Phaeophytin and Triterpenoids from *Brachystelma togoense* Schltr, a Nigerian Medicinal Herb

ABSTRACT

The medicinal herb *Brachystelma togoense* schtlr (Apocynaceae) is used traditionally for treatment of ailments. The secondary metabolites, phaeophytin *a*, α-amyrin and lupeol were isolated from the CH_2Cl_2 and MeOH extracts of *Brachystelma togoense*. The structures were elucidated using ^{1}H, ^{13}C and 2D NMR. These phytochemicals have previously being reported to have various biological activities such as anti-inflammatory, anti-fungal and anti-cancer. The presence of phaeophytin *a*, α-amyrin and lupeol in *Brachystelma togoense* justified the use of the plant for medicinal purpose in Nigeria.

Keywords: Secondary metabolites; phaeophytin *a*; α-amyrin; lupeol; *Brachystelma togoense* schtlr

1. INTRODUCTION

Brachystelma was first described by Robert Brown in 1822. The genus *Brachystelma* R. Br. (Apocynaceae: Asclepiadoideae) is represented by about 100-120 species (1). It is an erect perennial herb, growing up to 30 cm high. The genus *Brachystelma* is chiefly distributed in South Africa, South-East Asia and Australasia (2). A total of 18 species are known in India (3) and out of them, 3 species in Maharashtra. *Brachystelma* is found from Ghana to Nigeria, in lowlands to montane areas(4). The raw tuber is said to be edible (4). Many of the tuberous *Brachystelma* are known to be used medicinally for the treatment of headache, stomachache and colds in children(5). *Brachystelma togoense* has being medicinally used for the treatment of dysentery, cough and cold, wounds, stomach ache, typhoid and erectile dysfunction.

2. MATERIAL AND METHOD

2.1 Collection

The aerial parts of *Brachystelma togoense* was collected during April 2018 from the Ugbokolo forest in Okpokwu, which is the local government area of Benue State-Nigeria. The plant was collect and stored in a plastic container before it was air-dried. The collected specimen was positively identified by Mr. Namadi Sanusi, a botanistat Ahmadu Bello University, Zaria as *Brachystelma togoense*. A specimen (no. 25856) had been retained at the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria (Figure 1).

2.2 Extraction and isolation

The air-dried *B. togoense* was manually reduced to powder using mortar and pestil. Exactly 1000 g of the powdered plant material was extracted on a shaker at room temperature using 100 % dichloromethane (CH_2Cl_2) for 72 h. The extracts were concentrated using a rotary evaporator at 40^o C resulting in a brown gum-like texture (32 g). The same procedure was used for methanol (MeOH) which yielded a brown gum-like texture (36 g). The CH_2Cl_2 and MeOH extracts were separated by flash chromatography (Biotage system) over silica gel using three solvents. Firstly, a hexane/ CH_2Cl_2, gradient starting with 100 % hexane and gradually increasing the polarity to 100 % CH_2Cl_2. Secondly, CH_2Cl_2/EtOH/Ac from a 100 % CH_2Cl_2 to 50 % EtOH/Ac and to 100 % EtOH/Ac to yield various fractions (fr. 1-100). Fr.20 was spotted on the TLC plate using 100 % CH_2Cl_2 and appeared a pure compound **1** (51.0 mg). The same procedure was repeated for the MeOH extract yielding compounds **2** (32.0 mg) and **3** (28.0 mg) which were spotted as pure compounds using CH_2Cl_2 /EtOH/Ac (7:3) from fr.30.

2.2 General experimental procedure

NMR spectra were recorded in $CDCl_3$ on a 400MHz or 500 MHz Bruker AVANCE III NMR instrument at room temperature. HREIMS were recorded on an Agilent Technologies 6550 iFunnel Q-TOF LC/MS with samples dissolved in CH_2Cl_2. Infrared spectra were recorded using a Perkin-Elmar (2000 FTIR) spectrometer on NaCl plates.

3. Results and Discussion

The following following compounds phaophytin *a* (51.0 mg; 0.16 %), α-amyrin (32.0 mg; 0.10 %) and lupeol (28.0 mg; 0.09 %) were isolated from *Brachystelma togoense* using flash chromatography (biotage system). These compounds (Figure 2) were elucidated based on comparison of previous data (6–8).

Phaeophytin-*a* was isolated as a dark green solid from the CH_2Cl_2 extract of the aerial parts of *B. togoense* that was previously described (6). The IR spectrum showed absorbance bands for vinyl proton (3056 cm^{-1}) and sp^3 CH (2987, 2932 cm^{-1}) and carbonyl (1736 cm^{-1}) groups. A molecular ion could not be seen in the HRMS spectrometer despite repeated attempts.

From the ^1H and ^{13}C NMR spectra, it was evident that phaeophytin-*a* belonged to the phaeophytin class. This was particularly evident by the downfield shifts at δ_H 9.32 s, 9.48 s and 8.56 s which could be assigned as H-5, H-10 and H-20 respectively. The deshielded methyl groups proton resonances occurred at δ_H 3.19 (3H-2'), δ_H 3.3 (3H-7') and δ_H 3.38 (3H-12') and a methoxy group proton resonance occurred at δ_H 3.89 (3H-13^4). The presence of a C-20 phytol tail was evident from the presence of four methyl protons (δ_H 0.80 d, $J = 7.3$, δ_H 0.82 d, $J = 7.3$, δ_H 0.79 s, δ_H 1.61 s) and ester carbonyl resonance at δ_C 173.8 (C-13^3). A comparison of the NMR data of phaeophytin-*a* against literature values for phaeophytin *a* showed the enabled assignment of a keto group carbon resonances at δ_C 189.9 to C-13^1 (6,9). The ^1H and ^{13}C NMR spectra for compound **1** were assigned using HSQC and HMBC as given in table 1.

Amyrin (α) was isolated as a brown solid from the CH_2Cl_2 extract of the aerial parts of *B. togoense*, which had been isolated previously from the methanol extract of *Sacoglottis uchi* (7). The IR spectrum showed absorbance bands for hydroxyl (3055 cm^{-1}) and sp^3 CH (2987 cm^{-1}) in conjugation and unsymmetrical ethylenic double bond (1733 cm^{-1}) and olefinic carbon (1422 cm^{-1}) groups.

The molecular ion was not observed in the HRMS spectrum, however 30 carbons could be counted in the ^{13}C NMR spectrum, indicating the compound was a triterpenoid.

The ^1H and ^{13}C NMR spectra (spectrum 2.2 and 2.3) showed the presence of one trisubstituted double bond. A hydroxyl group was placed on C-3 confirmed by the C-3 (δ_C 79.3) resonance correlating with both the 3H-23 (δ_H 0.99 s), 3H-24 (δ_H 0.78 s) and H-5 (δ_H 0.73 d, J = 11.5) resonances. A further singlet (δ_H 0.79, 0.93, 0.99, 0.78 and 1.24) and two doublet (δ_H 0.86 d, J= 6.2 and δ_H 0.95 d, J= 6.2) methyl group proton resonances were present and the typical 12-olaenene double bond (δ_H 5.25, δ_C 126.1, δ_C 138.2) was seen. A comparison against literature data (7) confirmed that this compound was α-amyrin which has been isolated previously from the stem bark of *Sacoglottis uchi* (Humiriaceae)(7).

The configuration of the hydroxyl group at C-3 was confirmed as β by the coupling constant of H-3 (J = 5.1, 11.3). The configurations at the chiral centres were confirmed using the NOESY spectrum. The ^1H and ^{13}C NMR spectra for compound **2** were assigned using HSQC and HMBC as given in table 2.

Lupeol was isolated as a brown solid from the MeOH extract of the aerial parts of *B. togoense* which had been isolated previously from the hexane extract of *Magnolia salicifilia* (10) as well as synthesised (8). The IR spectrum showed an absorbance band for hydroxyl (3363 cm^{-1}). The molecular ion was no seen in the HRMS spectrum, however 30 carbons could be counted in the ^{13}C NMR spectrum indicating the compound was a triterpenoid.

The NMR spectra of lupeol showed the presence of an *iso*-propenyl group typical of the lupene-type of pentacyclic triterpenoids. Coupled 2H-29 methylene protons (δ_H 4.69 d, J = 2.1, δ_H 4.57 d, J = 2.4) and ^{13}C NMR resonances (δ_C 105.9, δ_C 151.2, δ_C 19.5) could be assigned to two H-29 and C-29, C-20 and C-30 respectively (11).

Lupeol was identified as the known 3β-hydroxylup-20(29)-ene, commonly referred to as lupeol. A literature search revealed that the ^{13}C NMR chemical shifts were similar to those of lupeol. The configurations at the chiral centres were confirmed using the NOESY spectrum. The ^{1}H and ^{13}C NMR spectra for compound **3** were assigned using HSQC and HMBC as given in table 3.

Previously, pheophytin *a* has been reported to possess antimicrobial activity against *Candida albicans* (ATCC 90028) and *C. albicans* (ATCC 76615) (12) as well as antioxidant activity (13). Amyrin (α) has been reported to exhibit antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *C. albicans*, *Staphylococcus aureus* and *Trichophyton mentagrophytes* (14). Antiprotozoal, anti-inflammatory, antitumor and antimicrobial activity had been reported for lupeol (15).

Conclusion

Phaeophytin *a*, α-amyrin and lupeol are reported here for the first time from *B. togoense.* This was also the first report of the phytochemical quantification in *B. togoense* in Nigeria. However, these secondary metabolites, i.e phaeophytin *a*, α-amyrin and lupeol were reported previously to show various biological activities. Therefore, the results of chemical compound analysis of *B. togoense* justified the ethnomedicinal uses of this plant in Nigeria.

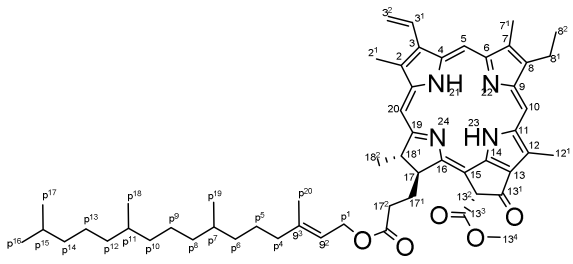
Acknowledgments

The author wishes to thank the Natural Product Research Group, University of Surrey, UK for the opportunity to carry out my research work using their laboratory, Chemicals and Instruments.

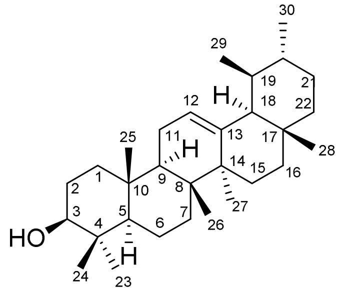
Competing Interests

Authors have declared that no competing interests exist.

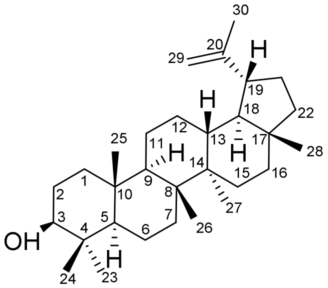
Figure 1: *Brachystelma togoense* in its natural habitat (16)



1



2



3

Fig.2: Structures of isolated compounds **1-3** from *B.togoense* schtlr

1. Phaeophytin *a*

2. α-Amyrin

3. Lupeol

References

1. Bruyns P V. Three New Species of *Brachystelma* (Apocynaceae, Asclepiadoideae, Ceropegieae) from South Tropical and Southern Africa. Vol. 19. SPIE; 2009. 5 p.
2. Ollerton J, Masinde S, Meve U, Picker M, Whittington A. Fly pollination in Ceropegia (Apocynaceae: Asclepiadoideae): biogeographic and phylogenetic perspectives. Annals of Botany. 2009;103(9):1501–14.
3. Britto SJ, Bruyns P V. Three new species of *Brachystelma* from Tamil Nadu, India. Haseltonia. 2016;(22):48–54.
4. Kew Royal Botanical Gardens. Electronic Plant Information Centre (ePIC) [Internet].

2019 [cited 2019 Feb 7]. Available from: http://epic.kew.org/index.htm

5. Rajaram MS, Rathod J, Dilip V. I S S N 2278 – 4357 Pharmacognostical Studies on the Tuber of Brachystelma Edulis Coll . and Helmsl . an Endemic To Peninsular , India . 2014;3(6):1958–65.

6. Schwikkard SL, Mulholland DA, Hutchings A. Phaeophytins from *Tapura fischeri*. Phytochemistry. 1998;49(8):2391–4.

7. Abreu VG da C, Corrêa GM, Lagos IA dos S, Silva RR, Alcântara AF de C. Pentacyclic triterpenes and steroids from the stem bark of *uchi* (Sacoglottis uchi, Humiriaceae). Acta Amazonica. 2013;43:525–8.

8. Cmoch P, Korda A, Rárová L, Oklešťková J, Strnad M, Luboradzki R, et al. Synthesis and structure–activity relationship study of cytotoxic lupane-type 3β-O-monodesmosidic saponins with an extended C-28 side chain. Tetrahedron [Internet]. 2014;70(17):2717–30. Available from: http://www.sciencedirect.com/science/article/pii/S0040402014003123

9. Matsuo A, Ono K, Hamasaki K, Nozaki H. Phaeophytins from a cell suspension culture of the liverwort *Plagiochila ovalifolia.* Phytochemistry [Internet]. 1996;42(2):427–30. Available from: http://www.sciencedirect.com/science/article/pii/0031942296000179

10. Silva ATM e, Magalhães CG, Duarte LP, Mussel W da N, Ruiz ALTG, Shiozawa L, et al. Lupeol and its esters: NMR, powder XRD data and in vitro evaluation of cancer cell growth. Brazilian Journal of Pharmaceutical Sciences [Internet]. 2018 Feb 1 [cited 2019 Feb 19];53(3). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-82502017000300621&lng=en&tlng=en

11. Silva ATM e, Magalhães CG, Duarte LP, Mussel W da N, Ruiz ALTG, Shiozawa L, et

al. Lupeol and its esters: NMR, powder XRD data and in vitro evaluation of cancer cell growth. Brazilian Journal of Pharmaceutical Sciences. 2018 Feb;53(3).

12. Gomes RA, Teles YCF, Pereira F de O, Rodrigues LA de S, Lima E de O, Agra M de F, et al. Phytoconstituents from *Sidastrum micranthum* (A. St.-Hil.) Fryxell (Malvaceae) and antimicrobial activity of pheophytin a. Brazilian Journal of Pharmaceutical Sciences [Internet]. 2015;51:861–7. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-82502015000400861&nrm=iso

13. Kusmita L, Puspitaningrum I, Limantara L. Identification, isolation and antioxidant activity of pheophytin from green tea (*Camellia sinensis* (L.) Kuntze). Procedia Chemistry [Internet]. 2015;14:232–8. Available from: https://dx.doi.org/10.1016/j.proche.2015.03.033

14. Ragasa CY, Puno MRA, Sengson JMAP, Shen C-C, Rideout JA, Raga DD. Bioactive triterpenes from *Diospyros blancoi*. 2009;23(13):1252–8. Available from: https://dx.doi.org/10.1080/14786410902951054

15. Gallo M, Miranda bullet, Sarachine J. Biological activities of lupeol. In: International Journal of Biomedical and Pharmaceutical Sciences. 2009. p. 46–66.

16. Erpenbach A. West African Plants - A Photo Guide - Brachystelma togoense Schltr. [Internet]. 2009 [cited 2019 Feb 18]. Available from: http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=4246

SUPPLIMENTARY INFORMATION

Table 1: Correlation table for NMR data of compound 1: phaeophytin-*a*

No.	^{13}C NMR (100 MHz) in CDCl$_3$	^{1}H NMR (400 MHz) CDCl$_3$ (*J* in Hz) (6)	^{1}H NMR (400 MHz) CDCl$_3$ (*J* in Hz)	HMBC (H→C)	COSY	NOESY
1	142.2 C	142.9	-	-	-	-
2	132.0 C	131.8	-	-	-	-
2^1	11.4 CH$_3$	12.1	3.19 s	1, 2, 3	-	3^1, $3^2\alpha$, 20
3	136.7 C	136.5	-	-	-	-
3^1	129.3 CH	129.0	7.97 dd, *J* = 11.5, 17.8	2, 3, 3^2, 4	$3^2\alpha$, $3^2\beta$	2^1, $3^2\alpha$, $3^2\beta$, 5
$3^2\alpha$	122.9 CH$_2$	122.8	6.28 dd, *J* = 1.2, 17.8	3, 3^1	3^1, $3^2\beta$	2^1, 3^1, $3^2\beta$, 5
$3^2\beta$			6.16 dd, *J* = 1.2, 11.5	3, 3^1	3^1, $3^2\alpha$	3^1, $3^2\alpha$
4	136.5 C	136.2	-	-	-	-
5	97.7 CH	97.3	9.32 s	3, 4, 7	-	3^1, $3^2\alpha$, $3^2\beta$, 7^1, 10
6	155.8 C	155.5	-	-	-	-
7	136.4 C	136.1	-	-	-	-
7^1	12.2 CH$_3$	11.2	3.30 s	7, 6, 8	-	
8	145.4 C	145.2	-	-	-	-
$8^1\alpha$	19.6 CH$_2$	19.7	3.64 d, *J* = 9.2	7, 8, 8^2, 9	$8^1\beta$, 8^2	7^1, 8^2, 10
$8^1\beta$			3.64 d, *J* = 9.2	7, 8, 8^2, 9	$8^1\alpha$, 8^2	7^1, 8^2, 10
8^2	17.6 CH$_3$	16.3	1.67 t, *J* = 7.6	8, 8^1	$8^1\alpha$, $8^1\beta$	7^1, $8^1\alpha$, $8^1\beta$, 10, 13^4
9	151.2 C	151.0	-	-	-	-
10	104.6 CH	104.4	9.48 s		-	5, $8^1\alpha$, $8^1\beta$, 8^2, 12^1, 13^4
11	138.1 C	137.9	-	-	-	-
12	129.2 C	129.1	-	-	-	-
12^1	12.3 CH$_3$	12.2	3.38 s	11, 12, 13	-	10
13	129.1 C	129.0	-	-	-	-
13^1	189.9 C	189.6	-	-	-	-
13^2	64.9 CH	64.7	6.27s	-	-	-
13^3	173.2 C	173.0	-	-	-	-
13^4	53.1 OCH$_3$	53.0	3.89 s	13^3	-	8^2, 10
14	149.9 C	150.0	-	-	-	-
15	105.4 C	105.2	-	-	-	-
16	161.5 C	161.3	-	-	-	-
17	51.4 CH	51.1	4.22 m	17^1, 17^2, 18^1, 19	$17^1\alpha$, $17^1\beta$	$17^1\alpha$, $17^1\beta$, 18^1
$17^1\alpha$	29.8 CH$_2$	29.8	2.49 m	16, 17, 17^2, 17^3, 18	17, $17^1\beta$, $17^2\alpha$, $17^2\beta$	17, $17^1\beta$, $17^2\beta$,
$17^1\beta$			2.22* m	17, 17^2, 17^3, 18	17, $17^1\alpha$, $17^2\alpha$, $17^2\beta$	17, $17^1\alpha$, $17^2\alpha$, 18
$17^2\alpha$	32.1 CH$_2$	31.2	1.26* m	17, 17^1, 17^3	$17^1\alpha$, $17^1\beta$, $17^2\beta$	$17^1\beta$
$17^2\beta$			1.26* m	17, 17^1, 17^3	$17^1\alpha$, $17^1\beta$, $17^2\alpha$	17, $17^1\alpha$, $17^1\beta$, $17^2\alpha$, 18
17^3	173.8 C	172.0	-	-	-	-
18	50.3 CH	50.1	4.47 d, *J*= 2.1	16, 17, 17^1, 18^1, 19	18^1	18^1, $17^1\beta$, $17^2\beta$, 20
18^1	22.8 CH$_3$	22.7	0.85 d, *J*= 6.6,	17, 18, 19	18	P1α, P1β,

						17, 18, 20
19	172.4 C	170.0	-	-	-	-
20	93.3 CH	93.1	8.56 s	1, 2, 18	-	2^1, 18^1, 18
P1α	61.7 CH_2	61.0	4.48 m	P2, P3, 17^3	P1β, P2, P20	18^1
P1β			4.48 m	P2, P3, 17^3	P1α, P2, P20	18^1
P2	117.9 CH	118.0	5.13 m	P4, P20	P1α, P1β, P20	
P3	143.1 C	142.0	-	-	-	-
P4α	40.0 CH_2	62.0	1.89 m	P2, P3, P20	P4β, P5α, P5β	
P4β			1.89 m	P2, P3, P20	P4α, P5α, P5β	
P5α	25.2 CH_2	39.5	1.26 m	P3, P4, P6	P4α, P4β, P5β, P6α, P6β	
P5β			1.26 m	P3, P4, P6	P4α, P4β, P5α, P6α, P6β	
P6α	36.9 CH_2	37.0	1.14* m	P4, P5, P7, P8, P19	P5α, P5β, P6β, P7	
P6β			1.01* m	P5, P7, P8, P19	P5α, P5β, P6α, P7	
P7	32.9 CH	37.0	1.32 m	P6, P8	P6α, P6β, P8α, P8β, P19	
P8α	37.6 CH_2	36.5	1.99* m	P6, P7, P9, P10, P19	P7, P8β, P9α, P9β	
P8β			1.00* m	P6, P7, P9, P10, P19	P7, P8α, P9α, P9β	
P9α	24.6 CH_2	25.0	1.31 m	P8, P10	P8α, P8β, P9β, P10α, P10β	
P9β			1.31 m	P8, P10	P8α, P8β, P9α, P10α, P10β	
P10α	37.5 CH_2	24.8	1.99* m	P8, P9, P11, P12, P18	P9α, P9β, P10β, P11	
P10β			1.00* m	P8, P9, P11, P12, P18	P9α, P9β, P10α, P11	
P11	32.8 CH	24.2	1.32* m	P9, P10, P12, P13, P18	P10α, P10β, P12α, P12β, P18	
P12α	37.4 CH_2	40.0	1.99* m	P10, P11, P13, P18	P11, P12β, P13α, P13β	
P12β			1.00* m	P10, P11, P13, P14, P18	P11, P12α, P13α, P13β	
P13α	24.9 CH_2	28.0	1.63 m	P11, P12, P14	P12α, P12β, P13β, P14α, P14β	
P13β			1.63 m	P11, P12, P14	P12α, P12β, P13α, P14α, P14β	
P14α	39.6 CH_2	32.5	1.19 m	P12, P13, P16, P17	P13α, P13β, P14β, P15	
P14β			1.10 m	P12, P13, P16, P17	P13α, P13β, P14α, P15	
P15	28.2 CH	32.5	1.49 sep $J = 6.6$	P13, P14, P16, P17	P14α, P14β, P16, P17	
P16	22.9 CH_3	22.7	0.81 d, $J = 7.3$	P14, P15, P17	P15, P17	
P17	23.3 CH_3	22.6	0.80 d, $J = 7.3$	P14, P15, P16	P15, P16	
P18	19.9 CH_3	19.6	0.82 d, $J = 7.3$	P10, P11, P12	P11	
P19	19.8 CH_3	19.4	0.79 d, $J = 9.2$	P6, P7, P8	P7	
P20	16.5 CH_3	16.2	1.61 d, $J = 4.2$	P2, P3, P4	P1α, P1β, P2	

*Overlapped proton resonances

Table 2: Correlation table for NMR data of compound 2: α-amyrin

C	^{13}C NMR(100MHz) in CDCl$_3$	^{13}C NMR(100MHz) in CDCl$_3$ (7)	^{1}H NMR (400MHz) CDCl$_3$ (*J* in Hz)	HMBC (H→C)	COSY	NOESY
1α	38.8 CH$_2$	38.7	1.66 m	2,3, 5, 9 10, 25	1β, 2α, 2β	2α,3
1β			1.63 m		1α, 2α,2β	2β
2α	27.4 CH$_2$	27.2	1.62 m	1, 3, 4, 10	1α, 1β, 2β,3	3α
2β			1.61 m		1α,1β, 2α,3	3β
3	79.3 CH	79.1	3.22 dd, *J* = 5.1, 11.3	1, 2, 4, 5, 23, 24	2α, 2β	23, 27, 30
4	38.9 C	38.9	-			-
5	55.4 CH	55.2	0.74 d, *J* = 11.7	1, 3, 4, 6, 7, 9, 10	6α, 6β	6α
6α	18.5 CH$_2$	18.4	1.55 m	4, 5, 7, 8	5,6β,7α,7β	5, 7α
6β			1.52 m		5,6α,7α,7β	7β
7α	33.2 CH$_2$	33.9	1.49 m	5, 6, 8, 9	6α,6β,7β	5, 6α
7β			1.32m		6α,6β,7α	6β
8	39.7 C	40.0	-		-	-
9	47.8CH	47.8	1.51 d, *J* = 9.1	1, 5, 7, 8, 10, 11, 12, 14, 25, 26	11α,11β	5, 24,25,26
10	37.2 C	37.2	-		-	-
11α	23.5 CH$_2$	23.1	1.92 d, *J* =3.6	8, 9, 10, 12, 13	9,11β,12	12α
11β			1.91 d, *J* =3.6		9,11 α,12	11β
12	126.1 CH	124.5	5.25 t, *J* = 4.5	9, 11, 13, 14, 18, 27	11α,11β	11α,18,27,30
13	138.2 C	139.9	-		-	-
14	42.2 C	42.3	-		-	-
15α	28.2 CH$_2$	28.0	1.0*	8, 13, 14, 16, 17, 27	15β,16α,16β	16α
15β					15α,16α,16β	16β
16α	24.4 CH$_2$	26.7	2.02 d, *J* =4.7	14, 15, 17, 18, 28	15 α,16α,16β	15α

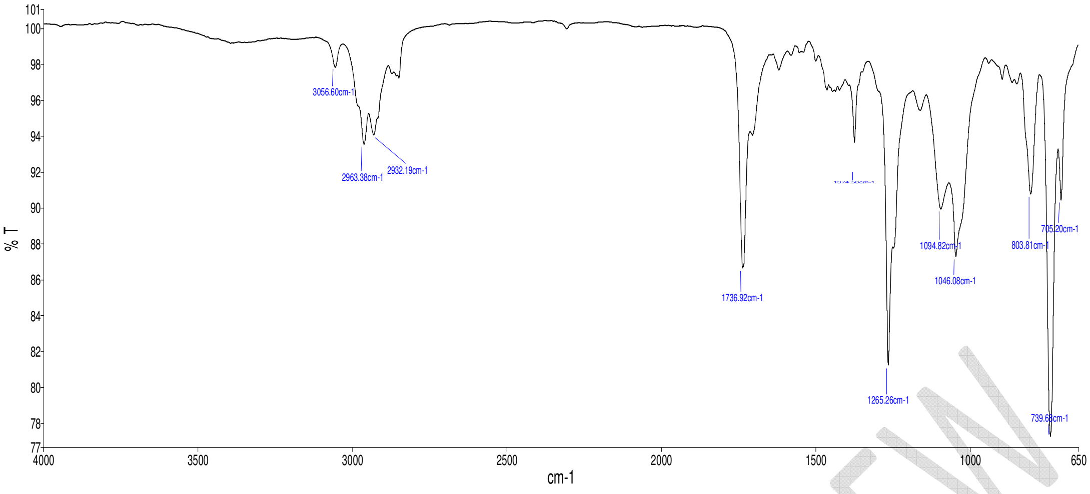
16β			1.66 m		15α,15β,16α	15β
17	48.1 C	33.9	-	-	-	-
18	52.9 CH	59.1	2.19 d, J = 11.4	12, 13, 14, 17, 20, 28	19	12, 23, 27, 30
19	39.3 CH	39.6	1.37 m	13, 17, 18, 20, 21, 29, 30	18	3, 18, 23,27, 30
20	39.0 CH	39.6	1.33 m	18, 19, 21, 22, 29, 30	19, 21α 21β	24, 25, 26, 28
21α	30.8 CH$_2$	31.5	1.52 d, J = 9.4	17,19,20,22	20,21β,22α,22β	22α
21β					20,21β,22α,22β	22β
22α	36.9 CH$_2$	41.5	1.74 m	16, 17, 18, 20, 28	21β,21α,22β	21α
22β			1.68 m		21α, 21β,22α	21β
23	28.4 CH$_3$	28.0	0.99 s	2, 3, 4, 5	-	-
24	15.8 CH$_3$	16.0	0.78 s	3, 4, 5	-	3-OH
25	15.7 CH$_3$	16.0	0.93 s	1, 2, 9, 10	-	24,26,28
26	17.3 CH$_3$	16.8	0.79 s	7, 8, 9, 14	-	24, 25, 28
27	22.9 CH$_3$	23.1	1.24 s	8, 13, 14, 15	-	23, 18, 30
28	23.8 CH$_3$	28.0	1.08 s	16, 17, 18, 22	-	23,25, 26, 29
29	17.2 CH$_3$	17.5	0.86 d, J = 6.2	18, 19, 20	-	24,25, 26, 28,
30	21.4 CH$_3$	21.1	0.95 d, J = 6.2	19, 20, 21	-	3α, 27, 30

*Overlapped proton resonances

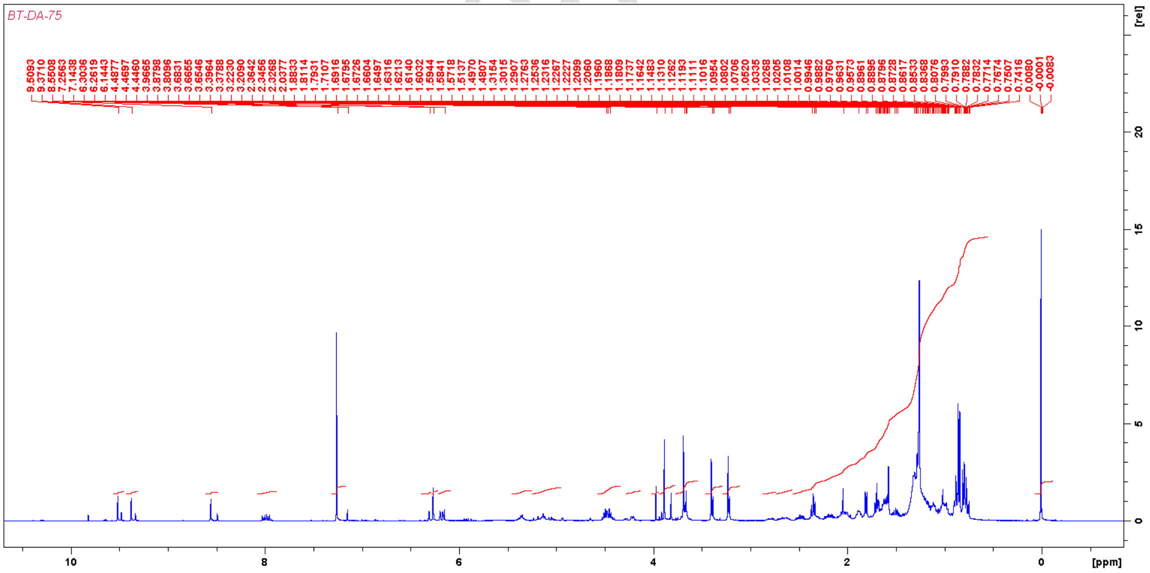
Table 3: Correlation table for NMR data of compound 3: Lupeol (3β-hydroxylup-20(29)-ene)

C	^{13}C NMR(100 MHz) in CDCl$_3$	^{13}C NMR(50 MHz) in CDCl$_3$ (11)	^{1}H NMR (400MHz) CDCl$_3$ (*J* in Hz)	HMBC (H→C)	COSY	NOESY
1α	38.9 CH$_2$	38.1	1.66 d, *J* = 3.8	2, 3, 5, 10, 25	1β, 2α, 2β	2α,3
1β			0.90 d, *J* =4.2		1α, 2α,2β	2β
2α	27.7 CH$_2$	27.4	2.02 d, *J* = 3.3	1, 3, 4, 10, 23	1α, 1β, 2β,3	3α
2β			1.03 m		1α,1β, 2α,3	3β
3	79.2 CH	79.1	3.19 dd, *J* = 5.1, 11.2	1, 2, 4, 5, 23, 24	2α, 2β	23, 27, 30
4	39.1 C	38.7	-	-		-
5	55.5 CH	55.3	0.68 d, *J* =10.5	4, 6, 7, 10, 23, 4, 25	6α, 6β	6α
6α	18.5 CH$_2$	18.3	1.52 d, *J* = 3.6	4, 5, 7, 8,1 0	5,6β,7α,7β	5, 7α
6β			1.39 d, *J* = 1.3		5,6α,7α,7β	7β
7α	34.5 CH$_2$	34.3	2.29 m	5, 6, 8, 9, 26	6α,6β,7β	5, 6α
7β			1.38 d, *J* = 1.4		6α,6β,7α	6β
8	41.1 C	40.9	-	-	-	-
9	50.7 CH	50.5	1.26 m	7, 8, 10, 15, 25, 26	11α,11β	5, 24,25,26
10	37.4 C	37.2	-	1, 2, 4, 5, 6, 8, 9, 11,25	-	-
11α	21.1 CH$_2$	20.9	1.41 d, *J* = 2.9	8, 9, 10, 12, 13	9,11β,12α,12β	12α
11β			1.26 m			11β
12α	25.4 CH$_2$	25.2	1.66 d, *J* = 2.3	9, 11, 13,1 4, 18	11α,11β,12β,13	11α,18,27,30
12β			1.66 d, *J* = 2.3		11α,11β,12α,13	-
13	38.3 CH	38.9	1.65 m	8, 11, 12, 14, 15, 17, 18,19	12α,12β	-
14	43.2 C	42.9	-	-	-	16α
15α	27.6 CH$_2$	27.5	1.68 m	8, 13, 14, 16, 17, 27	15β,16α,16β	16β
15β			1.59 d, *J* = 4.1		15 α,16α,16β	15α
16α	35.8 CH$_2$	35.6	1.67 d, *J* = 5.0	14, 15, 17, 18, 28	15α,15β,16α	15β
16β			1.38 d, *J* = 1.4		15α,15β,16 β	2α,3

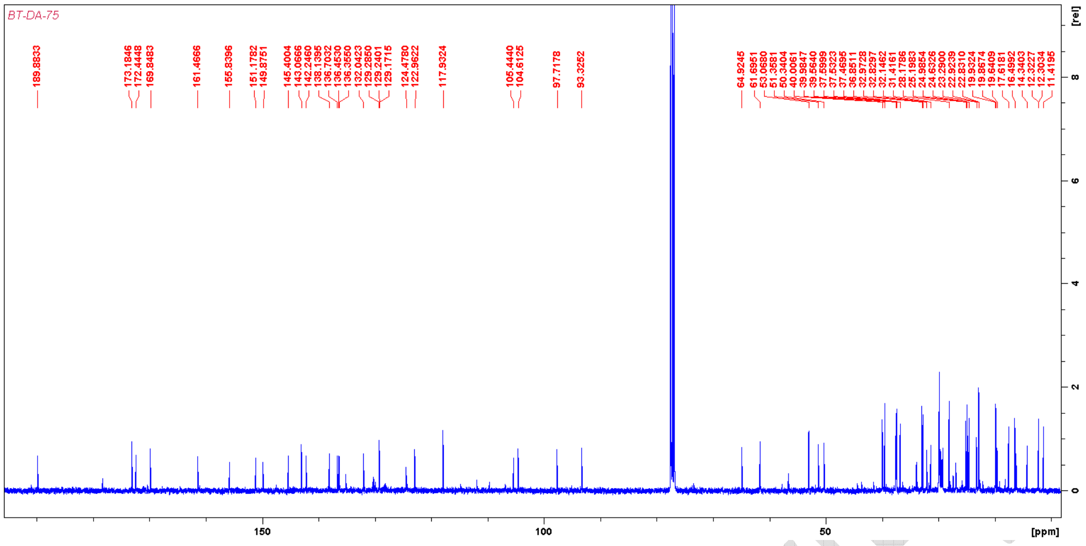
17	43.0 C	43.0	-	-	-	
18	48.5 CH	48.3	1.36 m	12, 13, 14, 19, 21, 20, 29	13,19	5,12, 20, 23, 27
19	48.2 CH	48.0	2.37 m	13, 17, 18, 20, 21, 22, 29, 30	18, 21α,21β	3, 18, 23,27
20	151.2 C	150.9	-	-	-	-
21α	30.0 CH_2	29.9	1.92 d, *J* = 4.9	17, 18, 19, 20, 22, 28	19,21β,22α,22β	22α
21β			1.76 d, *J* = 1.0		19,21α 22α,22β	22β
22α	40.2 CH	40.0	1.99 d, *J* = 3.3	16, 17, 19, 21, 28	22α, 21β, 22β	21α
22β			1.99 d, *J* =3.3		21α, 21β, 22α	21β
23	28.2 CH3	28.0	0.97 s	3, 4, 5, 23	-	3-OH, 25, 26, 28
24	15.6 CH_3	15.4	0.76 s	3, 4, 5, 24	-	3, 5, 9, 19, 27
25	16.3 CH_3	16.1	0.83 s	1, 2, 5, 9, 10	-	24,26,28
26	16.2 CH_3	16.0	1.03 s	7, 8, 9, 14	-	24, 25, 28
27	14.8 CH_3	14.6	0.94 s	8, 13, 14, 15	-	23, 18, 30
28	18.2 CH3	18.0	0.79 s	16, 17, 18, 22	-	23,25, 26, 29
29α	109.5 CH_2	109.3	4.69 d *J* = 2.1	19, 20, 30	29β	3α,19, 23,27
29β			4.57 q, *J* = 2.4		29α	3-OH, 24, 25, 26, 28
30	19.5 CH_3	19.3	1.68 s	19, 20, 29	-	



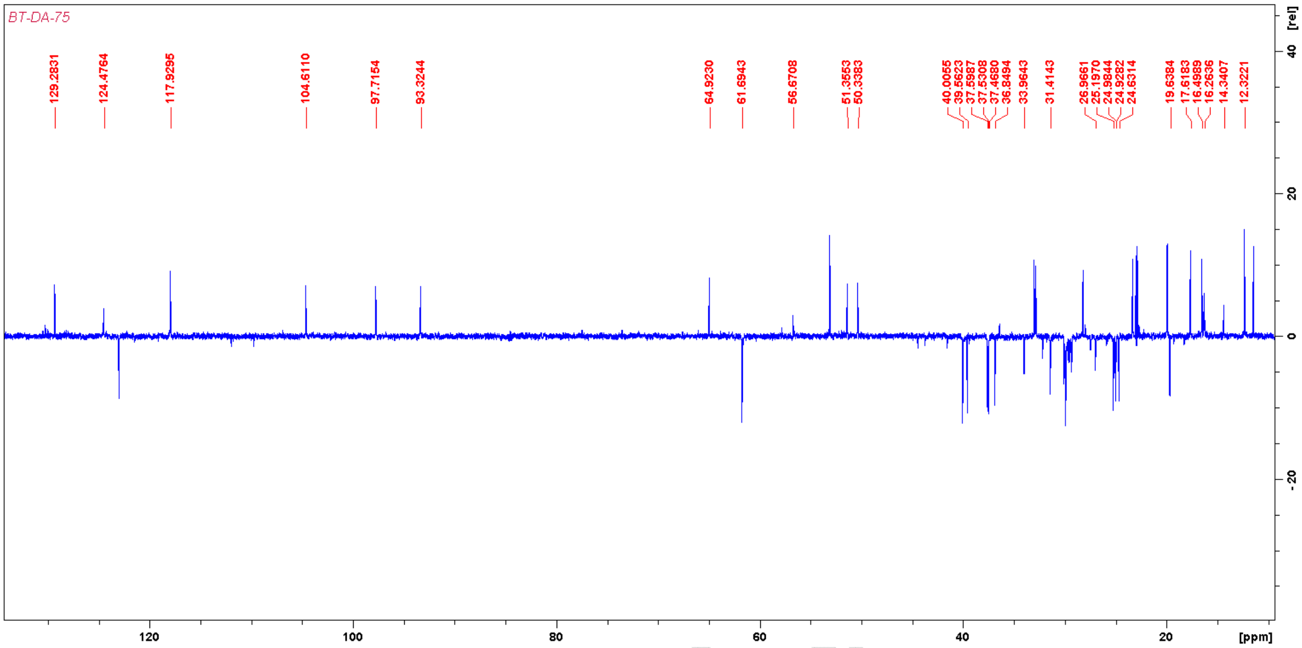
Spectrum 1.1: FTIR spectrum for compound 1



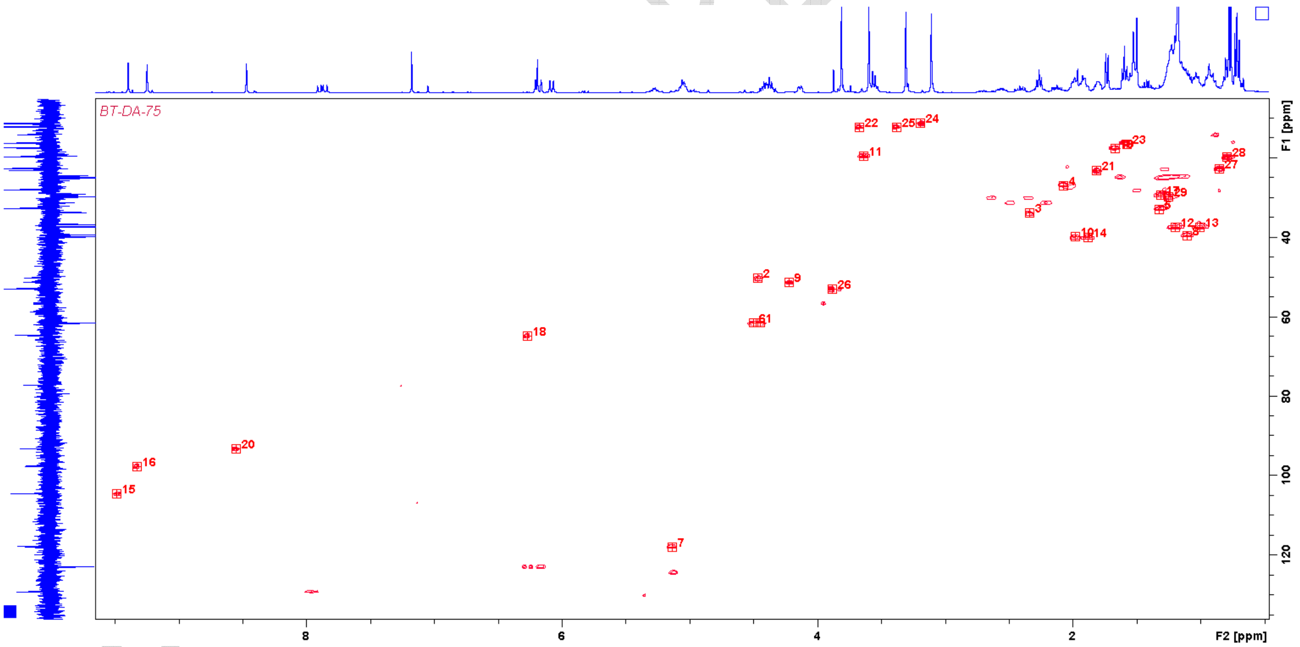
Spectrum 1.2: ^{1}H NMR spectrum for compound 1 in $CDCl_3$



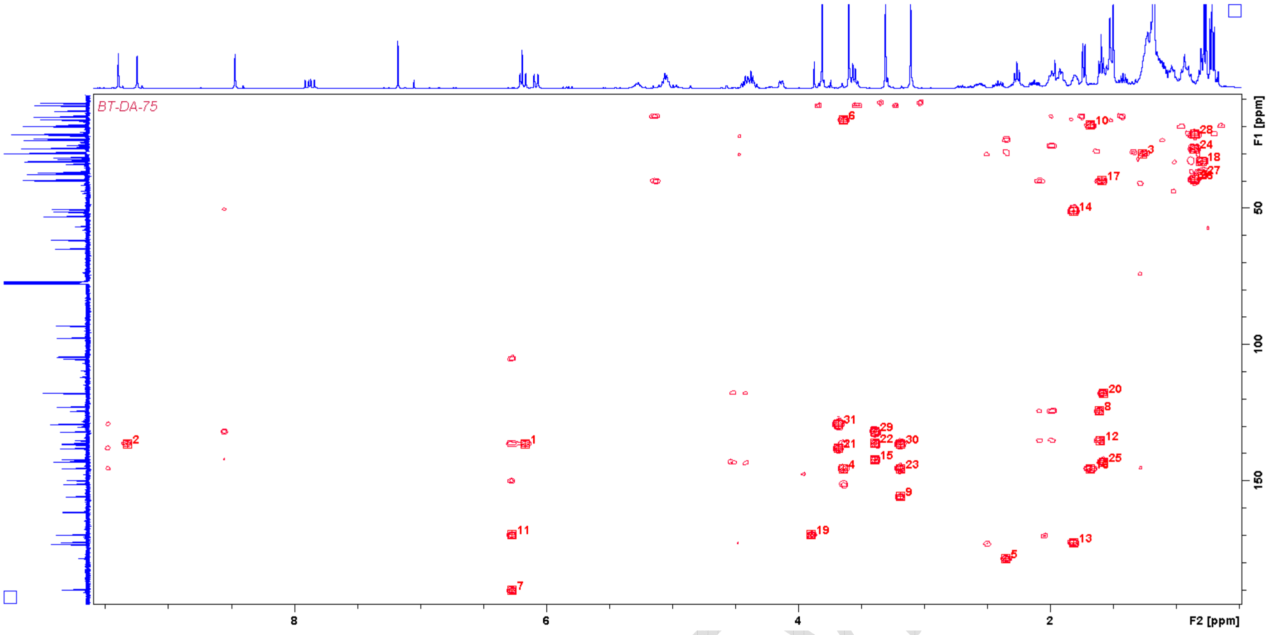
Spectrum 1.3: ^{13}C NMR spectrum for compound 1 in $CDCl_3$



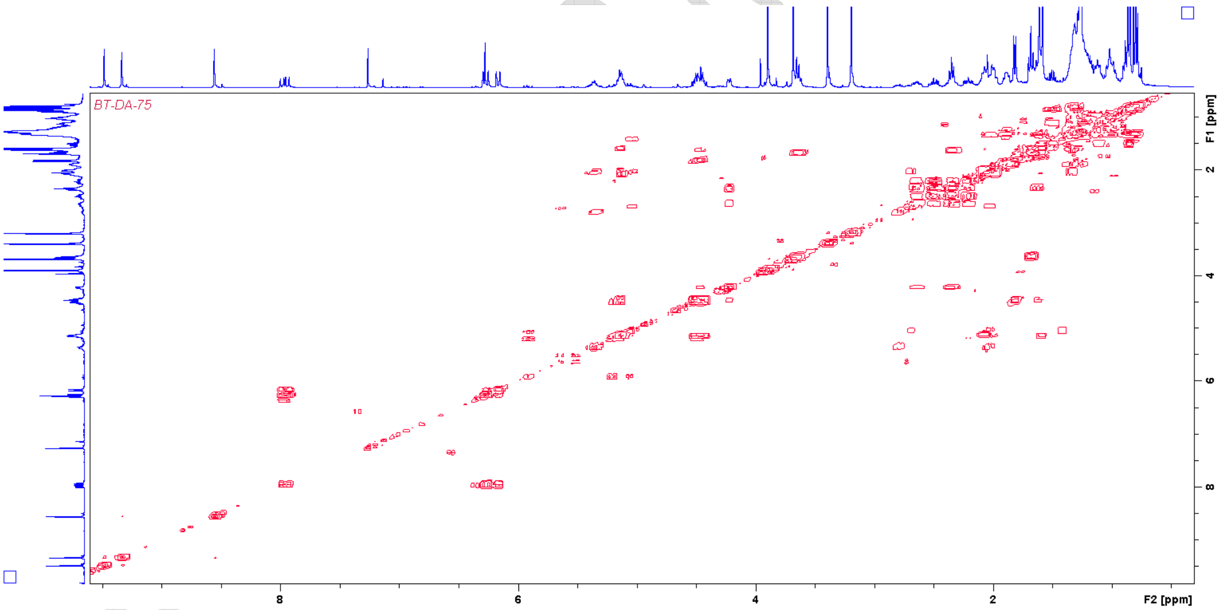
Spectrum 1.4: DEPT spectrum for compound 1 in $CDCl_3$



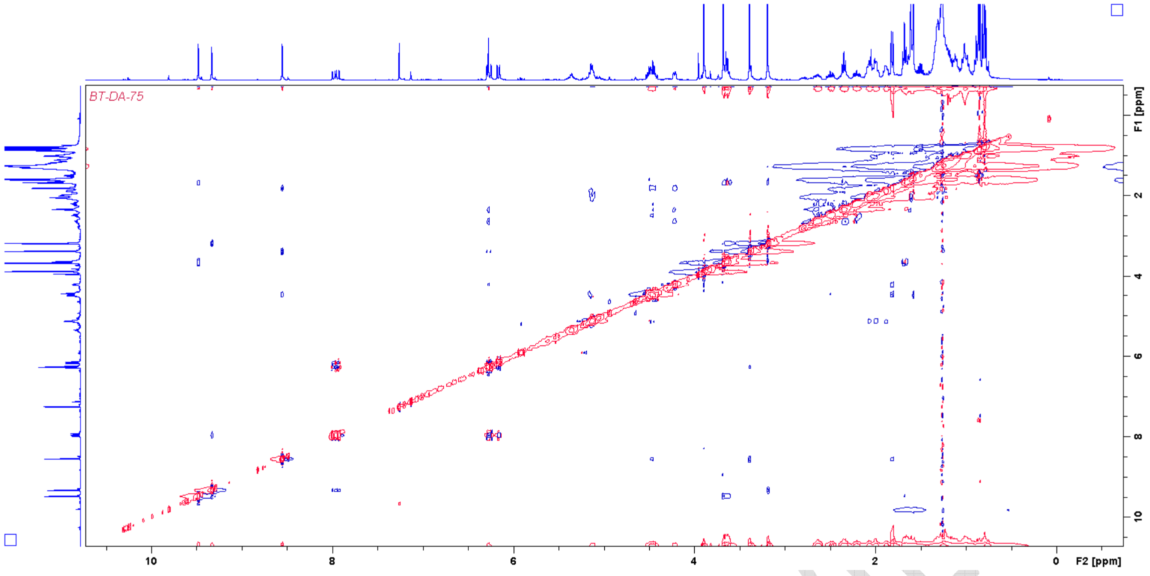
Spectrum 1.5: HSQCDEPT spectrum for compound 1 in $CDCl_3$



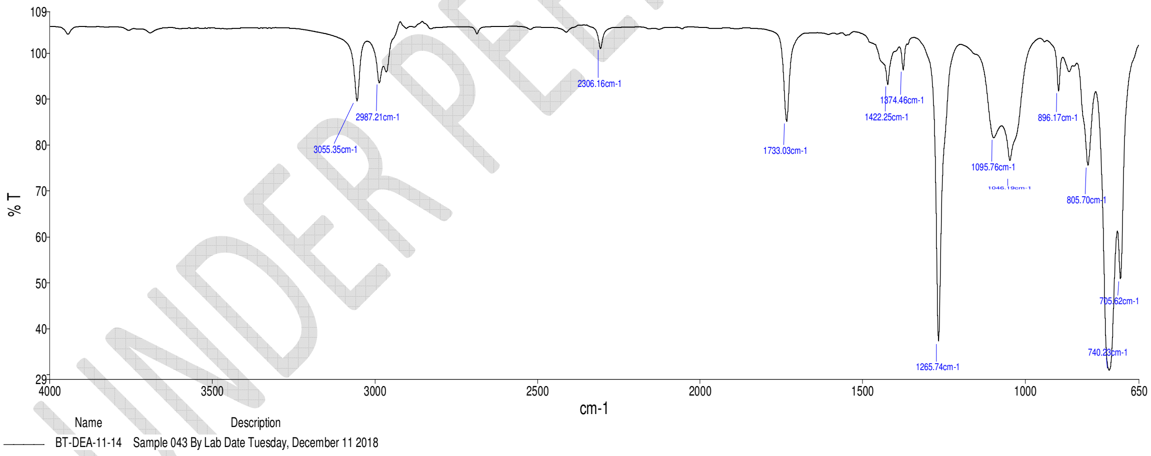
Spectrum 1.6: HMBC spectrum for compound 1 in $CDCl_3$



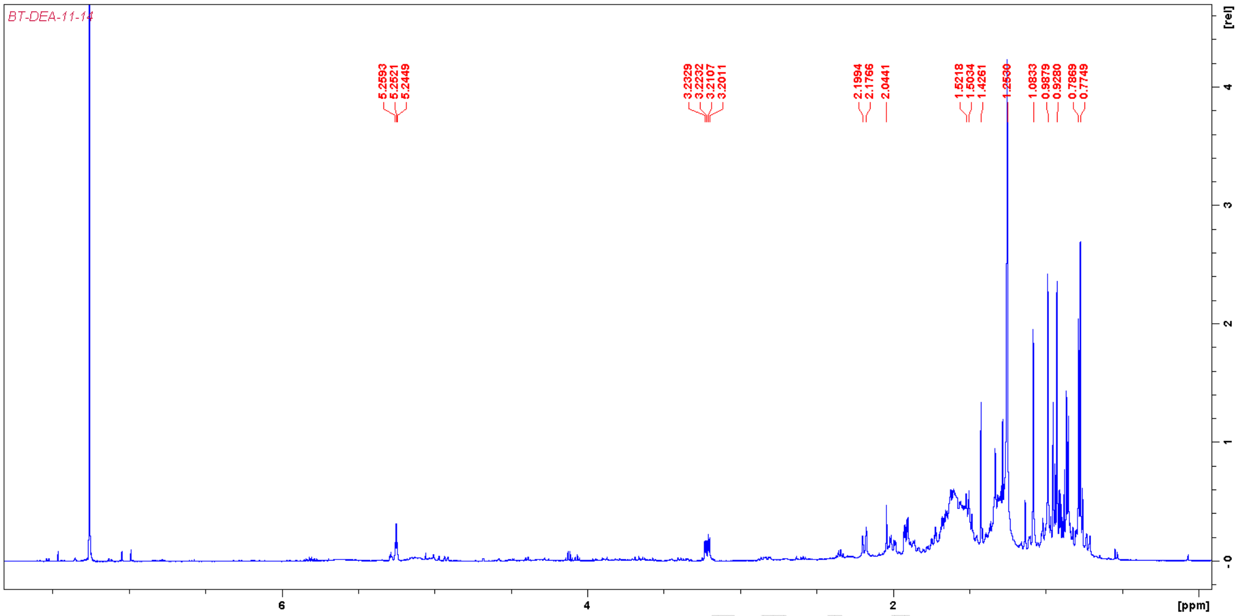
Spectrum 1.7: COSY spectrum for compound 3 in $CDCl_3$



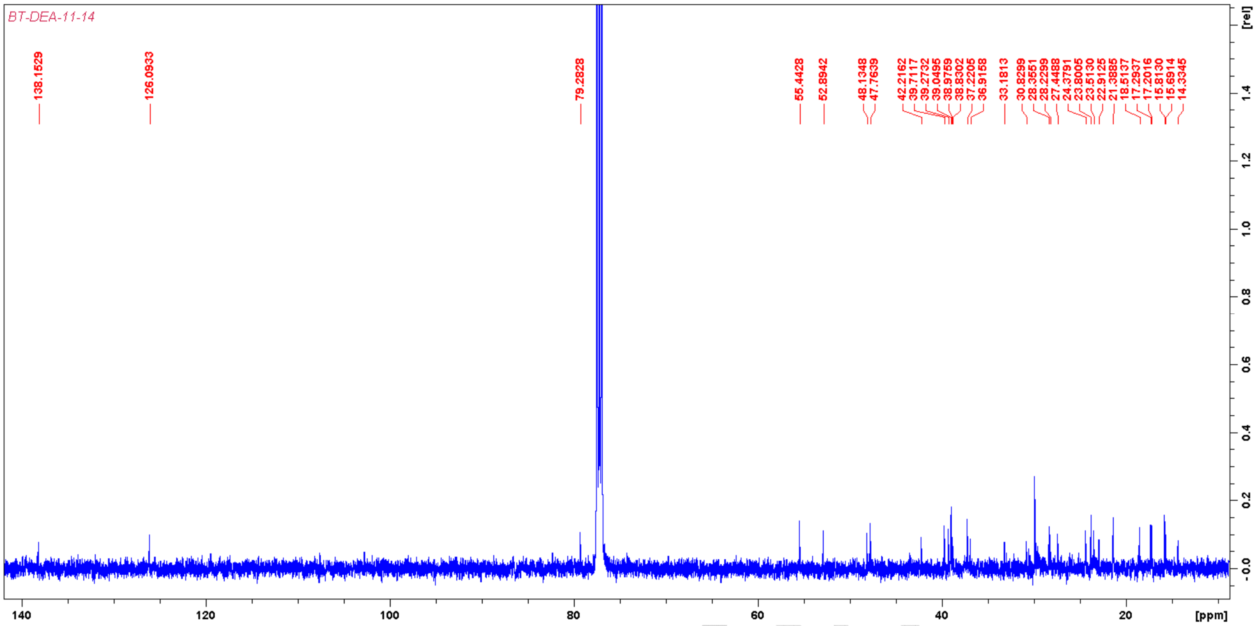
Spectrum 1.8: NOESY spectrum for compound 1 in $CDCl_3$



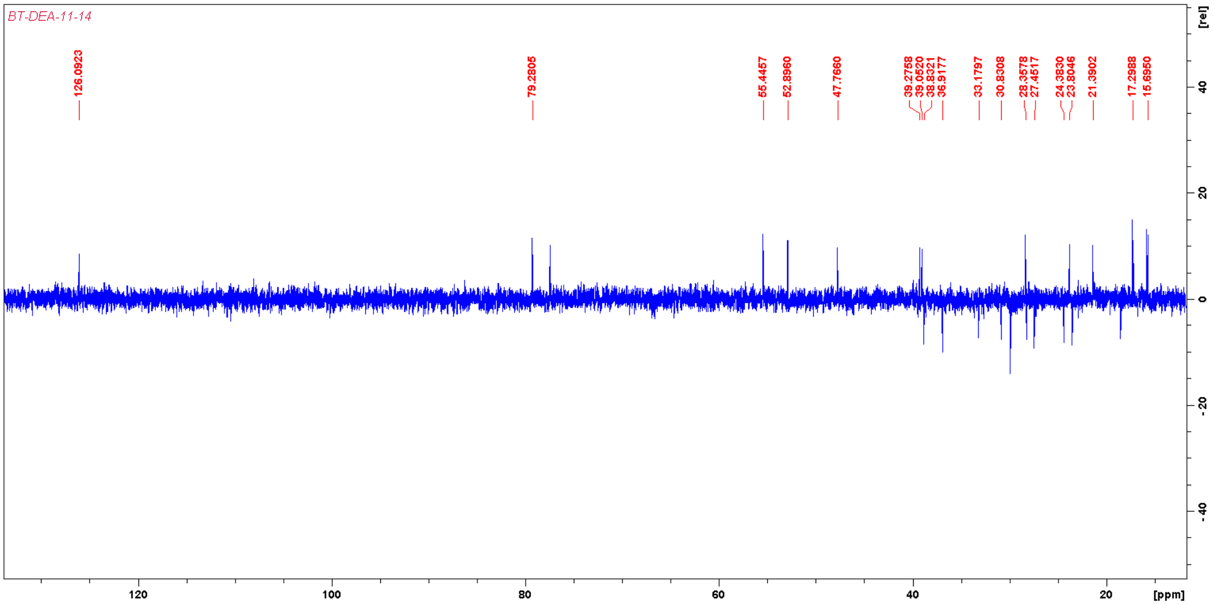
Spectrum 2.1: FTIR spectrum for compound 2



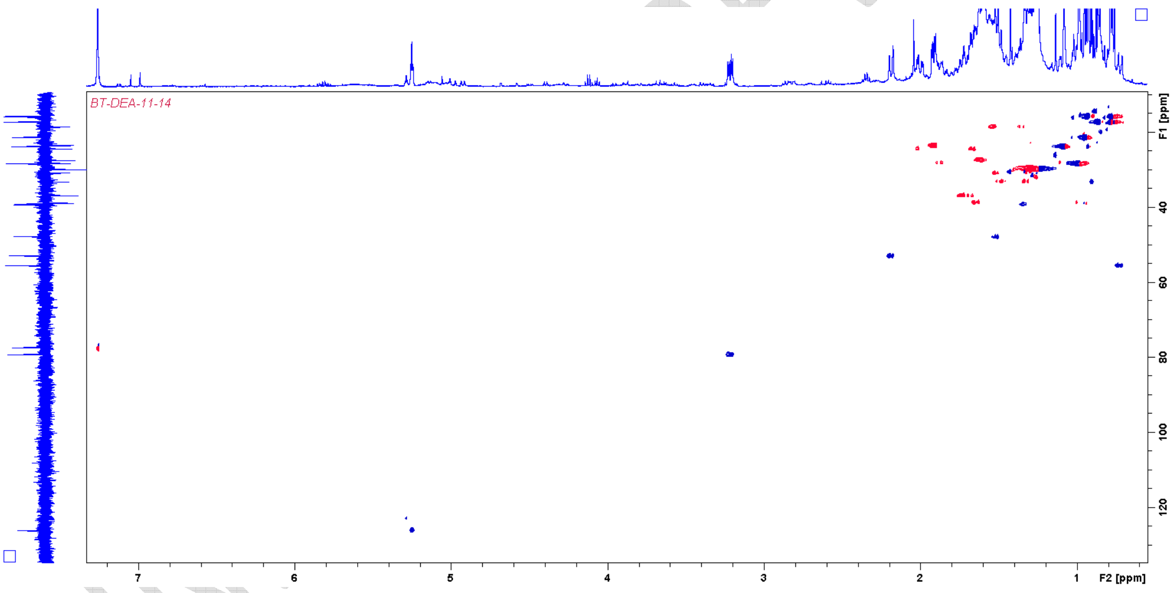
Spectrum 2.2: ^{1}H NMR spectrum for compound 2 in $CDCl_3$



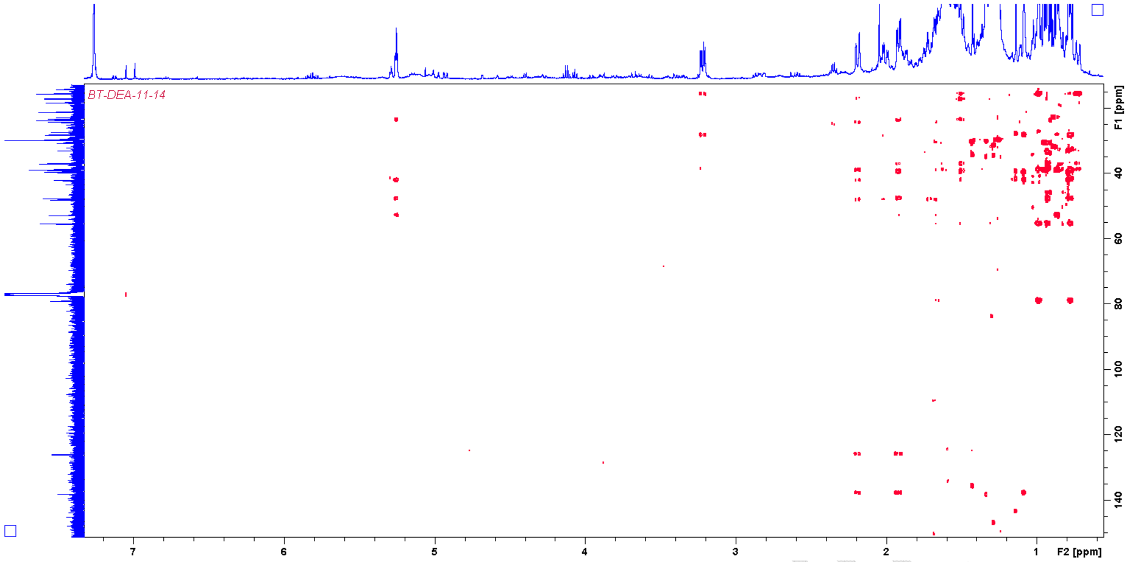
Spectrum 2.3: ^{13}C NMR spectrum for compound 2 in $CDCl_3$



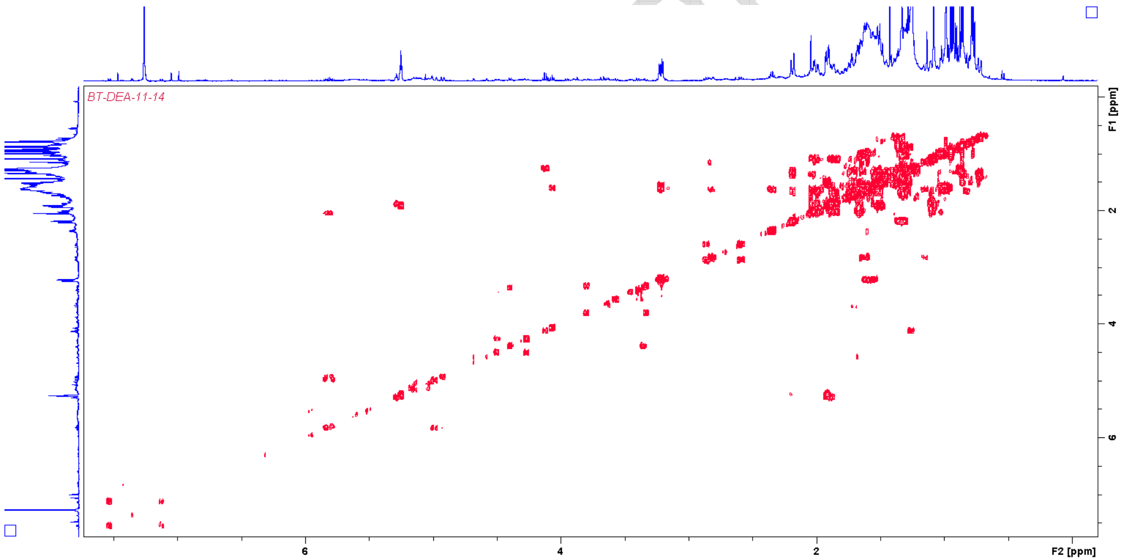
Spectrum 2.4: DEPT spectrum for compound 2 in CDCl$_3$



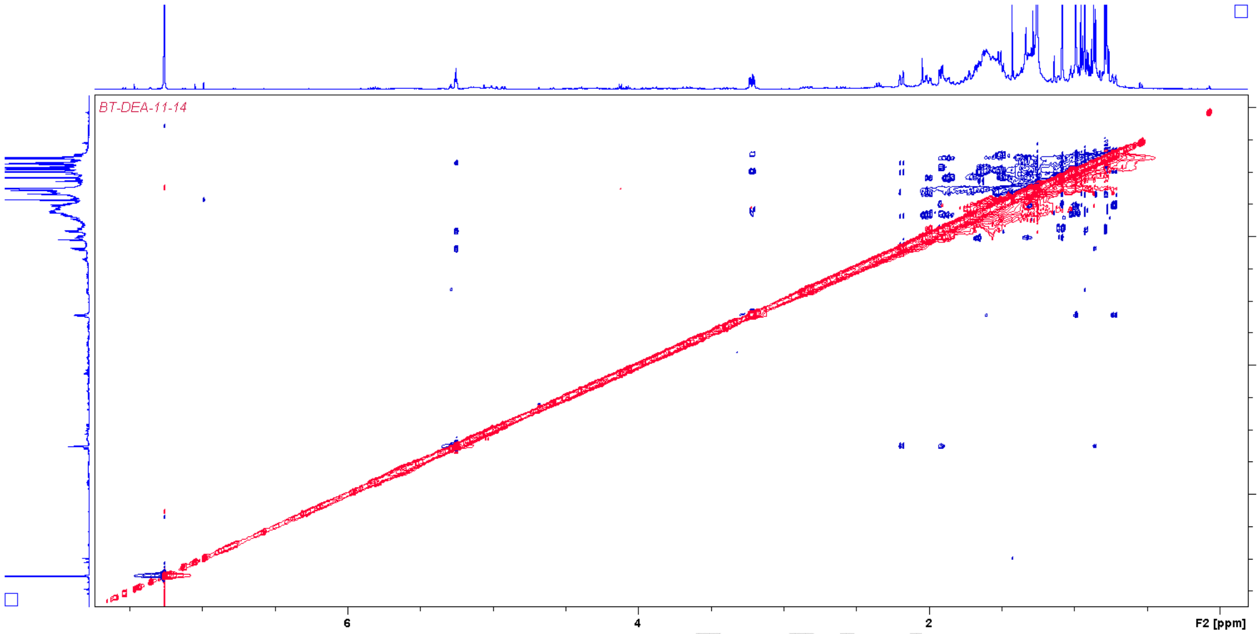
Spectrum 2.5: HSQCDEPT spectrum for compound 2 in CDCl$_3$



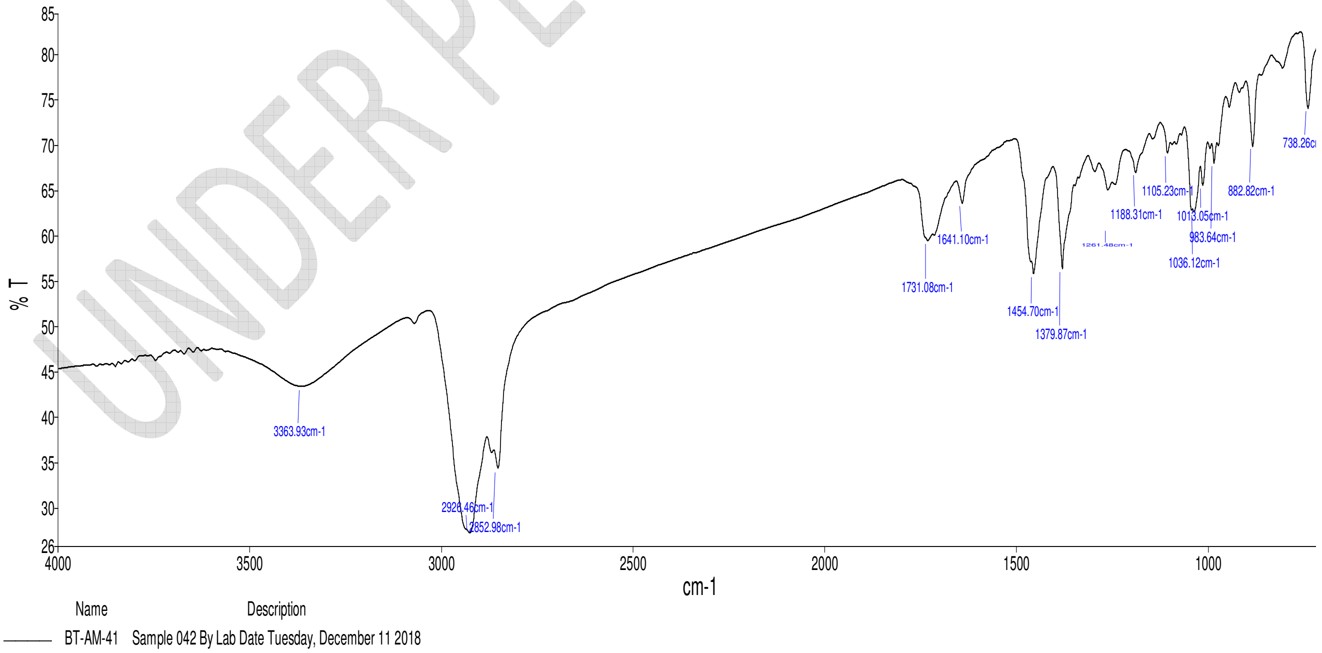
Spectrum 2.6: HMBC spectrum for compound 2 in $CDCl_3$



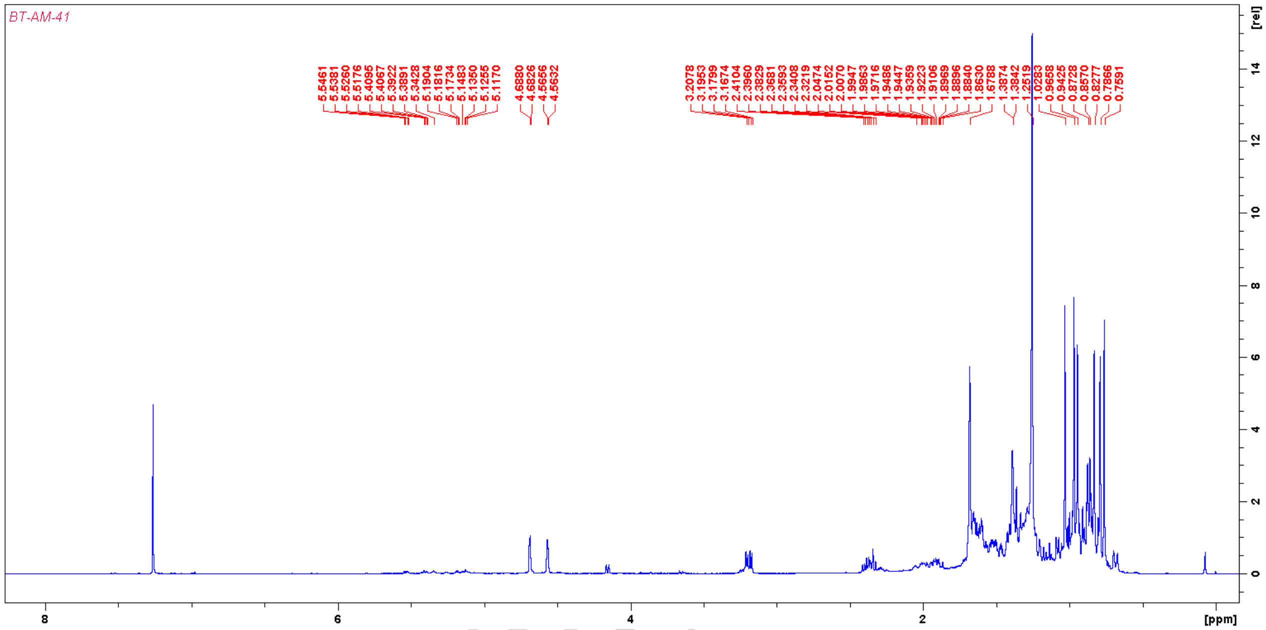
Spectrum 2.7: COSY spectrum for compound 2 in $CDCl_3$



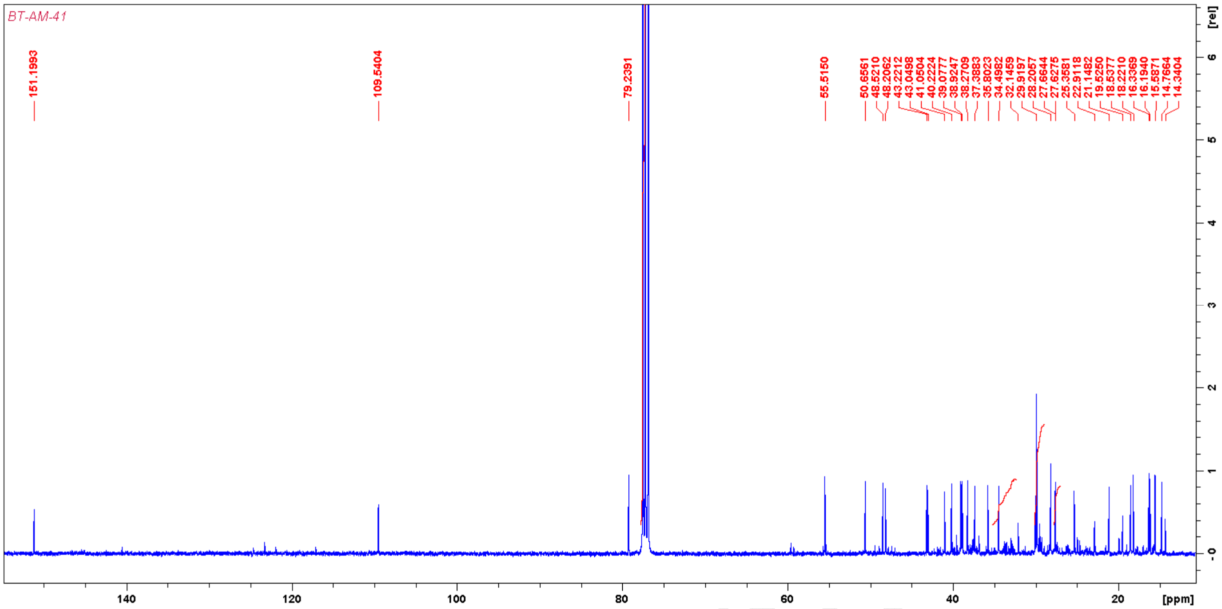
Spectrum 2.8: NOESY spectrum for compound 2 in $CDCl_3$



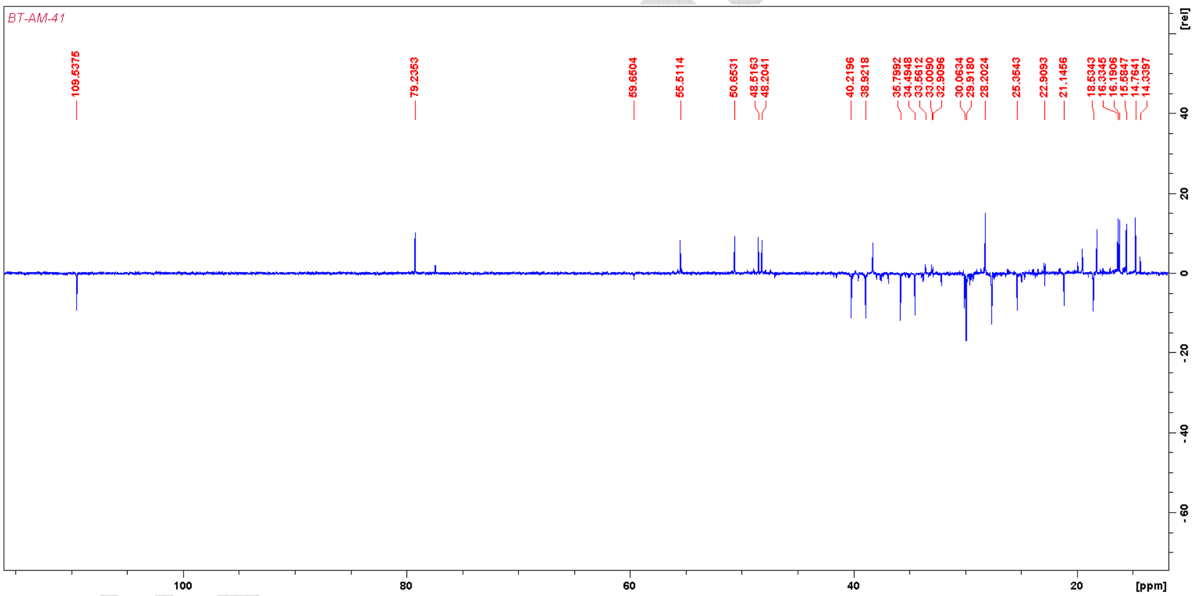
Spectrum 3.1: FTIR spectrum for compound 3



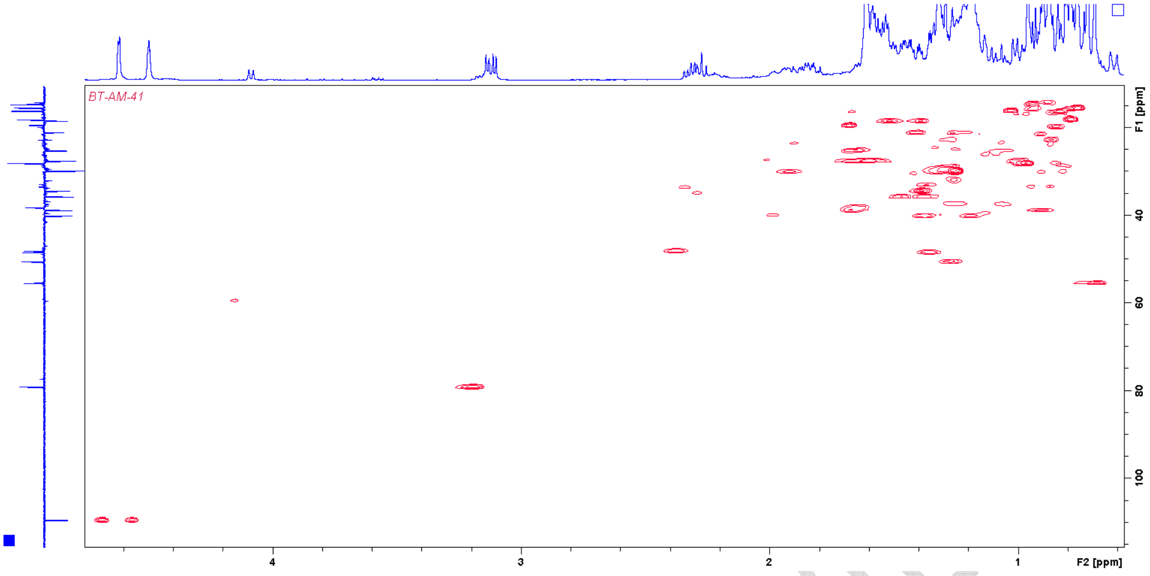
Spectrum 3.2: ^{1}H NMR spectrum for compound 3 in $CDCl_3$



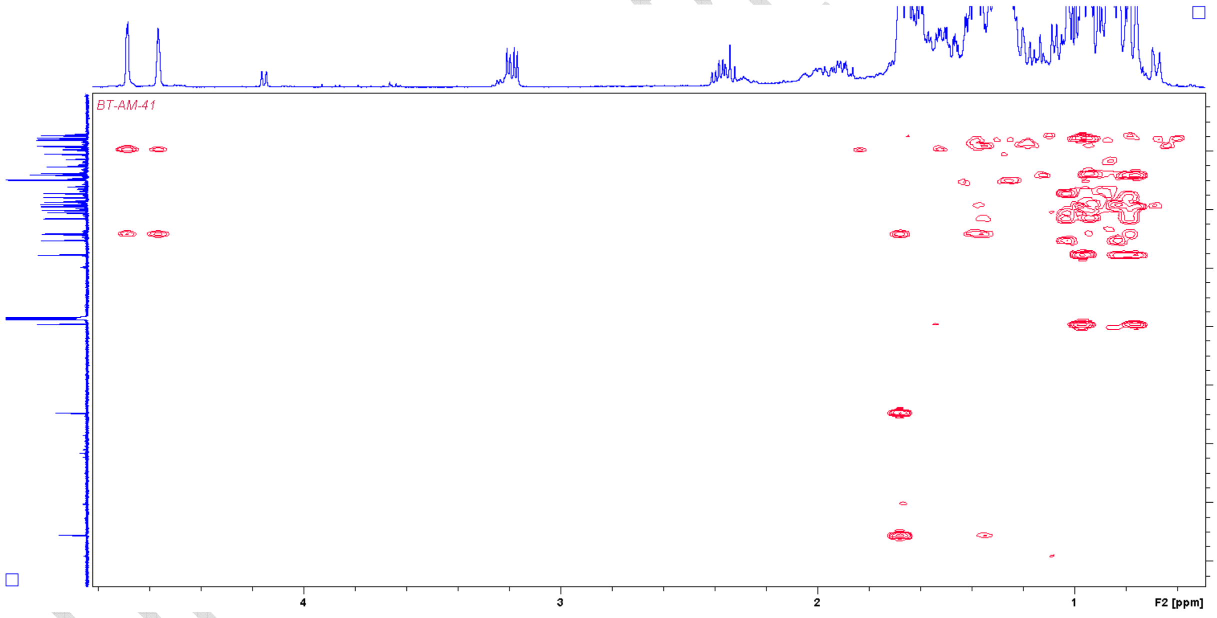
Spectrum 3.3: ^{13}C NMR spectrum for compound 3 in $CDCl_3$



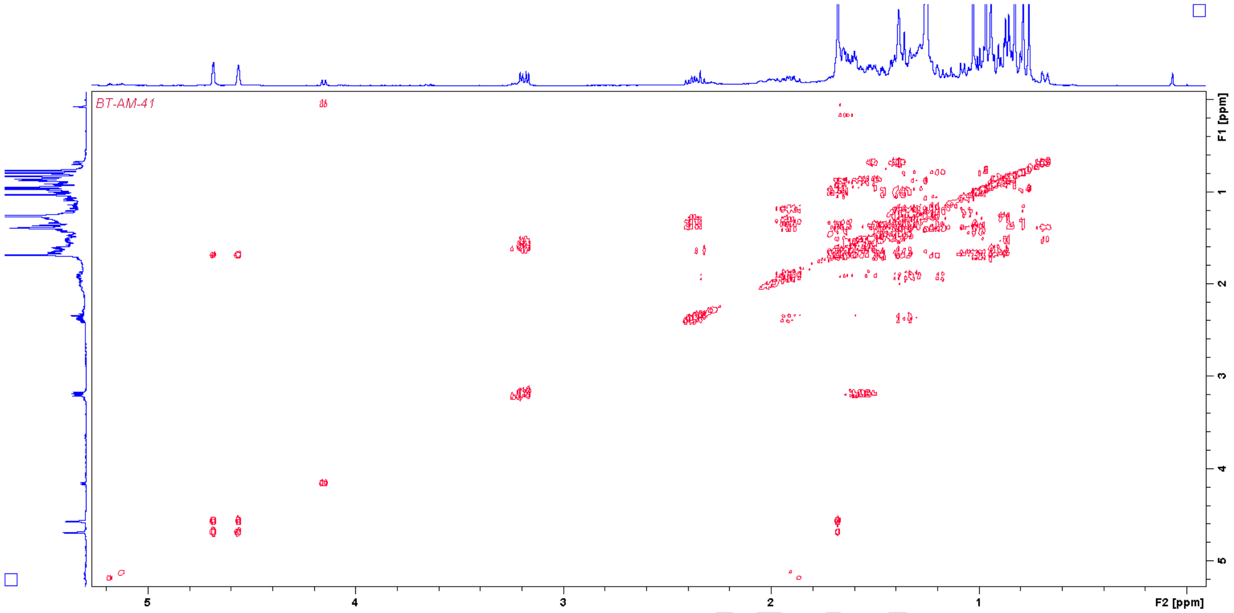
Spectrum 3.4: DEPT spectrum for compound 3 in $CDCl_3$



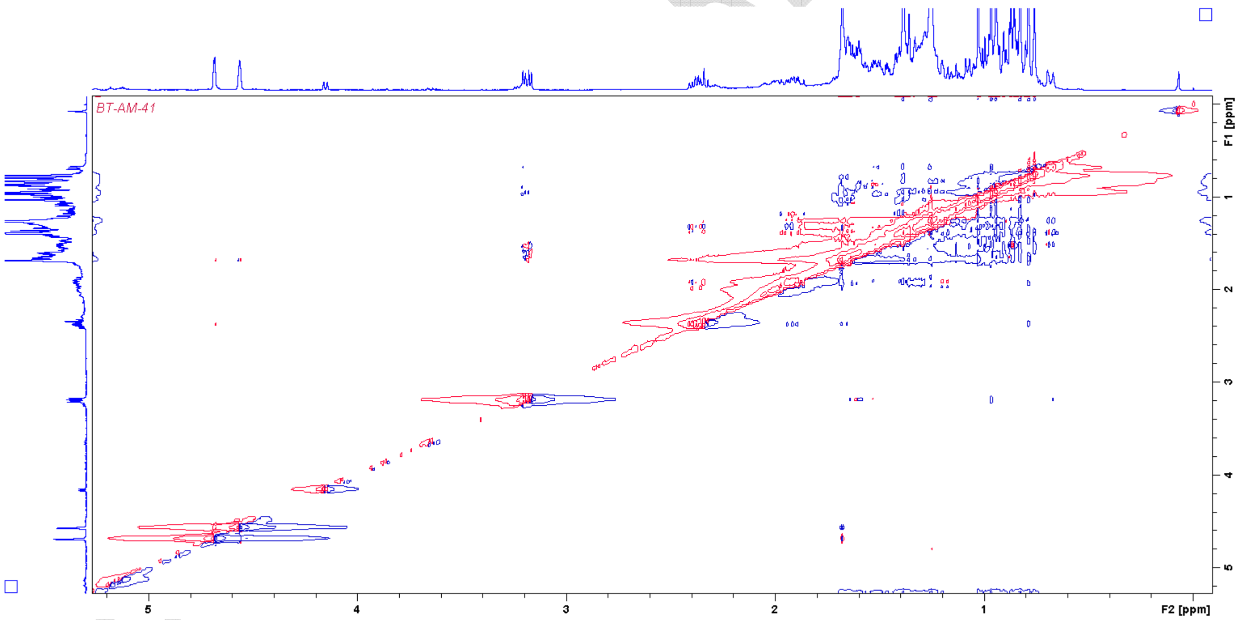
Spectrum 3.5: HSQCDEPT spectrum for compound 3 in $CDCl_3$



Spectrum 3.6: HMBC spectrum for compound 3 in $CDCl_3$



Spectrum 3.7: COSY spectrum for compound 3 in $CDCl_3$



Spectrum 3.8: NOESY spectrum for compound 3 in $CDCl_3$