

Synthesis of novel stilbene-coumarins derivatives, and antifungal screening of *Monotes kerstingii* specialized metabolites against *Fusarium oxysporum*

Ghislain Wabo Fotso^{1,4}, Bathelémy Ngameni^{2,3*}, Thomas E. Storr³, Bonaventure Tchaleu Ngadjui¹, Sibongile Mafu^{4**} and G. Richard Stephenson³

¹ Department of Organic Chemistry, Faculty of Science University of Yaoundé 1, P.O. Box 812 Yaoundé, Cameroon

² Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 1364, Yaoundé, Cameroon

³ School of Chemistry, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

⁴ Department of Biochemistry and Molecular Biology, University of Massachusetts

Correspondence: bath_ngameni@yahoo.fr; Tel.: +237 690 230 281 (B.N)
smafu@umass.edu, Tel.: +1 413 545 4083 (S.M)

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Abstract: *Fusarium* is one of the most toxigenic phytopathogen causing diseases and reduced agricultural productivity worldwide. Current chemical fungicides exhibit toxicity against non-target organisms, triggering negative environmental impact and danger to consumers. In order to explore the chemical diversity of plants for potential antifungal applications, crude extract and fractions from *Monotes kerstingii* were screened for their activity against two multi-resistant *Fusarium oxysporum* strains: *Fo32931* and *Fo4287*. Antifungal activity was evaluated by the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts using kinetic OD_{600 nm} reading by a spectrophotometer. The *n*-butanol fraction showed the best activity against *Fo4287*. We screened eleven previously reported natural compounds isolated from different fractions and a stilbene-coumarin 5-[(1*E*)-2-(4-hydroxyphenyl)ethenyl]-4,7-dimethoxy-3-methyl-2*H*-1-benzopyran-2-one (**1**) was the most active compound against both strains. Compound **1** was employed as a nucleophile with a selection of electrophilic derivatizing agents to synthesize five novel stilbene-coumarin analogues. These semisynthetic derivatives showed moderate activity against *Fo32931* with only the prenylated derivative exhibiting activity comparable to the natural stilbene coumarin (**1**), demonstrating the key role of the phenolic group.

Keywords: *Monotes kerstingii*, antifungal activity, *Fusarium oxysporum*, semisynthesis, stilbene-coumarins.

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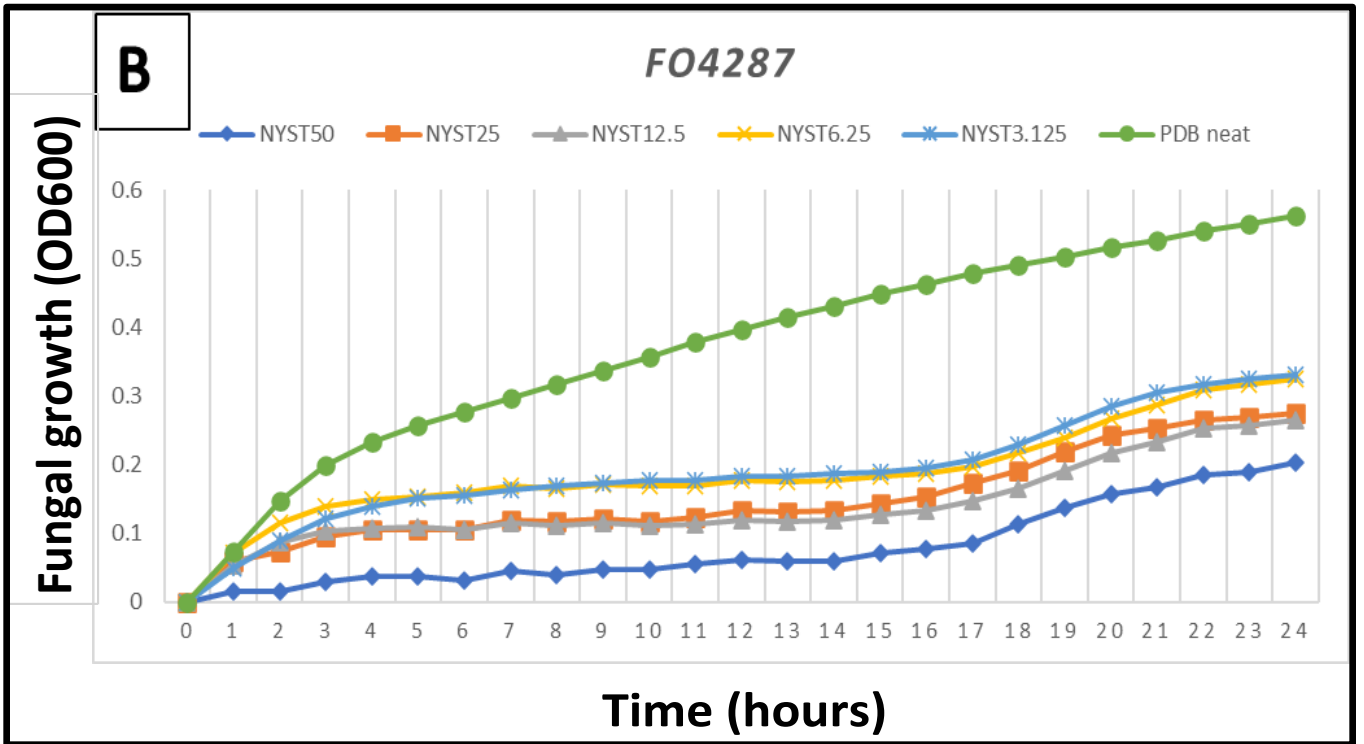
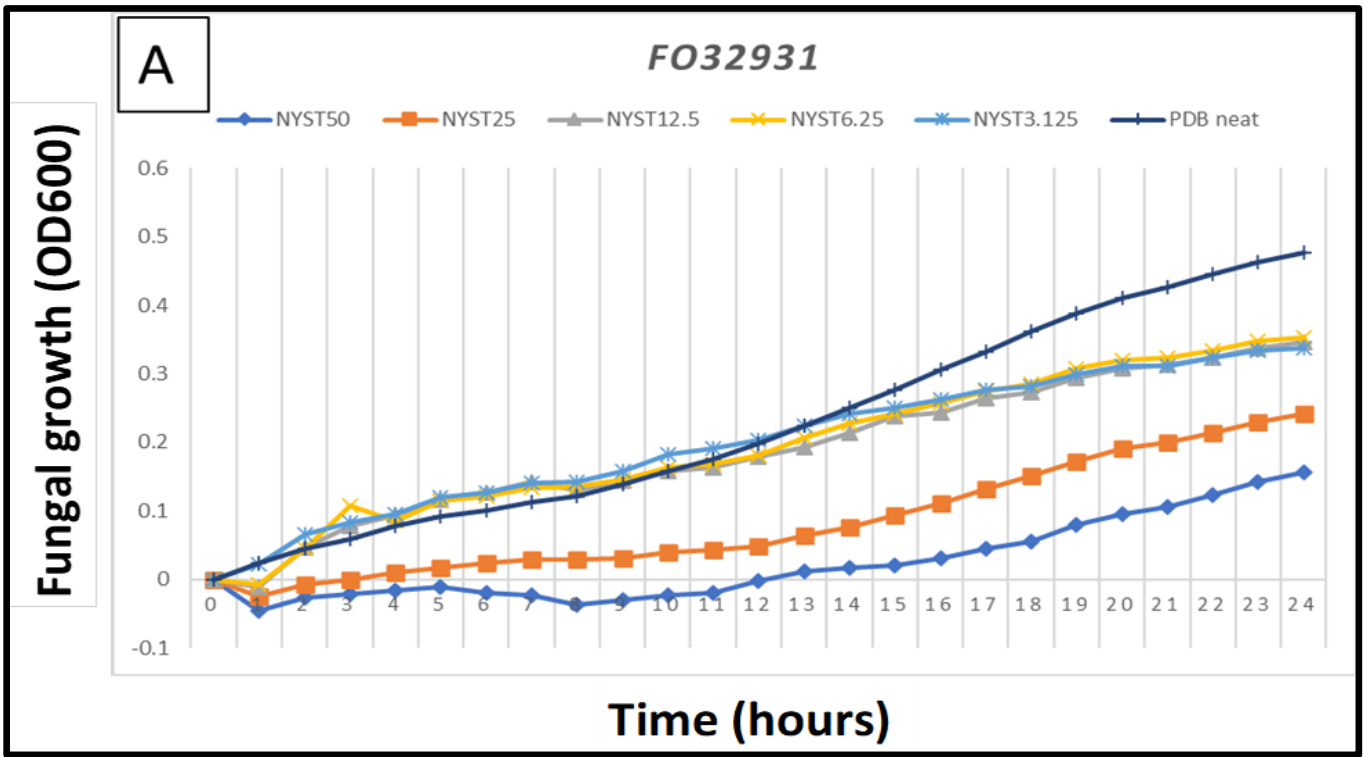


Figure S1: Fungal growth curves of *Fo32931* (A) and *Fo4287* (B) in PDB neat (negative control) and Nystatin at different concentrations (positive control)

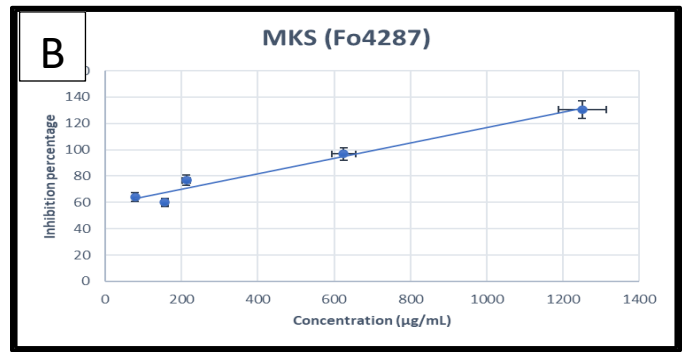
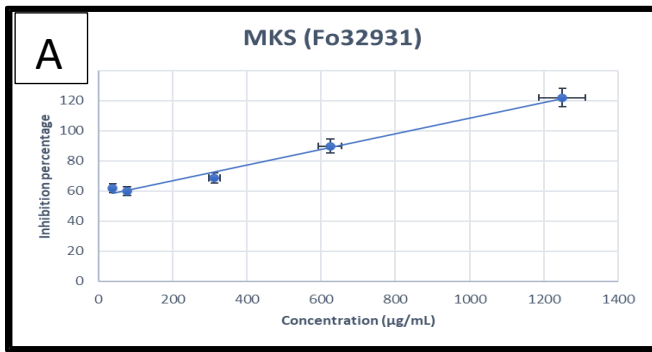


Figure S2: MIC of *Monotes kerstingii* stem bark crude extract against *Fo32931* (A) and *Fo4287* (B)

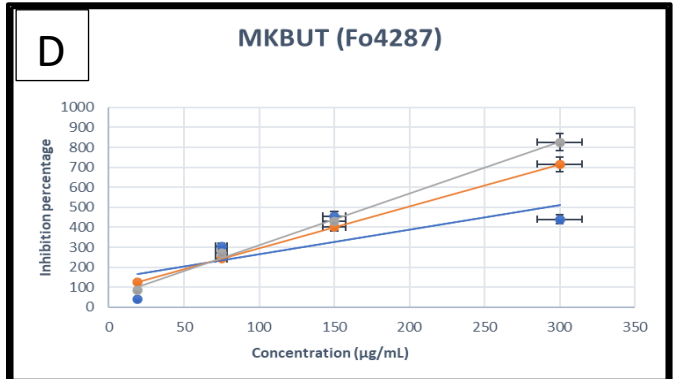
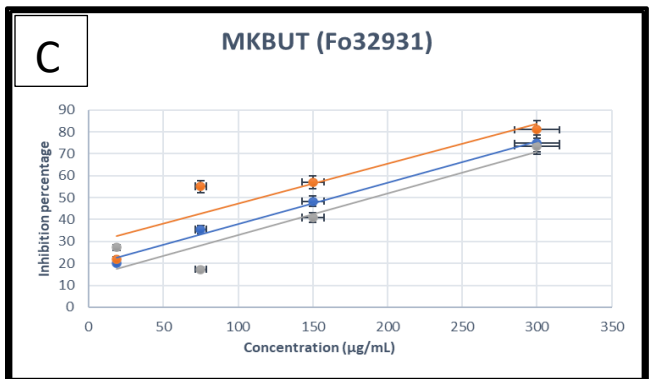
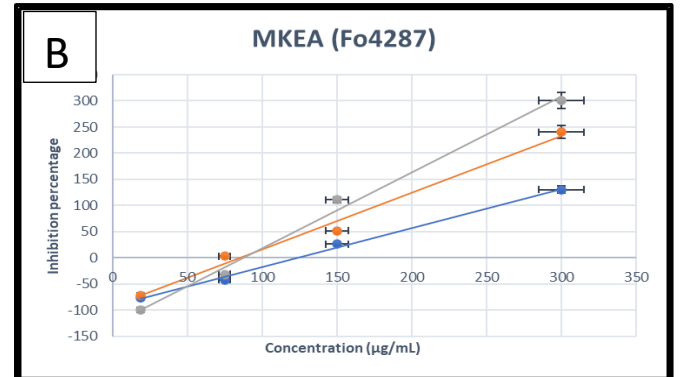
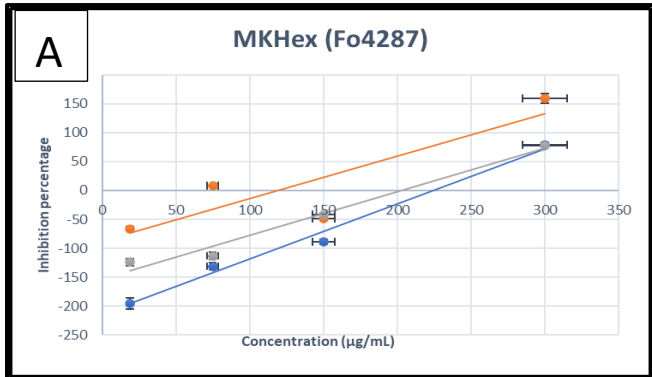


Figure S3: MIC of hexane fraction against *Fo4287* (A), ethyl acetate fraction against *Fo4287* (B), n-butanol fraction against *Fo32931* (C) and against *Fo4287* (D).

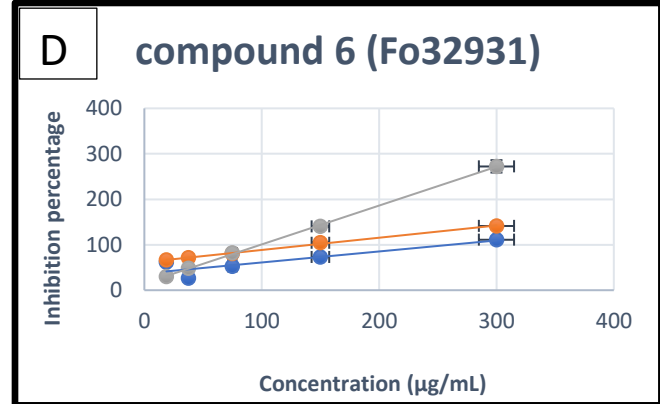
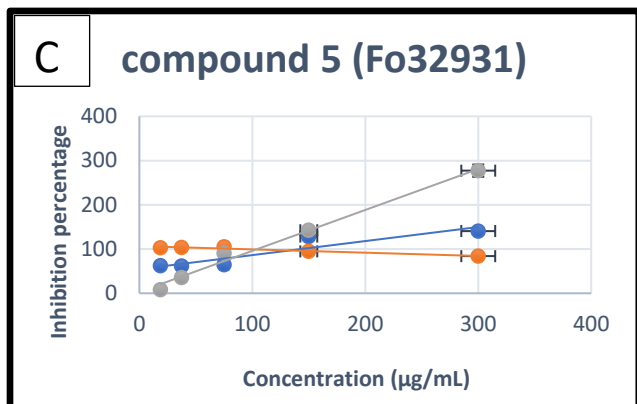
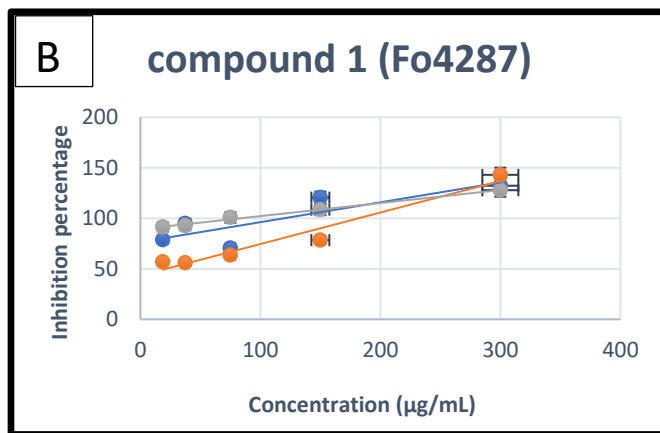
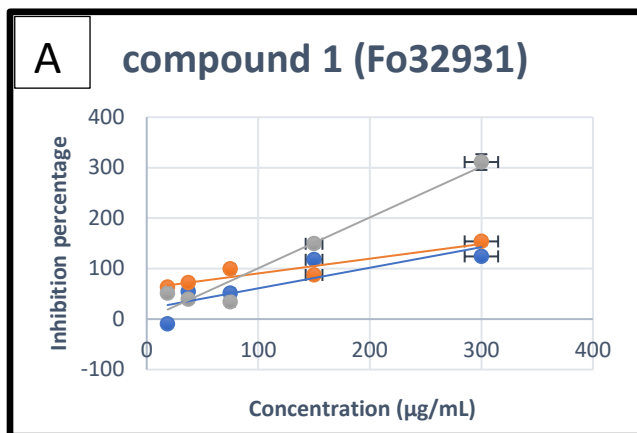


Figure S4: MIC of the most active natural compounds: stilbene-coumarin **1** (A) and (B); ellagic acid derivatives **5** and **6** (C) and (D) against *Fo32931* and *Fo4287*

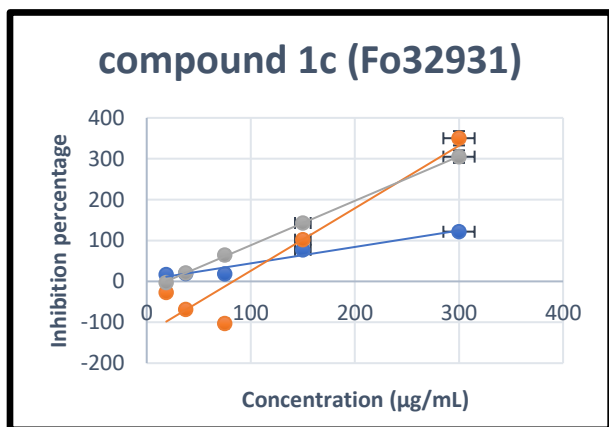
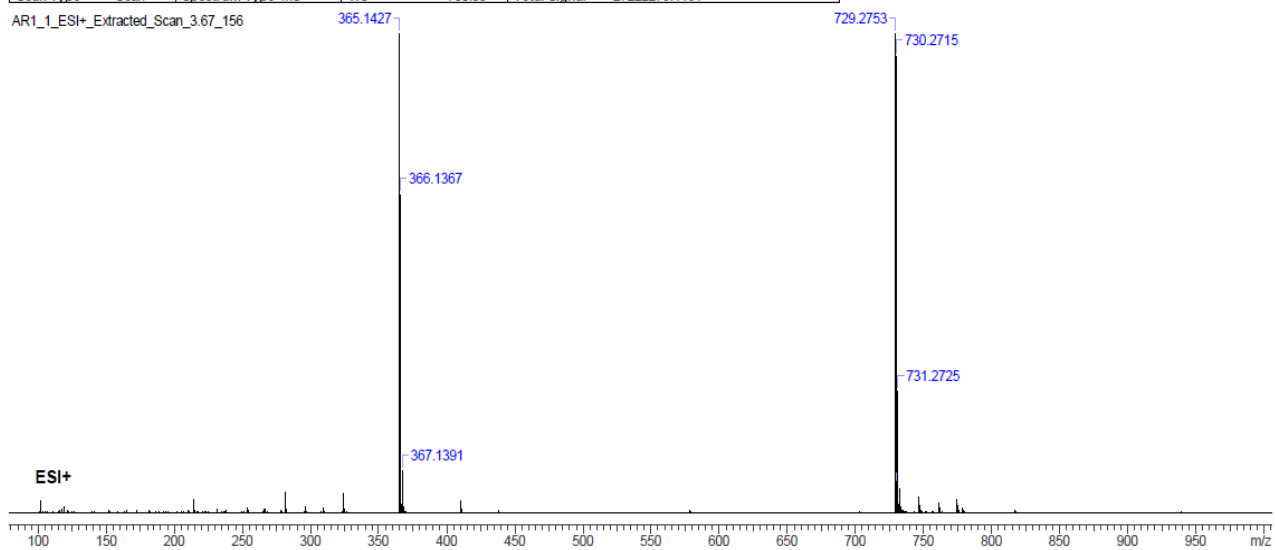


Figure S5: MIC of the most active semisynthetic compound **1c** against *Fo32931*

fragmentor voltage 130

Comment	130 V	Count	3017	Data Type	MS	Date	2019-08-23	File Name	D:\190823HRMS\AR1.d		
Instrument	Instrument 1	Ion Mode	ESI+	Plot Type	Stick	Retention Time	3.671	Sample	AR1	Scan	156
Scan Type	Scan	Spectrum Type	MS	TIC	750.33	Total Signal	2722279.1161				



Expected Mass (m/z)	Observed Mass (m/z)	Error (ppm)
365.1389	365.1427	10.407

Figure S6: HRESIMS spectrum of compound 1a

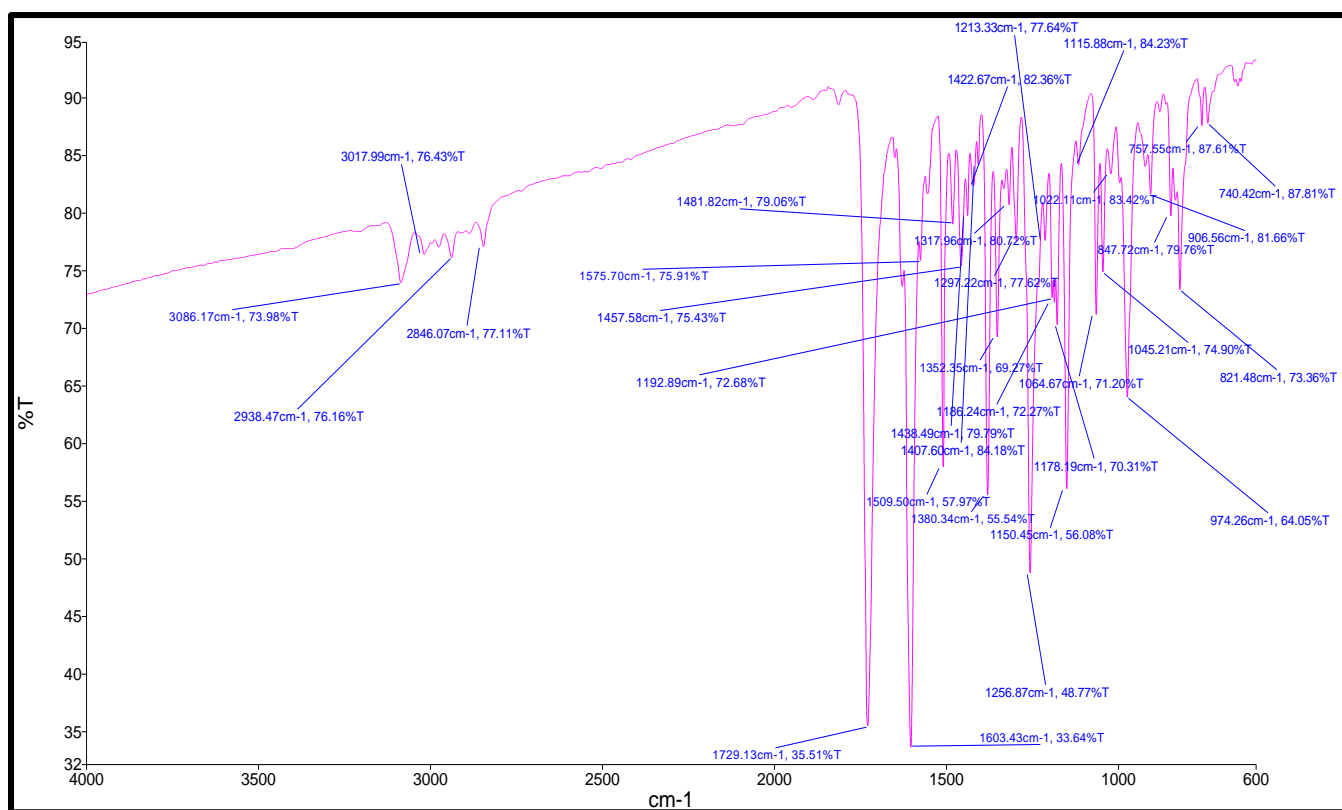


Figure S7: IR spectrum of compound 1a

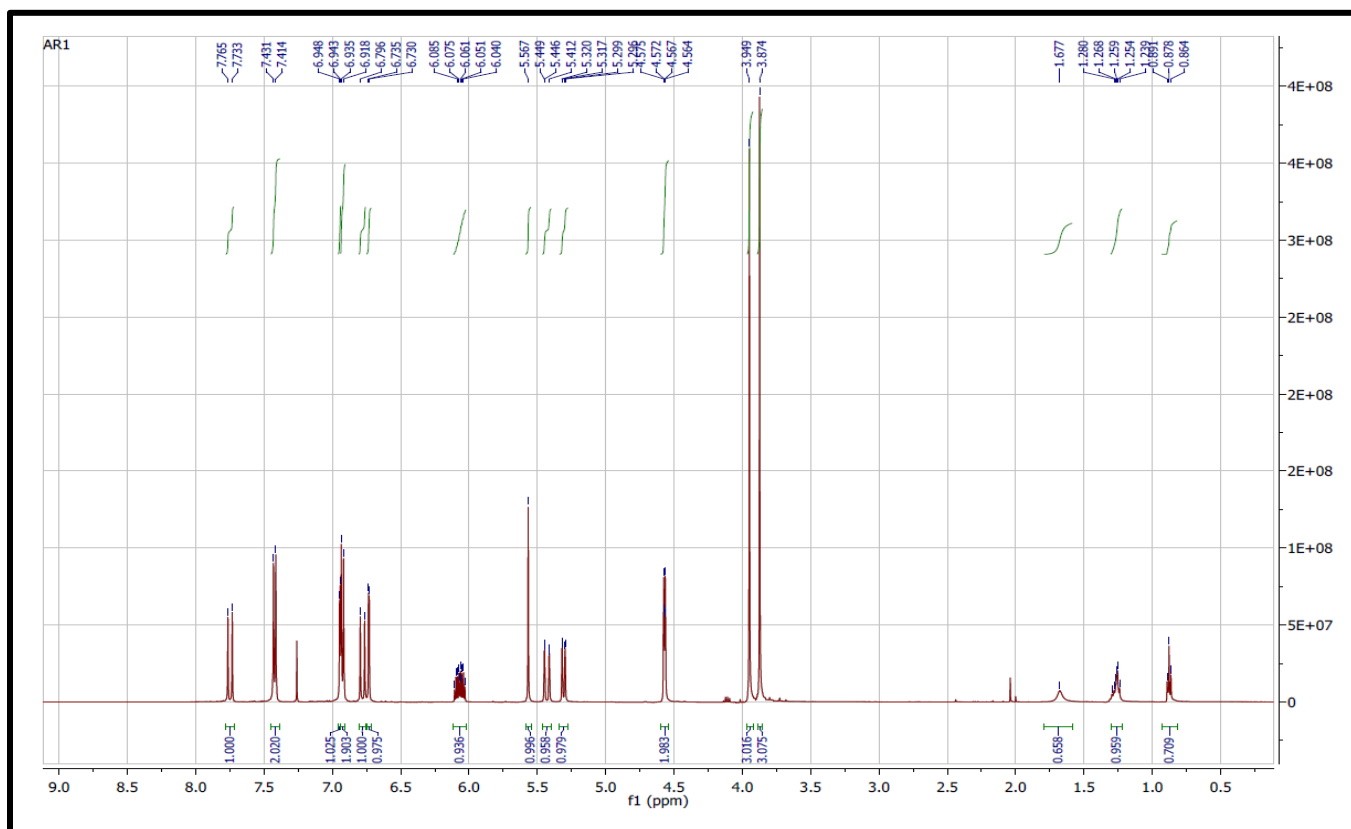


Figure S8: ^1H NMR (CDCl_3 , 500 MHz) spectrum of compound 1a

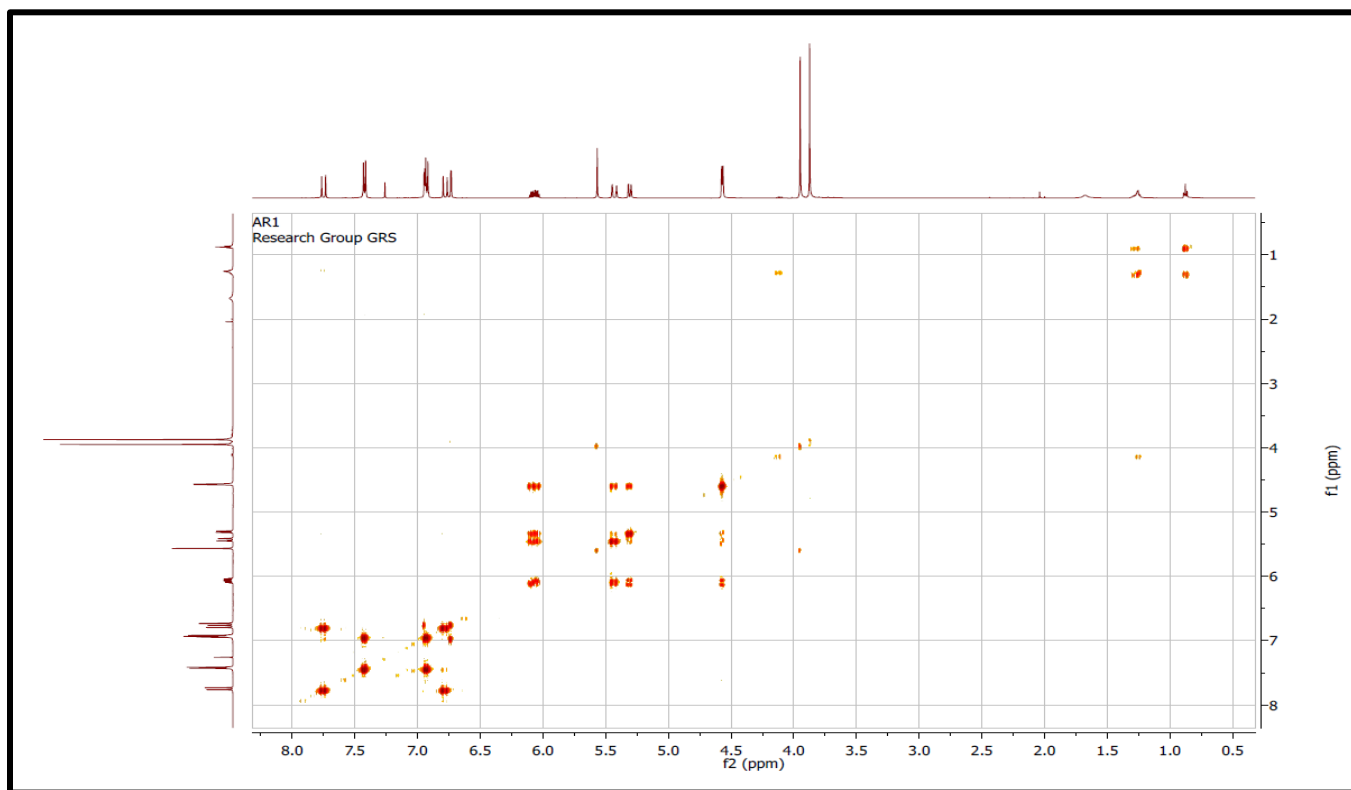


Figure S9: COSY spectrum of compound 1a

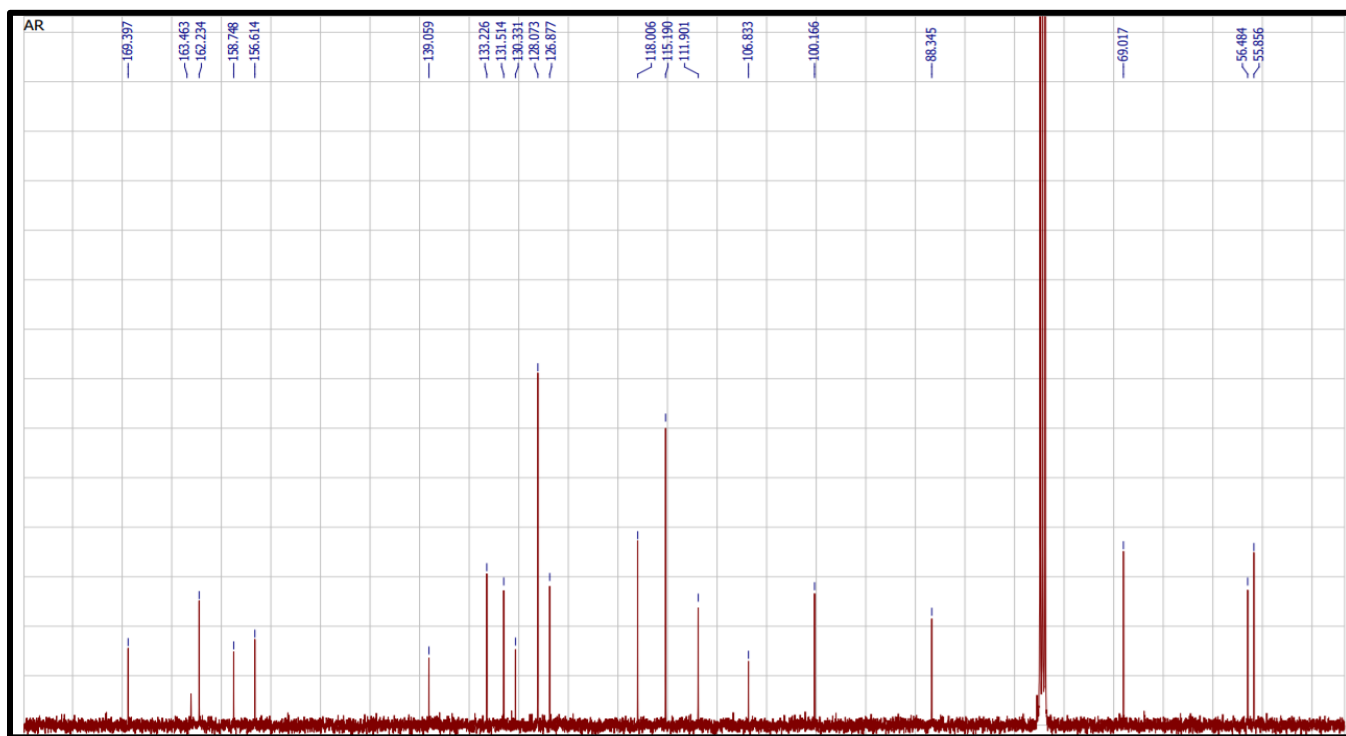


Figure S10: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of compound **1a**

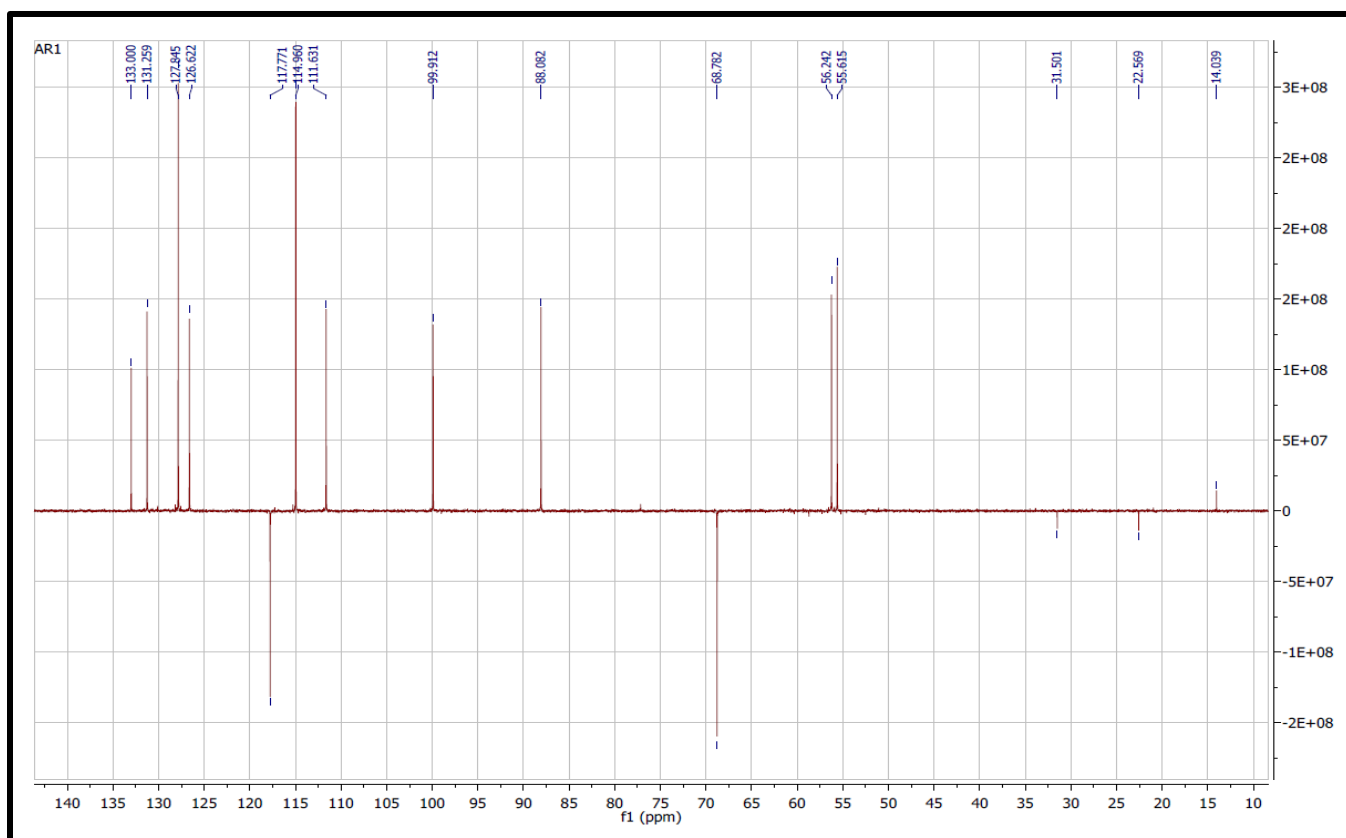


Figure S11: DEPT 135 spectrum of compound **1a**

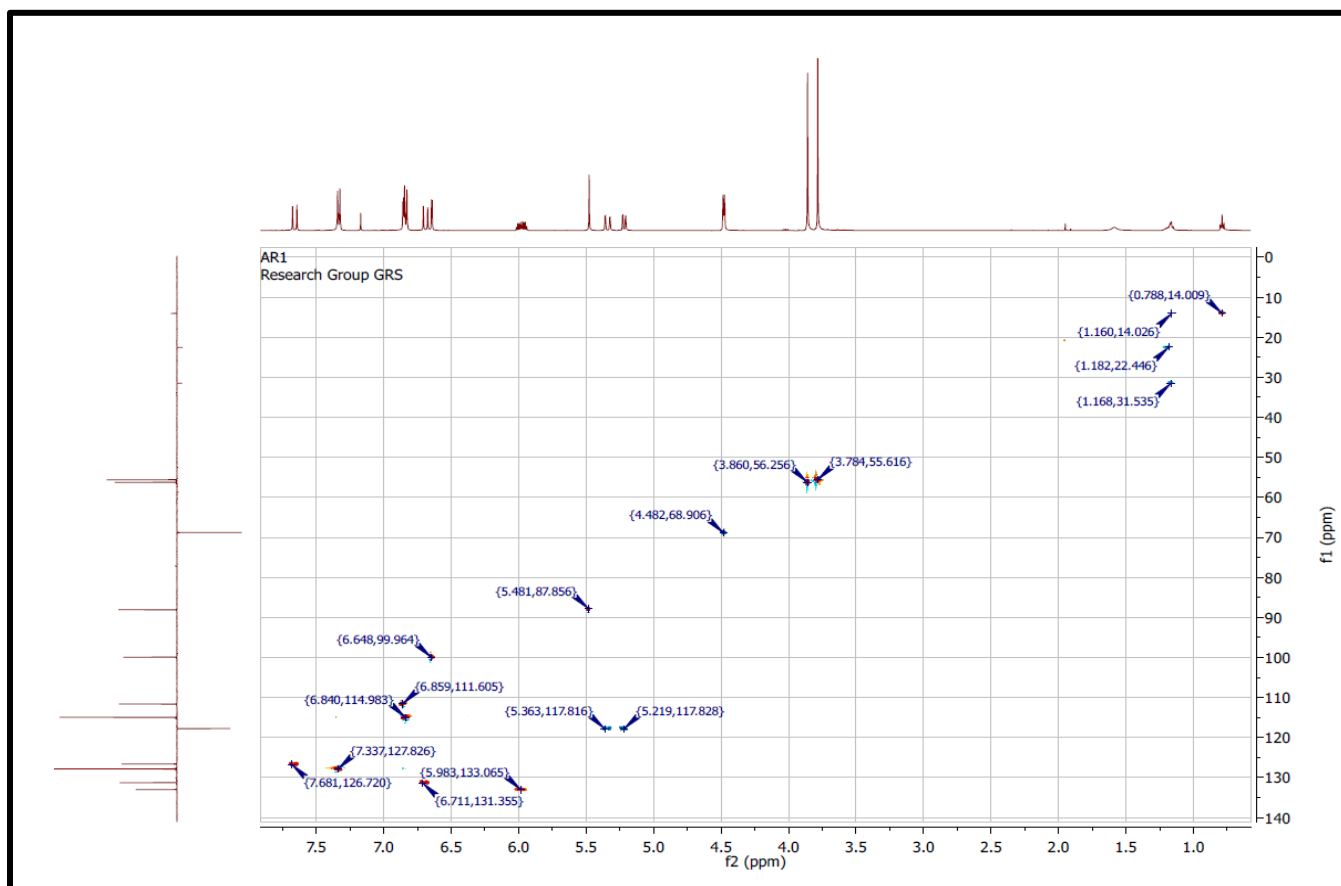


Figure S12: HSQC spectrum of compound 1a

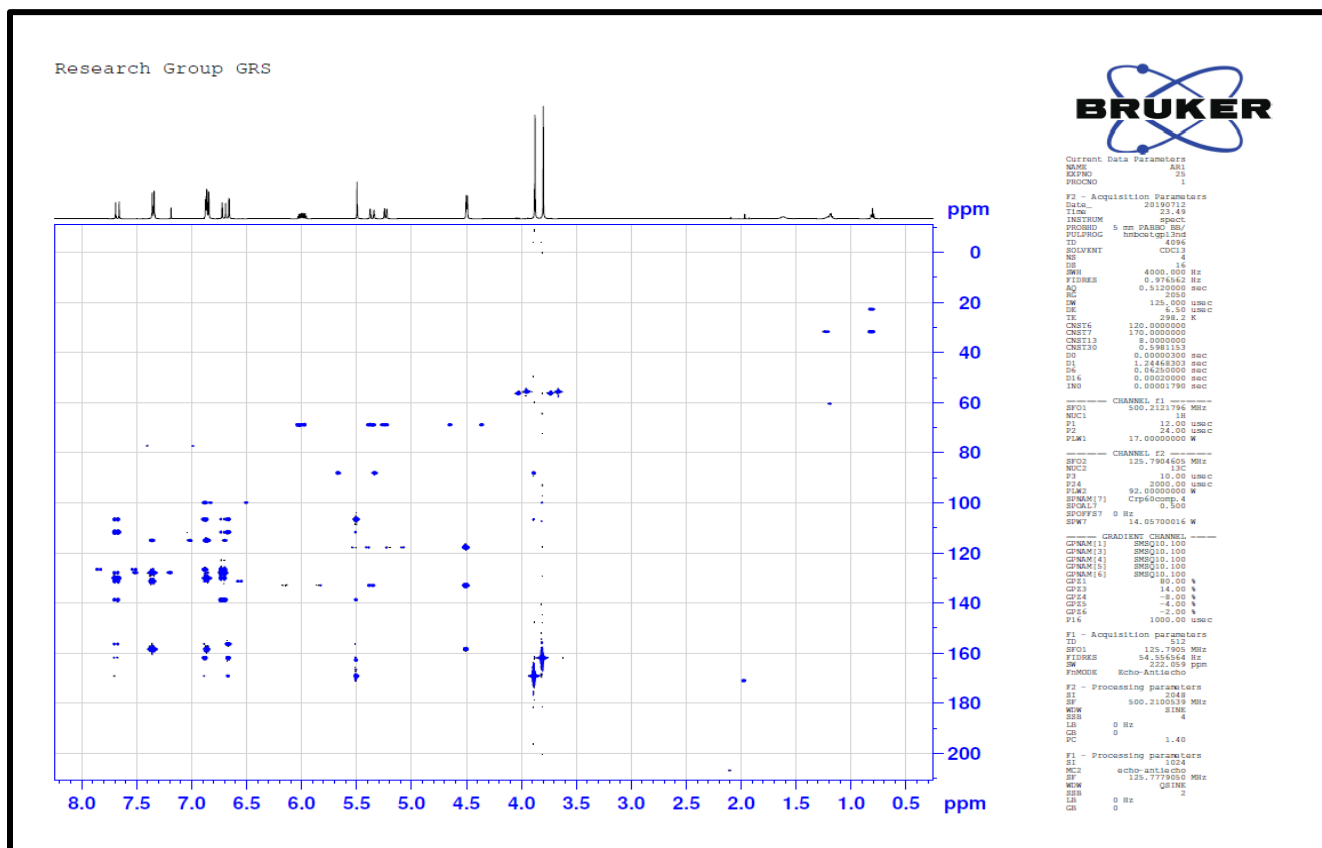


Figure S13: HMBC spectrum of compound 1a

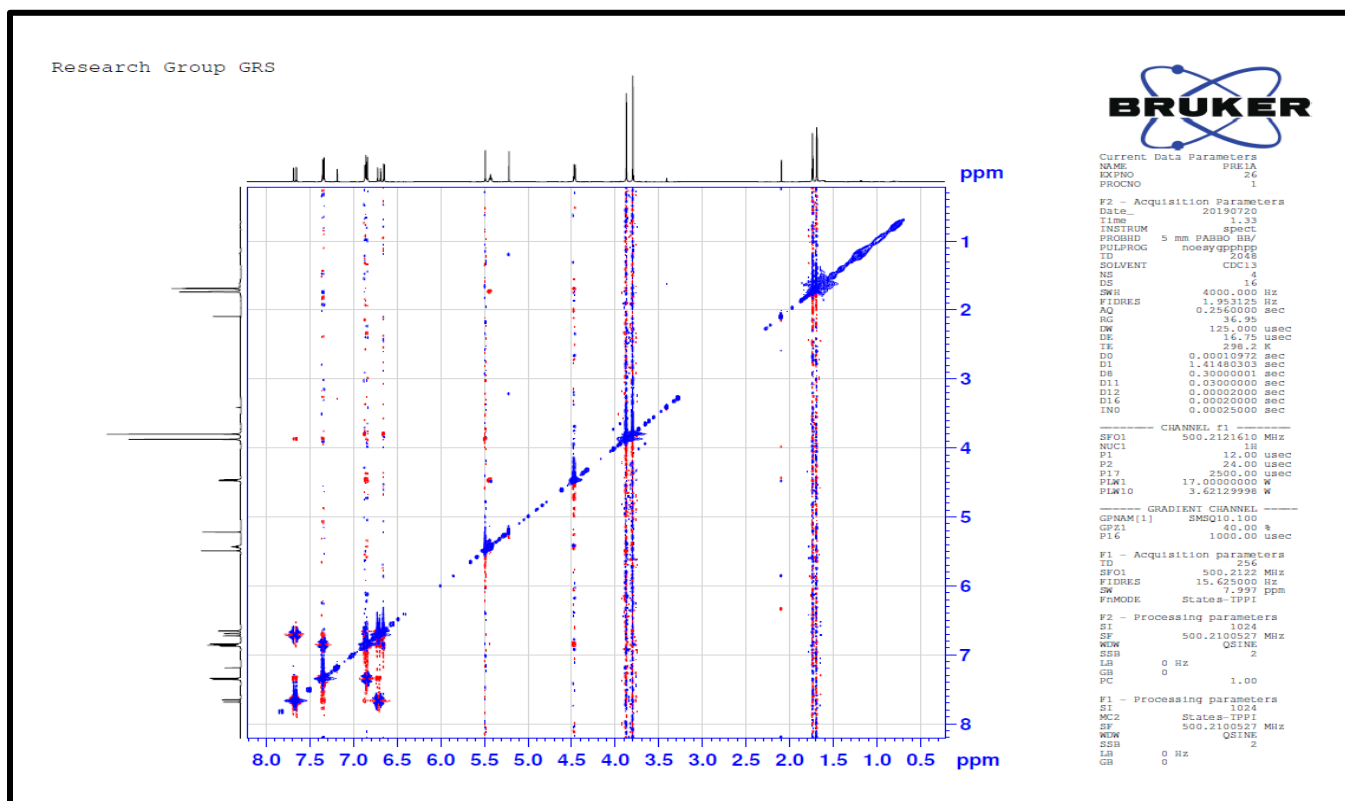


Figure S14: ROESY spectrum of compound 1a

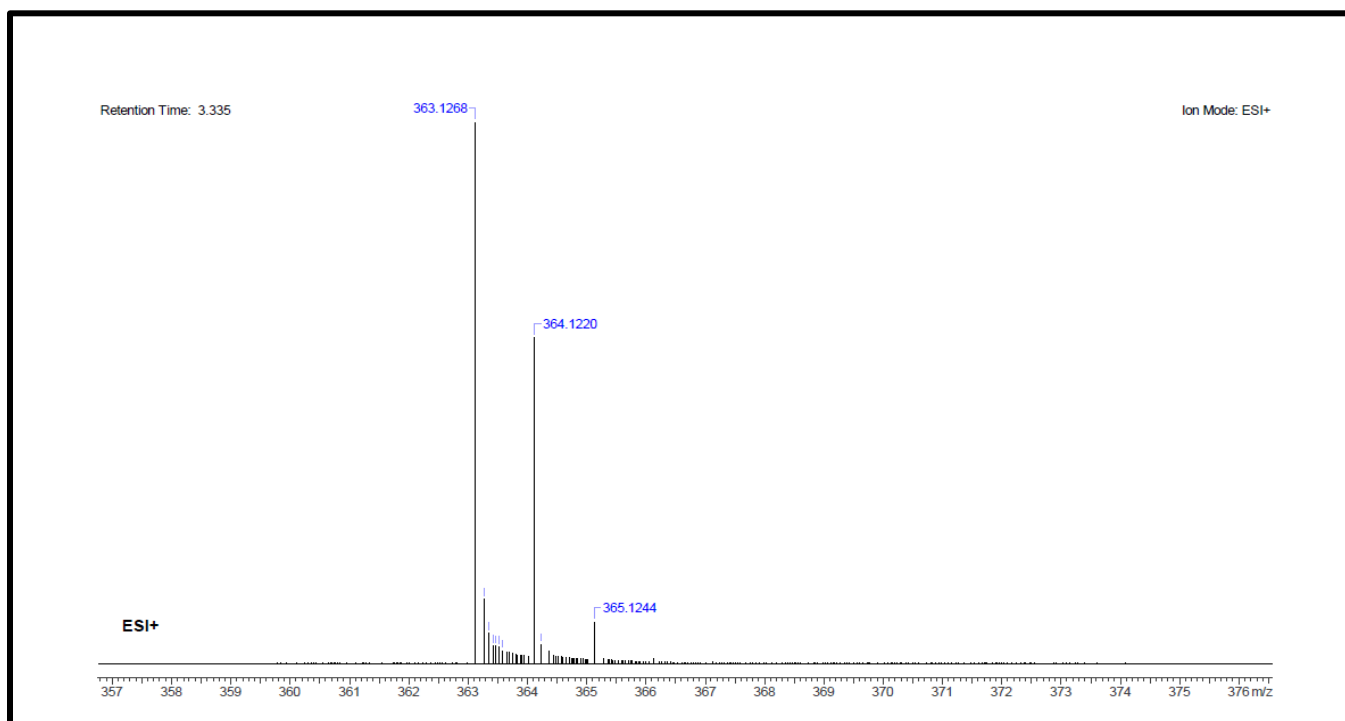


Figure S15: HRESIMS spectrum of compound 1b

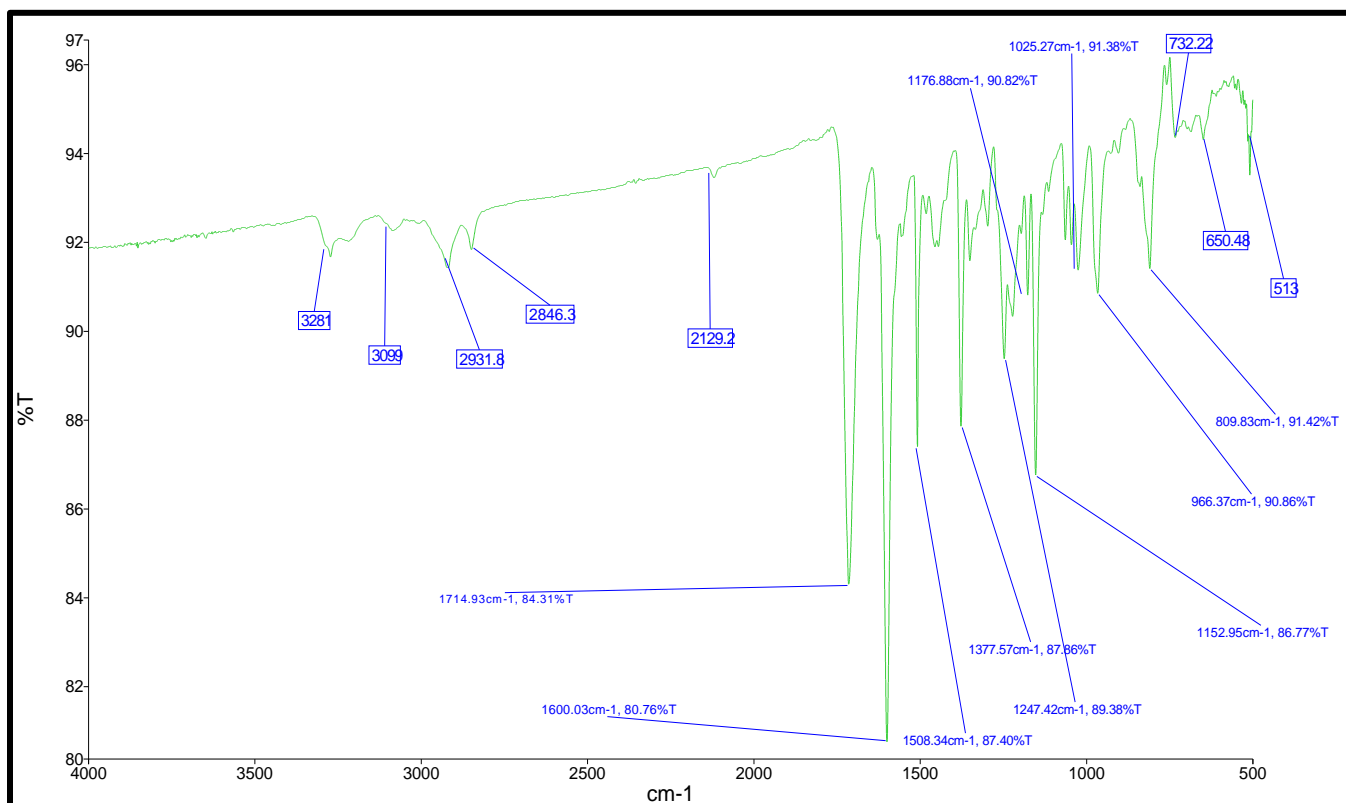


Figure S16: IR spectrum of compound 1b

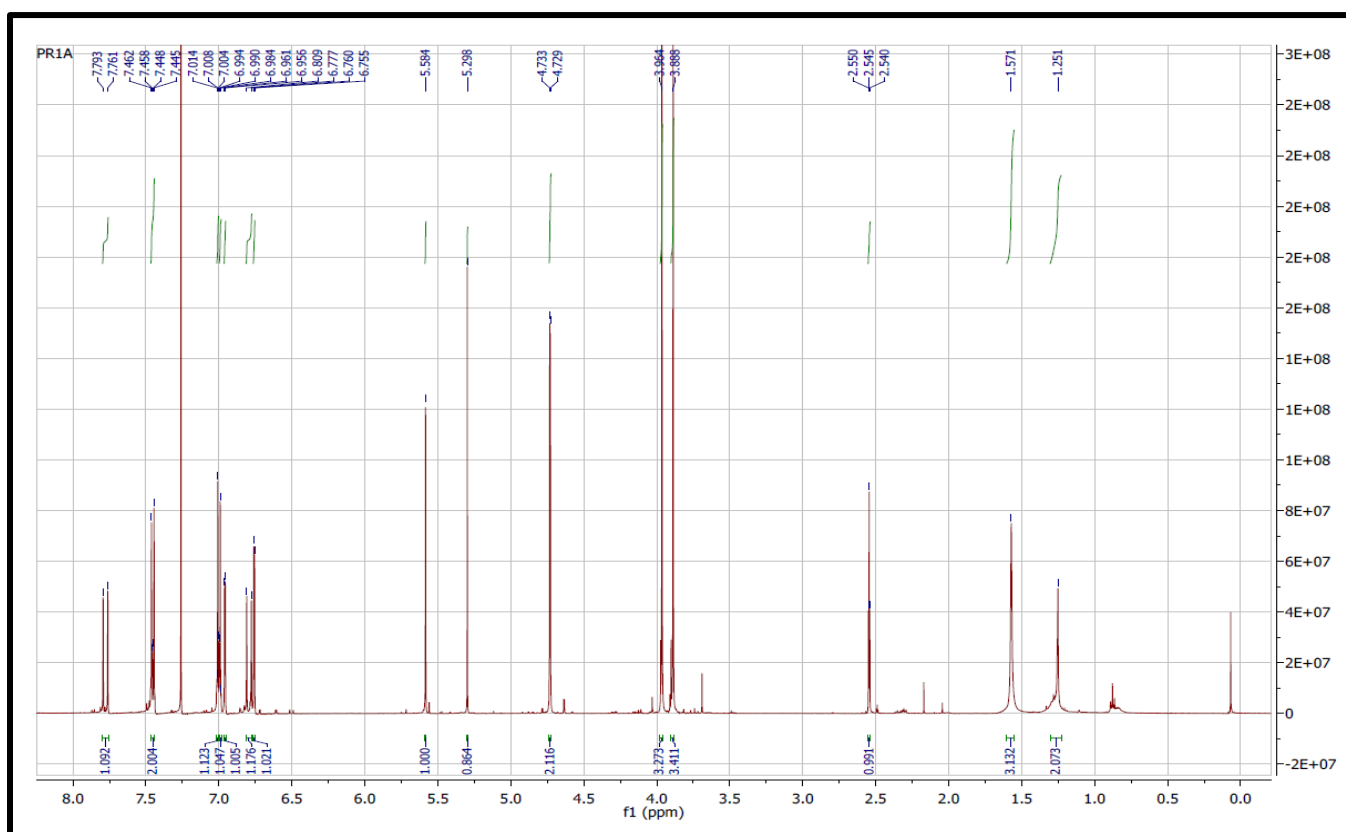


Figure S17: ¹H NMR spectrum (CDCl₃, 500 MHz) of compound 1b

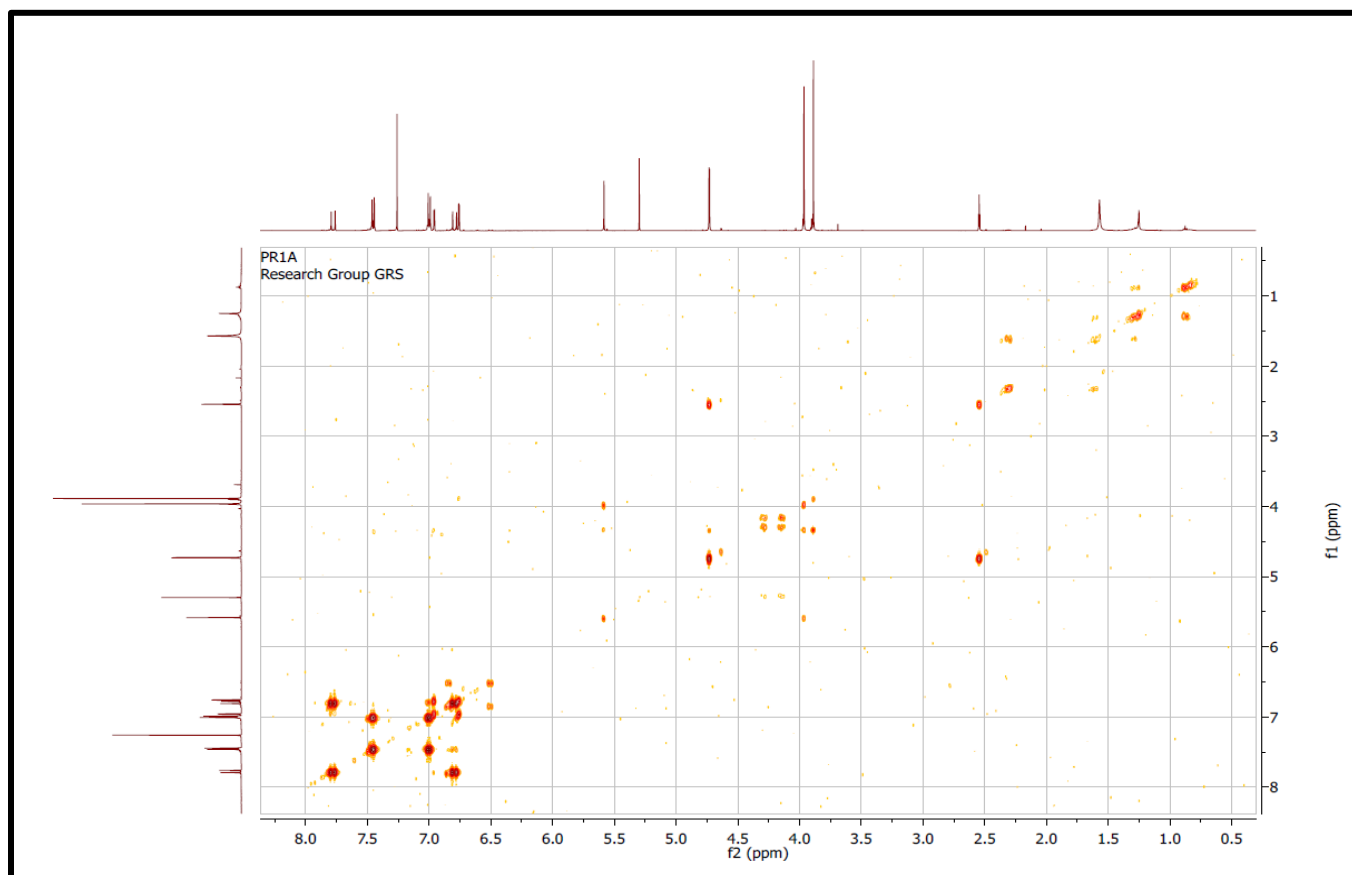


Figure S18: COSY spectrum of compound **1b**

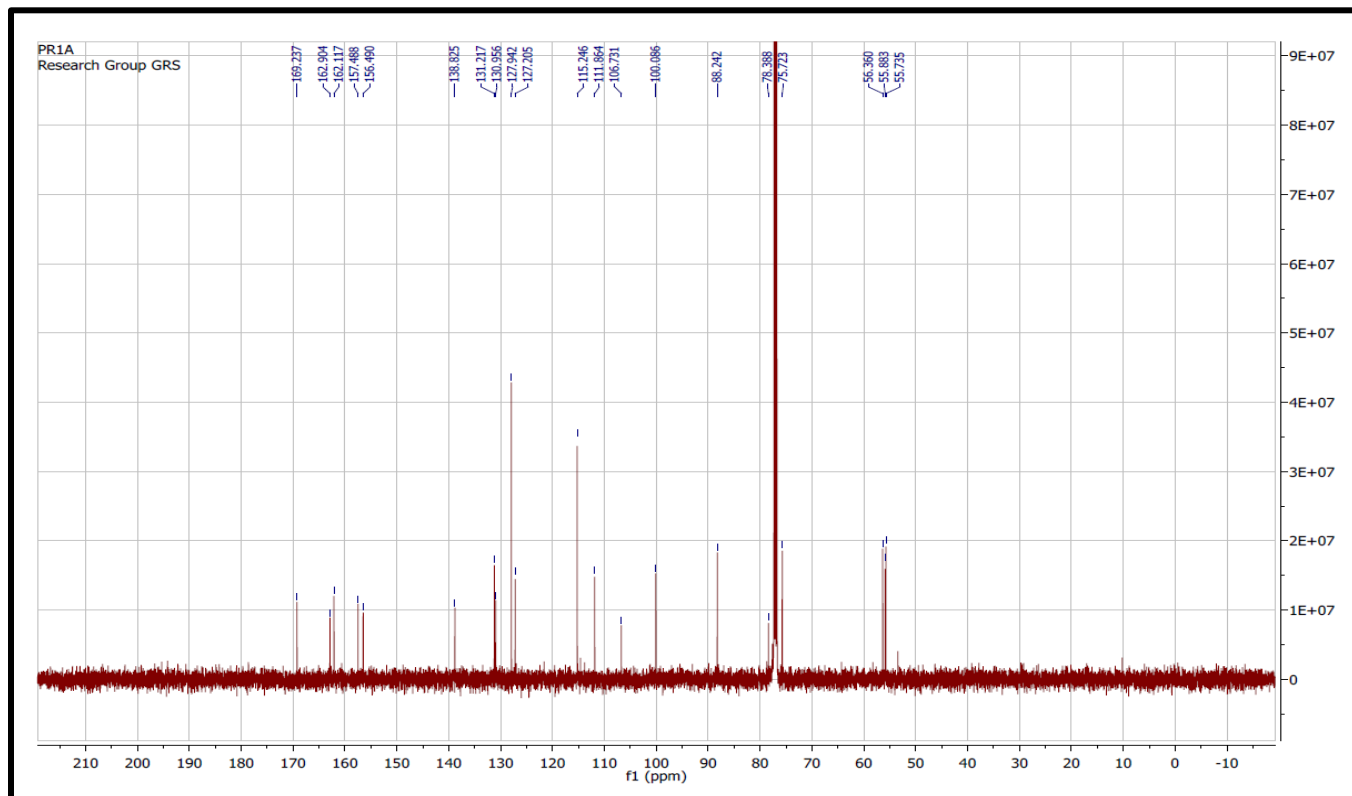


Figure S19: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of compound **1b**

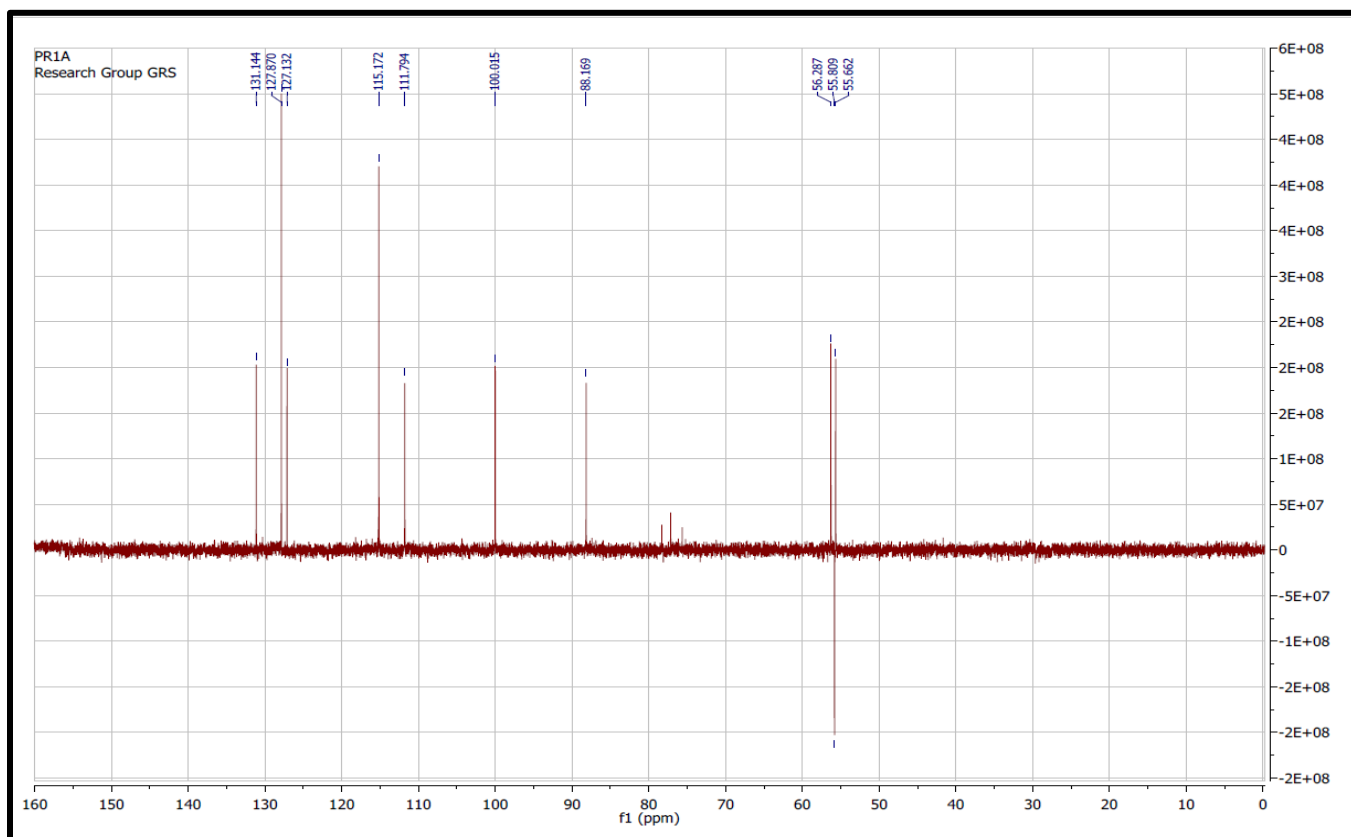


Figure S20: DEPT 135 spectrum of compound **1b**

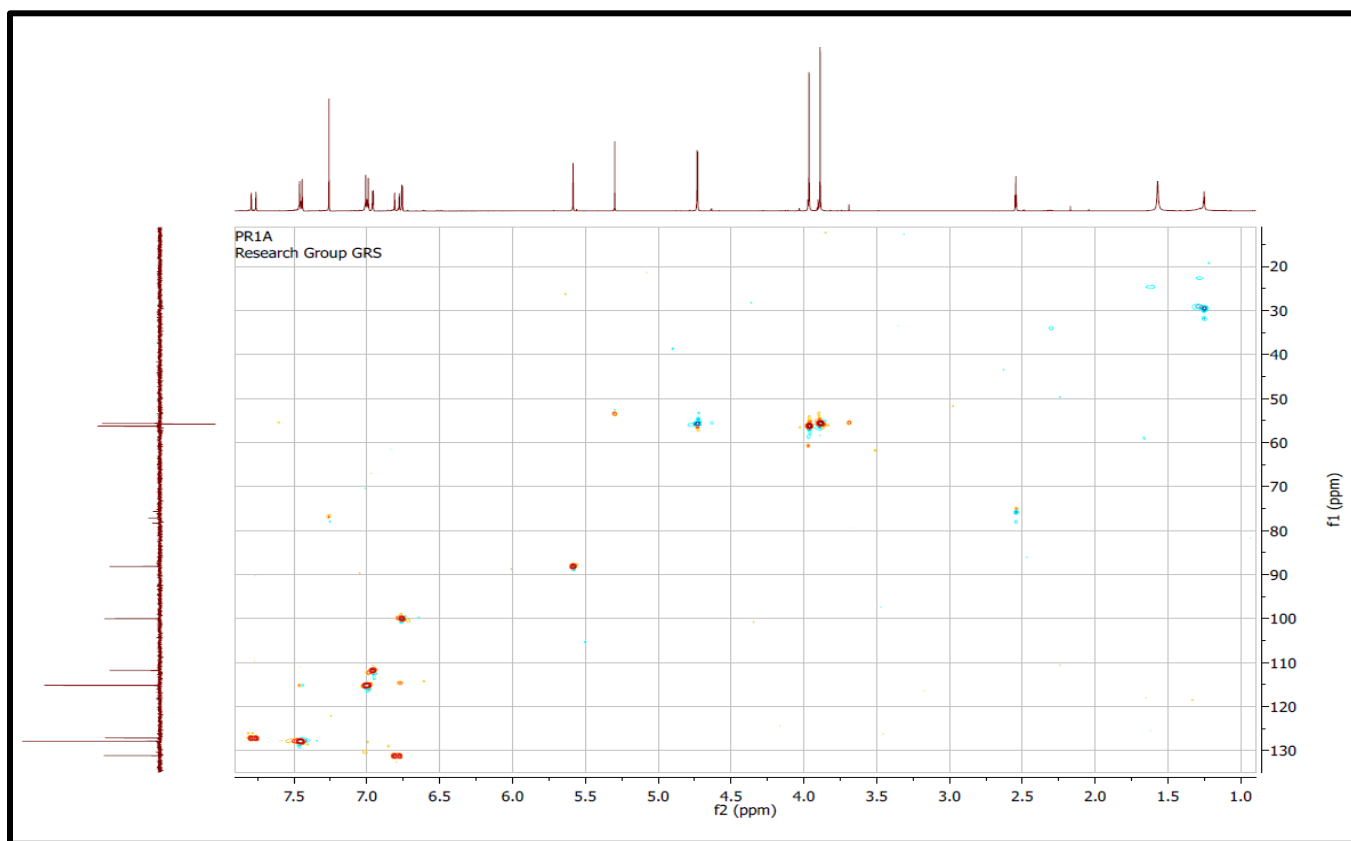


Figure S21: HSQC spectrum of compound **1b**

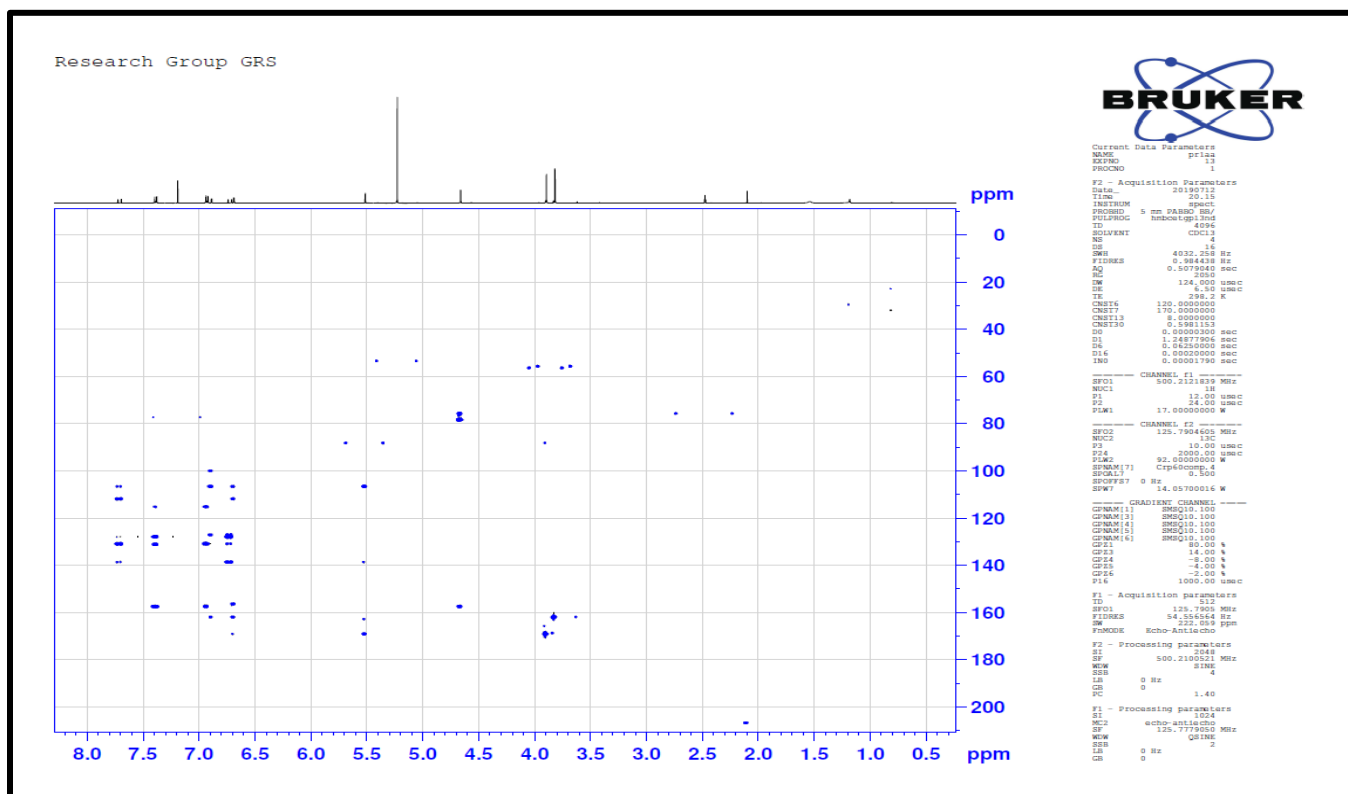


Figure S22: HMBC spectrum of compound **1b**

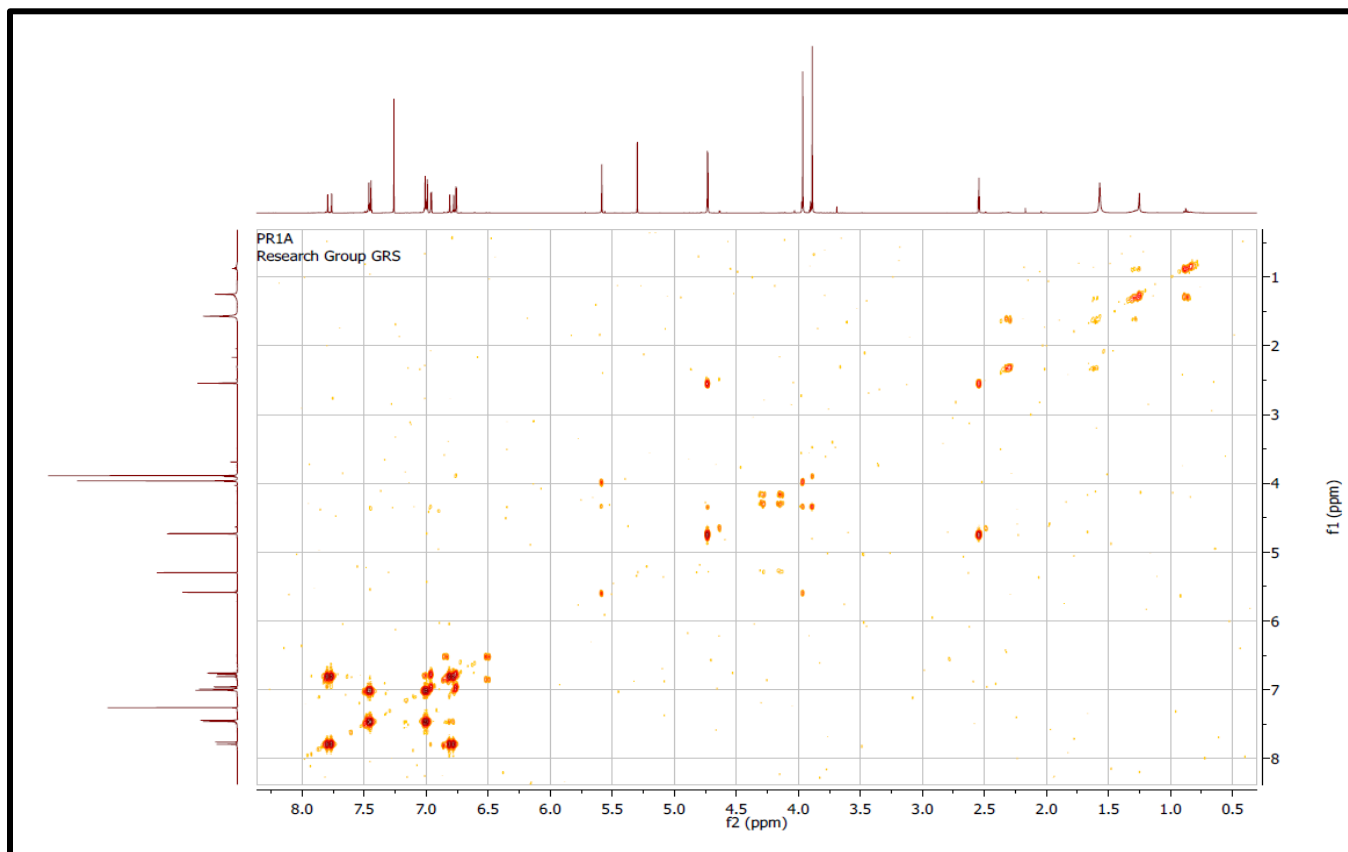
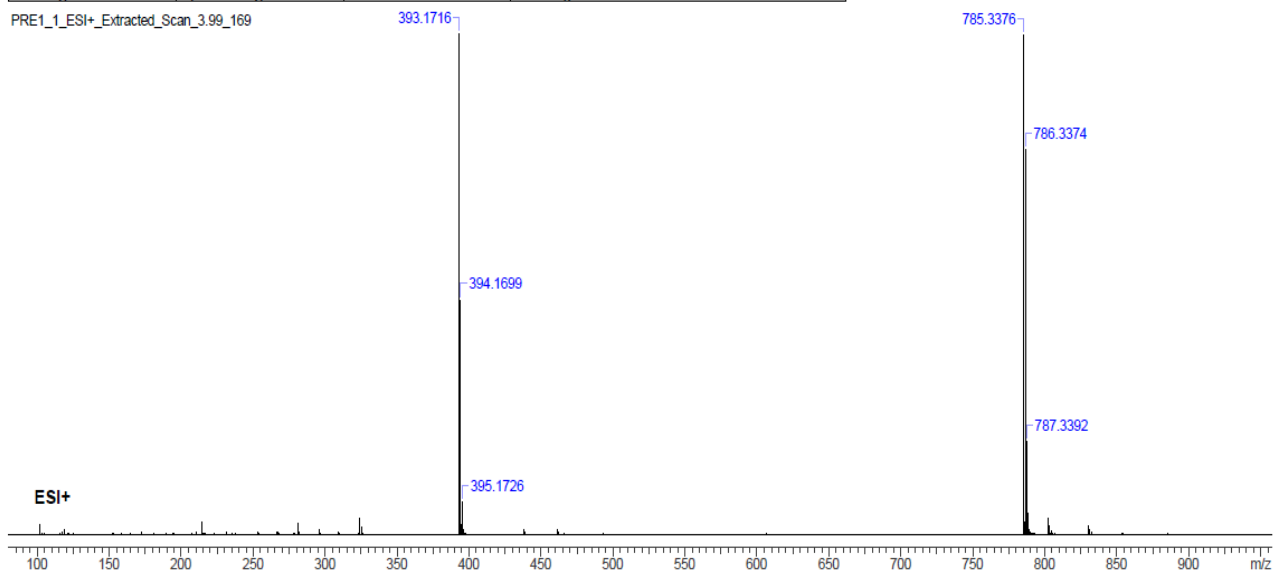


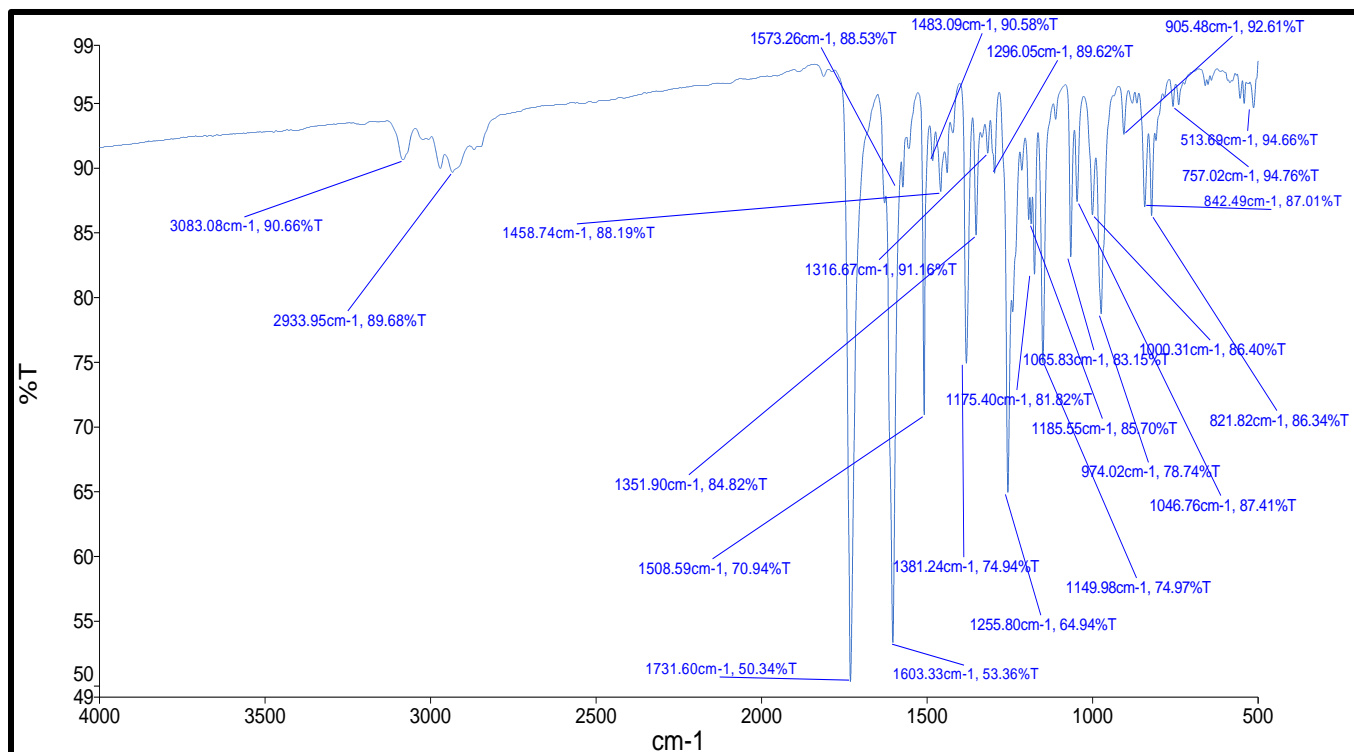
Figure S23: NOESY spectrum of compound **1b**

fragmentor voltage 130

Comment	130 V	Count	2483	Data Type	MS	Date	2019-08-23	File Name	D:\190823HRMSPRE1.d
Instrument	Instrument 1	Ion Mode	ESI+	Plot Type	Stick	Retention Time	3.985	Sample	PRE1
Scan Type	Scan	Spectrum Type	MS	TIC	609.22	Total Signal	22093927 9365	Scan	169



Expected Mass (<i>m/z</i>)	Observed Mass (<i>m/z</i>)	(Error (ppm))
393.1702	393.1716	3.560799

Figure S24: HRESIMS spectrum of compound **1c**Figure S25: IR spectrum of compound **1c**

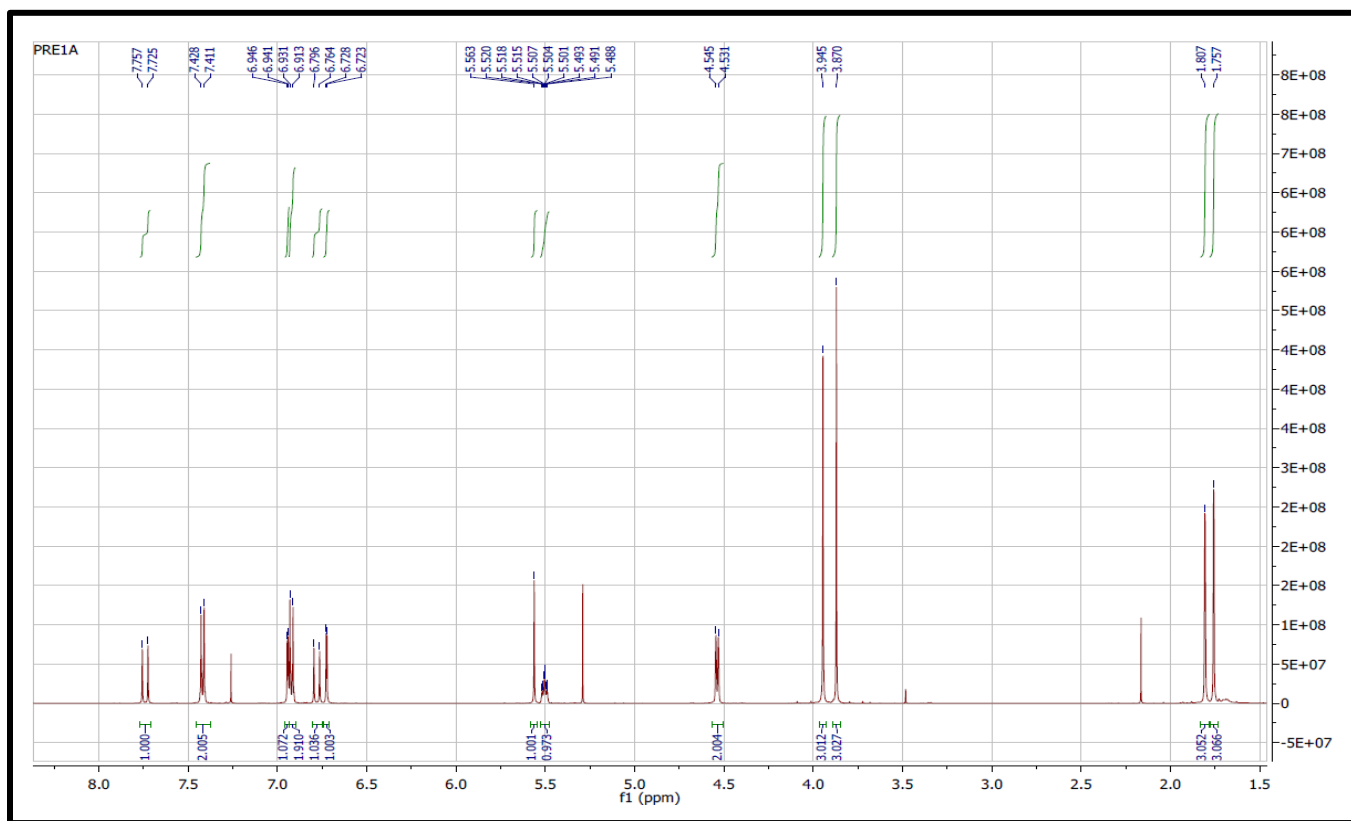


Figure S26: ^1H NMR spectrum (CDCl_3 , 500 MHz) of compound **1c**

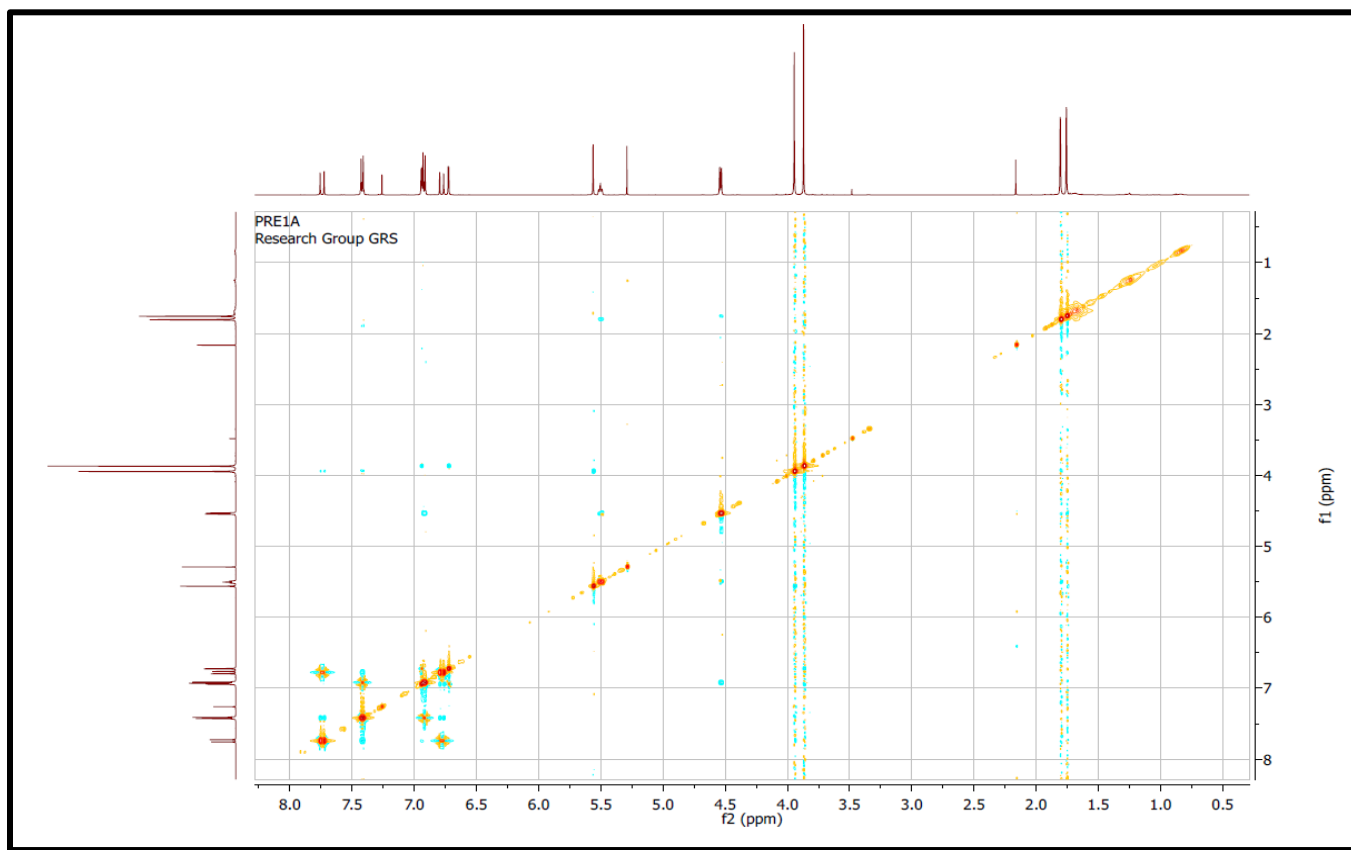


Figure S27: COSY spectrum of compound **1c**

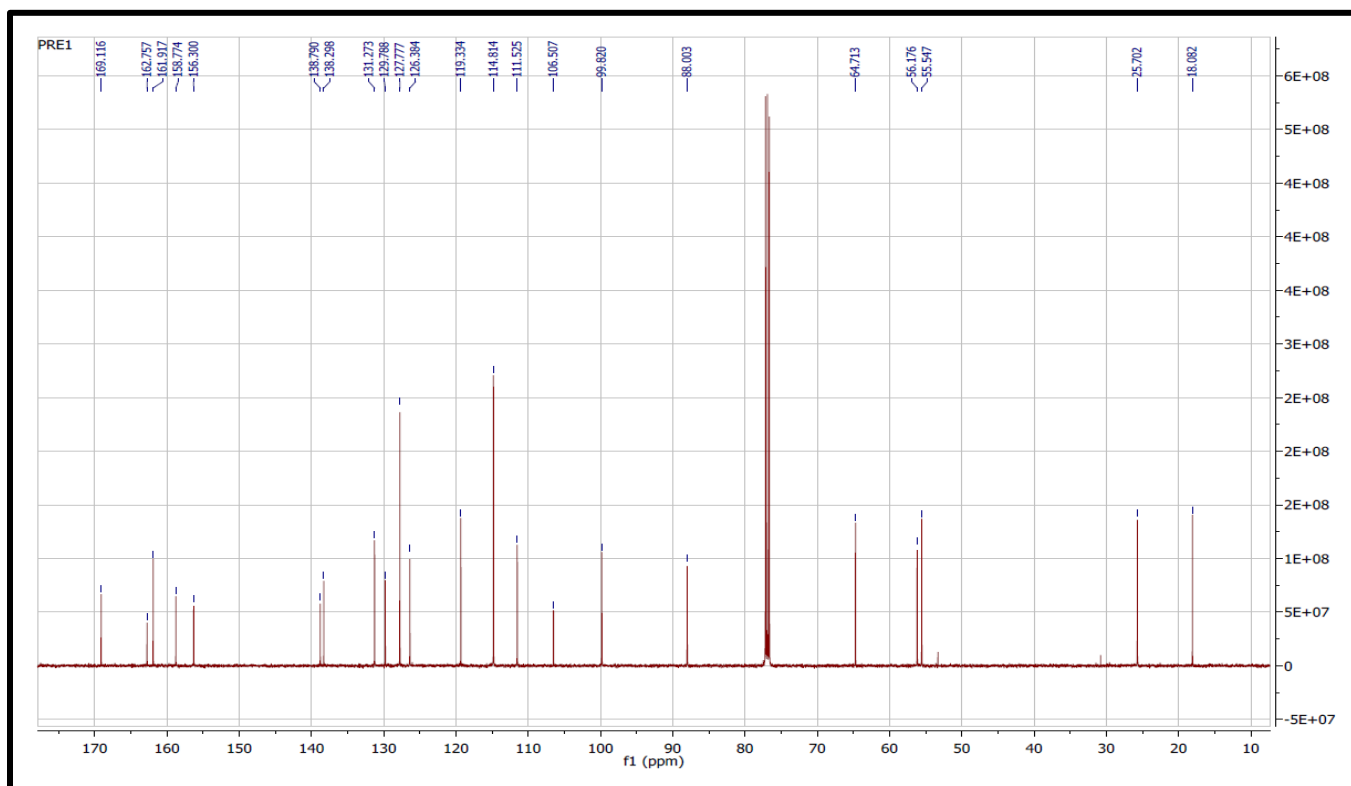


Figure S28: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of compound **1c**

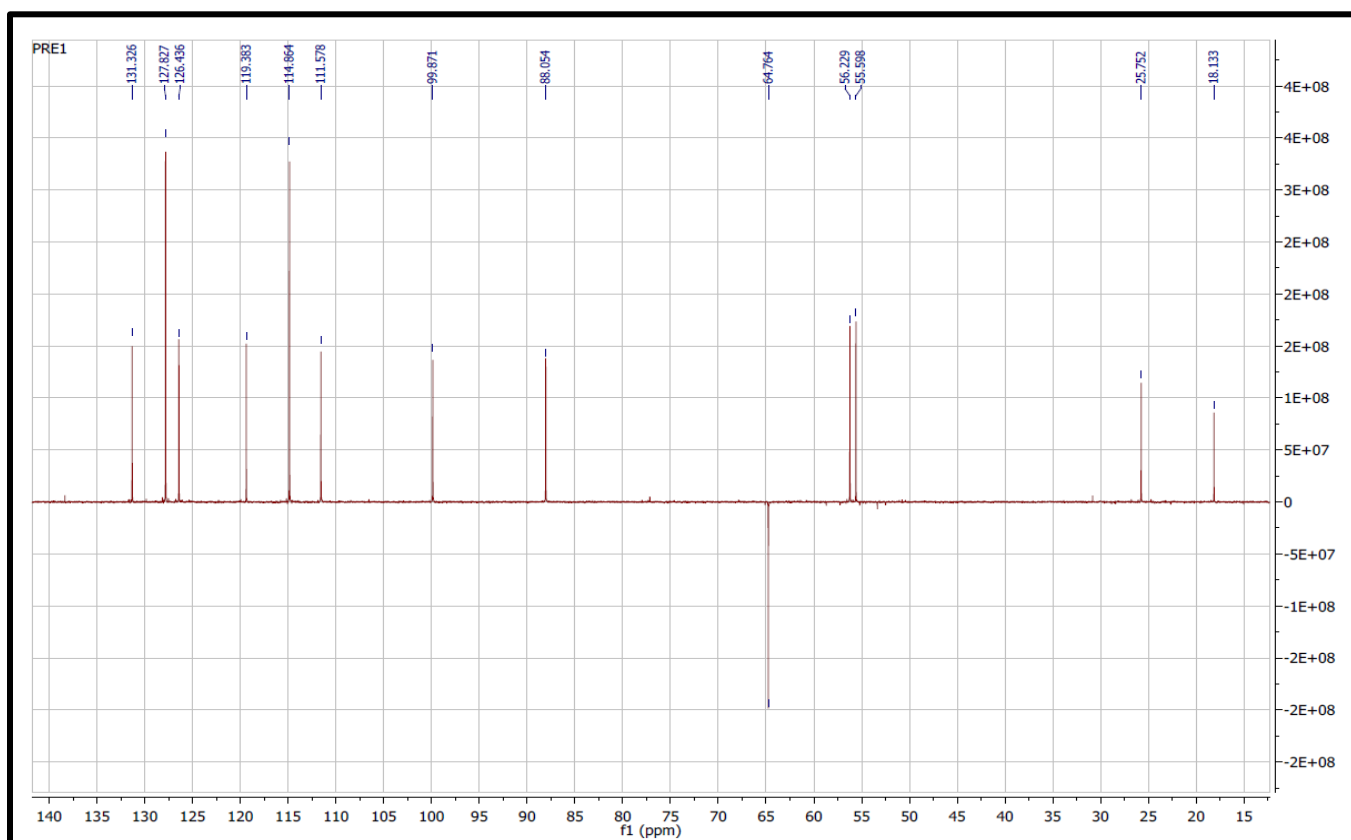


Figure S29: DEPT 135 spectrum of compound **1c**

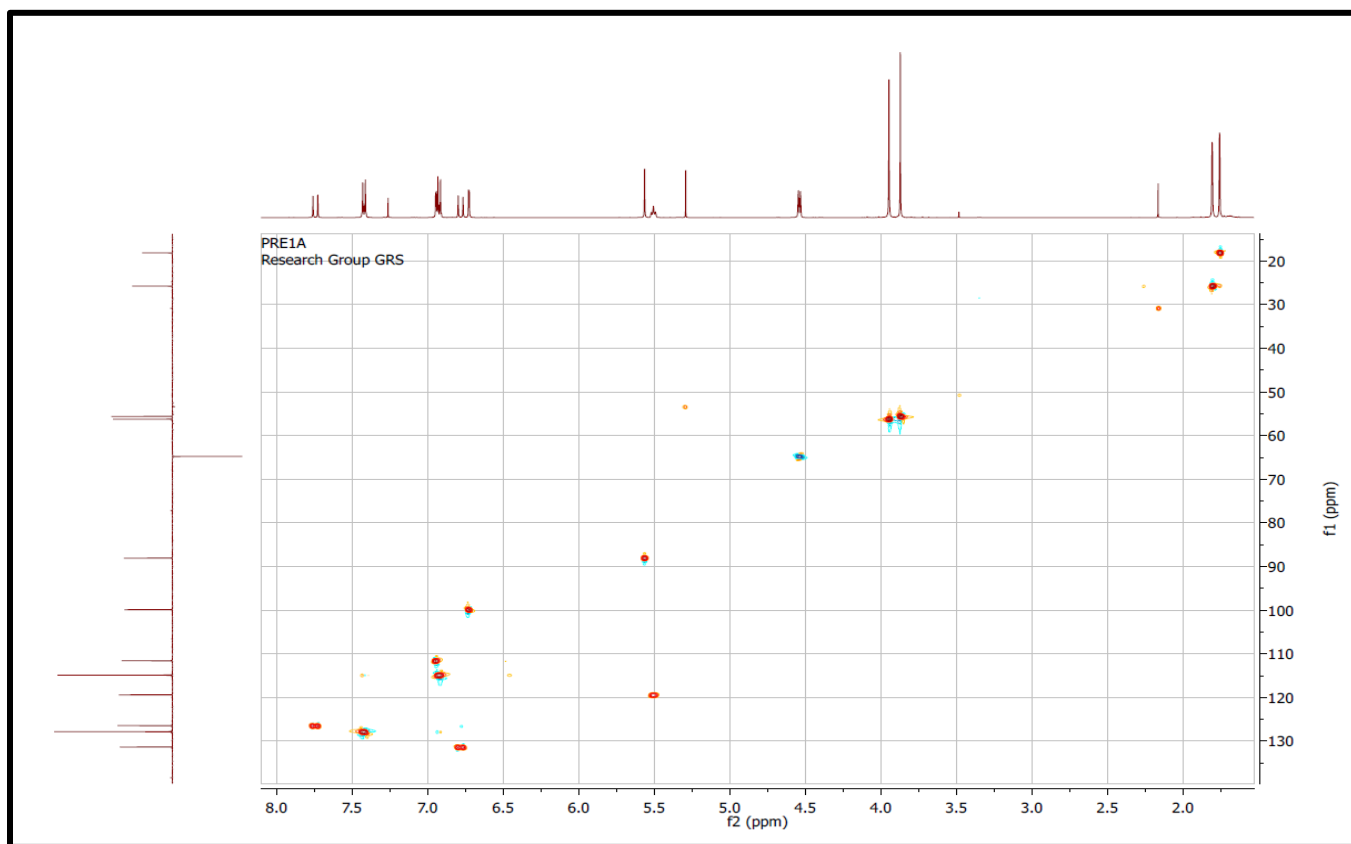


Figure S30: HSQC spectrum of compound **1c**

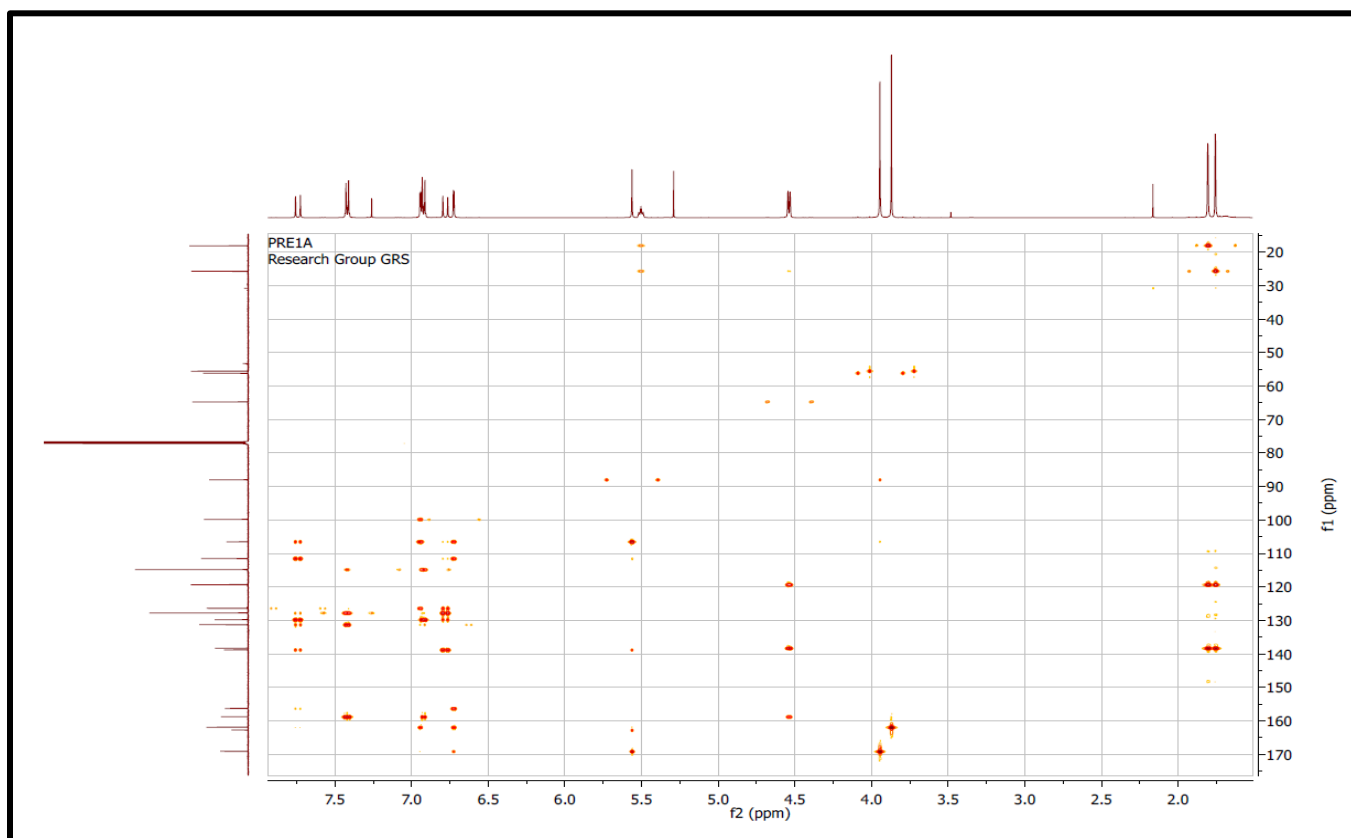


Figure S31: HMBC spectrum of compound **1c**

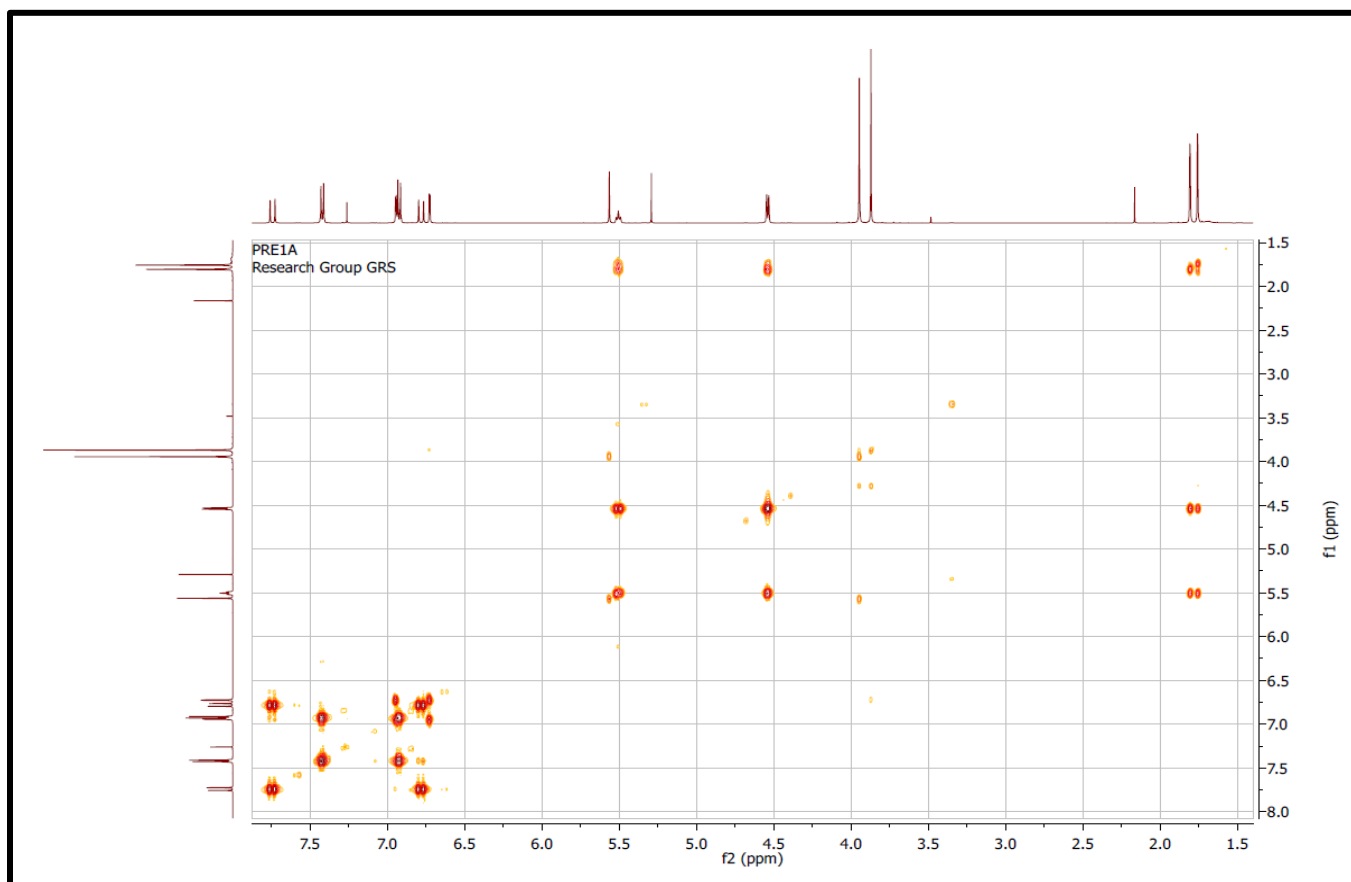


Figure S32: NOESY spectrum of compound 1c

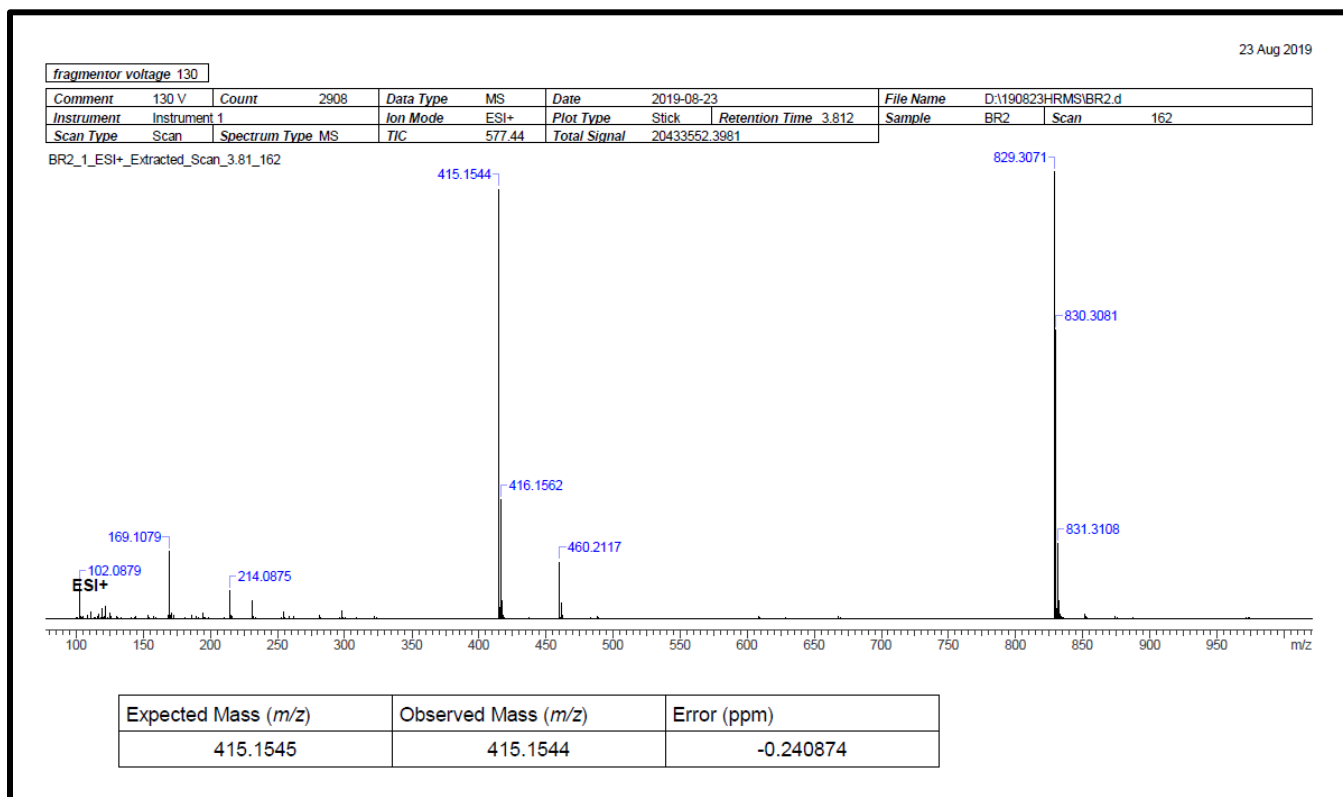


Figure S33: HRESIMS spectrum of compound 1d

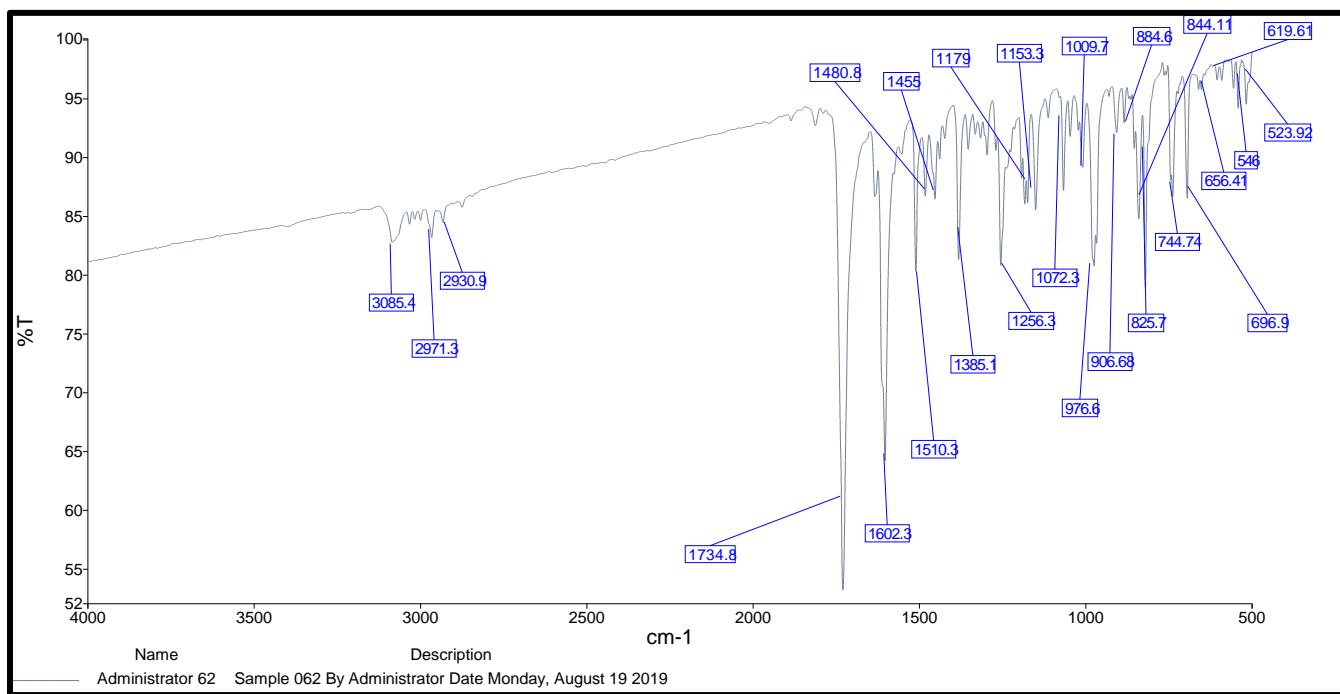


Figure S34: IR spectrum of compound 1d

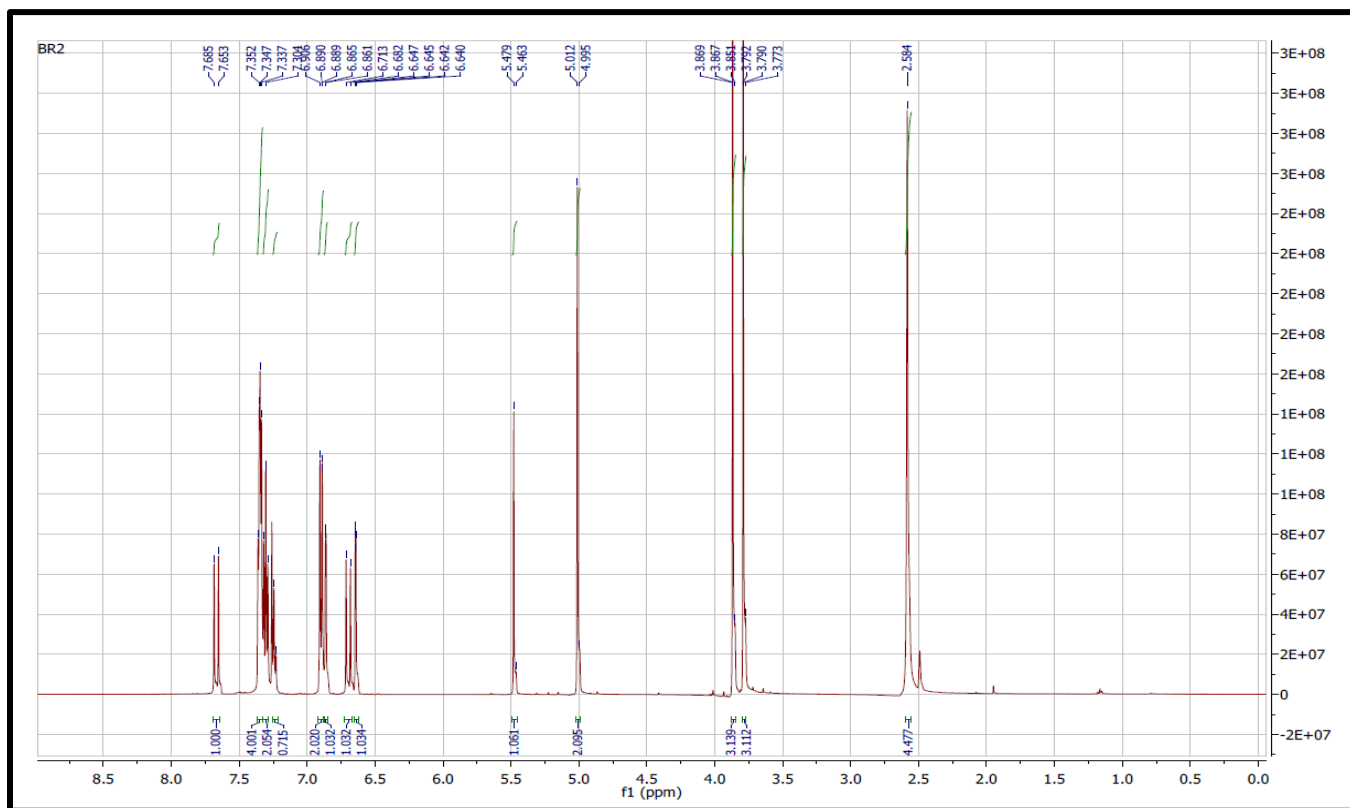


Figure S35: ¹H NMR spectrum (CDCl₃, 500 MHz) of compound 1d

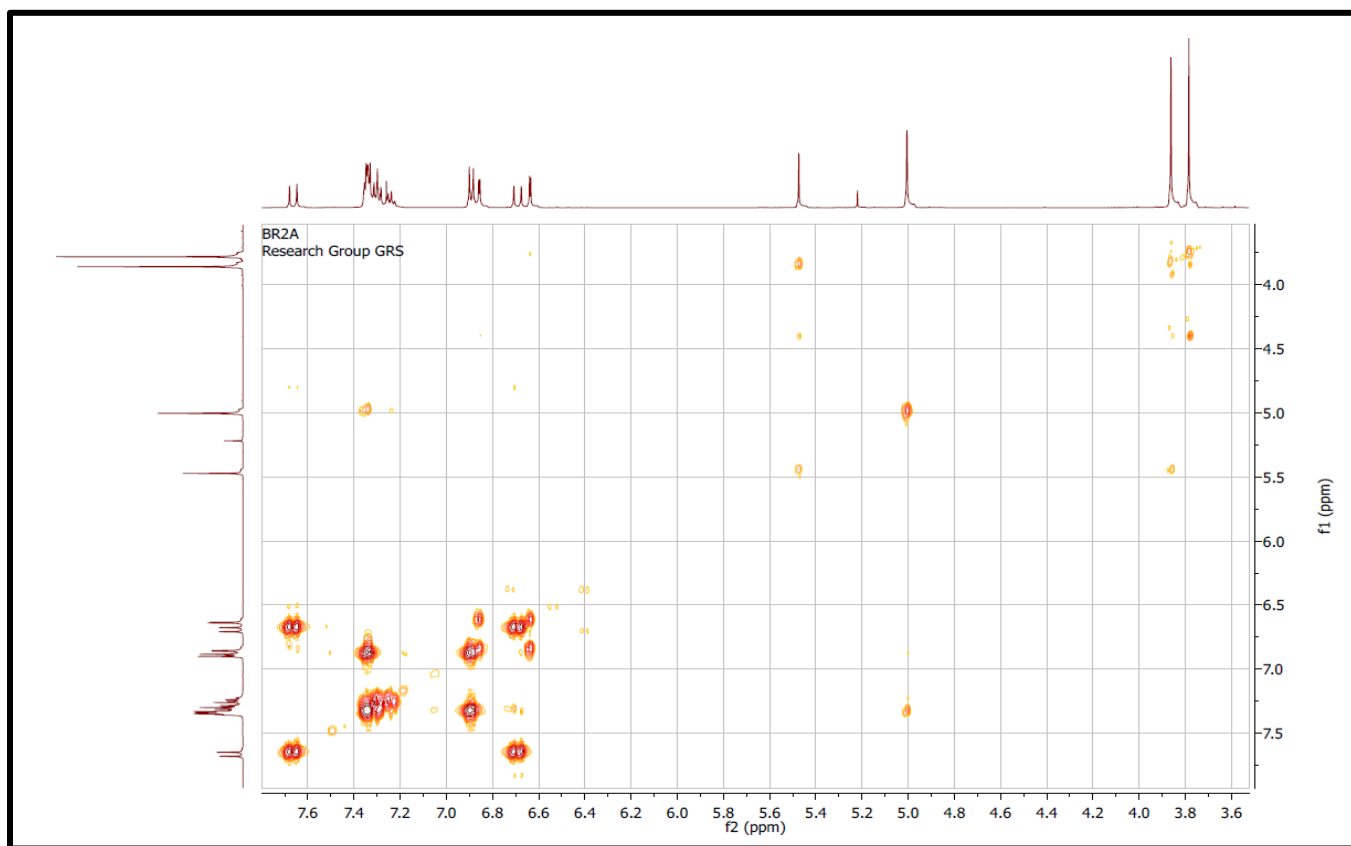


Figure S36: COSY spectrum of compound **1d**

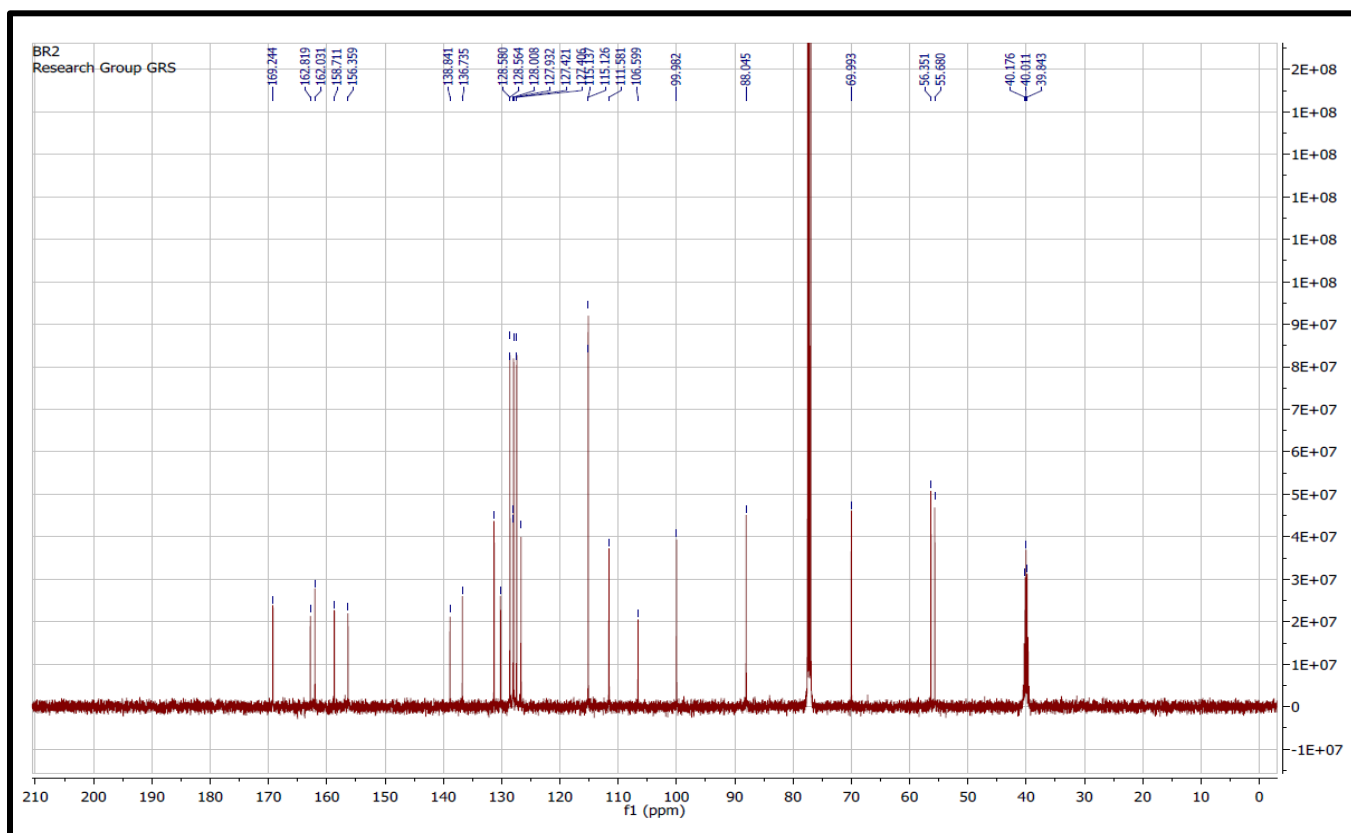


Figure S37: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of compound **1d**

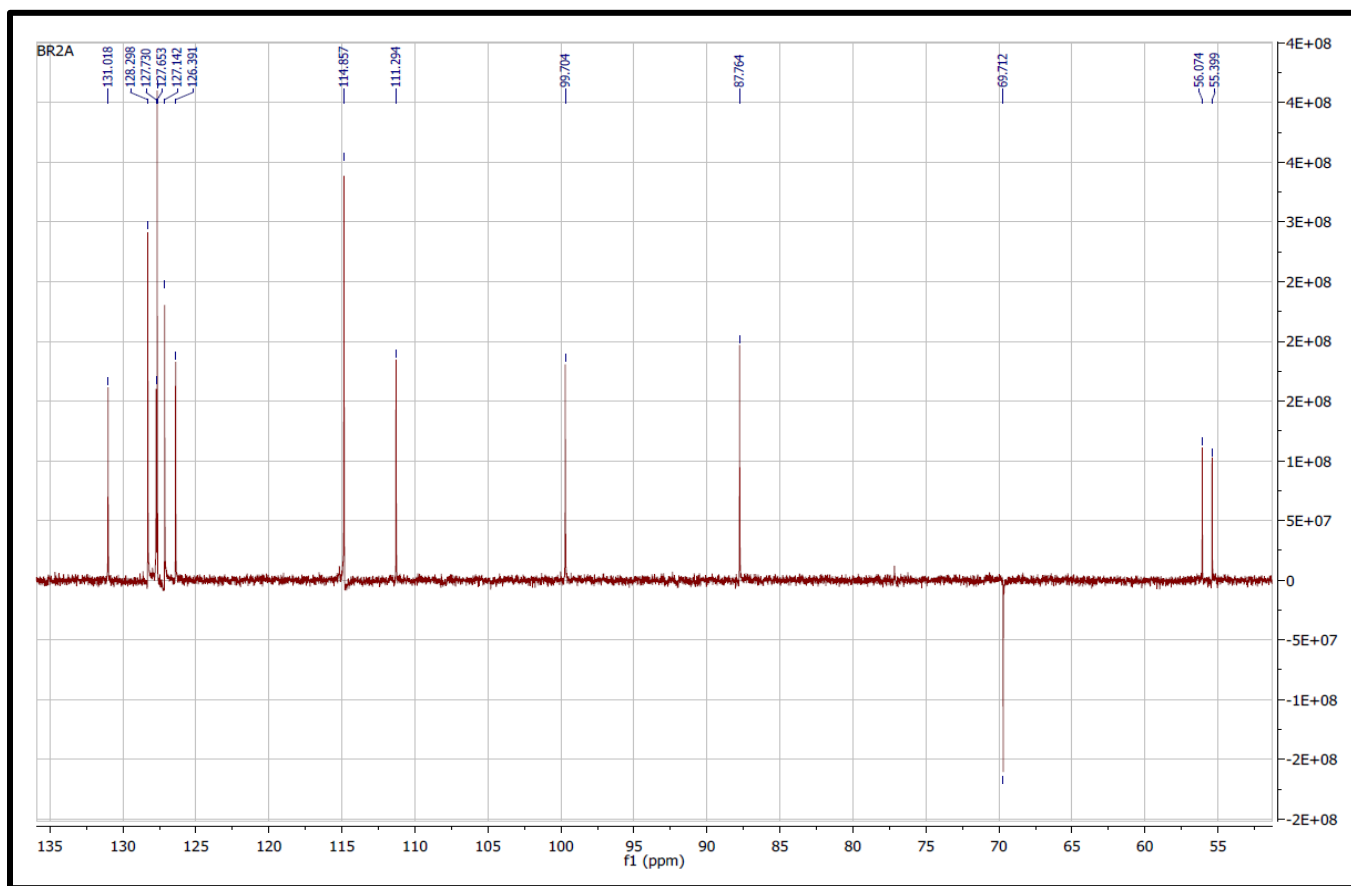


Figure S38: DEPT 135 spectrum of compound **1d**

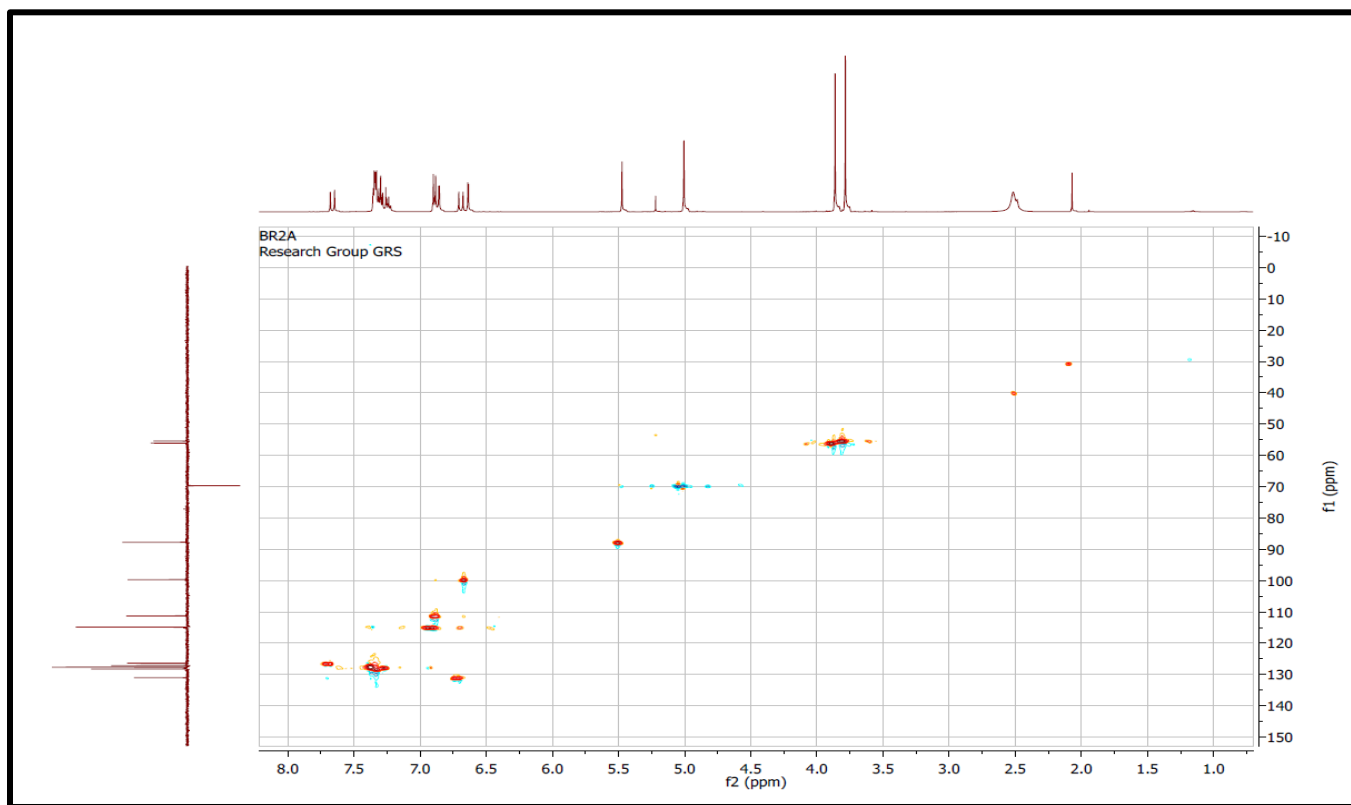


Figure S39: HSQC spectrum of compound **1d**

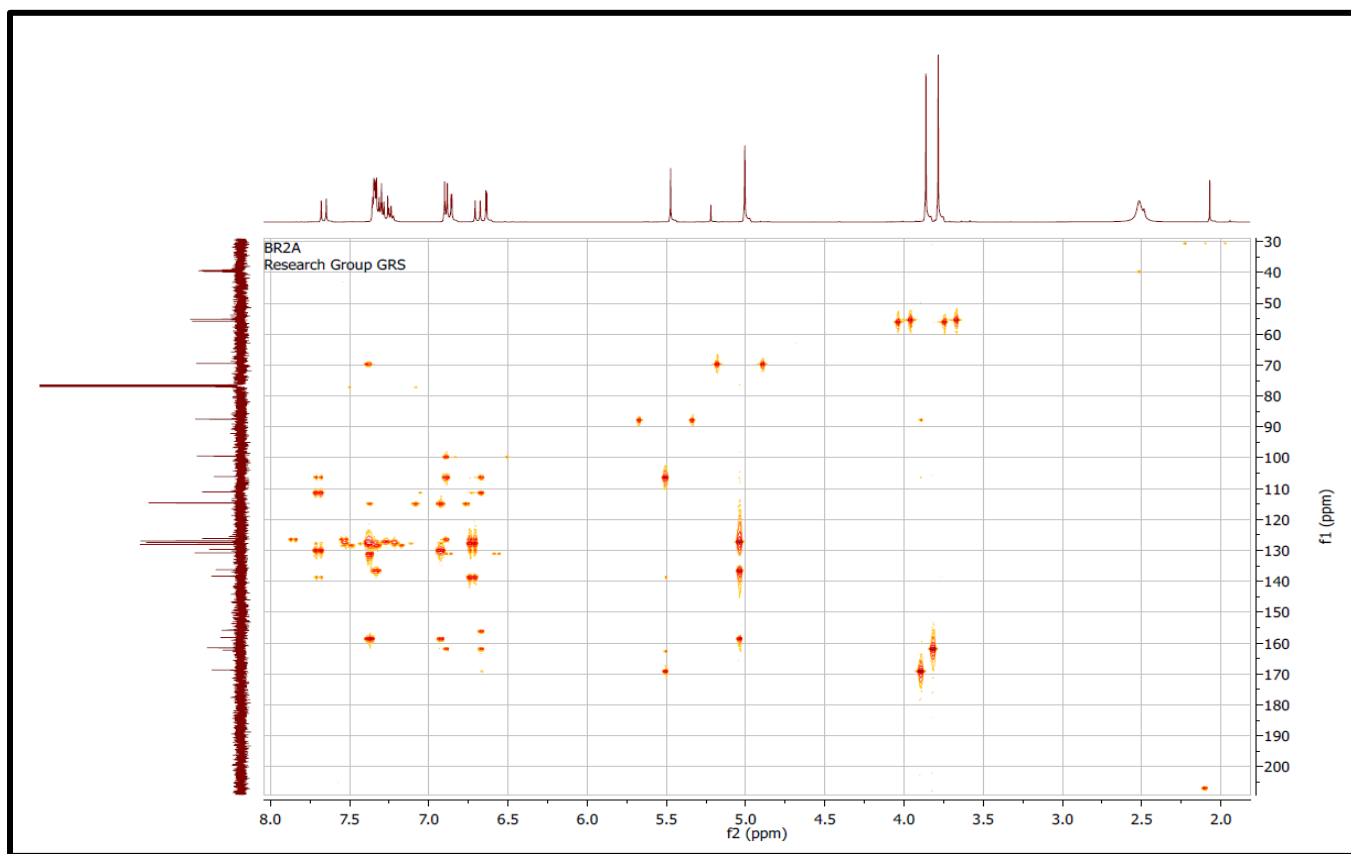


Figure S40: HMBC spectrum of compound 1d

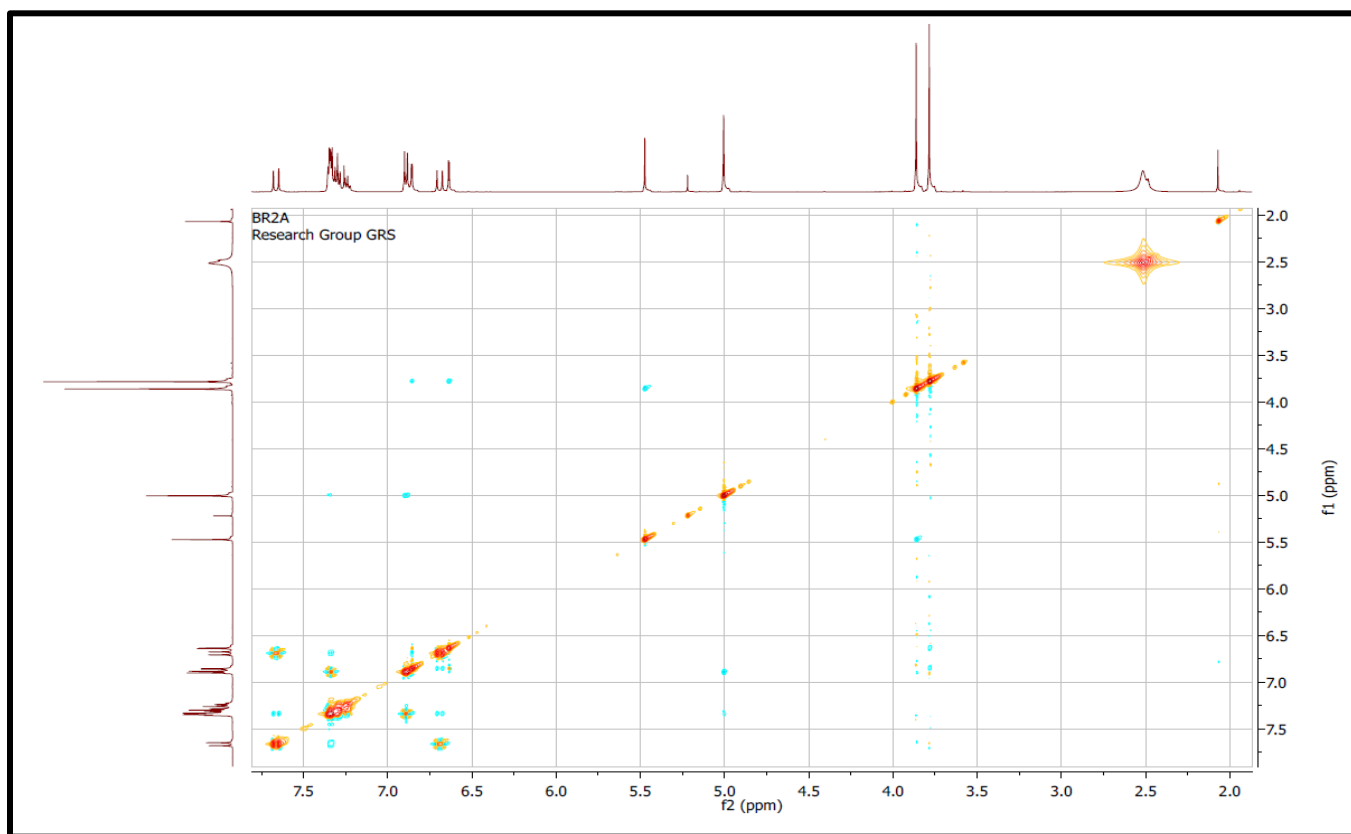


Figure S41: NOESY spectrum of compound 1d

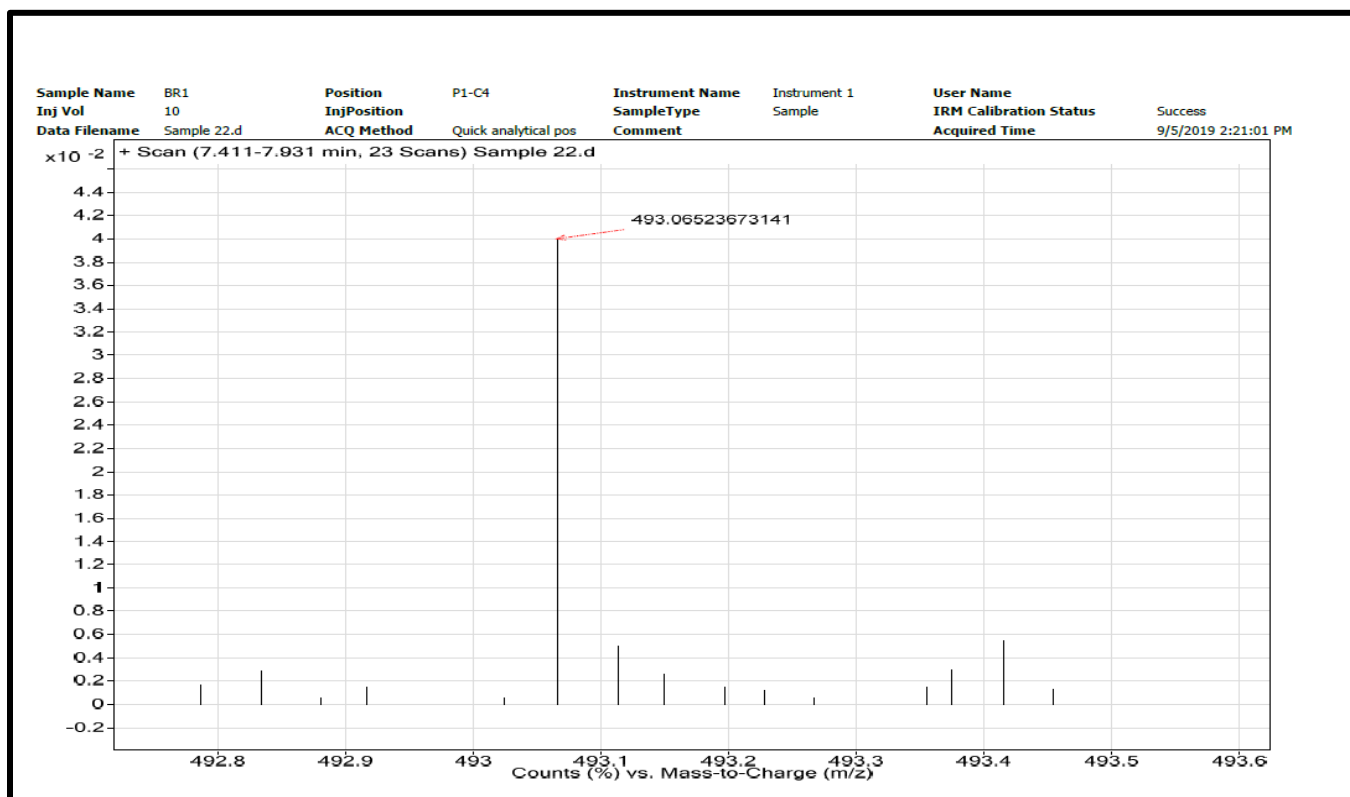


Figure S42: HRESIMS spectrum of compound 1e

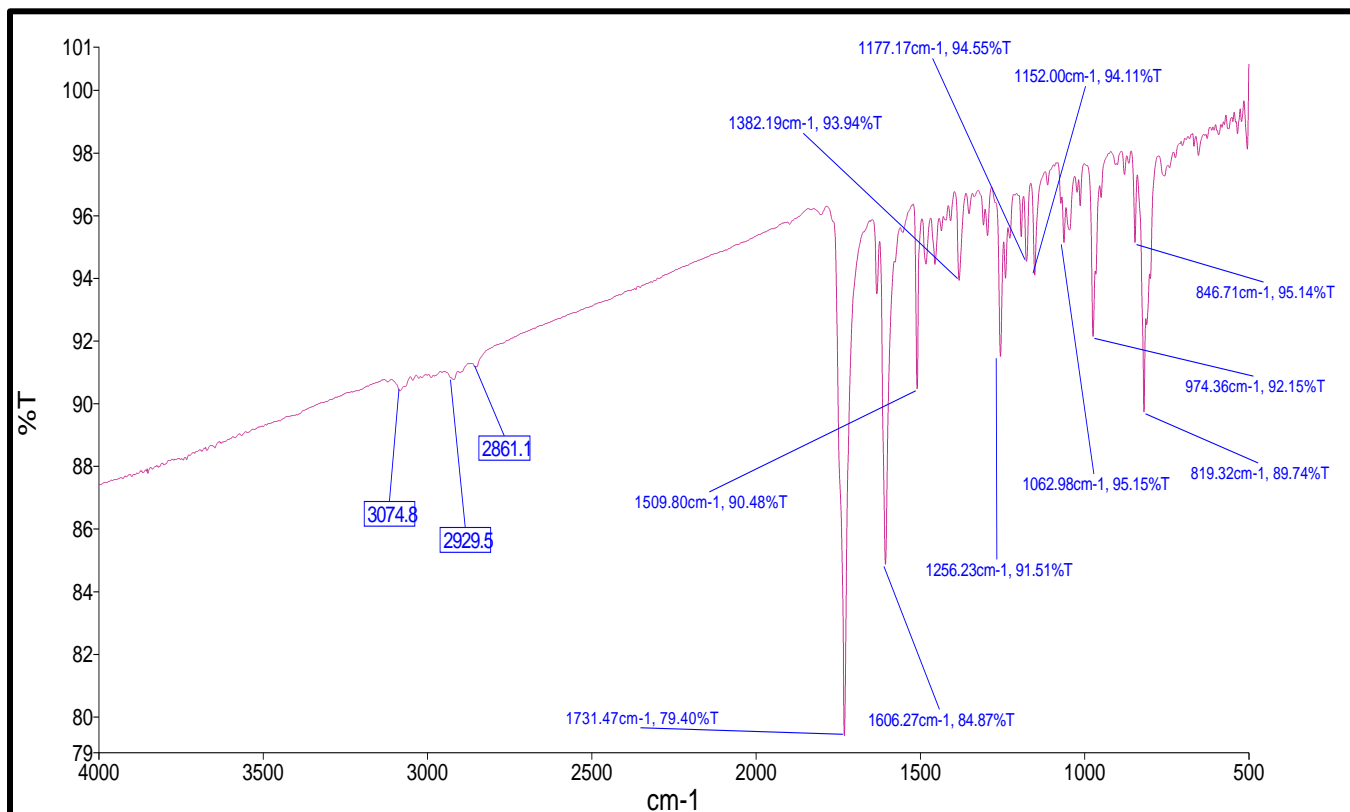


Figure S43: IR spectrum of compound 1e

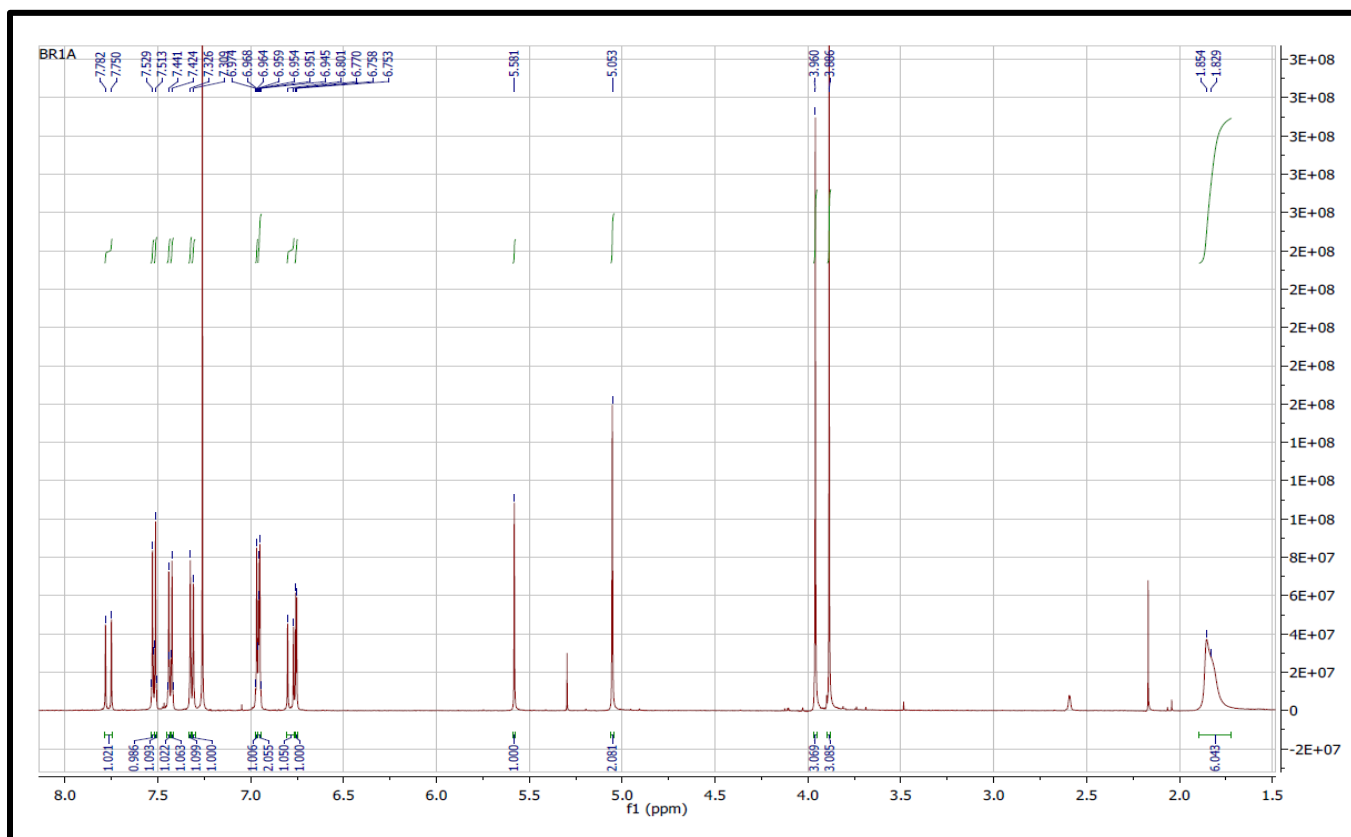


Figure S44: ^1H NMR spectrum (CDCl_3 , 500 MHz) of compound **1e**

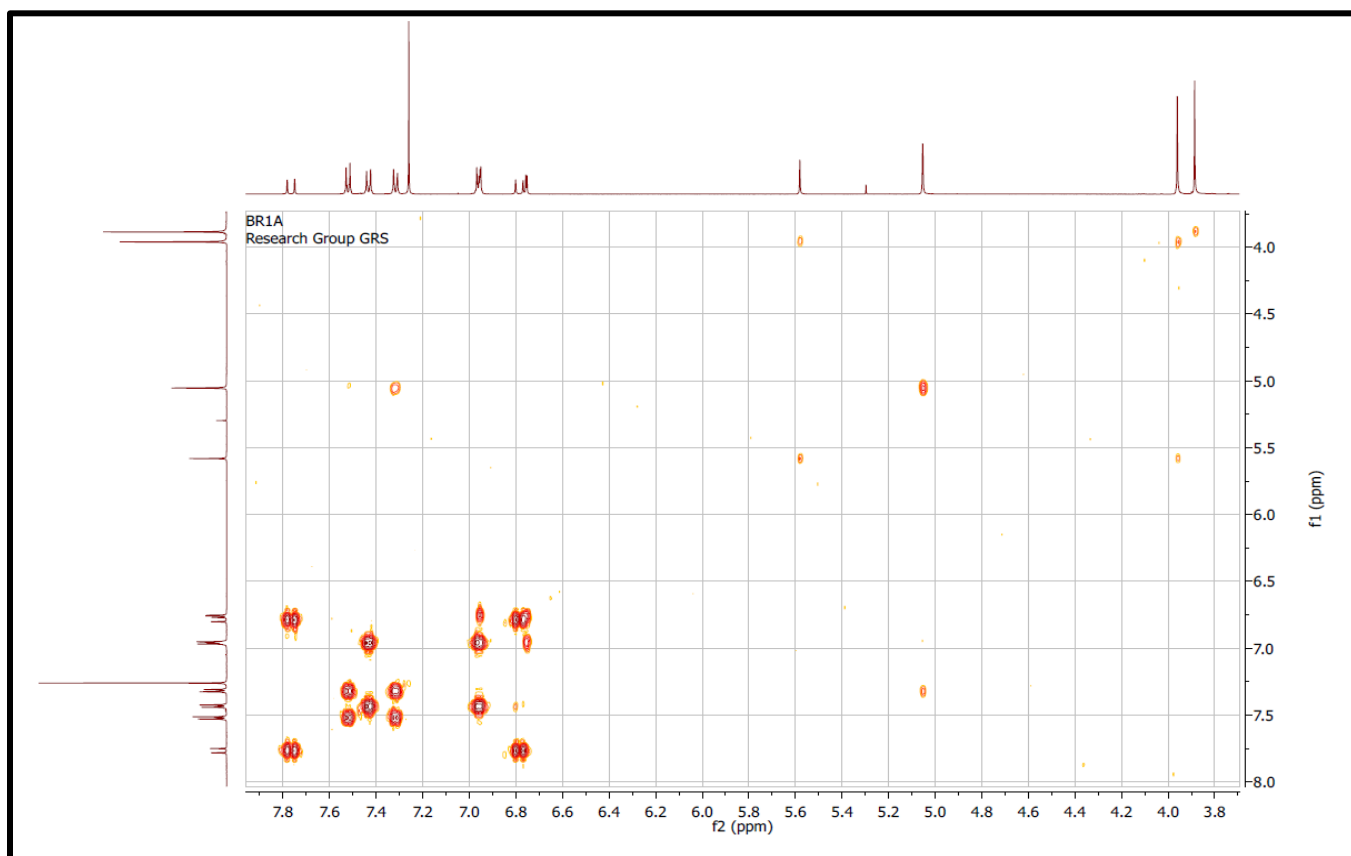


Figure S45: COSY spectrum of compound **1e**

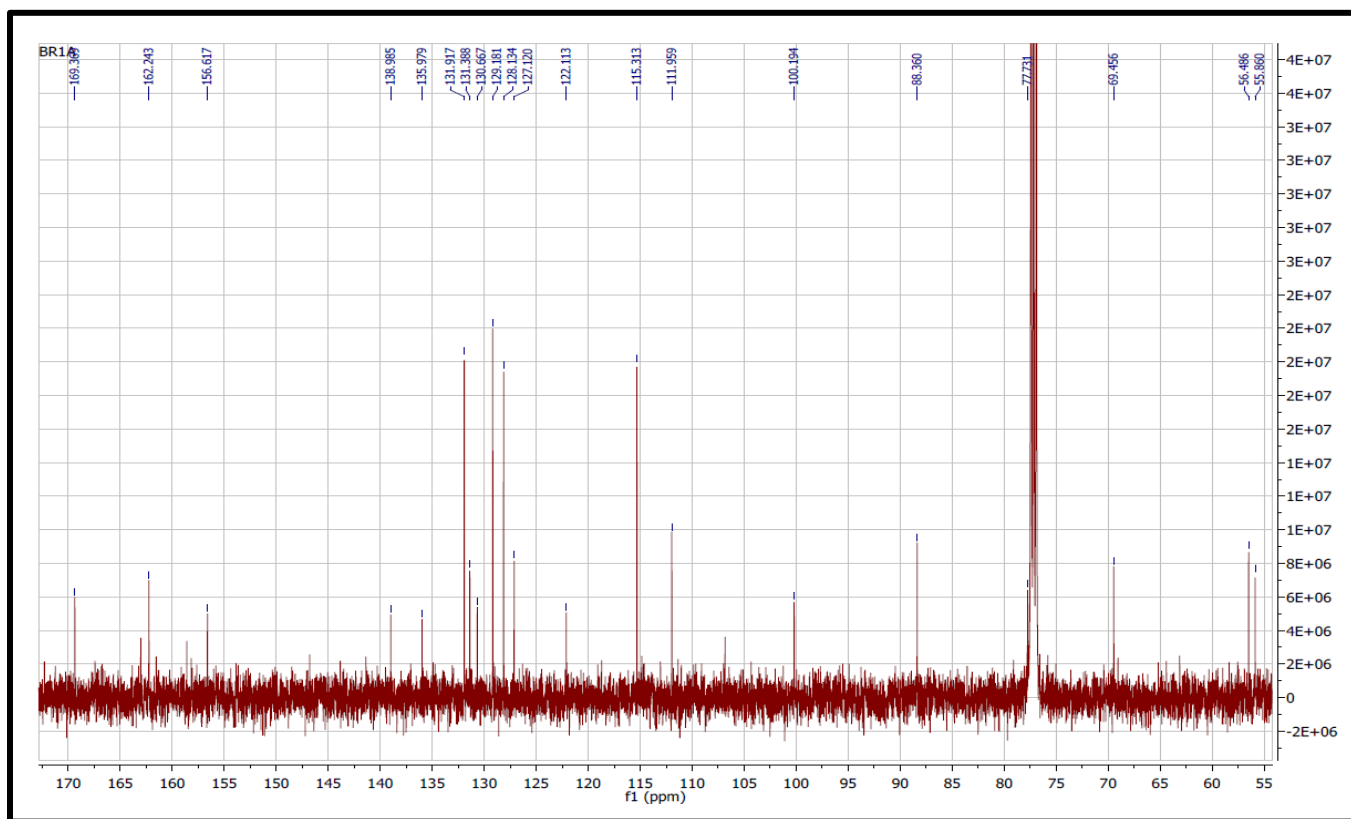


Figure S46: ^{13}C NMR spectrum (CDCl_3 , 125 MHz) of compound **1e**

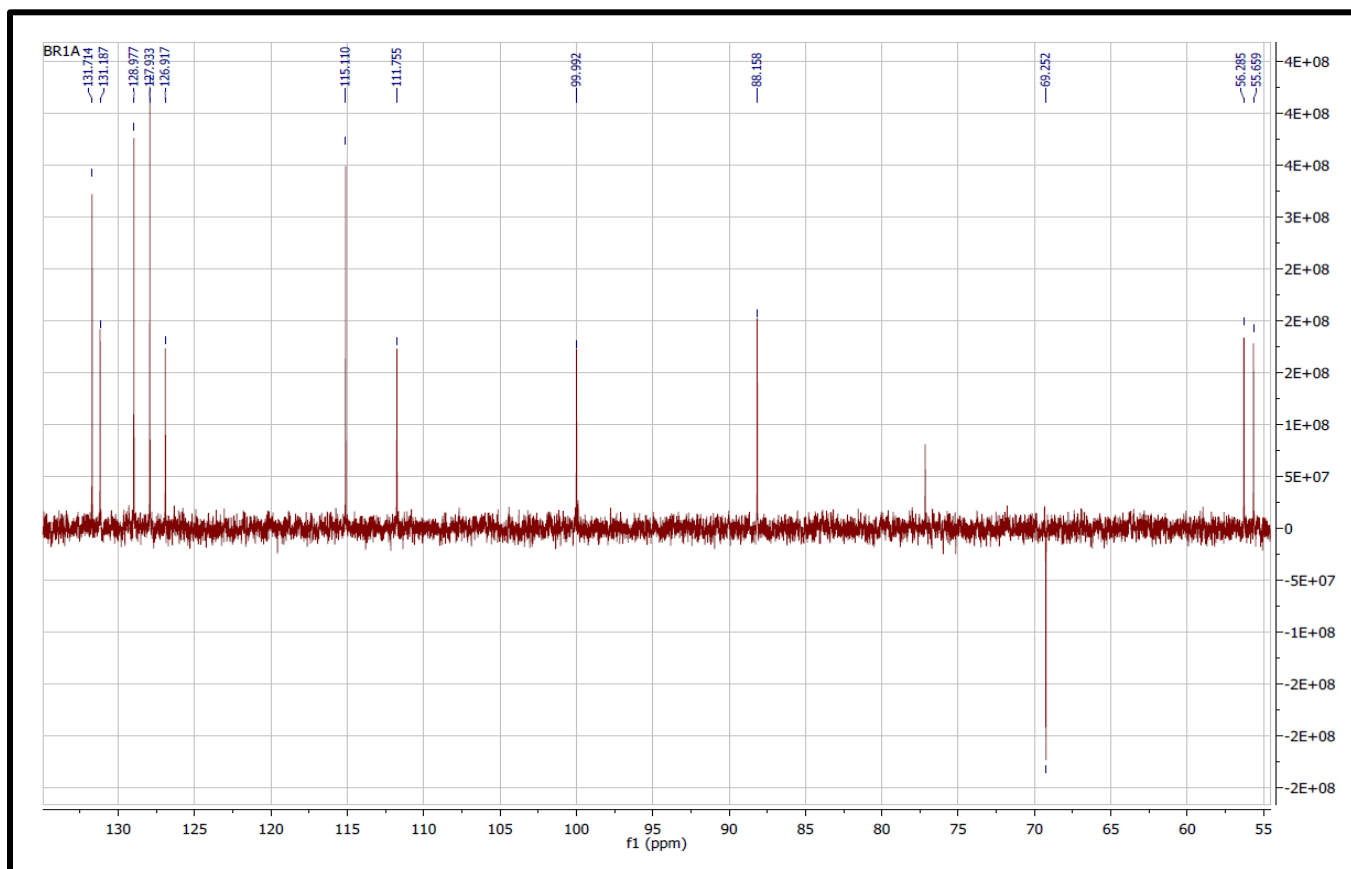


Figure S47: DEPT 135 spectrum of compound **1e**

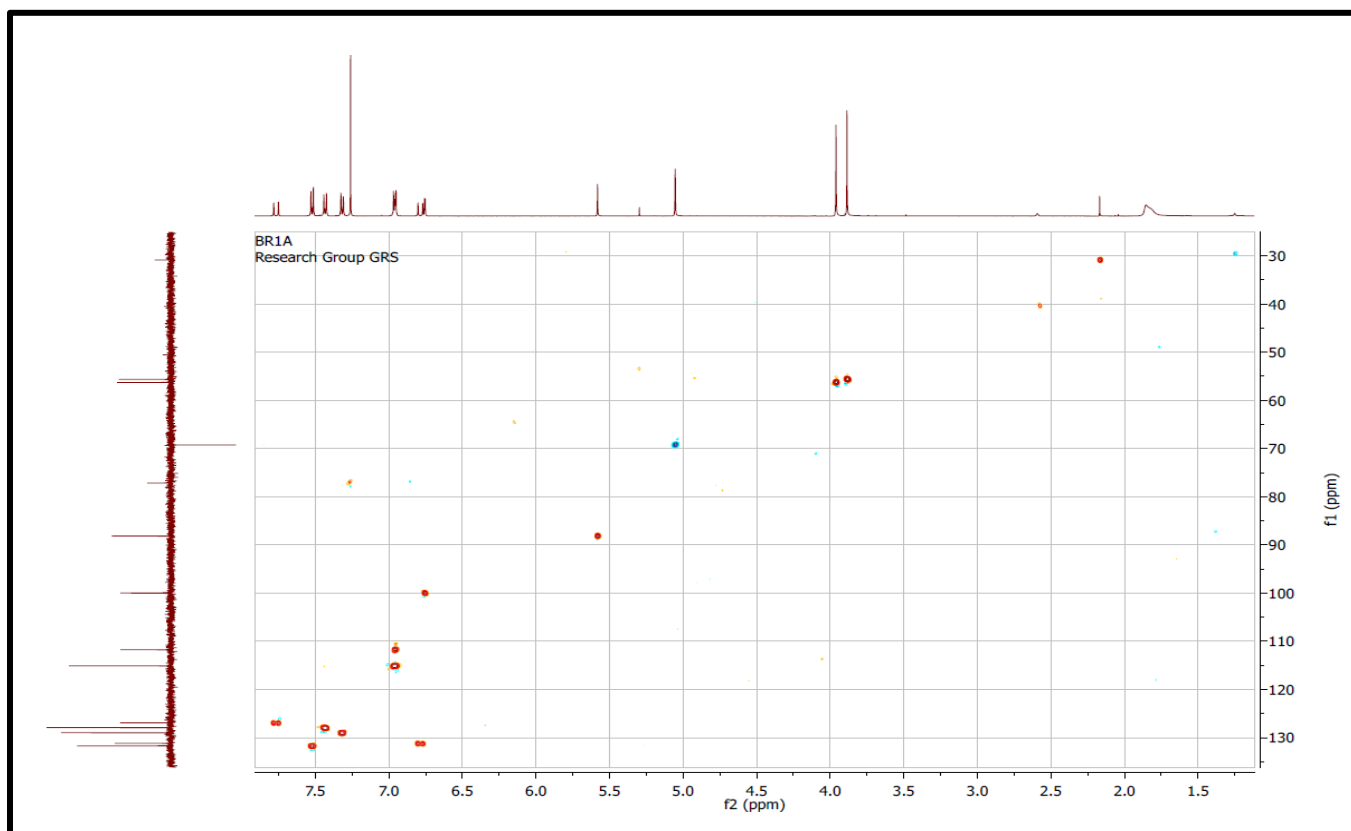


Figure S48: HSQC spectrum of compound **1e**

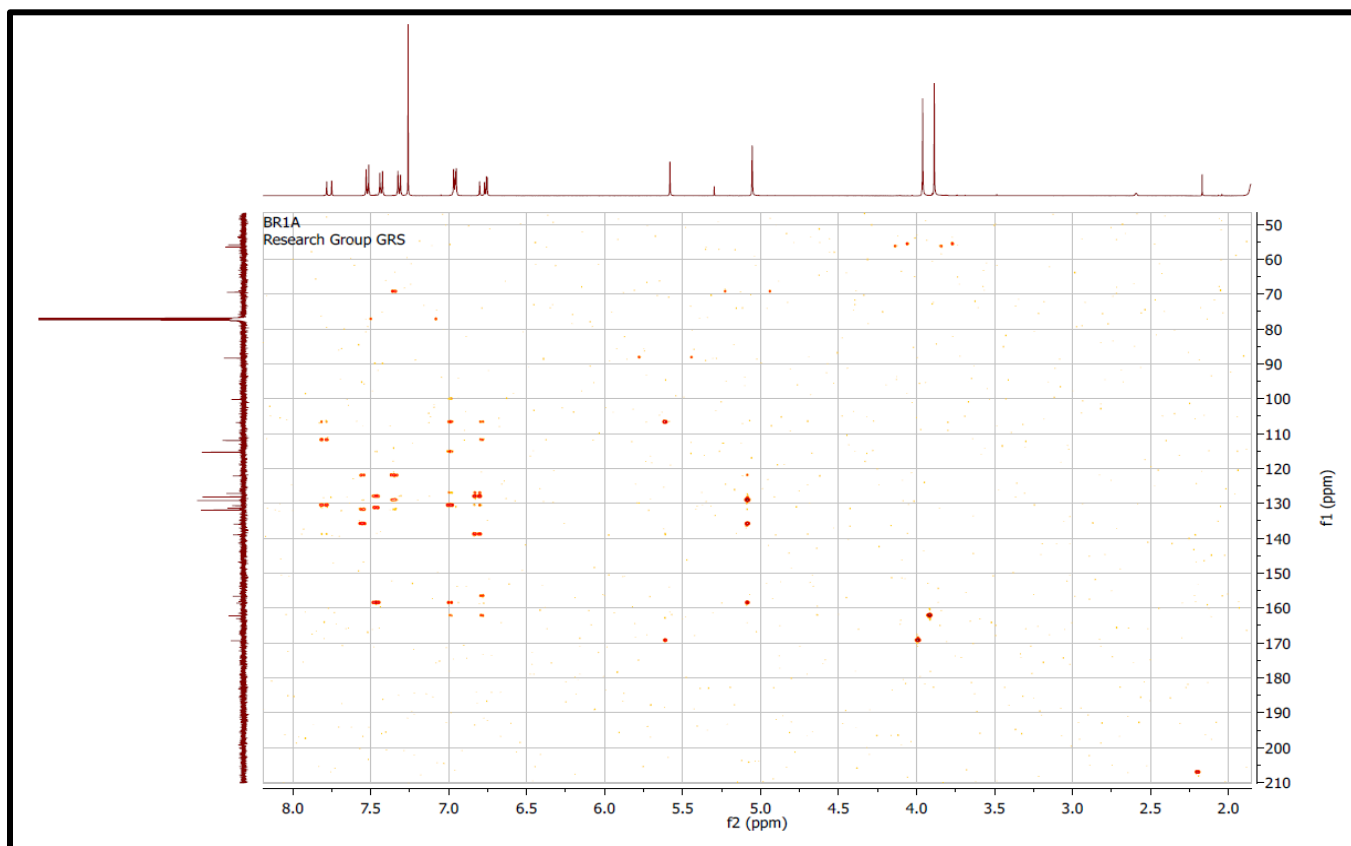


Figure S49: HMBC spectrum of compound **1e**