https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 1 (2025); 372-388
e-ISSN:0198-2734 p-ISSN:0198-5680
doi.org/10.33003/chemclas_2025_0901/030

Isolation and Characterization of Pheophytin B from Ethyl Acetate Extracts of *Musa acuminata calla* (*Banana blossom*)

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Abstract

The research aims to isolate the secondary metabolites in order to discover the bioactive compound(s) present. *Banana blossom* was extracted using ethyl acetate as solvent. The crude extract was subjected to isolation using column chromatography. Fractions obtains were collected from the column and subjected to thin layer chromatography to confirm their purity for separation of the component combination by applying a thin stationary phase supported by an inert backing, yielding BF 59 fraction. The fraction BF 59 was characterized using FTIR, NMR and HR-LCMS. Results of FTIR analysis show absorption bands at 2929.80, that is characteristic of N-H stretching. Absorption at 970.52 is due to cyclic olefinic –HC= CH- structure, 1683.18 is a bending frequency for C=O, 2335.00 is a bending frequency for O=C-O and 2861.87 assigned to C-H structure. 1451.24 is a bending frequency for cyclic CH_3 and 2861.87 for –CH. The absorption frequency at 724.02.22 is typical of aromatic. The ^{1}H NMR spectrum was typical of a pheophorbide derivative with a phytol chain. The spectrum showed four deshielded protons at δ_H11.06(1H, *s*), 10.26(1H, *s*), 9.53(1H, *s*) and 8.56(1H, *s*) and two methyls between 3.00~4.00 ppm. The rest of the protons were for other methyl groups and methine as well as methylene protons. The ^{13}C NMR spectrum gave 55 carbon signals including an aldehyde carbonyl carbon at δ_C187.63, and ester carbonyl carbons at 174.0, 170.7, and 169.0. There were also C=N carbons at 159.0, 146.5 and 163.1 indicating the presence of a porphyrin group and possibly a pheophytin class of compound. Based on these correlations and comparison with literature data, fraction BF-59 was identified as pheophytin B. The HR-LCMS revealed a quasi-molecular ion $[M+H]^+$ at *m/z*885.5554 (Calc. 885.5530), indicating a molecular formula of $C_{55}H_{72}O_6N_4$. . Findings from the study established the isolation of pheophytin B from *Musa acuminata* calla that provide a scientific rationale for the plant part as a potential source of antimicrobial agent which in turn contribute to drug discovery and development in the pharmaceutical sector.

Key words: Bioactive compound, Chromatography, Diabetes, Extract, *Musa acuminata*

Introduction

Bananas are the one of the most widely consumed fruits in the world because of its taste, nutritional value and potential health benefits. In terms of monetary value, it is the fourth most valuable food crop on the planet. Bananas are consumed in greater quantities in the United States than apples and oranges combined [1]. The banana plant, which is commonly recognized as a gift from nature to mankind, is best known for its fruit, which has huge

nutritional and health benefits, but it also has the potential to be regarded as a functional food or superfood due to its high nutrient content and therapeutic value [2]. When compared to other food plants, the banana plant stands out since it is one of the few plants in the world whose entire structure may be used for food or non-food uses. Fruits, the most prevalent form of consumption, are consumed both raw and cooked. In addition to being delicious, fruits are also incredibly nutritious. They are high in potassium, fiber, magnesium, vitamin C, B6, and antioxidants. Banana leaves are used for a variety of purposes in different countries. For example, in India, fresh banana leaves are used as plates to serve food at weddings and sacred thread ceremonies, whereas in China, fresh banana leaves are used to wrap meat and heat it at low temperatures because they believe it adds more flavor [2].

Banana pseudo stem (so called because it has an appearance of tree trunk) can be utilized for its fibre content which can be used as natural threads. The pulp can be used to manufacture rope, paper, place mats and other [3]. Potassium, calcium, magnesium, phosphorus, and iron are all abundant in it. Vitamins A, C, and B6 are also present. Apart from being high in nutrients, it also has therapeutic benefits, such as the juice of the banana pseudo stem, which is thought to help diabetic people control their blood sugar levels. It has also been discovered that it heals stomach ulcers and relieves urinary discomfort [4]. Banana peels can be used for non-food purposes as a cattle feed or as a natural fertilizer as the peels are rich in nutrients which in turn enrich the soil and make it more fertile. Just like the other parts of banana plant there is one more part which is very nutritious and possesses various health benefits, banana blossom (aka banana flower or banana heart).

Musa paradisiaca (banana flower) is a highly nutritious edible flower. It is nutrient-dense and antioxidant-rich, with numerous health benefits. Banana blooms are typically dried to extend their shelf life [5]. In southern Indian states many people consume this blossom as a curry consumed with rice and wheat bread. According to [5], the research found that banana blossom contains abundant dietary fibre (5.74 g/100 g) which helps to maintain our body health, to reduce the cholesterol level and protect our body from obesity.

Banana blossoms are strong in antioxidants including phenolic and flavonoids, which help to enhance human resistance to a number of illnesses. The flower of the banana blossom is extracted using chloroform, water, and ethanol. According to [5], banana blossom has a high nutritional content as well as health benefits, such as treating bronchitis, dysentery, and ulcers. Banana blossom can be used as a food ingredient instead of being discarded as agricultural waste. [6] states that banana plants are cultivated in many countries and are larger producer with a production to 88 million metric tons. Banana blossom is considered as a by-product of banana cultivation. Banana plant as such is beneficial in many ways. The plant gives anti spoilage effect to the food products. Fibres extracted from the pseudo stem are used for decorative purpose.

Ruvini *et al*. [7] also states that banana blossoms of various varieties are low in protein. Banana roots are used for culinary purpose in Malaysia, Indonesia, India and Sri Lanka. It is also quoted by [7] that the flowers can be used to treat dysentery, ulcer and many more. Banana blossoms can also be used as an alternative to diabetes treatment by acting as an enzyme inhibitor derived from plant extract. The blossom are also said to have antioxidative and antibacterial properties, according to previous study [5, 8].

Materials and Methods

Plant collection

Banana Blossom was collected from Song Local Government Area of Adamawa State. The plant part was authenticated and voucher number assigned by the Department of Biology (Botany), Faculty of Life Sciences, Modibbo Adama University Yola, Nigeria.

Sample Preparation

The Banana Blossom was thoroughly washed with tap water to remove dusts and impurities, while some impurities are carefully picked and thereafter rinsed with tap water again. The plant is air dried at ambient temperatures to a constant weight over a period of four weeks. It was pulverized powdered using pestle and mortar.

Sample Extraction

The extraction was carryout using 1 kg of powdered sample, poured into a glass container and macerated successively with 1200 mL each of distilled hexane, ethyl acetate and methanol. Each extraction cycle was carried out for three days with occasional shaking after which it was filtered and the filtrate evaporated at room temperature to obtain crude extracts [9].

Column Chromatography

Extracts of banana flower 6 g of distilled hexane, ethyl acetate, and methanol extracts were independently adsorbed onto Celite by dissolving the extract in tiny volumes of solvent and thoroughly mixed with 10 g of Celite, and the adsorbed extract was crushed into powdery form after drying completely. A small piece of cotton wool was placed at the bottom of the column andtapped gently with a rubber applicator. Slurry of silica gel was made by mixing 50 g (230-400 mesh ASTM) with n-hexane and stirred with a glass rod. This allowed to cool for about fifteen minutes then quickly transferred into the column. More of the solvent was added to rinse the slurry down the column and tapped with a rubber applicator to make the bed compact and remove any air bubbles. A beaker was placed under the column, and then the tap was allowed to run until the solvent got close to the top level of the bed. The sample was then loaded into the column carefully while the tap was closed. To start elution, a solvent mixture was added. 20 cm^3 vials was used to collect fractions from the column [9].

Thin Layer Chromatography (TLC)

The hexane, ethyl acetate and methanol extracts of the plant was subjected to thin layer chromatographic analysis to find the solvent system that will give better separation of the components therein. This will achieve use of pre-coated TLC plates. A micro quantity of the sample

solution was spotted on TLC pre-coated (MERCK) plates, and developed with various ratios of organic solvents (hexane, ethyl acetate, methanol, chloroform) taking into consideration their polarity. The solvent systems that separate the components to high degree of resolution were considered. The plates were visualized under visible and UV-light (366 and 254 nm). The plates were sprayed with 10% sulphuric acid in methanol and heated at 100 ºC for 1-5 minutes. Appropriate fractions are collected from the column which was monitored by TLC. The fractions with the same retention factor Rf values ($\frac{\text{distance move by solvent}}{\text{distance move by sample}}$) was pooled together, concentrated and the spots and observation were [9, 10]

Spectroscopic Measurement

Spectroscopic analysis of the final products of the fractions was carried-out. Characterization using NMR-spectrophotometer at SIPBS, University of Strathclyde, Glasgow, UK. on a JEOL-LA-400MHz FT-NMR spectrophotometer.

Result and Discussion

The ethyl acetate extract (6.8 g) was adsorbed unto Celite and subjected to column using 30g of silica gel (GF 254 MERCK). The column was eluted using hexane and ethyl acetate (40 mL) in gradient. The 130 fractions obtain were examined by TLC and similar fractions were combined based on their TLC profiles. The BF 59 was pure as presented by TLC plate. Below are results from BF 59 extracted from the spectrum figures.

Table 1: FT-IR absorption bands and description

FT-IR absorption bands	Analysis of the observed absorption	Description of absorption
2929.80	N-H	Amine
1683.18	C=O	Conjugated aldehyde
970.52	HC= CH-	Alkene
2335.00	O=C-O	Ester
2861.87	C-H	Alkane
1451.24	C-H	Methyl group
724.02.22	C-H	Benzene derivative

The compound gave a molecular ion $[M^+H]^+$ at m/z 885 in its HR-LCMS corresponding to the molecular formula $C_{55}H_{72}N_4O_6$ (calc. 885). FT-IR spectroscopic analysis was observed absorption bands are 2929.80 that is characteristic of N-H stretching. Absorption at 970.52 is due to cyclic olefinic –HC= CH- structure, 1683.18 is a bending frequency for C=O, 2335.00 is a bending frequency for O=C-O, 1451.24 is a bending frequency for cyclic CH_3 and 2861.87 for –CH. The absorption frequency at 724.02.22 aromatic (Table 1).

Table 2: Experimental and literature data for Pheophytin B. ^{13}C NMR

Position	[11]	[12]	Experimental data
1	143.25	143.54	143.54
2	132.00	132.31	132.80
2^{1}	12.10	12.26	12.06
3	136.80	137.75	137.93
3^{1}	128.60	128.62	128.63
3^{2}	123.90	123.57	123.53
4	136.70	136.16	137.54
5	103.20	101.36	101.51
6	151.20	151.22	151.11
7	132.80	132.46	132.83
7^{1}	187.70	187.77	187.63
8	159.00	159.48	159.33
8^{1}	19.10	19.11	19.02
8^{2}	19.50	19.41	19.35
9	146.50	147.16	146.79
10	106.00	107.00	106.89
11	140.50	138.00	138.52
12	131.70	132,19	132.18
12^{1}	12.40	12.07	12.48
13	131.70	129.69	128.63
13^{1}	187.90	189.50	189. 50
13^{2}	64.00	64.53	64.57
13^{3}	169.00	169.25	169.30
13^{4}	52.90	52.99	52.98
14	151.20	150.80	150.70
15	103.30	104.90	104.95
16	163.10	164.00	164.05
17	50.60	51.27	51.36
17^{1}	31.90	31.16	31.25
17^{2}	29.80	29.68	29.66
17^{3}	170.70	174.014	174.01
18	44.14	50.08	50.13
18^{1}	22.80	23.06	22.61
19	174.80	172.80	172.80
20	93. 68	93.35	93.35

Continuation of Table 2: Experimental and literature data for Pheophytin B. ^{13}C NMR

Position	[11]	[12]	Experimental data
P 1	62.00	61.51	61.55
P 2	117.80	117.57	117.68
P 3	143.50	142.97	143.66
P 4	39.50	39.77	39.35
P 5	25.00	24.75	25.00
P 6	36.90	36.6	36.64
P 7	32.10	32.72	32.73
P 8	37.60	37.35	37.39
P 9	21.60	24.96	24.77
P 10	37.60	37.22	37.42
P 11	32.10	32.58	32.62
P 12	37.60	37.27	37.42
P 13	24.40	24.38	24.77
P 14	39.90	31.91	39.81
P 15	27.90	27.94	27.96
P 16	22.70	22.70	22.70
P 17	22.90	22.60	22.61
P 111	19.70	19.70	19.35
P 71	19.63	19.63	19.72
P 31	16.27	16.27	16.29

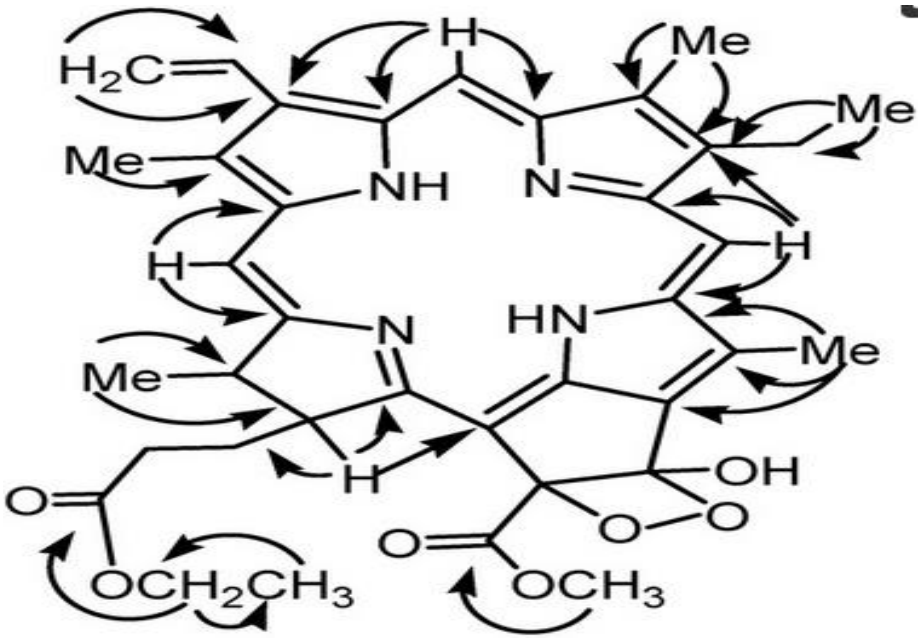
The ^{1}H NMR spectrum (Table 3) was typical of a pheophorbide derivative with a phytol chain. The spectrum showed four deshielded protons at δ_H11.07(1H, *s*), 10.26(1H, *s*), 9.54(1H, *s*) and 8.58(1H, *s*) and two methyls between 3.00~4.00 ppm. The rest of the protons were for other methyl groups and methine as well as methylene protons.The ^{13}C NMR spectrum Table 2gave 55 carbonsignals including an aldehyde carbonyl carbon at δ_C187.60, and ester carbonyl carbons at 174.00, 170.70, and 169.00. There were also C=N carbons at 159.00, 146.50 and 163.10 indicating the presence of a porphyrin group and possibly a pheophytin class of compound.

The 2D NMR spectra were used to confirm the structure as follows: COSY correlations showed couplings between neighboring protons and identified the presence of ethylene and ethyl side chains as well as five methyl doublets indicating the presence of five CH_3-CH groups.

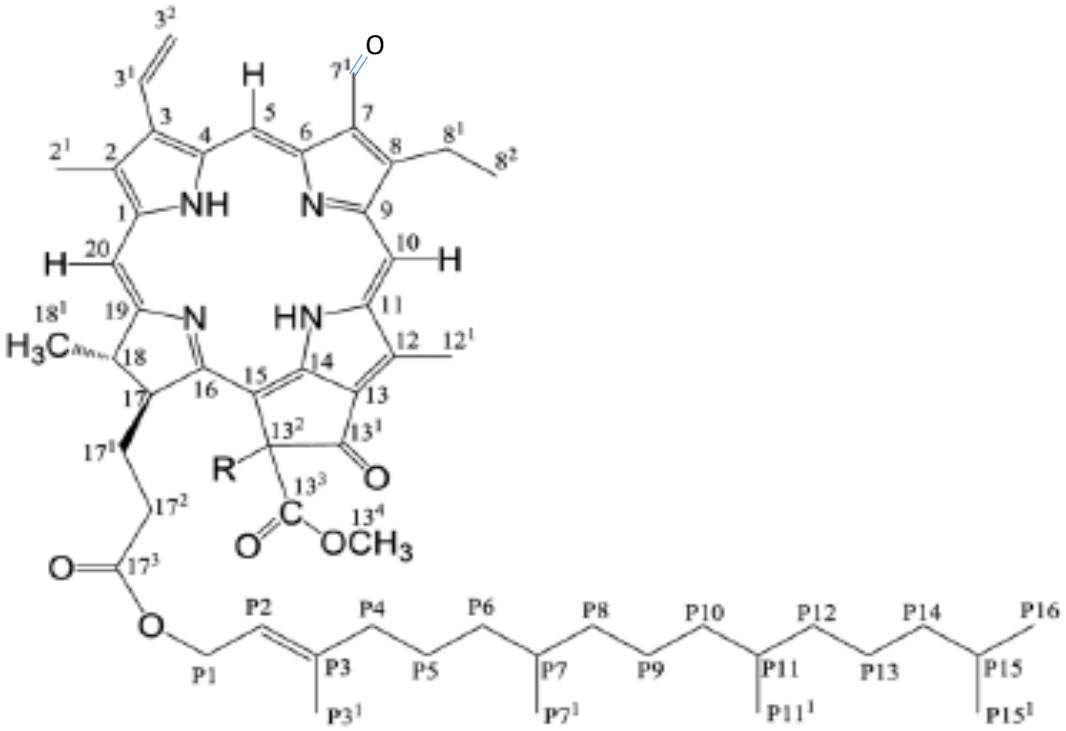
The HSQC spectrum identified the carbons bearing the respective protons in the compound especially the aldehyde proton at 11.07 coupling to the aldehyde carbonyl at 187.60 ppm and this coupled with the HMBC correlations were further used to confirm the structure. The proton at 11.07 (H-7^1) showed long-range correlations to C-6, and C-7 while the proton singlet at 6.25 (H-13^2) showed correlations to C-13^1, C-13^3 and C-14).

Table 3: Experimental and literature data for Pheophytin B. ^{1}H NMR

Position	[11]	[12]	Experimental data
1	–	–	–
2	–	–	–
2^{1}	3.60 (s)	3.37 (s)	3.38 (s)
3	–	–	–
3^{1}	8.00 (dd-J=17.65 Hz e 11.56 Hz)	8,00 (dd-J=17.65 Hz e 11.56 Hz)	8.56 (dd-J=17.65 Hz e 11.56 Hz)
3^{2}	6.20 (*Trans*) (d-J=17.83 Hz) e 6.22 (*Cis*) (d,J=10.90Hz)	6.37 (*Trans*) (d-J=17.83 Hz) e 6.22 (*Cis*) (d,J=10.90Hz)	6.39 (*Trans*) (d-J=17.83 Hz) e 6.22 (*Cis*) (d,J=10.90Hz)
4	–	–	–
5	10.30 (s)	10.36 (s)	9.53 (s)
6	–	–	–
7		–	
7^{1}	11.10 (s)	11.13 (s)	11.0a6 (s)
8	–	–	–
8^{1}	3.75 (s,2H)	3.82 (s,2H)	3.87 (s,2H)
8^{2}	1.80 (m)	1.72 (m)	1.70 (m)
9	–	–	–
10	10.26 (s)	9.64(s)	10.26 (s)
11	–	–	–
12	–	–	–
12^{1}	3.68 (s)	3.68(s)	3.68 (s)
13	–	–	–
13^{1}	–	–	–
13^{2}	7.50 (s)	6.23(s)	6.25 (s)
13^{3}	–	–	–
13^{4}	3.50 (s)	3.90 (s)	3.91 (s)
14	–	–	–
15	–		–
16	–	–	–
17	4.23 (m)	4.16 (m)	4.23 (m)
17^{1}	–	–	–
17^{2}	–	–	–
17^{3}	–	–	–
18	4.40 (m)	4.46 (m)	4.47 (m)
18^{1}	1.60 (s)	1.82(s)	1.82 (s)
19	–	–	–
20	8.64 (s)	8.53 (s)	8.63 (s)



HMBC (→) correlations Pheophytin B



Pheophytin B. structure

There were correlations to the ester carbonyls indicating a methyl ester and a phytol chain ester hence the compound must be a pheophytin. Based on these correlations and comparison with literature data, fraction BF-59 was identified aspheophytin B. The NMR chemical shifts were in agreement with previous reports [13].

Conclusion

From ethyl acetate exacts of *Musa acuminata calla* Pheophytin B was isolated using column chromatography and characterized by FT-IR, 1D NMR, and 2D NMR. Based on the findings of the study, it was established that the isolated pheophytin B provide a scientific rationale for the plant part as a potential source of antimicrobial agent which in turn contribute to drug discovery and development in the pharmaceutical sector.

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Appendices

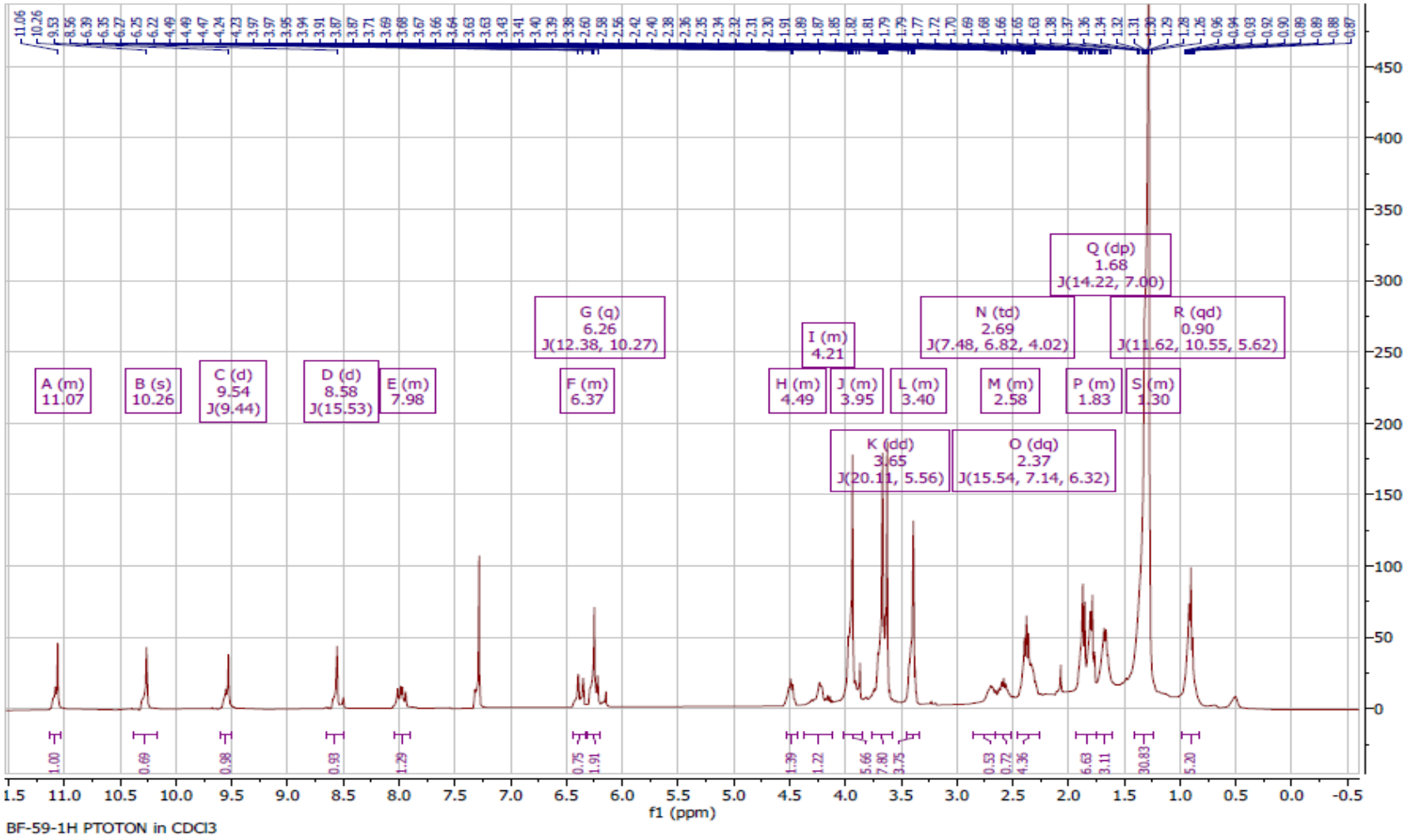

Figure 1. Proton NMR of Pheophytin B

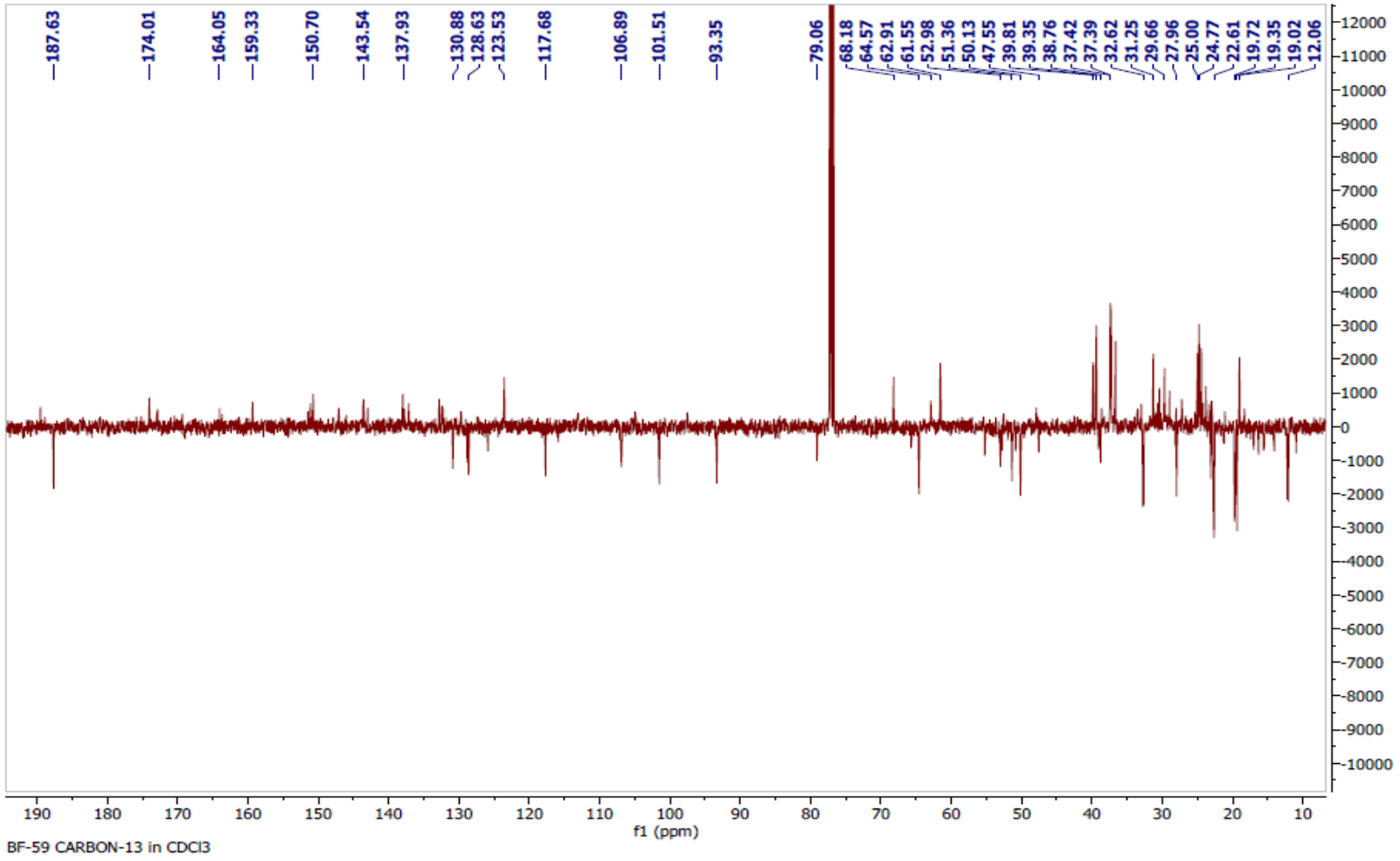

Figure 2. ^{13}C NMR of Pheophytin B

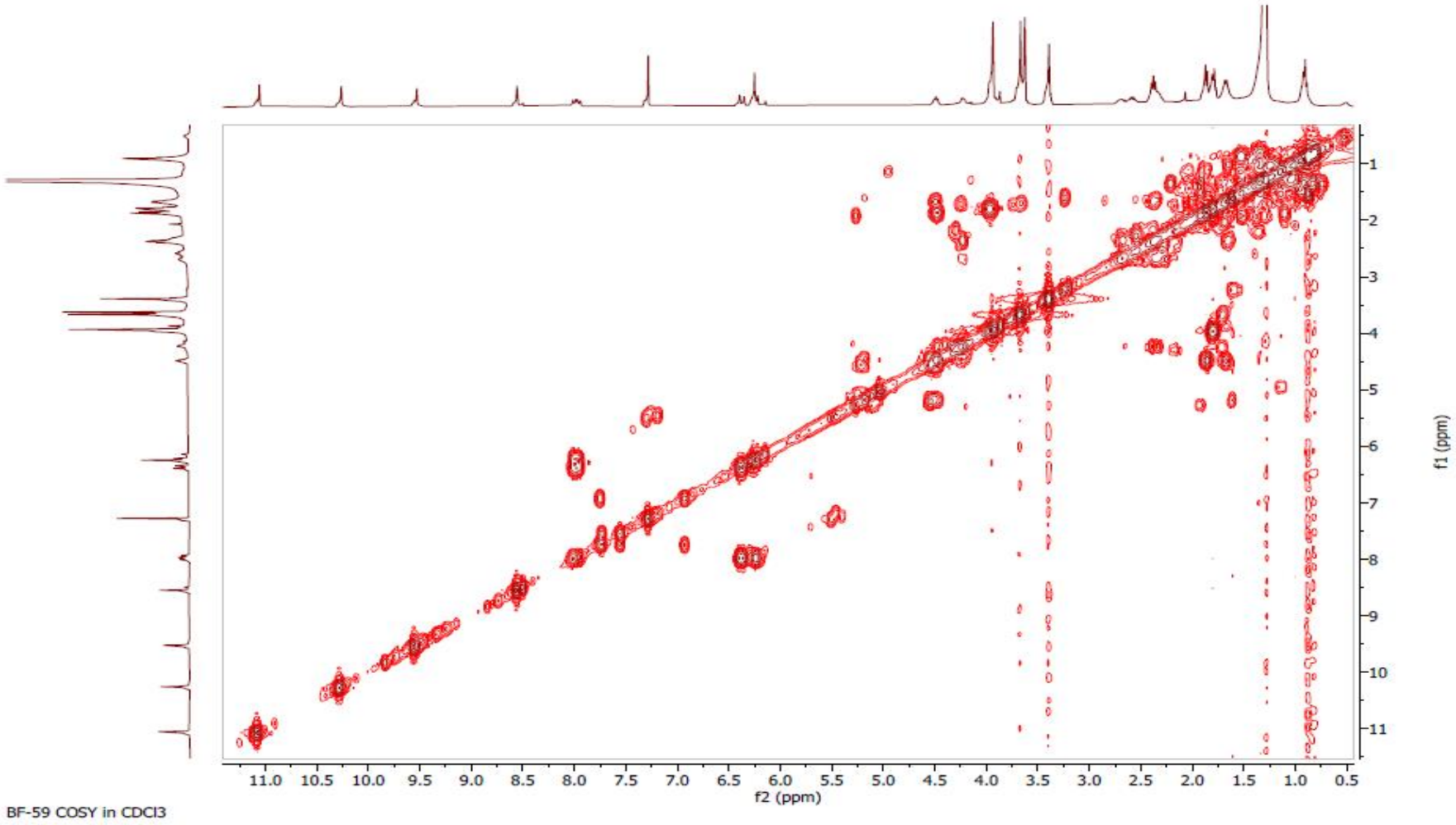

Figure 3. COSY 2D NMR of Pheophytin B

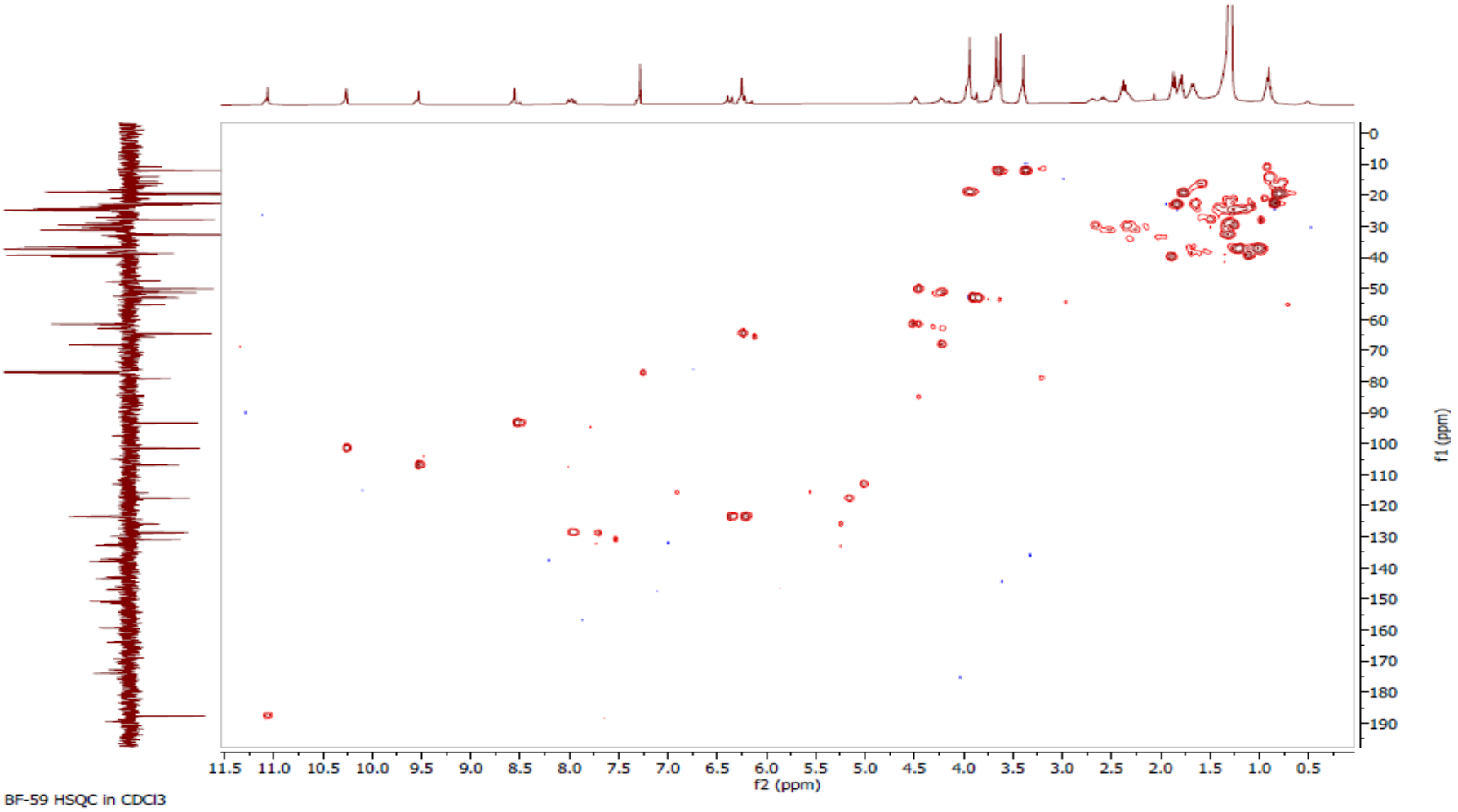

Figure 4. HSQC 2D NMR of Pheophytin B

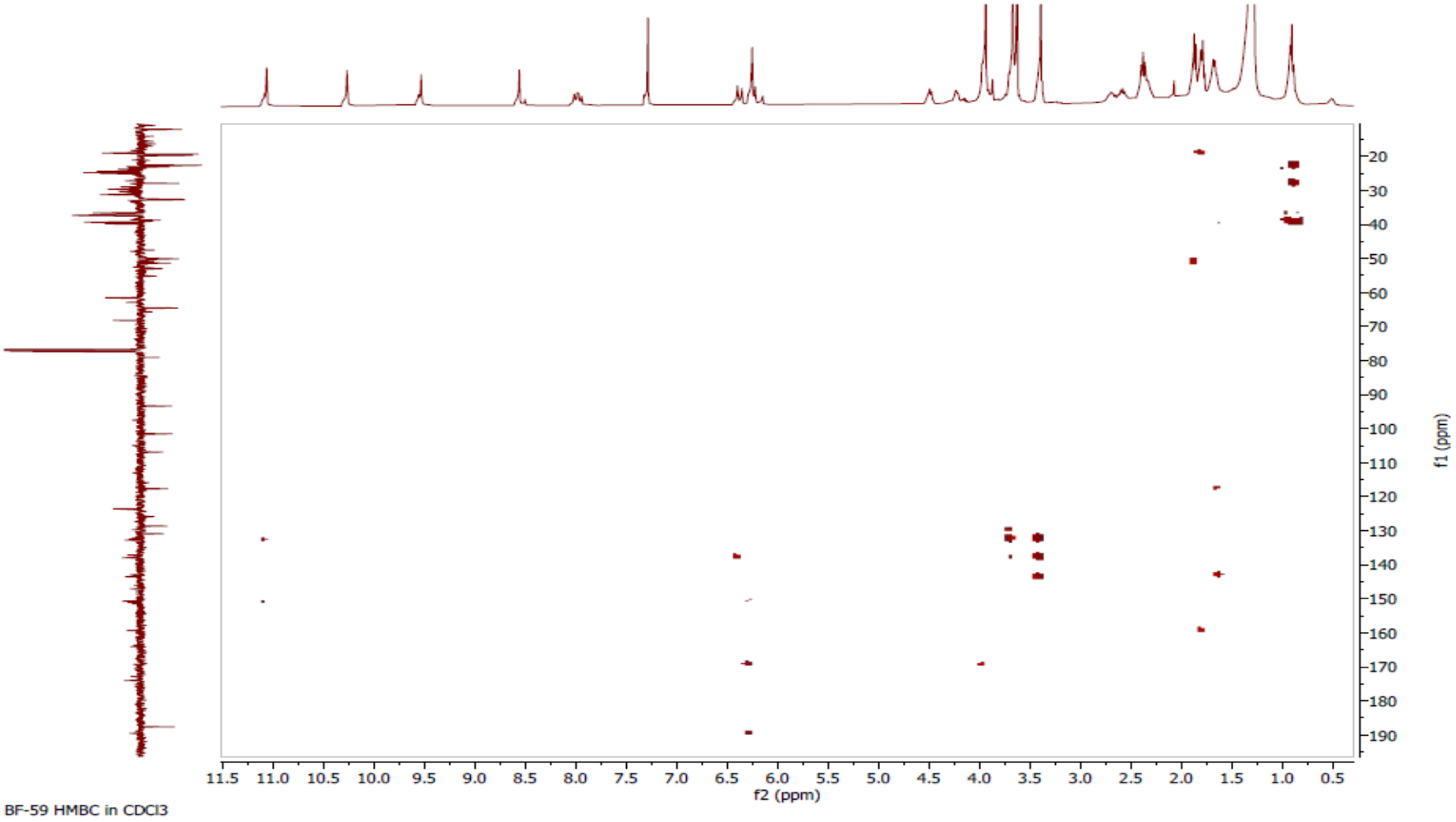

Figure 5. HMBC 2D NMR of Pheophytin B

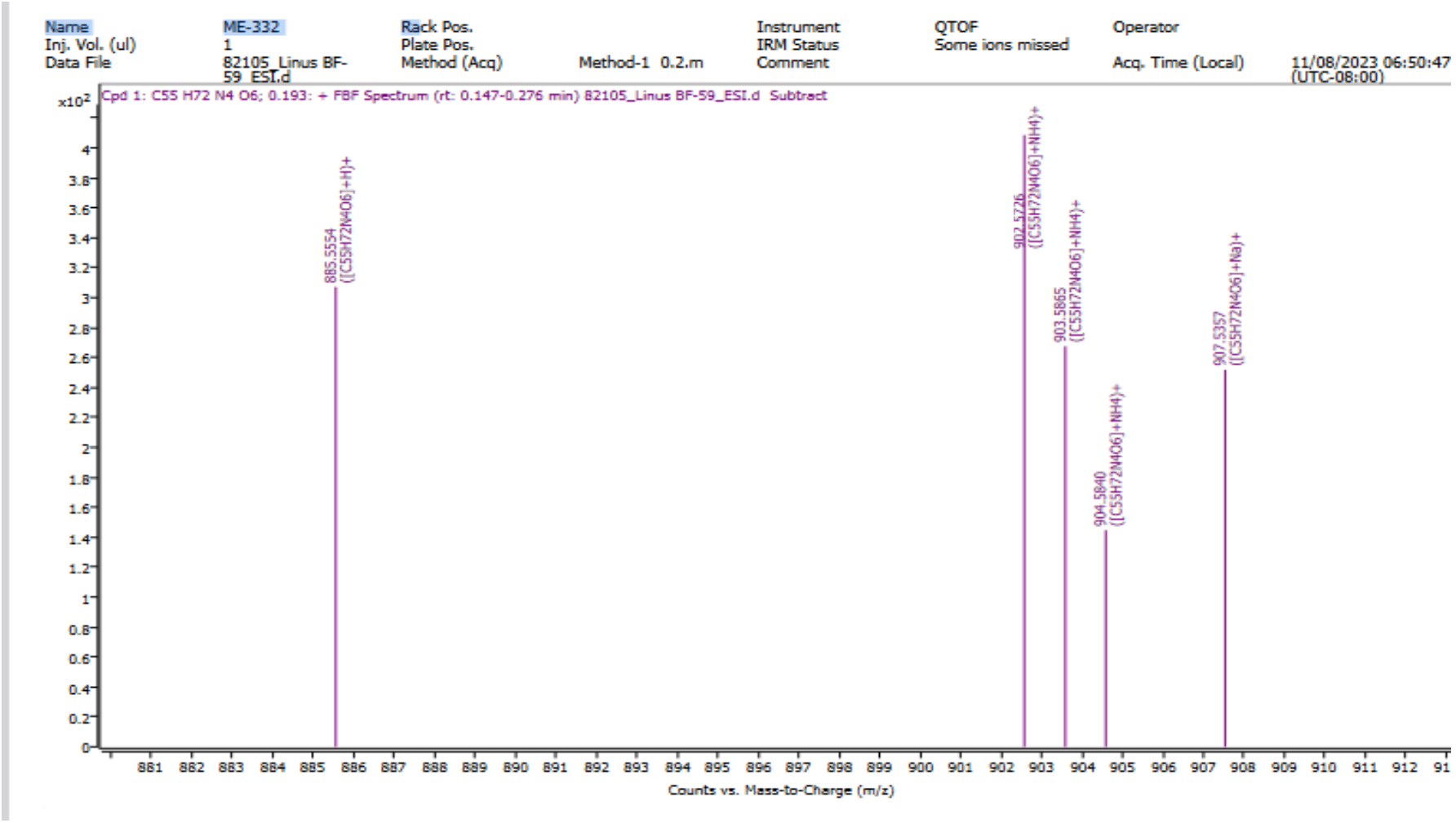

Figure 6. Mass spectrometry 2D NMR of Pheophytin B

Manual Peak Pick Results

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Date	Fri Oct 13 14:05:26 2023

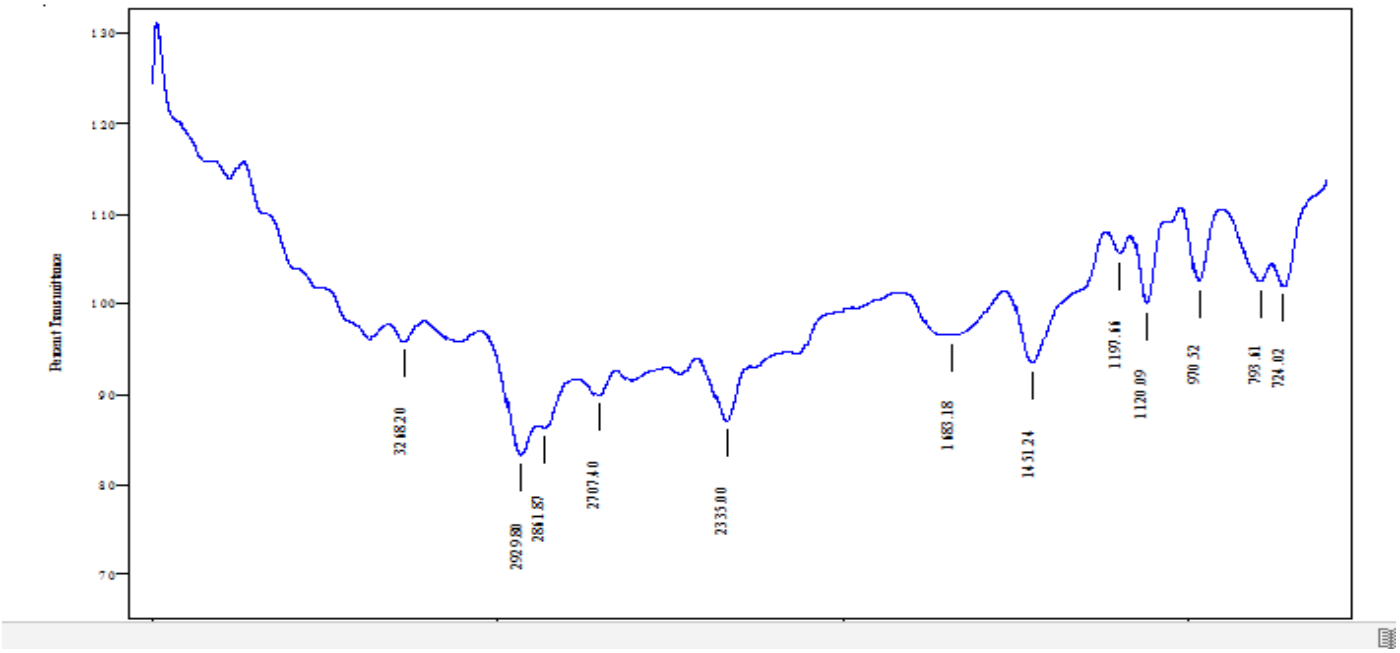

Figure 7. FT-IR of pheophytin B