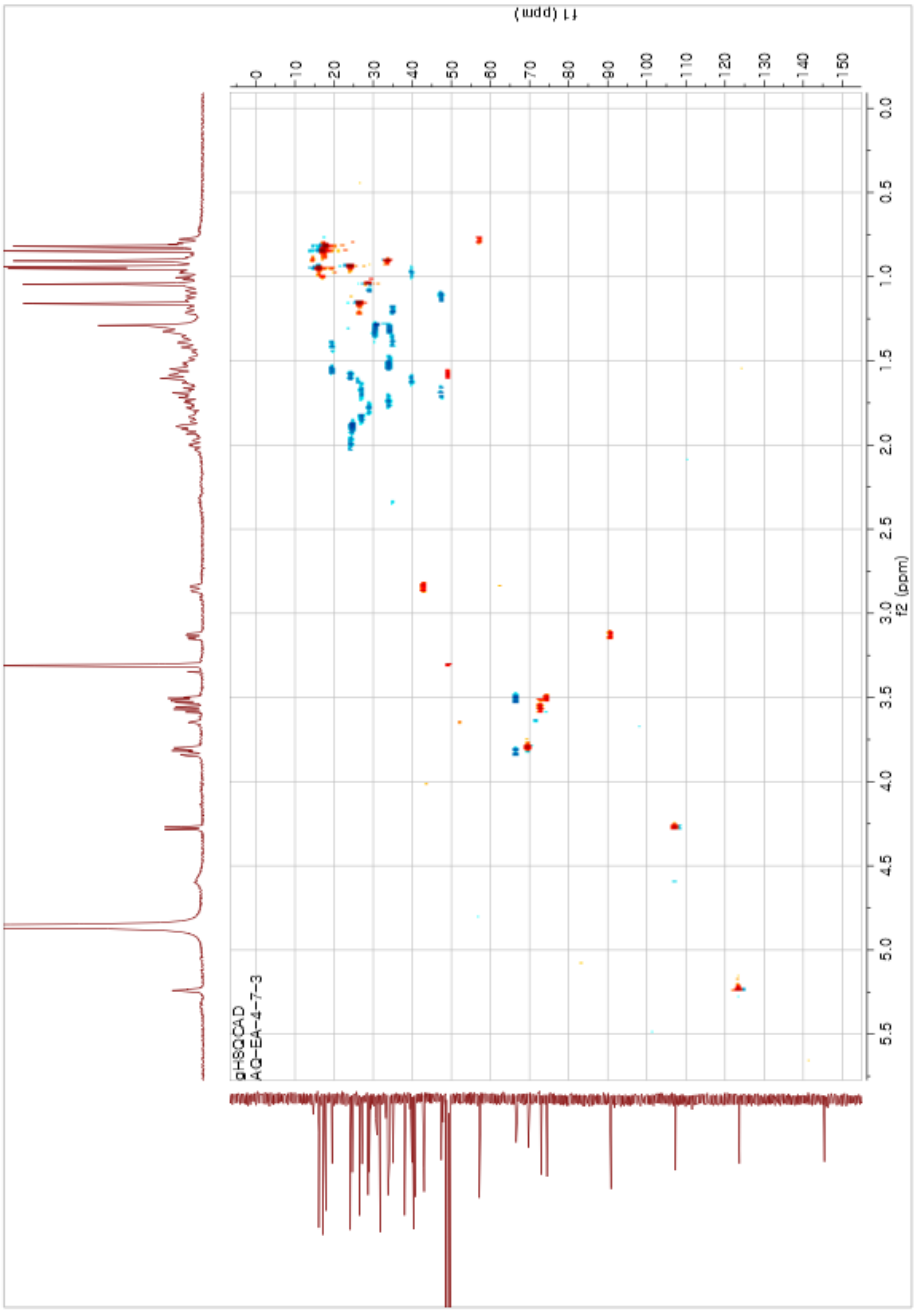

Fig.24: HSQC spectrum of compound 6 (500 MHz, CD_3OD)

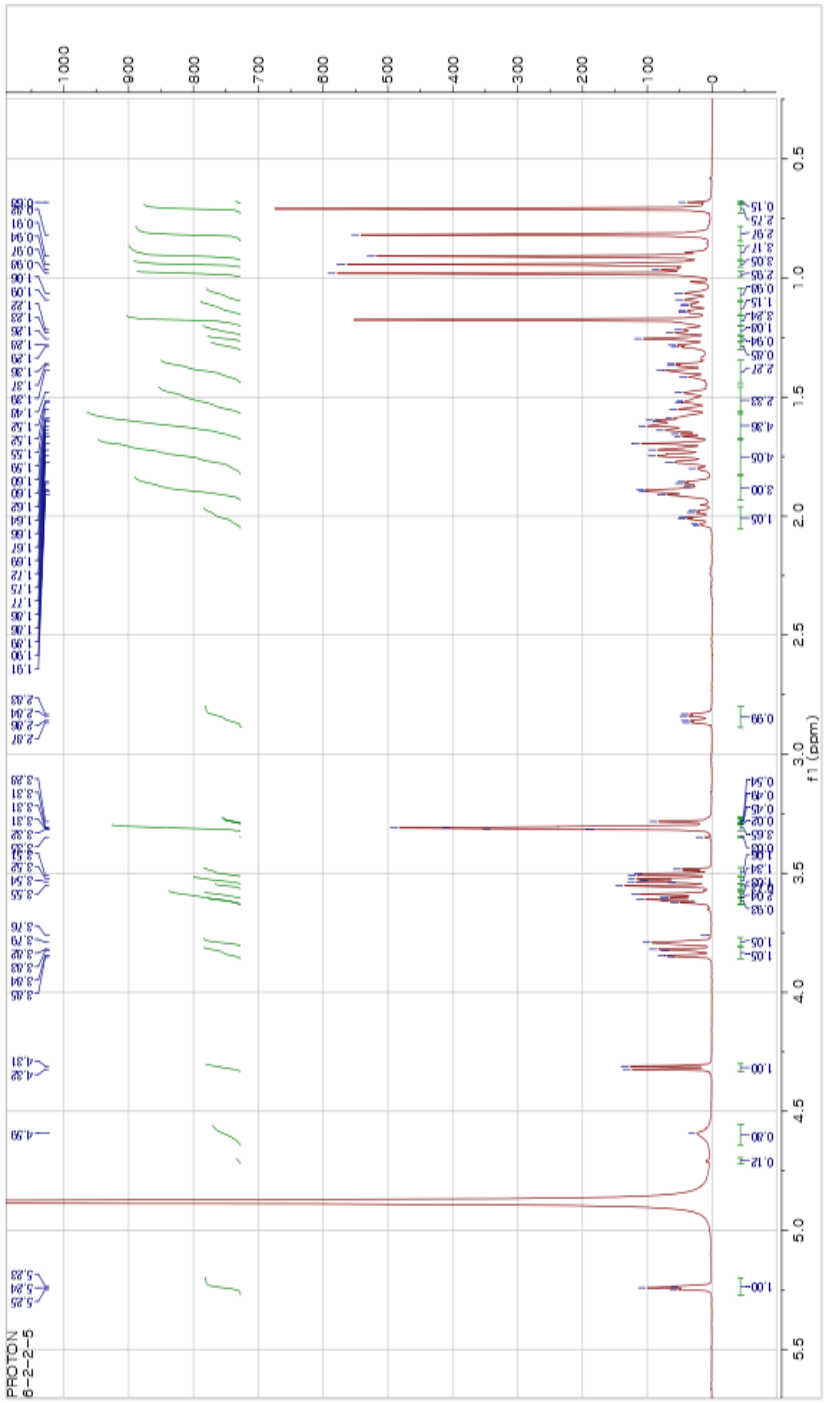

Fig.25: ^{1}H-NMR spectrum of compound 7 (500 MHz, CD_3OD)

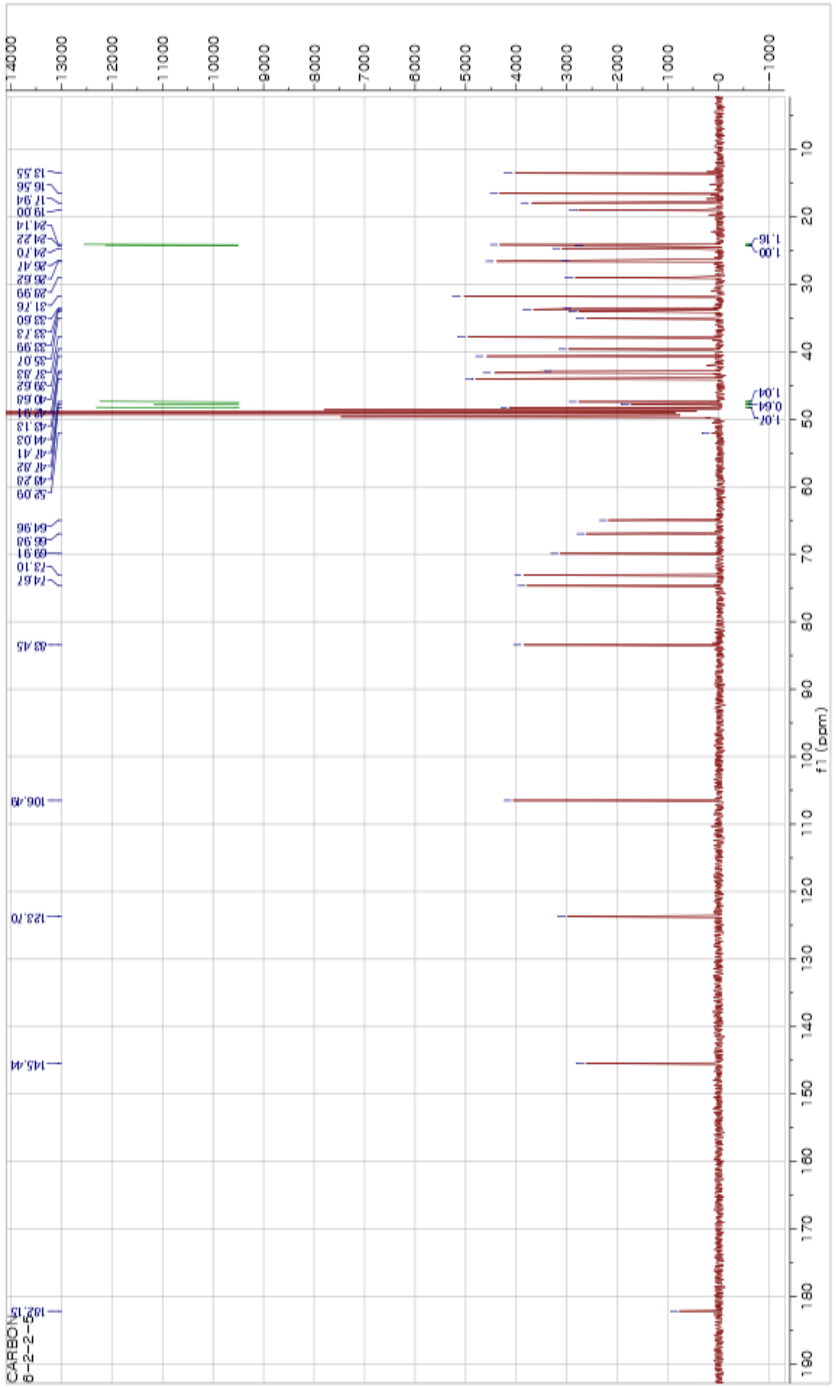

Fig. 26: ^{13}C-NMR spectrum of compound 7 (500 MHz, CD_3OD)

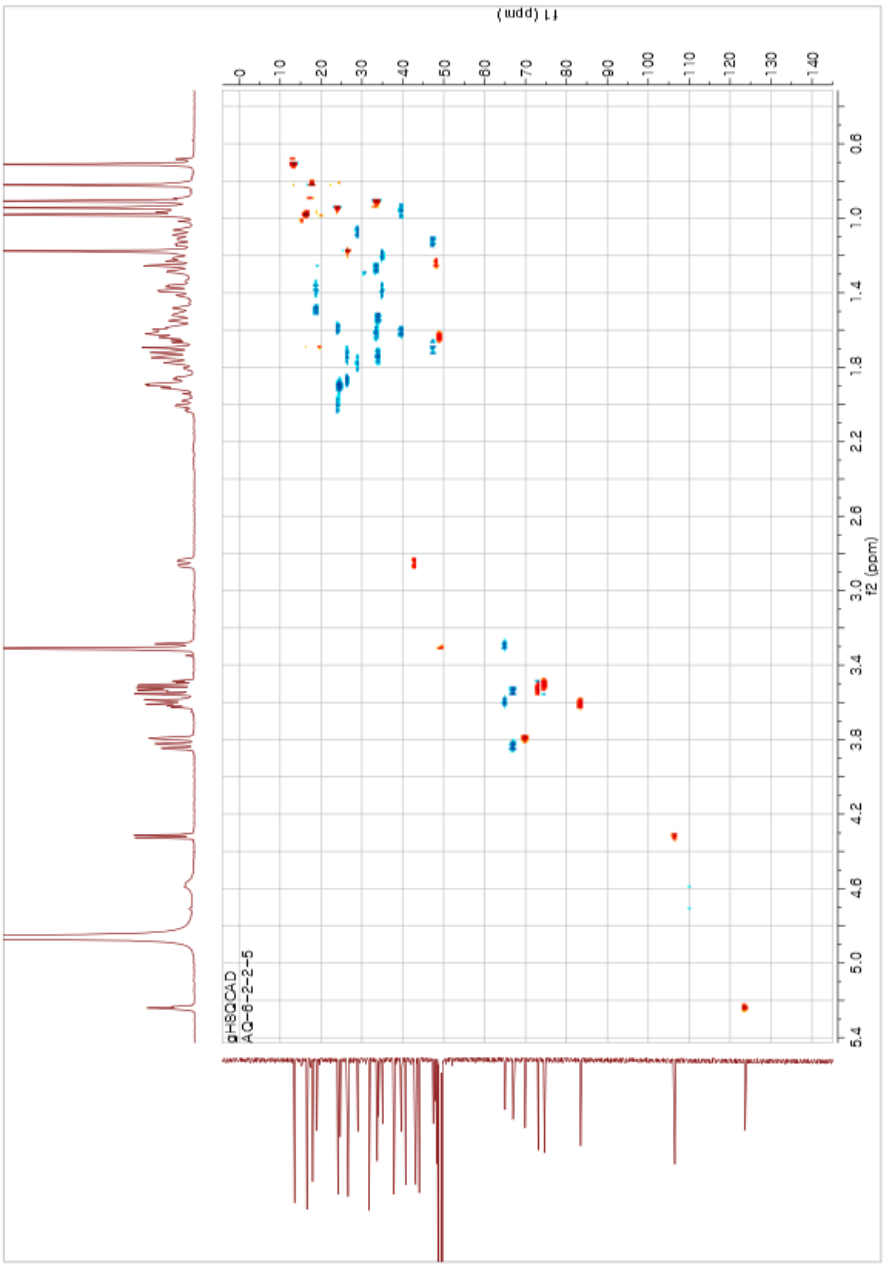

Fig.27: HSQC spectrum of compound 7 (500 MHz, CD_3OD)

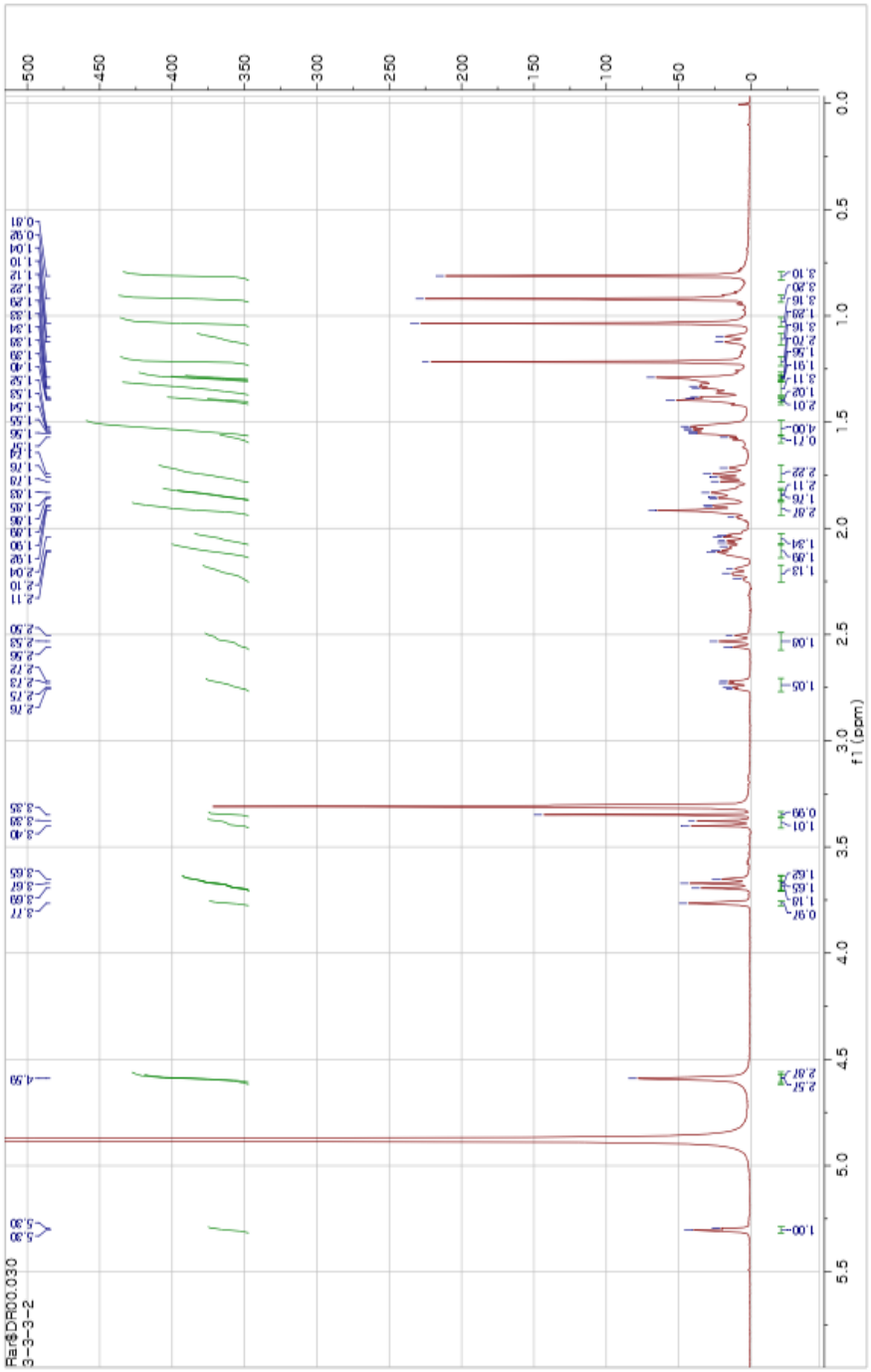

Fig.28: ^{1}H-NMR spectrum of compound 8 (500 MHz, CD_3OD)

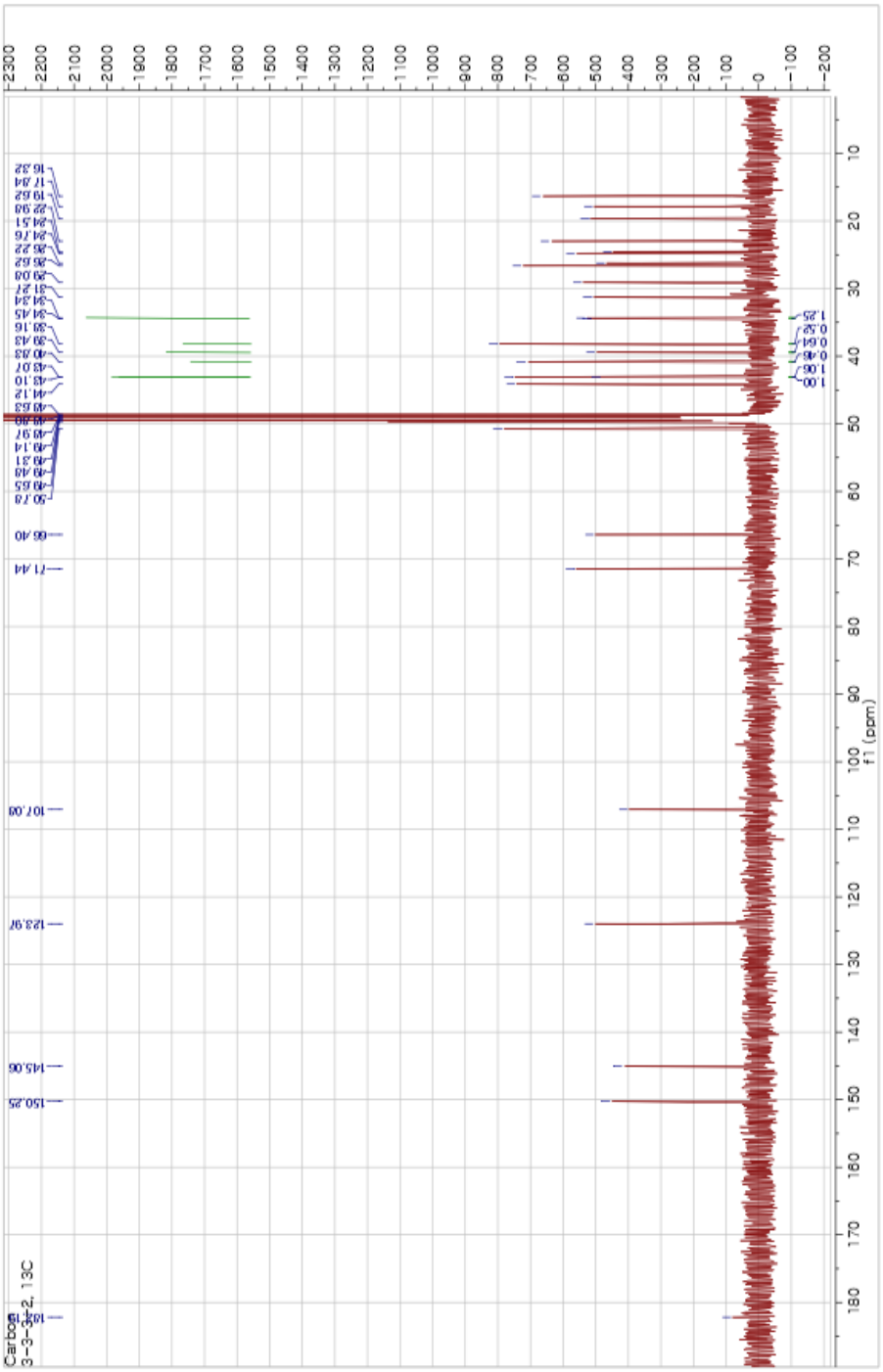

Fig. 29: ^{13}C-NMR spectrum of compound 8 (500 MHz, CD_3OD)

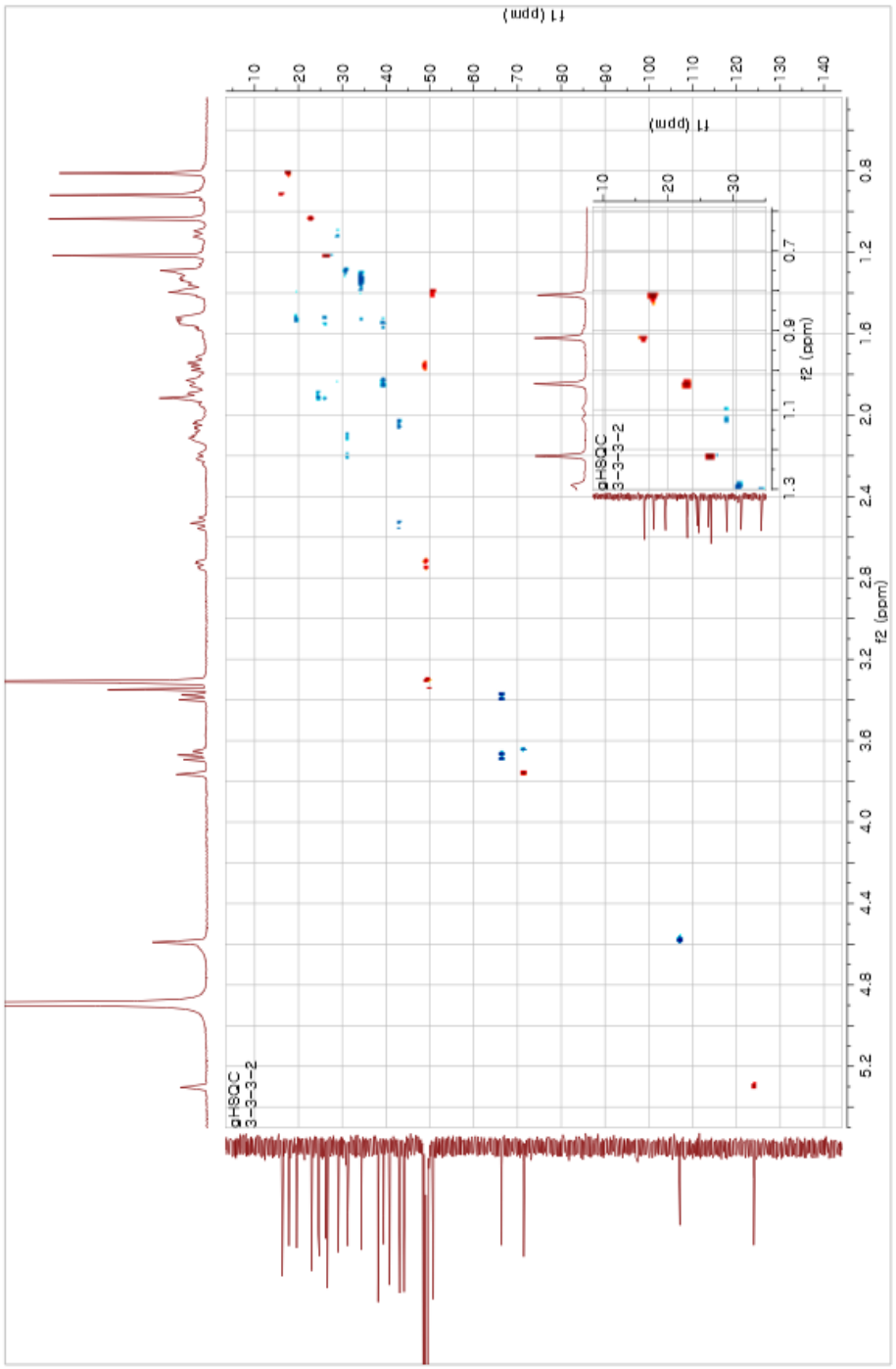

Fig.30: HSQC spectrum of compound 8 (500 MHz, CD_3OD)

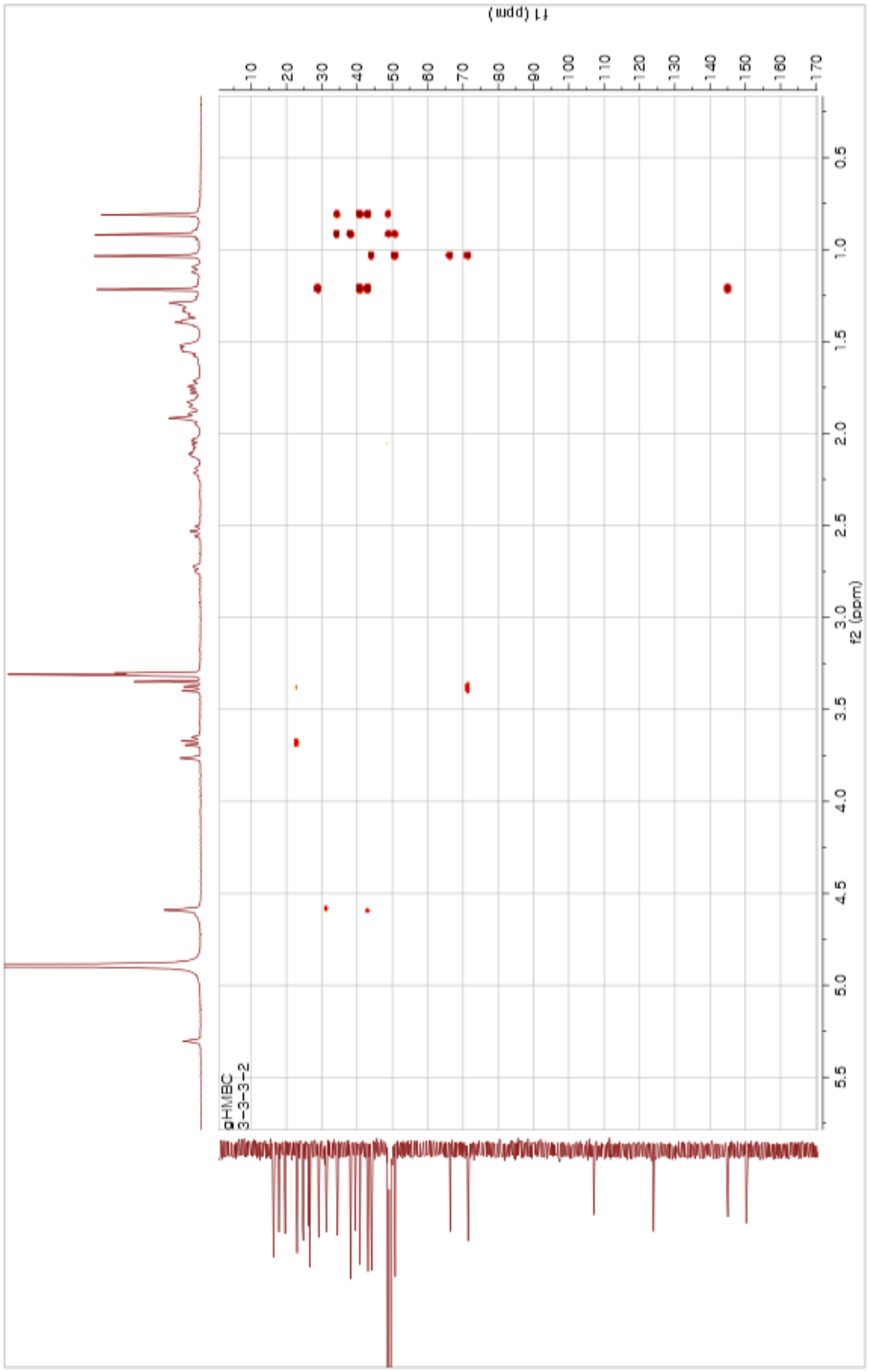

Fig.31: HMBC spectrum of compound 8 (500 MHz, CD_3OD)

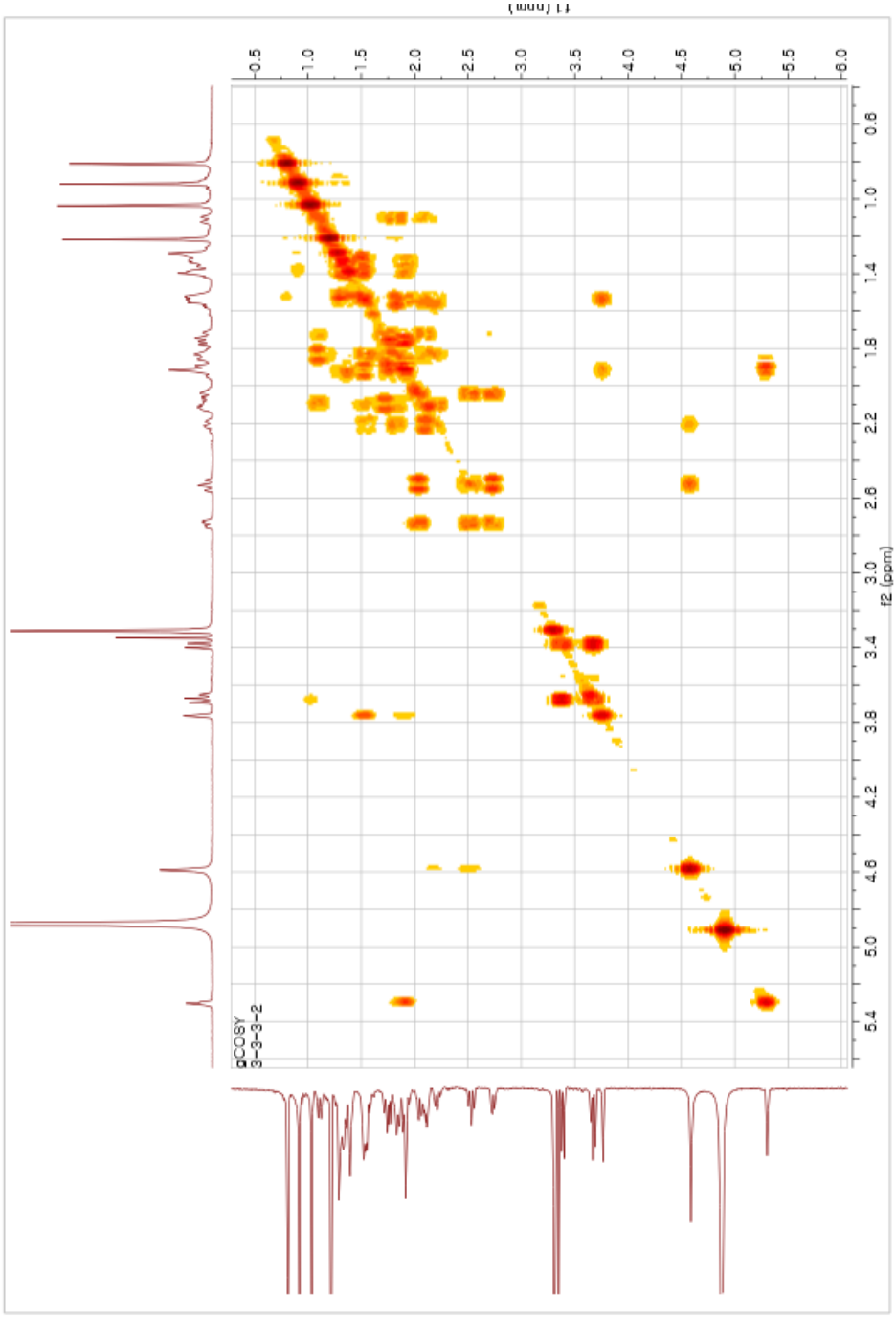

Fig.32: ^{1}H-^{1}H COSY spectrum of compound 8 (500 MHz, CD_3OD)

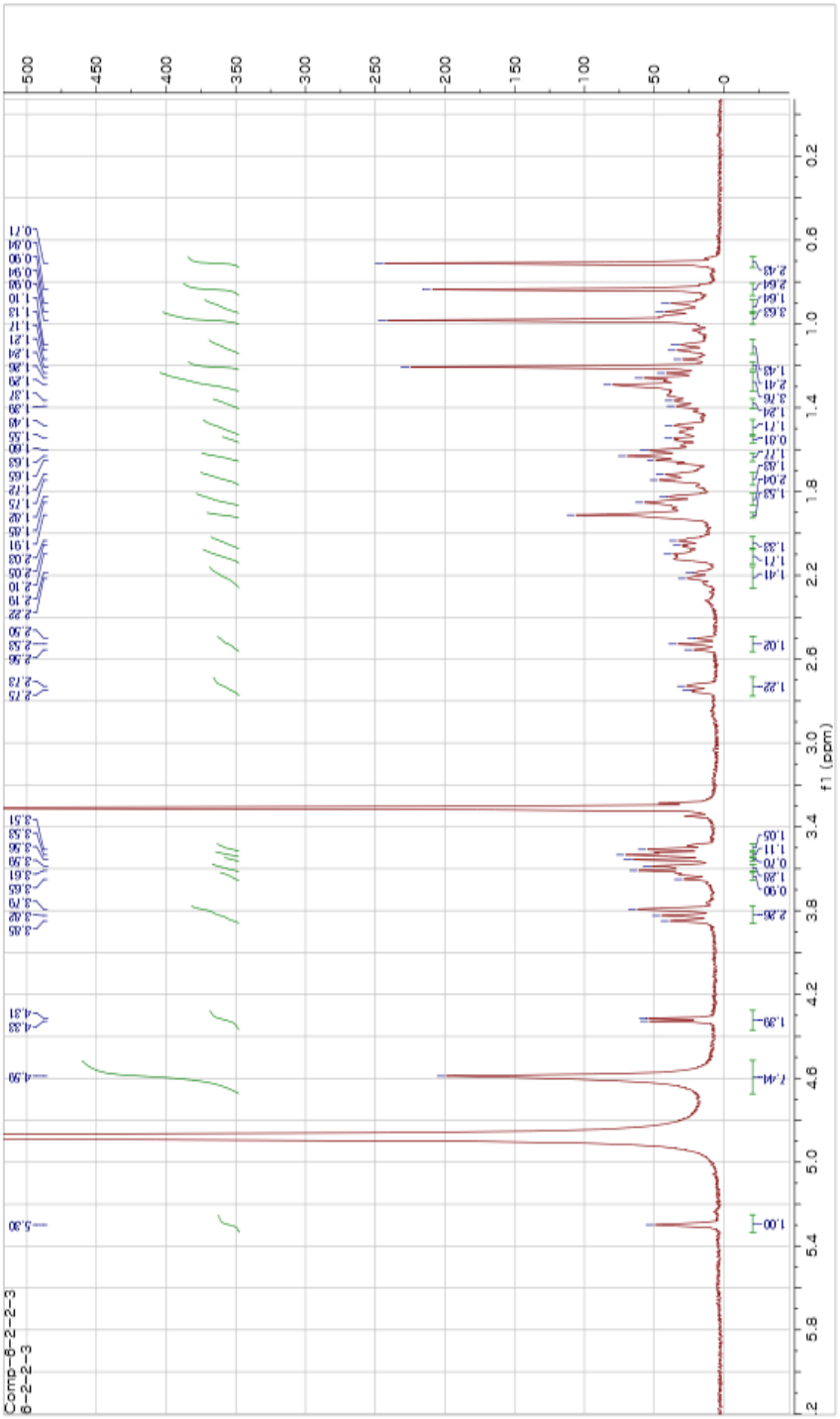

Fig.33: ^{1}H-NMR spectrum of compound 9 (500 MHz, CD_3OD)

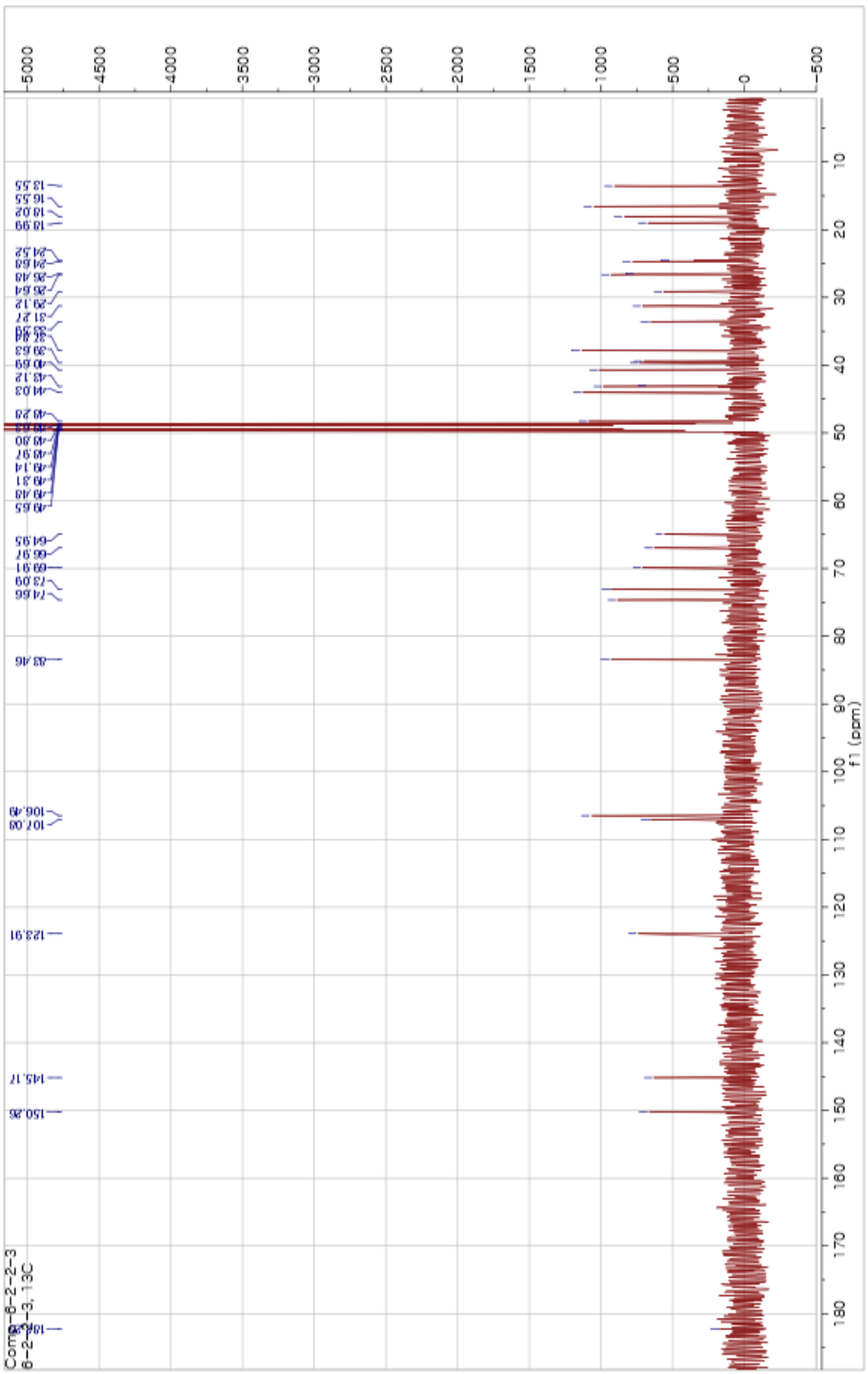

Fig. 34: ^{13}C-NMR spectrum of compound 9 (500 MHz, CD_3OD)

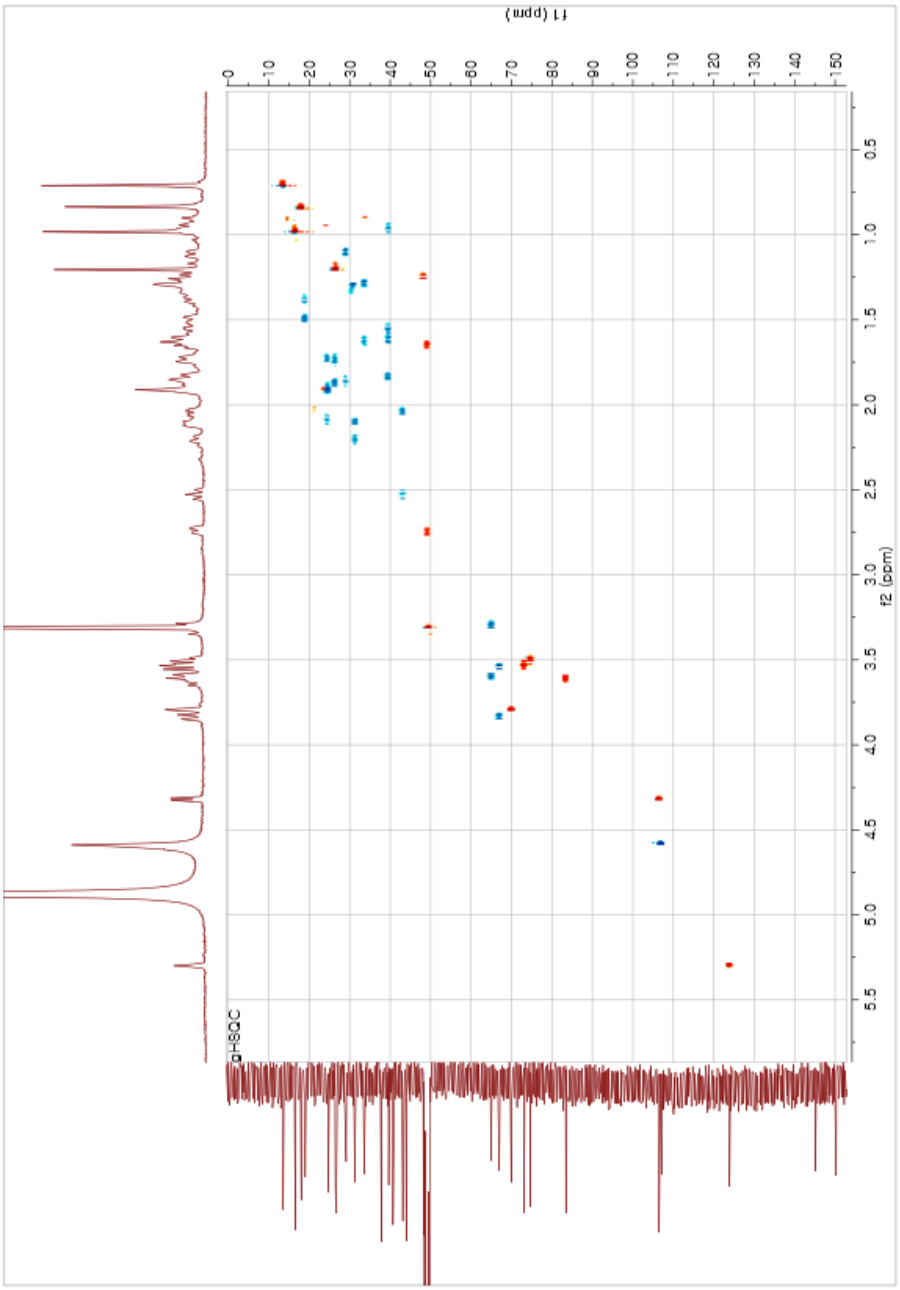

Fig.35: HSQC spectrum of compound 9 (500 MHz, CD_3OD)