Supplementary Information for

Megastigmanes isolated from *Boehmeria nivea* leaves and their immunomodulatory activity on IL–1β and IL–10 production in raw 264.7 macrophages

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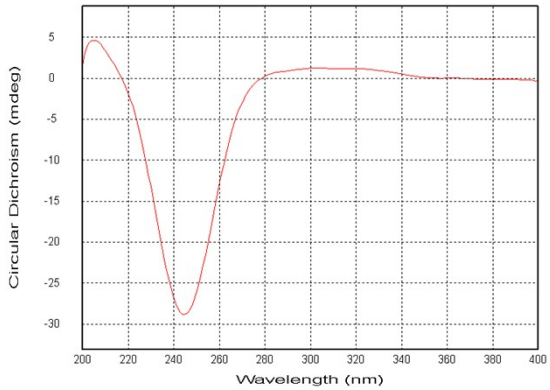


Figure S1. ECD spectra of **1** in MeOH

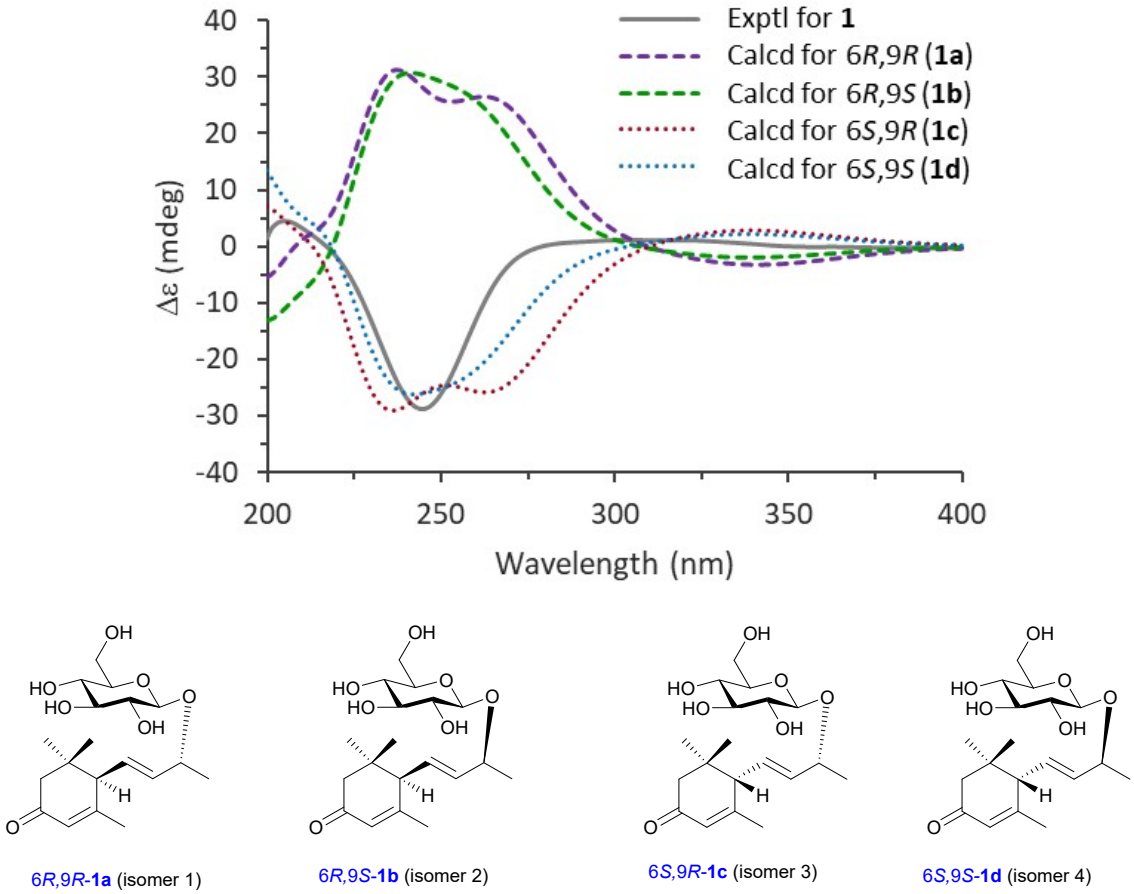
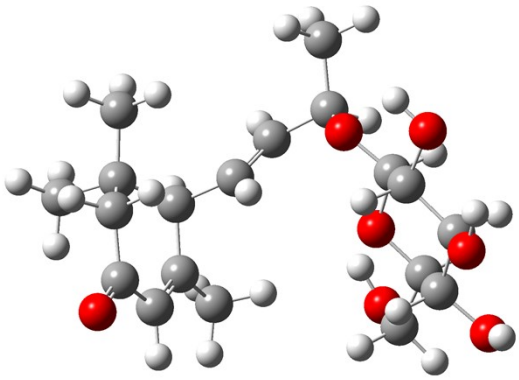
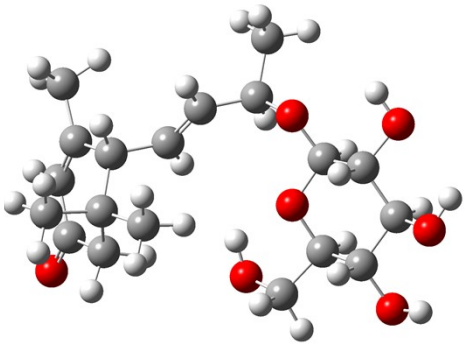
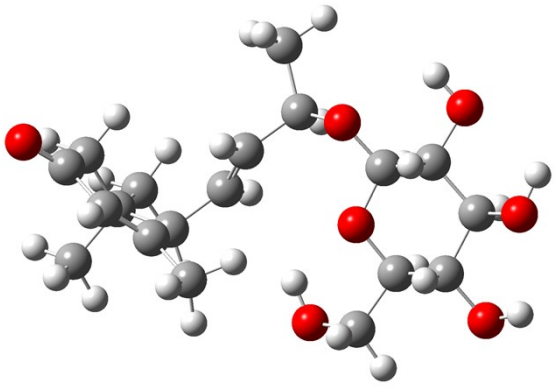
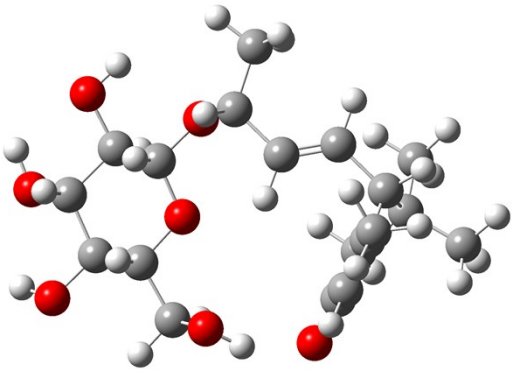
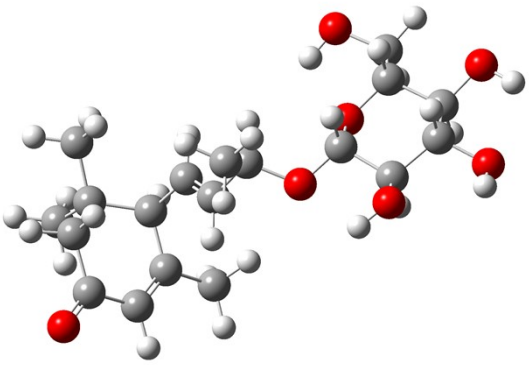
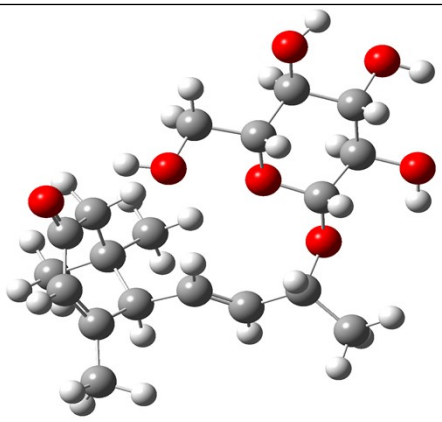
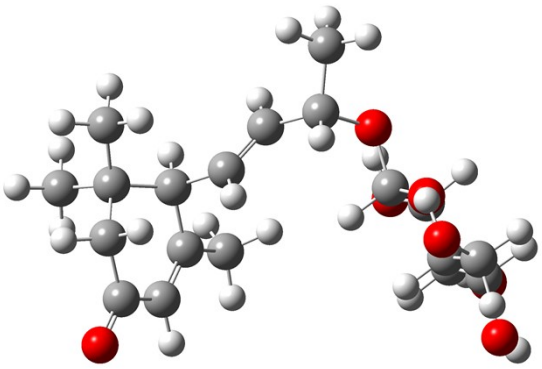
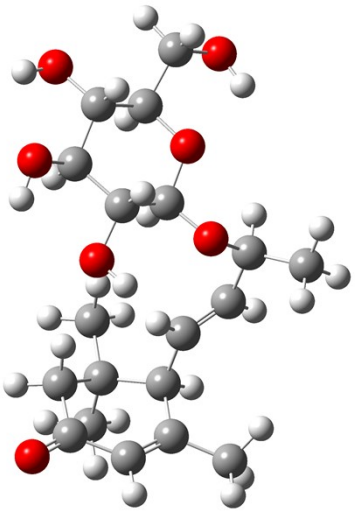
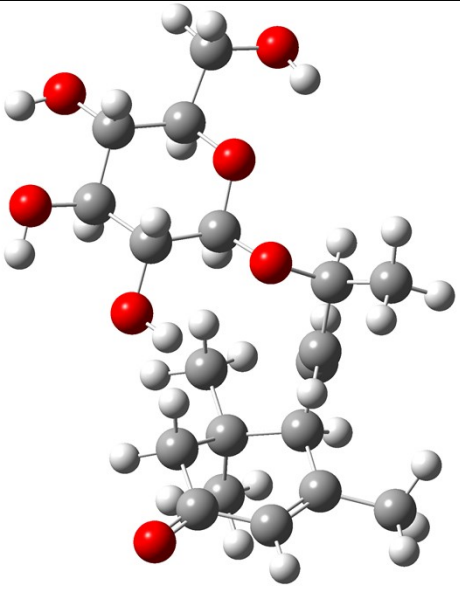
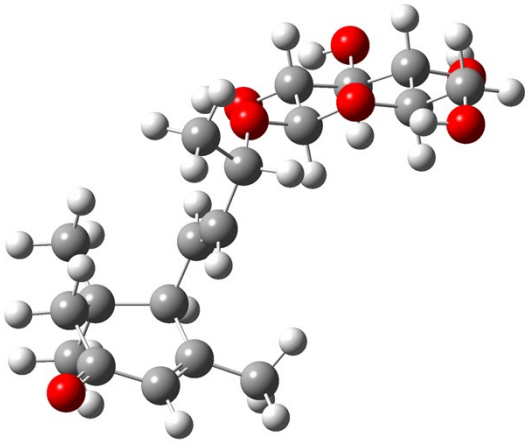
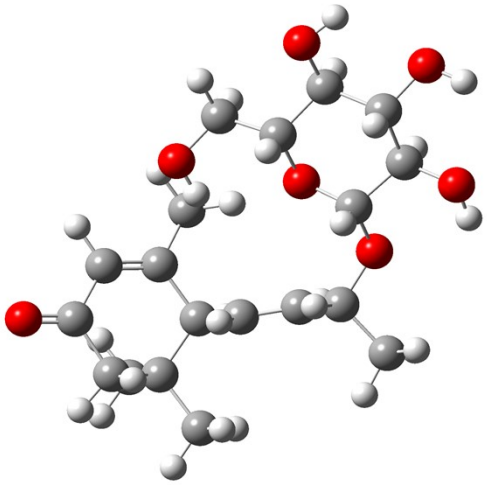
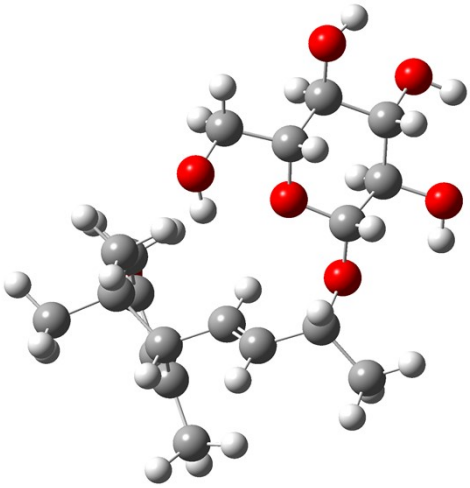
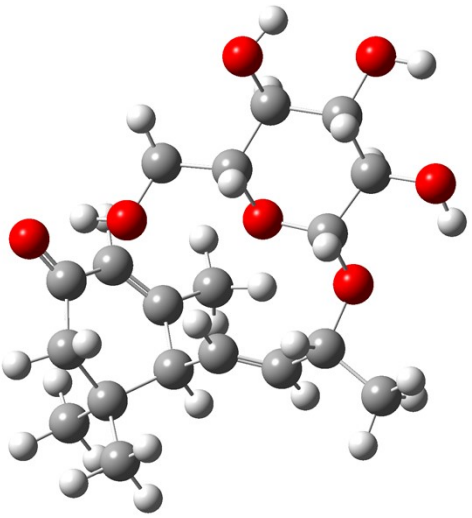


Figure S2. ECD spectra of experimental **1** and calcd for 4 isomers B3LYP_6-31G(d,p)_IEFPCM_MeOH

 **1a-1**	 **1a-2**
 **1a-3**	 **1a-4**
 **1a-5**	 **1a-6**

 **1b-1**	 **1b-2**
 **1b-3**	 **1b-4**
 **1c-1**	 **1c-2**



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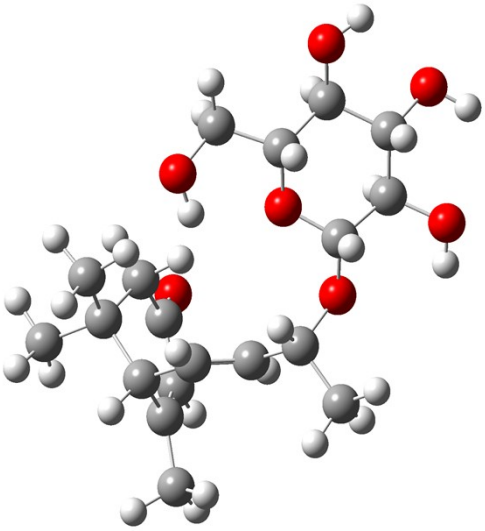

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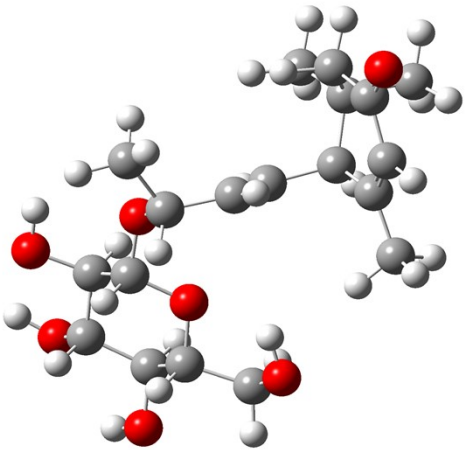

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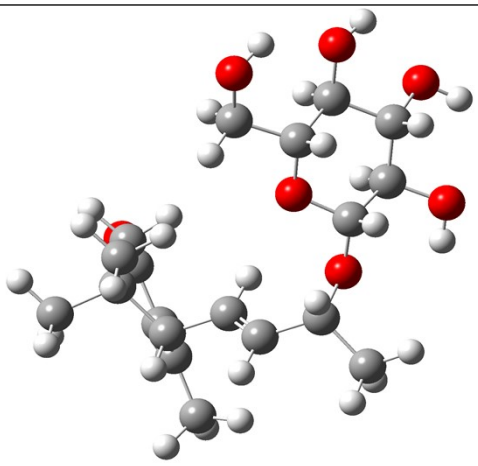

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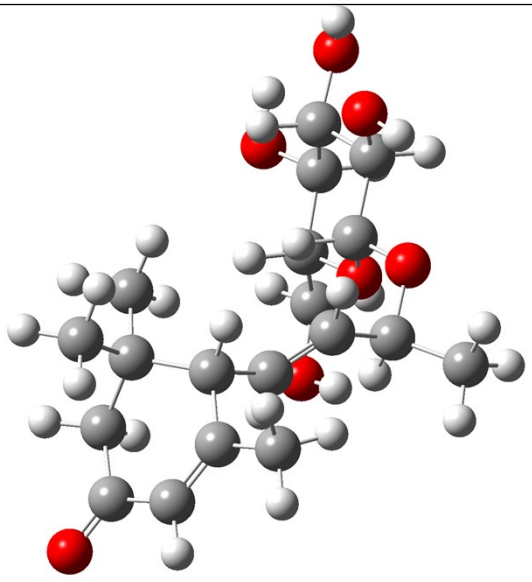

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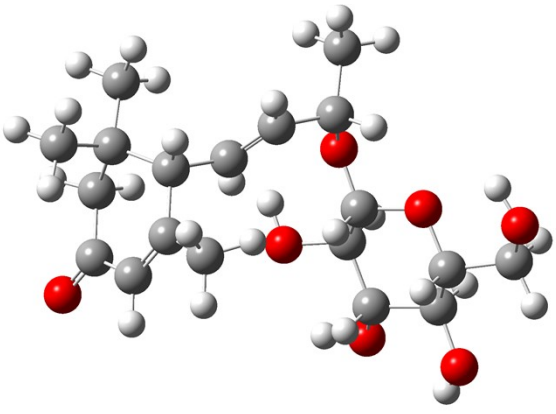

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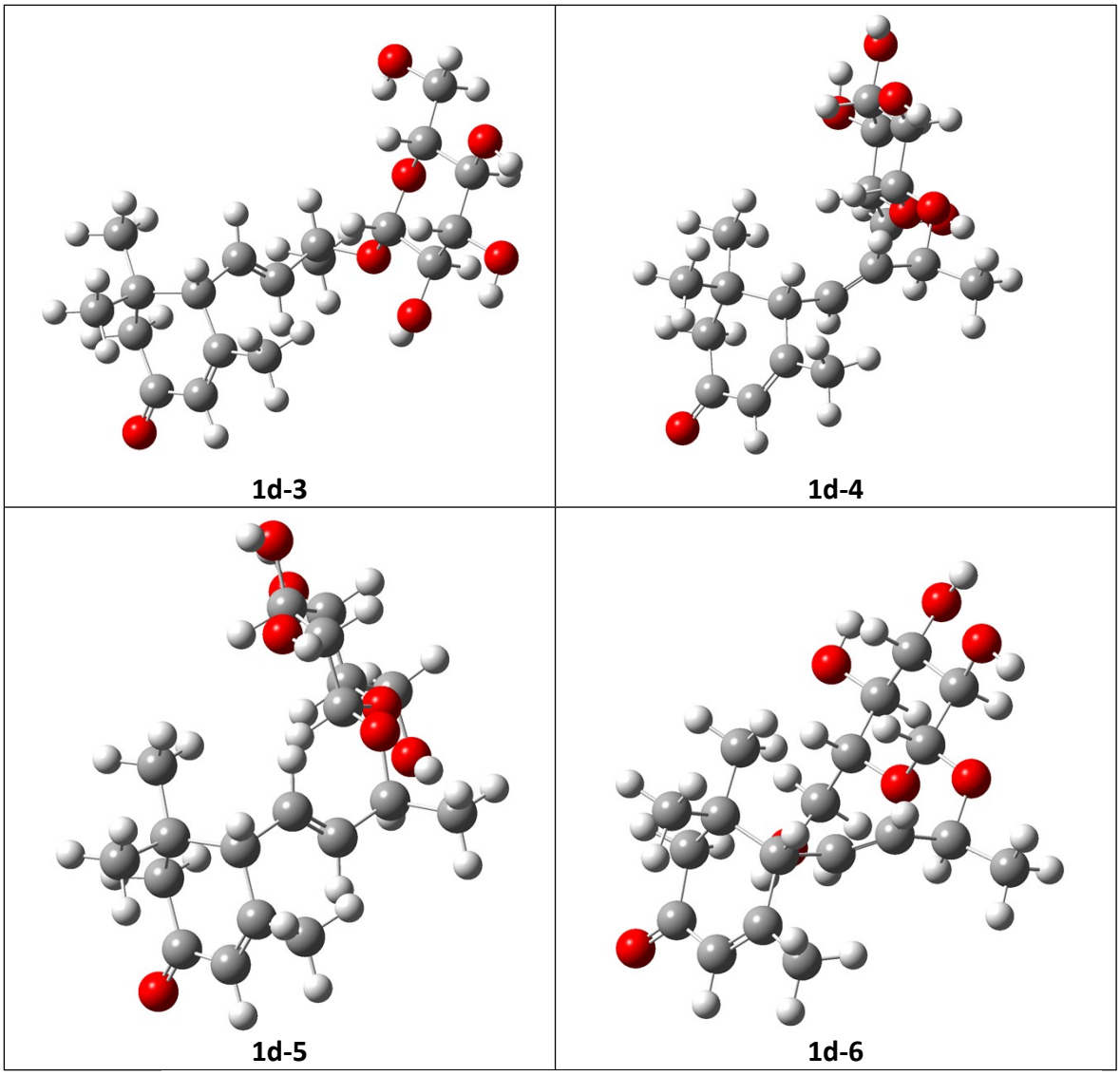


Figure S3. Optimized conformers of compounds 1a-1d with DFT/B3LYP/6-31G(d) in gas phase

Settings	Type of data (shifts)
Default	Shielding tensors

Functional	Solvent?	Basis Set
mPW1PW91	PCM	6-31+G(d,p)

Isomer №			1	2	3	4
DP4+ (%)		H data	0.08%	0.01%	0.07%	99.83%
		C data	34.22%	0.00%	65.78%	0.00%
		All data	37.37%	0.00%	62.63%	0.00%
Type	sp2?	Exp	1	2	3	4

	isomer 1	isomer 2	isomer 3	isomer 4
	6R9R (a)	6R9S (b)	6S9R (c)	6S9S (d)
Default parameters	1	2	3	4
sDP4+ (H data)	0.12%	0.11%	0.09%	99.68%
sDP4+ (C data)	47.11%	0.05%	52.84%	0.00%
sDP4+ (all data)	54.35%	0.05%	44.34%	1.25%
uDP4+ (H data)	26.25%	3.39%	31.46%	38.91%
uDP4+ (C data)	36.85%	0.00%	63.15%	0.00%
uDP4+ (all data)	32.74%	0.00%	67.26%	0.00%
DP4+ (H data)	0.08%	0.01%	0.07%	99.83%
DP4+ (C data)	34.22%	0.00%	65.78%	0.00%
DP4+ (all data)	37.37%	0.00%	62.63%	0.00%

Settings	Type of data (shifts)
Default	Shielding tensors

Functional	Solvent?	Basis Set
mPW1PW91	PCM	6-311G(d,p)

Isomer №			1	2	3	4
DP4+ (%)		H data	0.02%	0.45%	99.53%	0.00%
		C data	0.03%	0.03%	99.93%	0.00%
		All data	0.00%	0.00%	100.00%	0.00%
Type	sp2?	Exp	1	2	3	4

Figure S4. DP4+ probability analysis of 4 isomers (**1a-1d)** at mPW1PW91/6-31+G(d,p) level of theory using PCM solvent model in $CDCl_3$

	isomer 1	isomer 2	isomer 3	isomer 4
	6R9R (a)	6R9S (b)	6S9R (c)	6S9S (d)
Default parameters	1	2	3	4
sDP4+ (H data)	1.77%	9.47%	86.39%	2.37%
sDP4+ (C data)	0.63%	0.63%	98.54%	0.20%
sDP4+ (all data)	0.01%	0.07%	99.91%	0.01%
uDP4+ (H data)	0.88%	3.96%	95.12%	0.05%
uDP4+ (C data)	4.85%	4.85%	89.91%	0.38%
uDP4+ (all data)	0.05%	0.22%	99.73%	0.00%
DP4+ (H data)	0.02%	0.45%	99.53%	0.00%
DP4+ (C data)	0.03%	0.03%	99.93%	0.00%
DP4+ (all data)	0.00%	0.00%	100.00%	0.00%

Figure S5. DP4+ probability analysis of 4 isomers (**1a-1d)** at mPW1PW91/6-311G(d,p) level of theory using PCM solvent model in $CDCl_3$

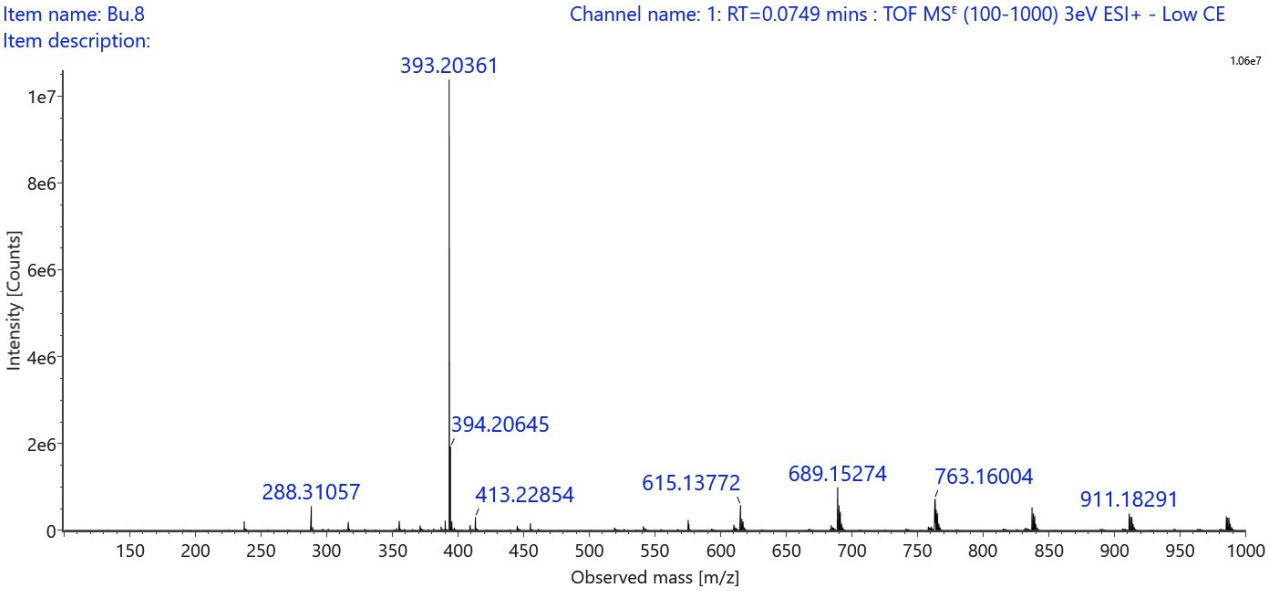


Figure S6. HR-MS spectrum of **1**

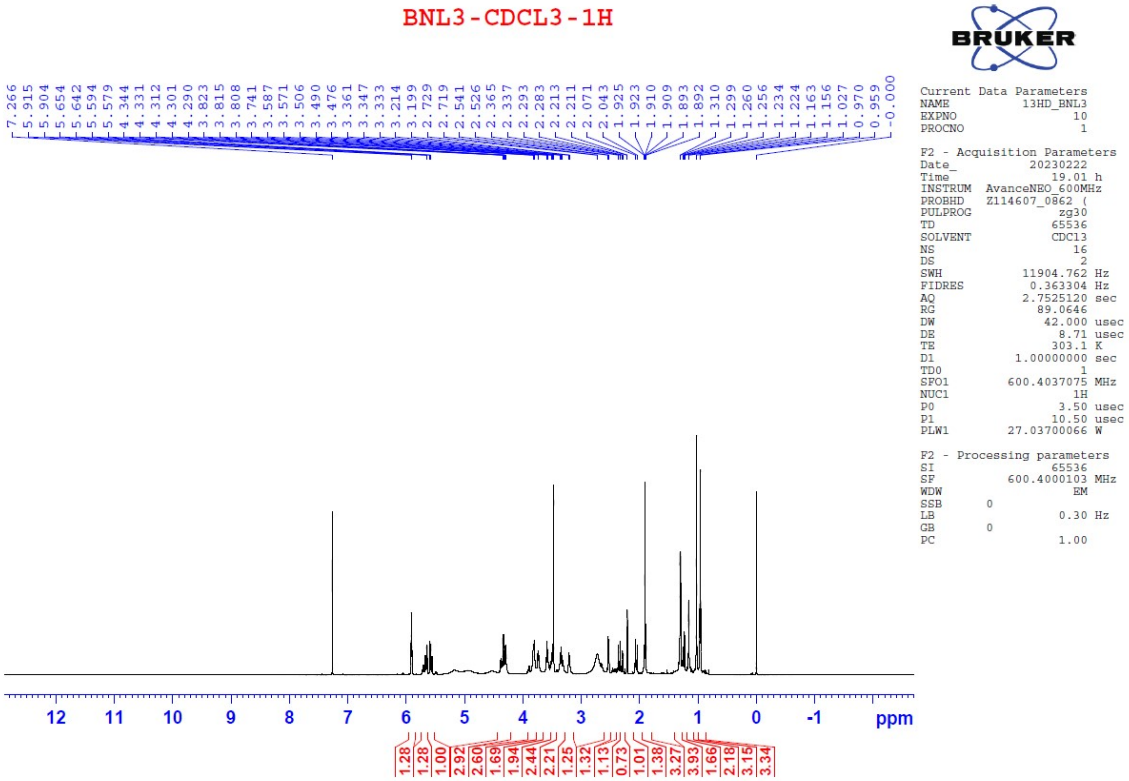


Figure S7. ^{1}H-NMR spectrum of **1** in $CDCl_3$. 600 MHz

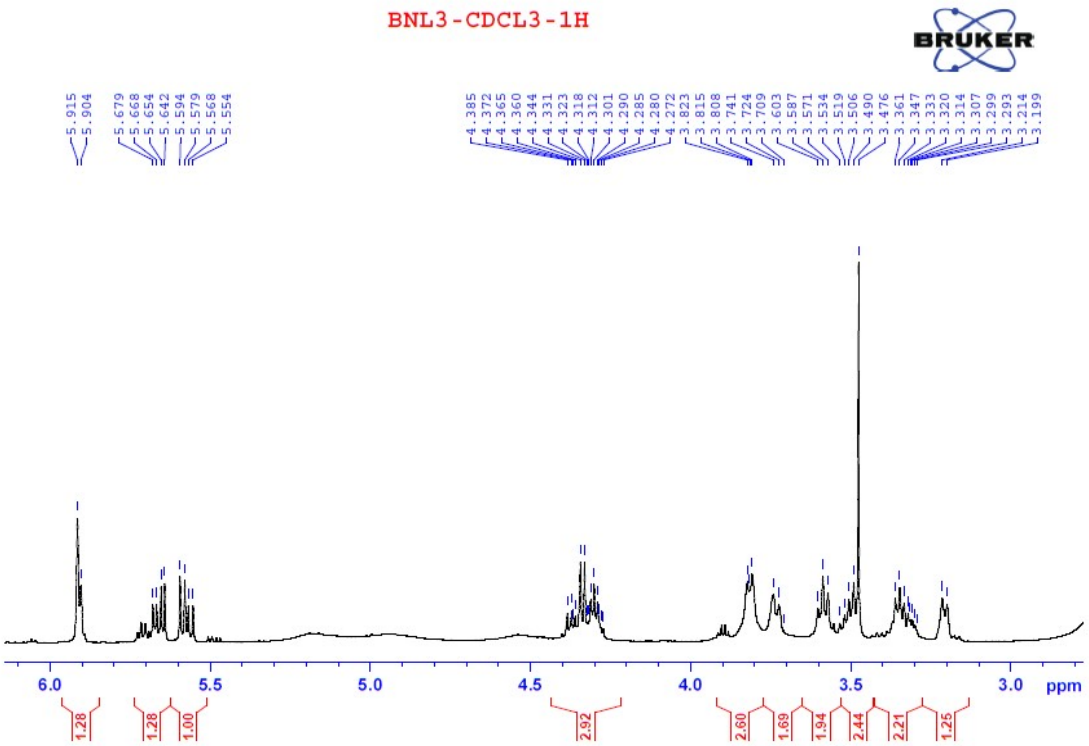


Figure S8. ^{1}H-NMR spectrum of **1** from 3.0 to 6.0 ppm region

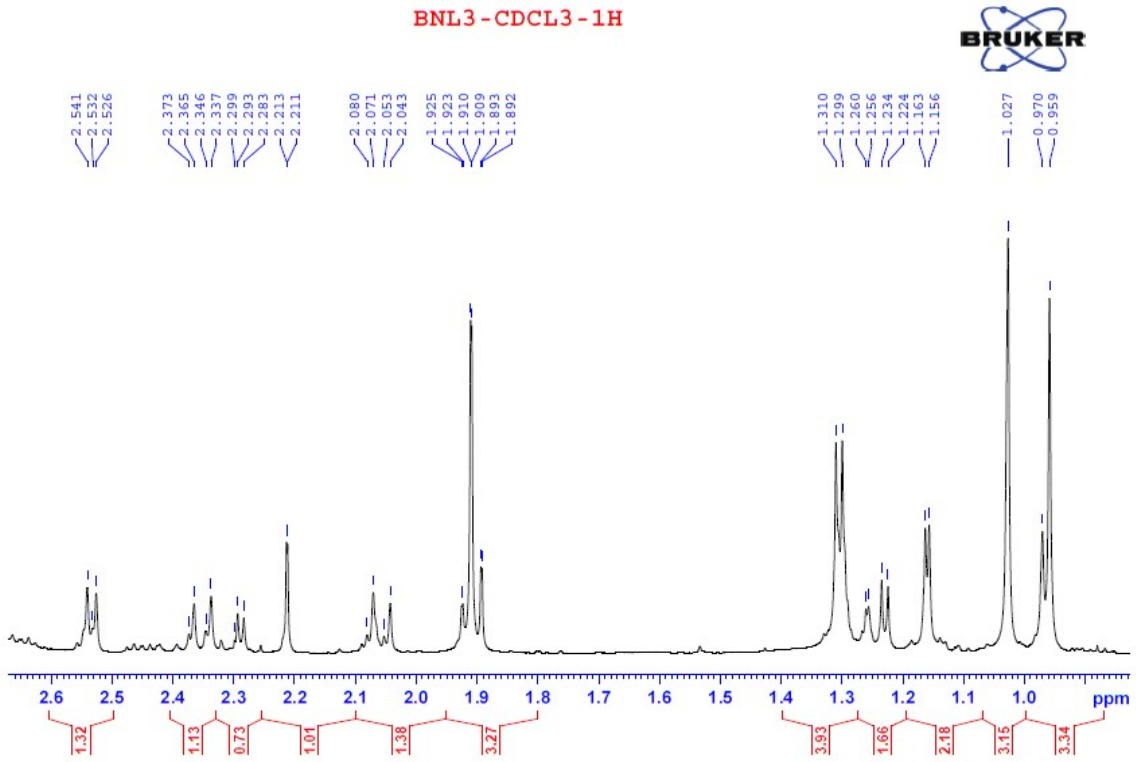


Figure S9. ^{1}H-NMR spectrum of **1** from 0.5 to 2.6 ppm region

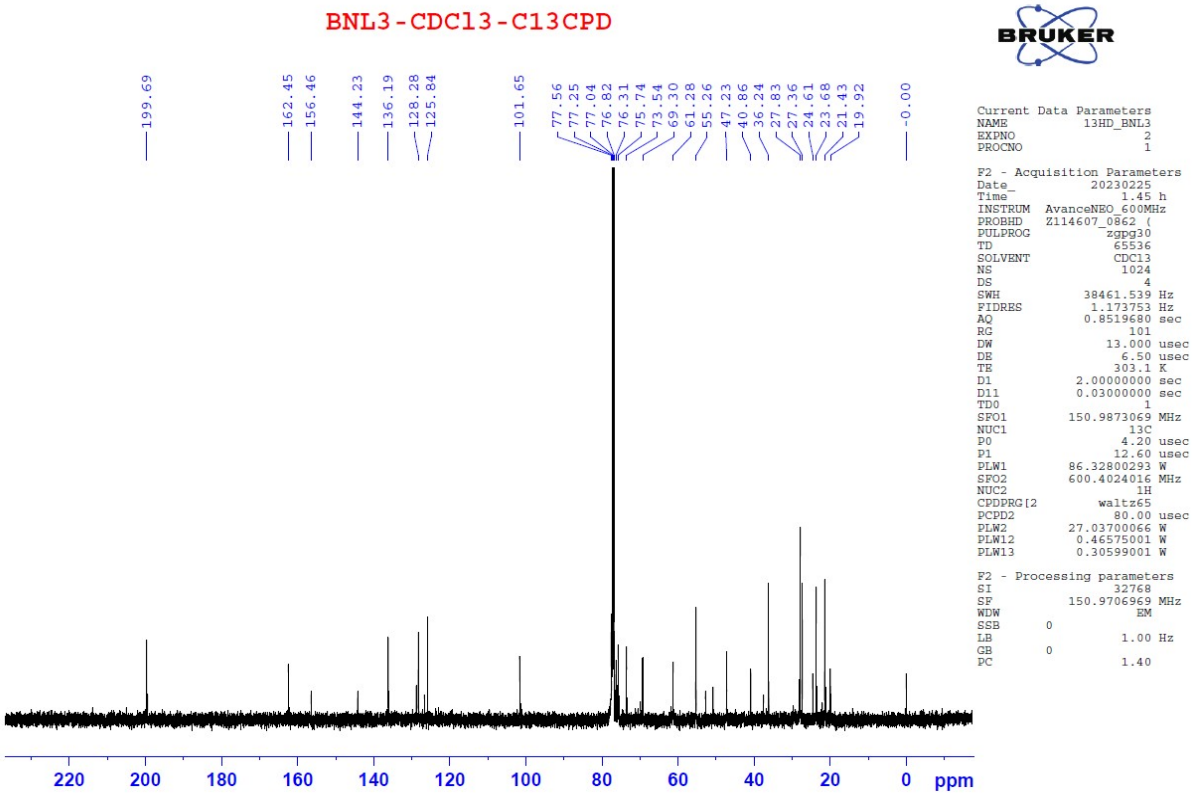


Figure S10. ^{13}C-NMR spectrum of **1** in $CDCl_3$. 150 MHz

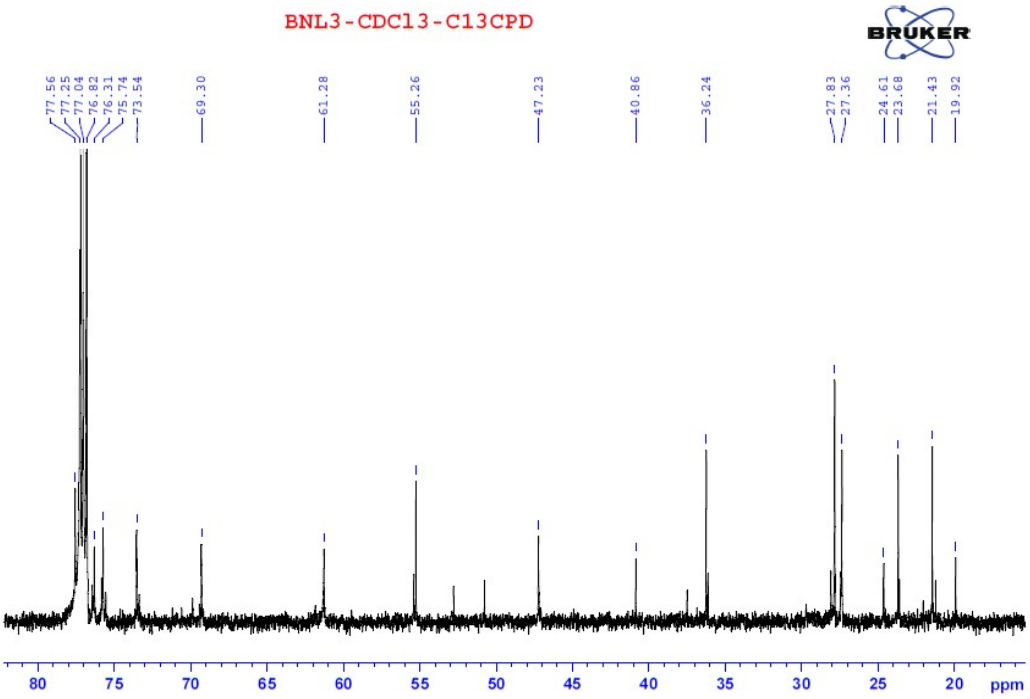


Figure S11. ^{13}C-NMR spectrum of **1** from 15 to 80 ppm region

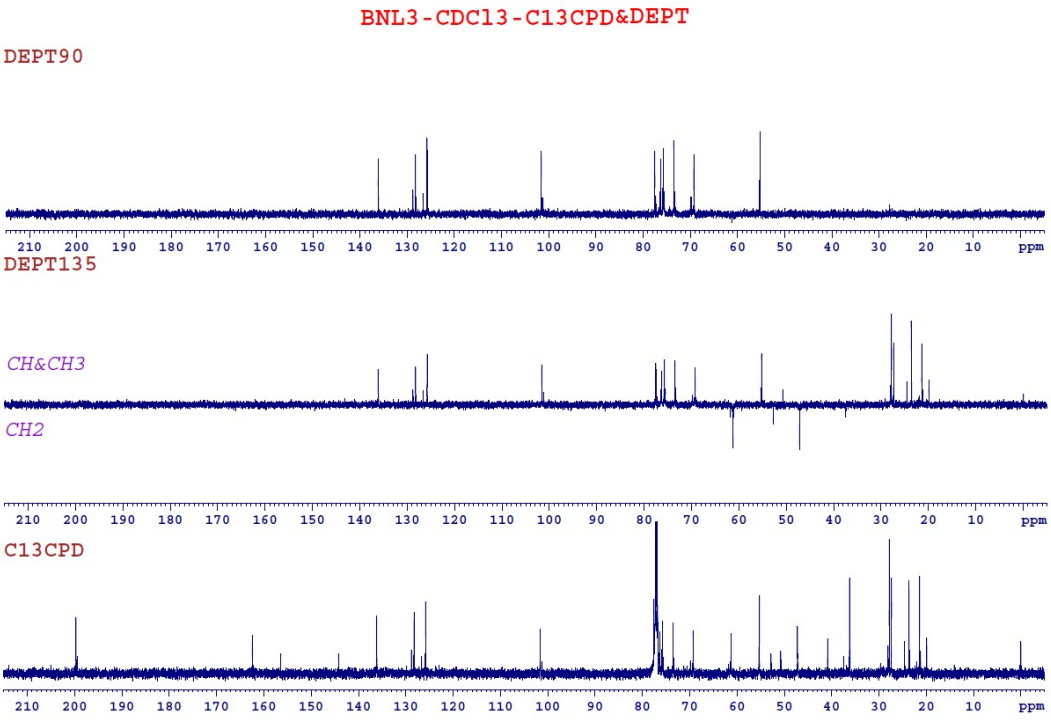


Figure S12. DEPT spectrum of **1**

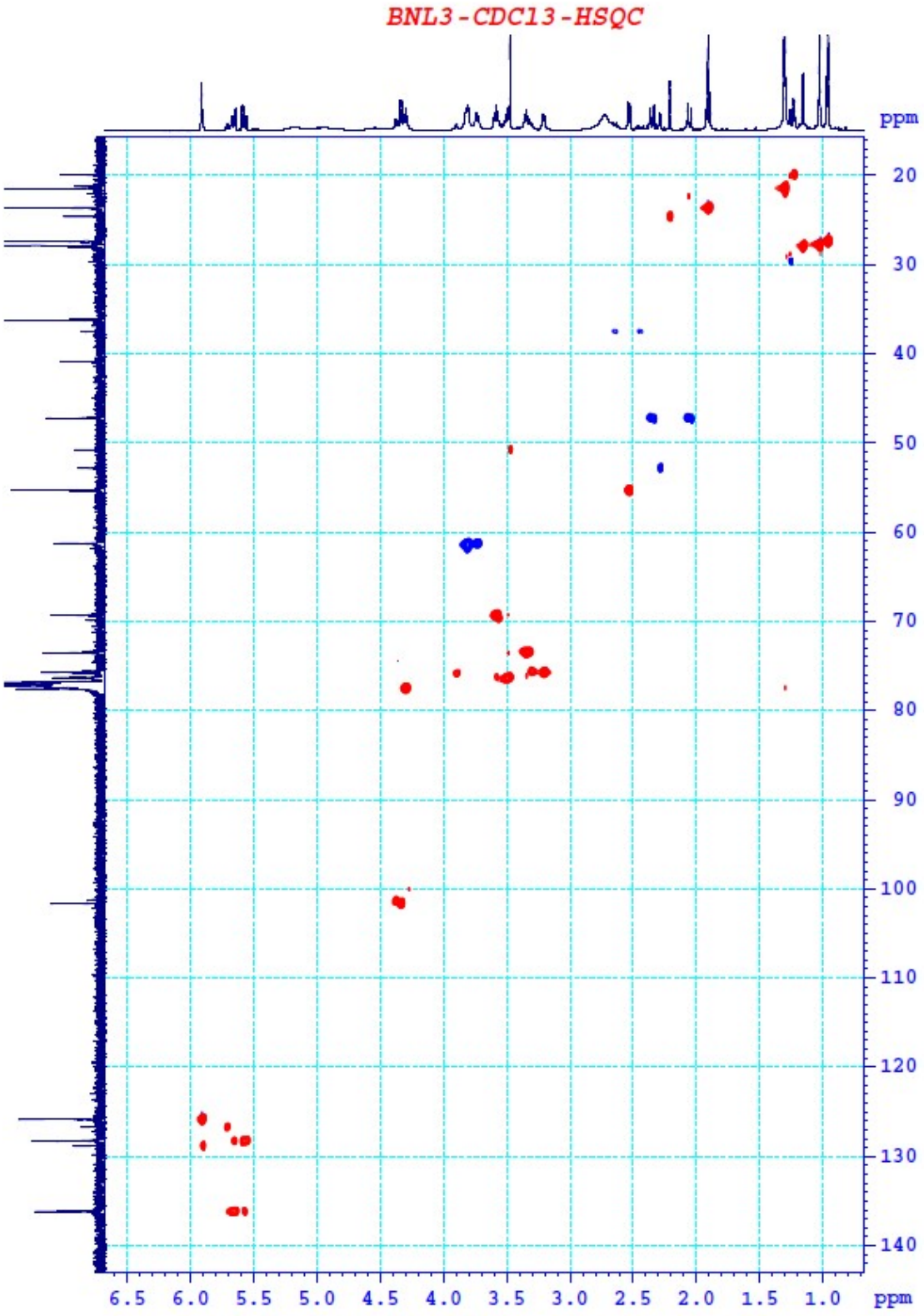


Figure S13. HSQC spectrum of **1**

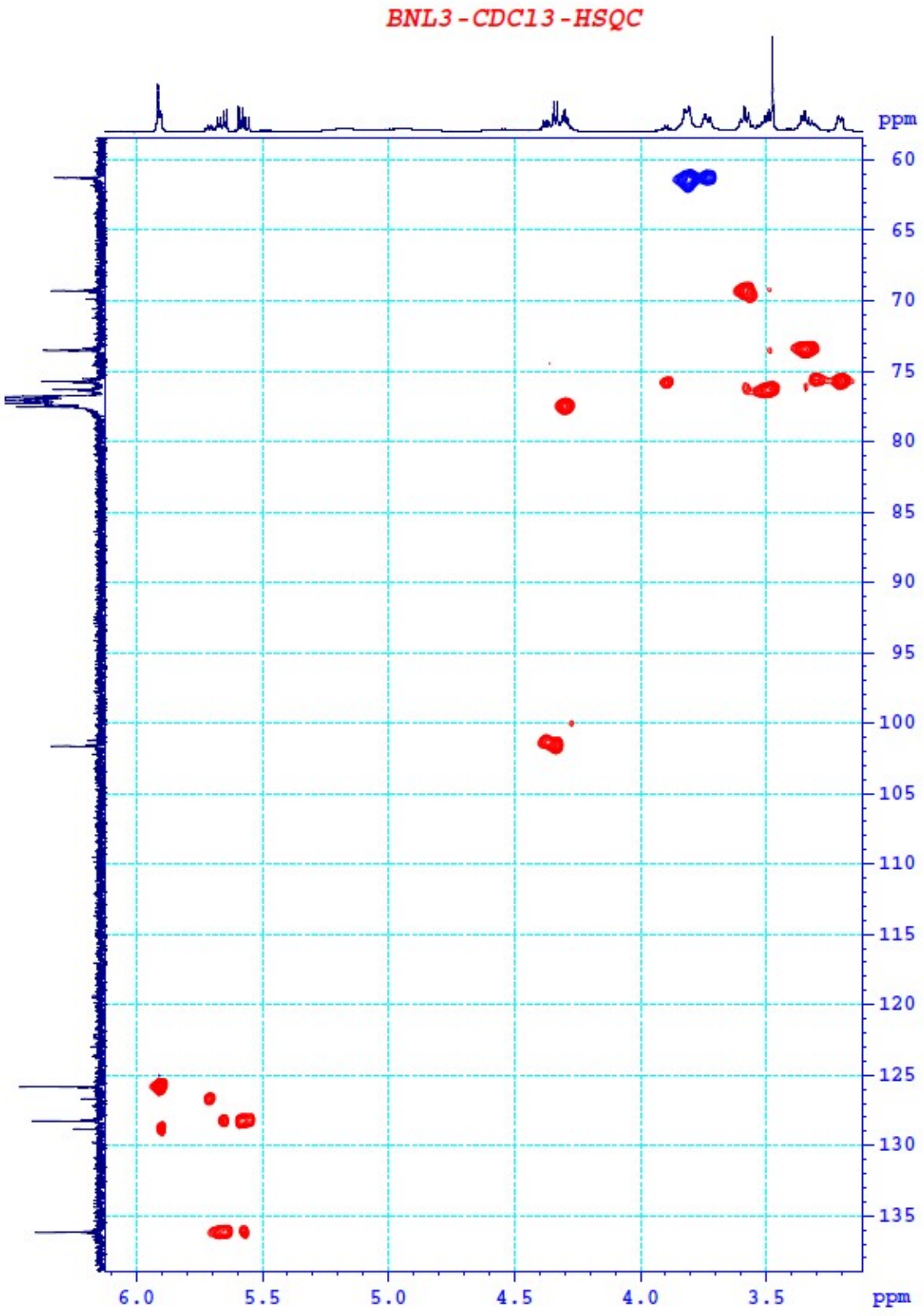


Figure S14. HSQC spectrum of **1** from 3.0 – 6.0 and 60 – 115 ppm region

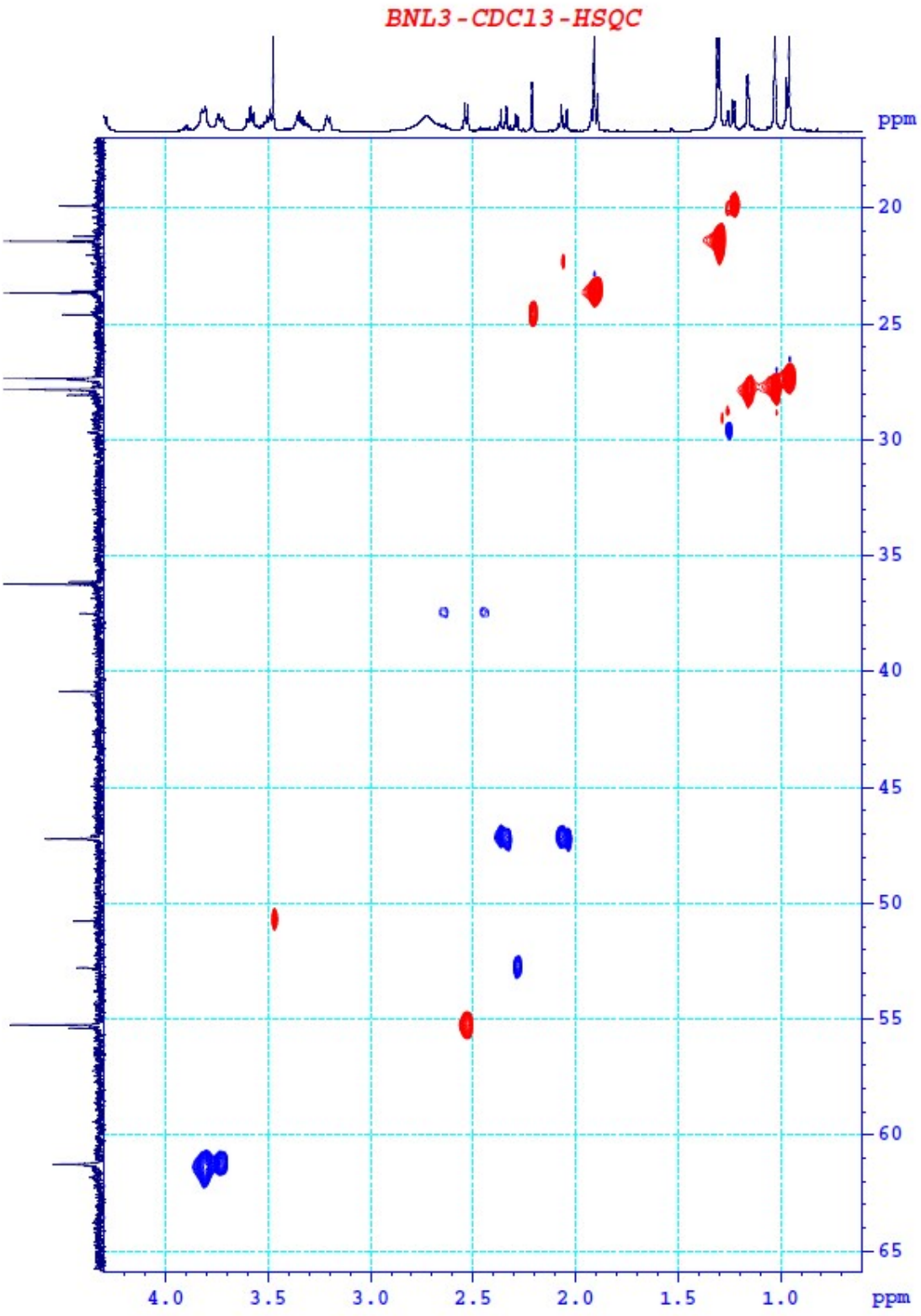


Figure S15. HSQC spectrum of **1** from 0 – 4.0 and 20 – 65 ppm region

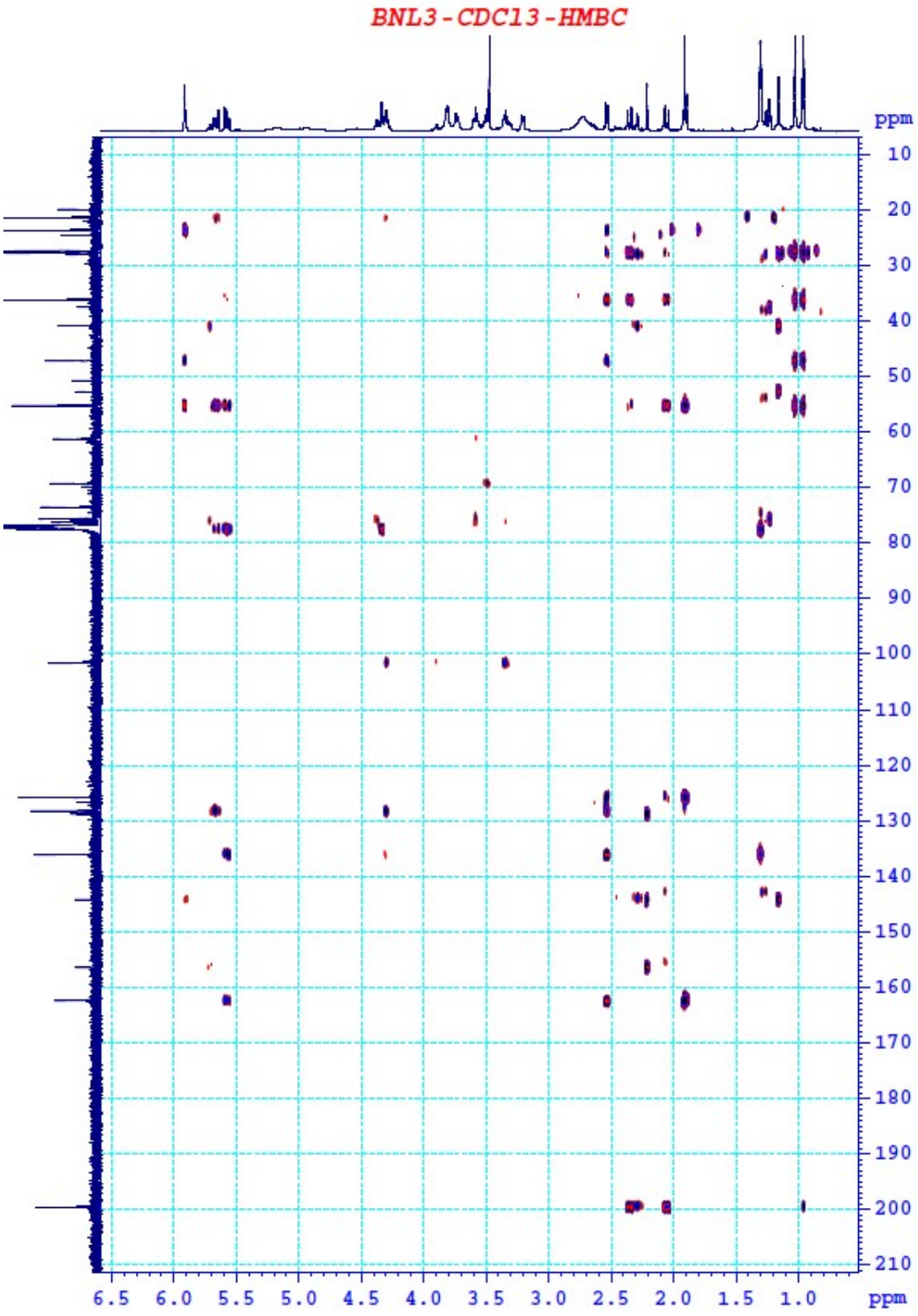


Figure S16. HMBC spectrum of **1**

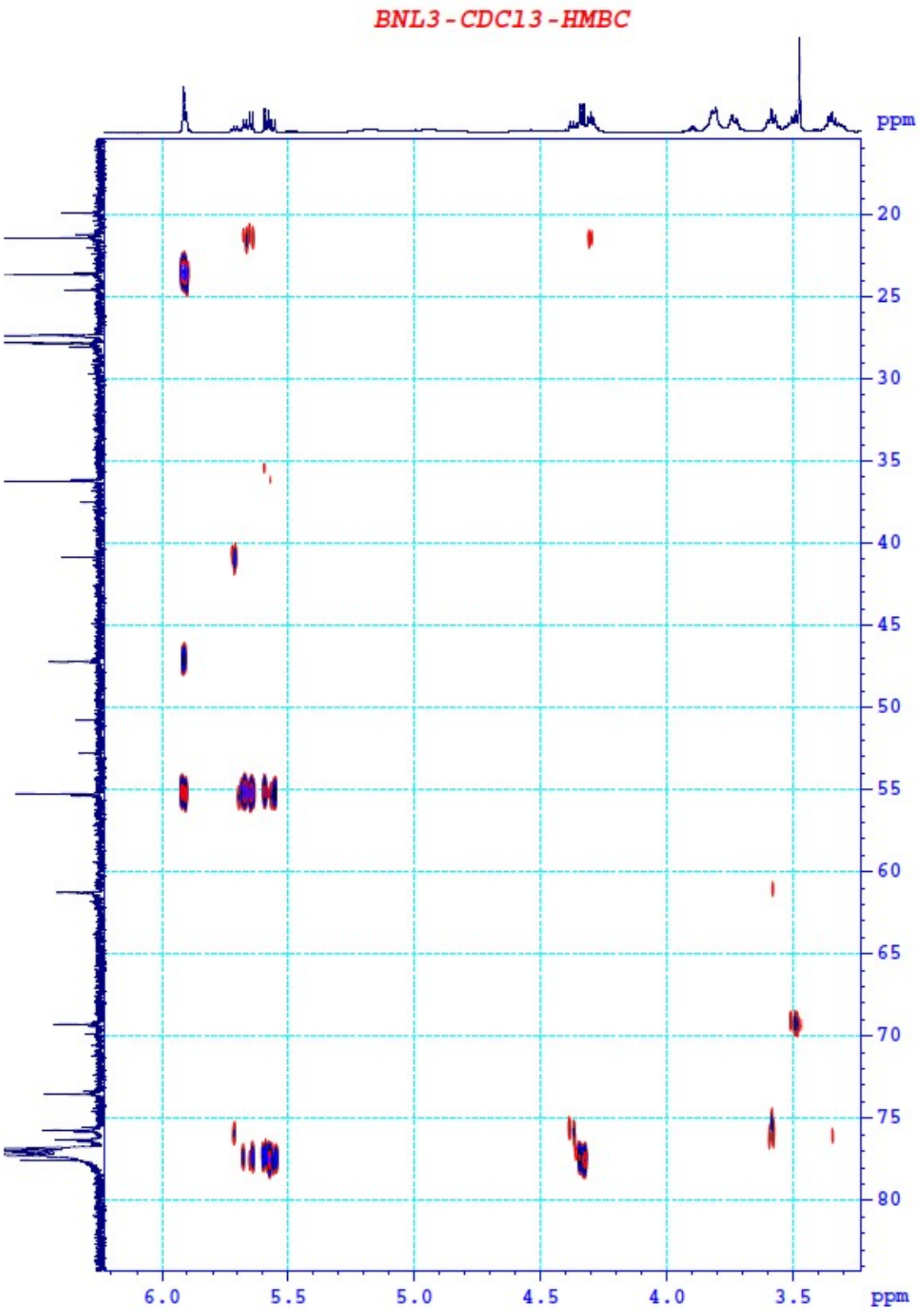


Figure S17. HMBC spectrum of **1** from 3.5 – 6.0 and 20 – 80 ppm region

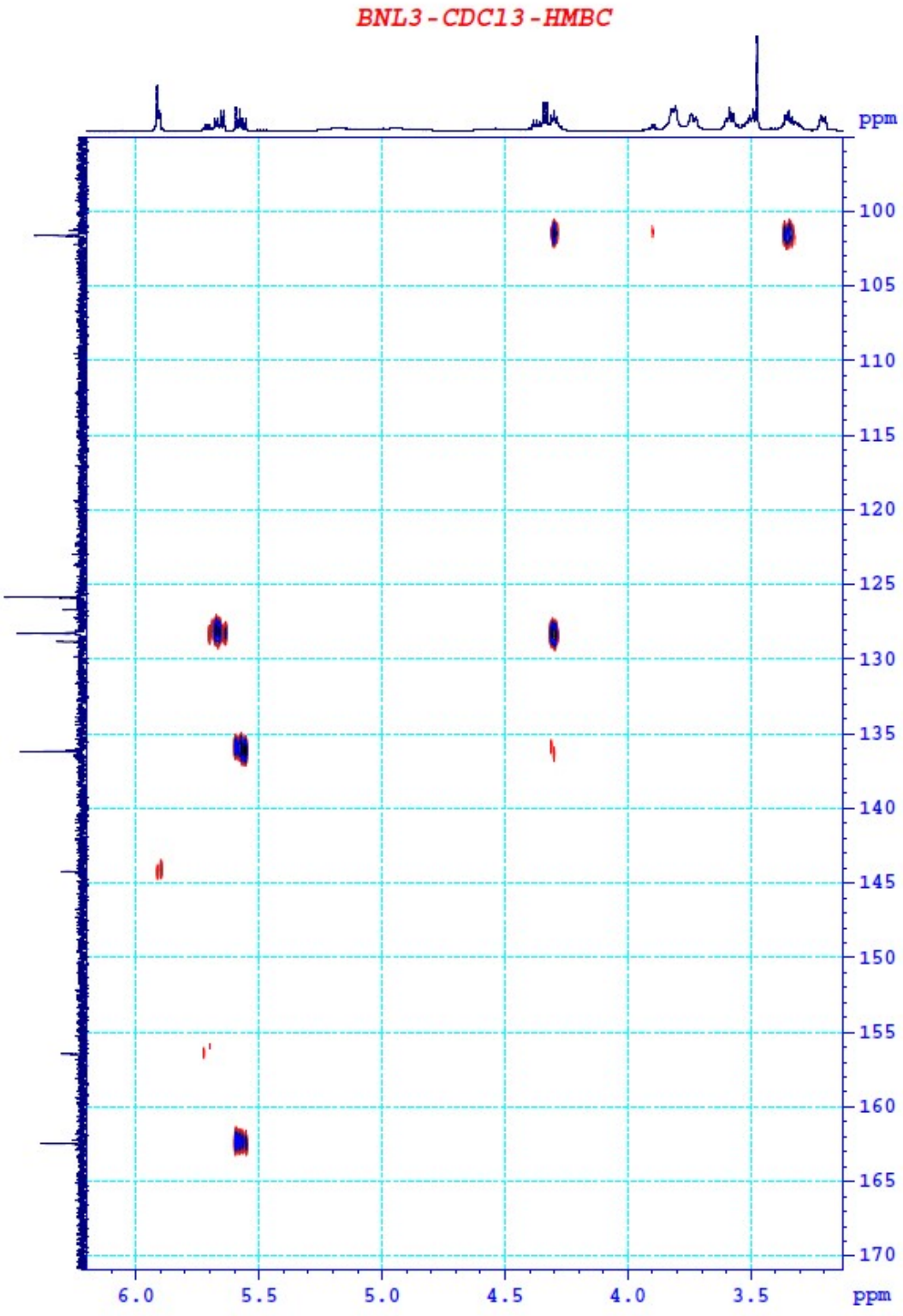


Figure S18. HMBC spectrum of **1** from 3.0 - 6.0 and 100-170 ppm region

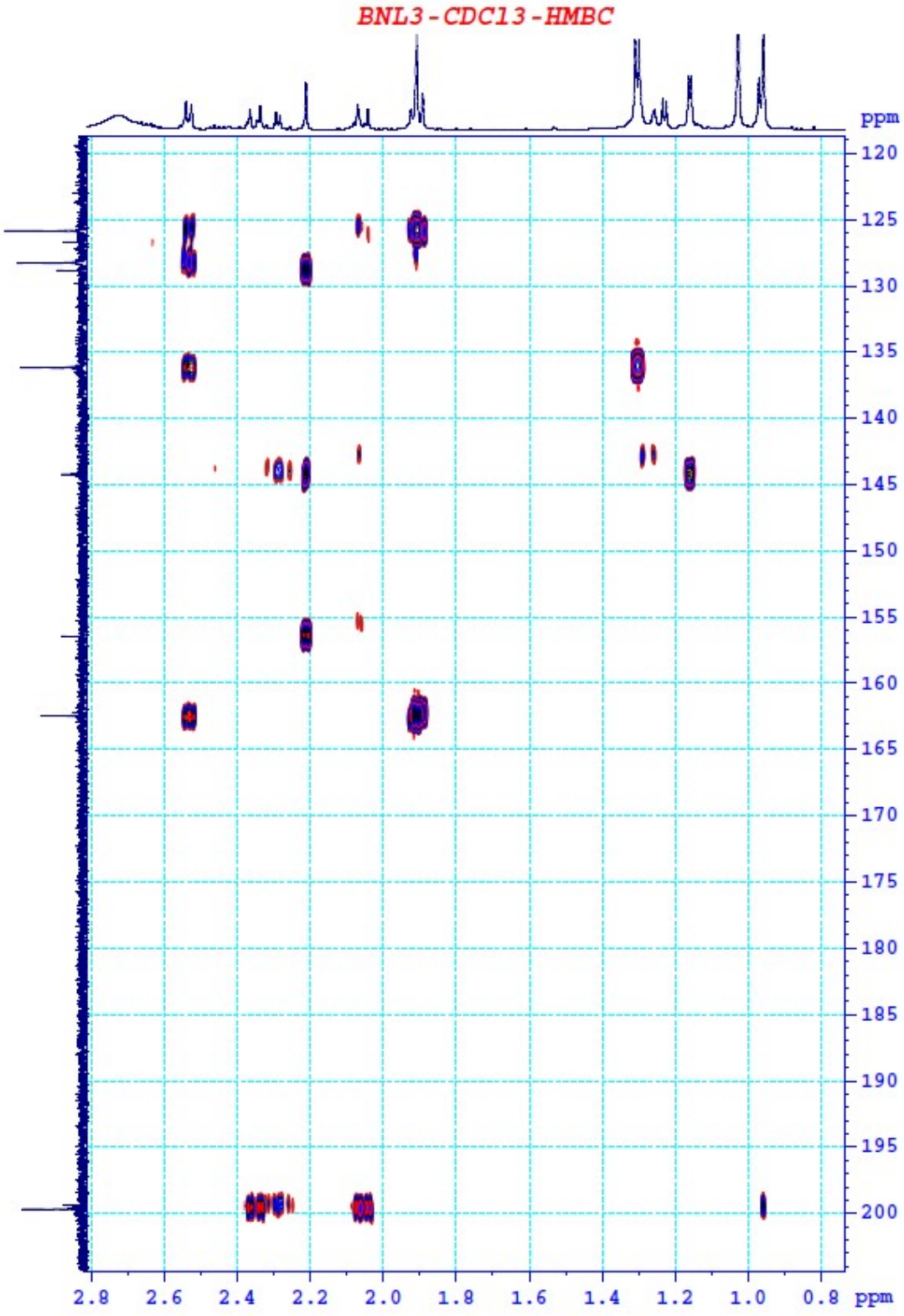


Figure S19. HMBC spectrum of **1** from 0.8 -2.8 and 120-200 region

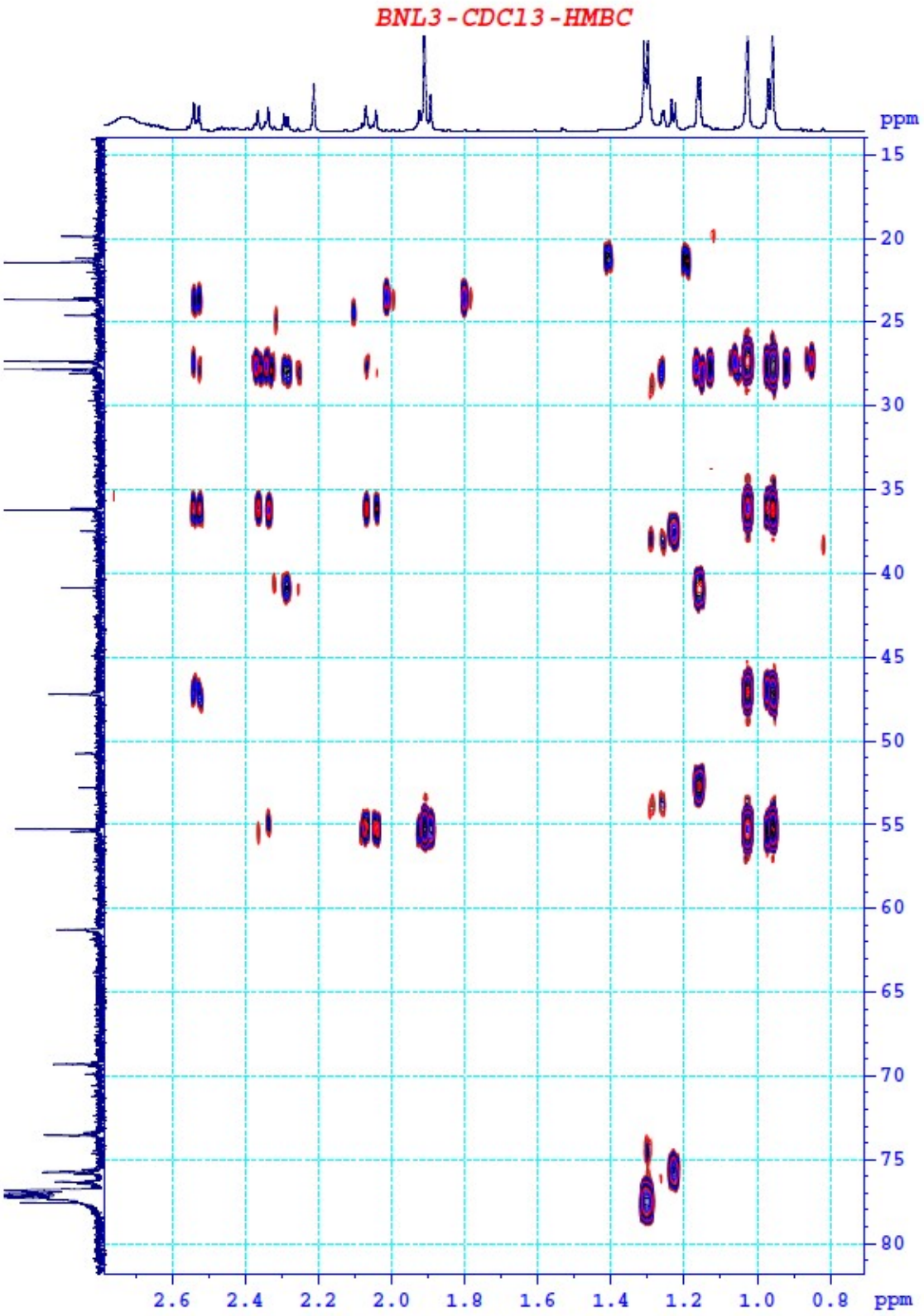


Figure S20. HMBC spectrum of **1** from 0.8-2.6 and 15-80 ppm region

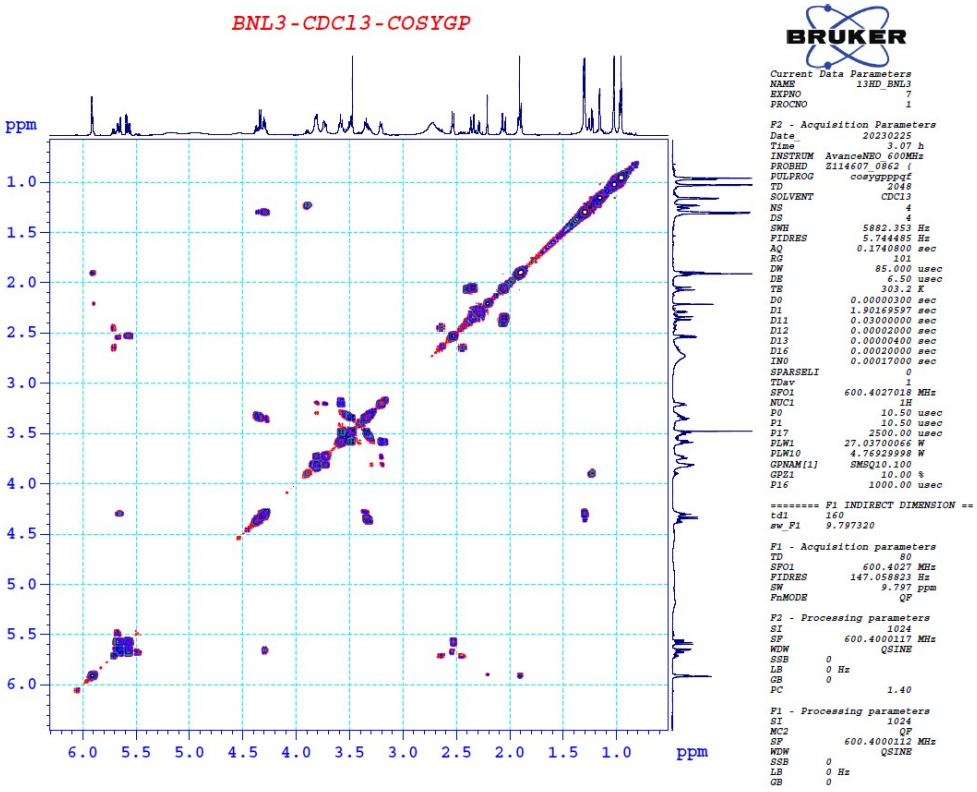


Figure S21. COSY spectrum of **1**

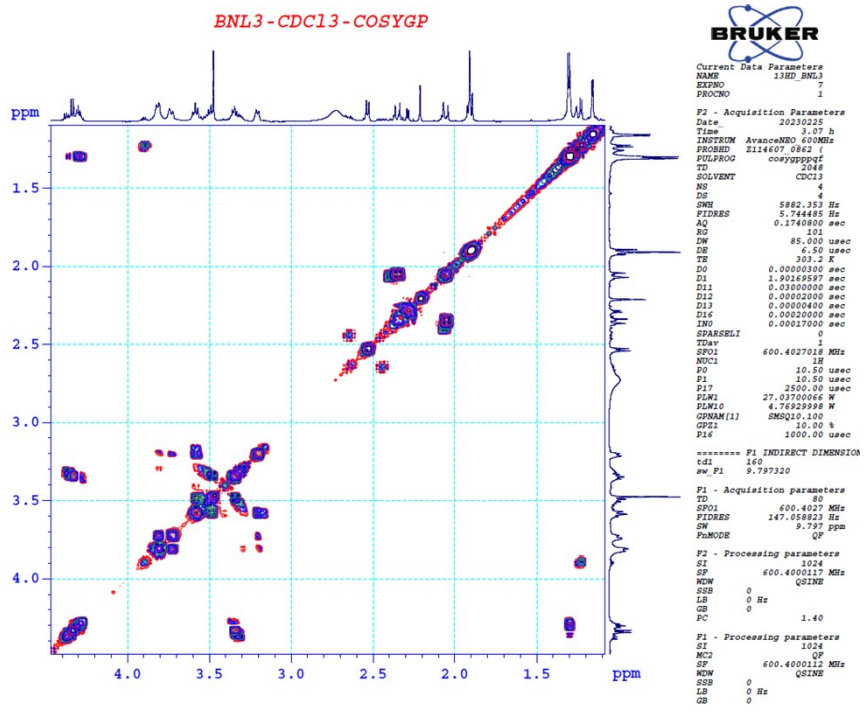


Figure S22. COSY spectrum of **1** from 1.0 to 4.0 ppm region

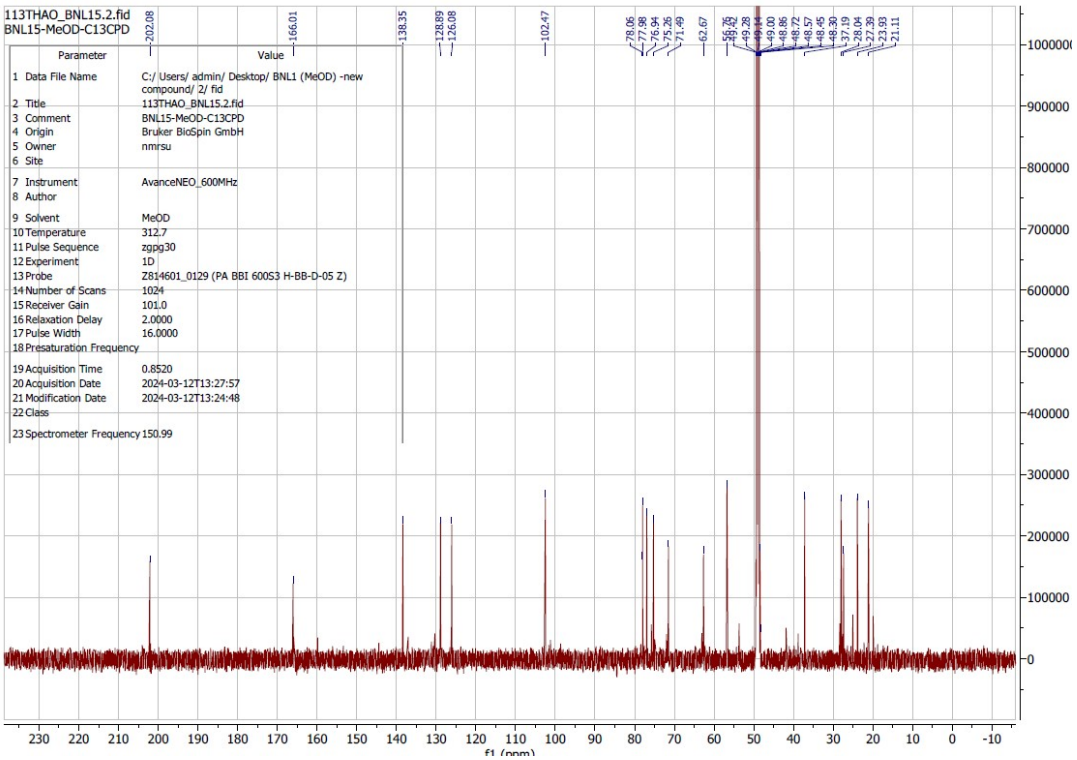
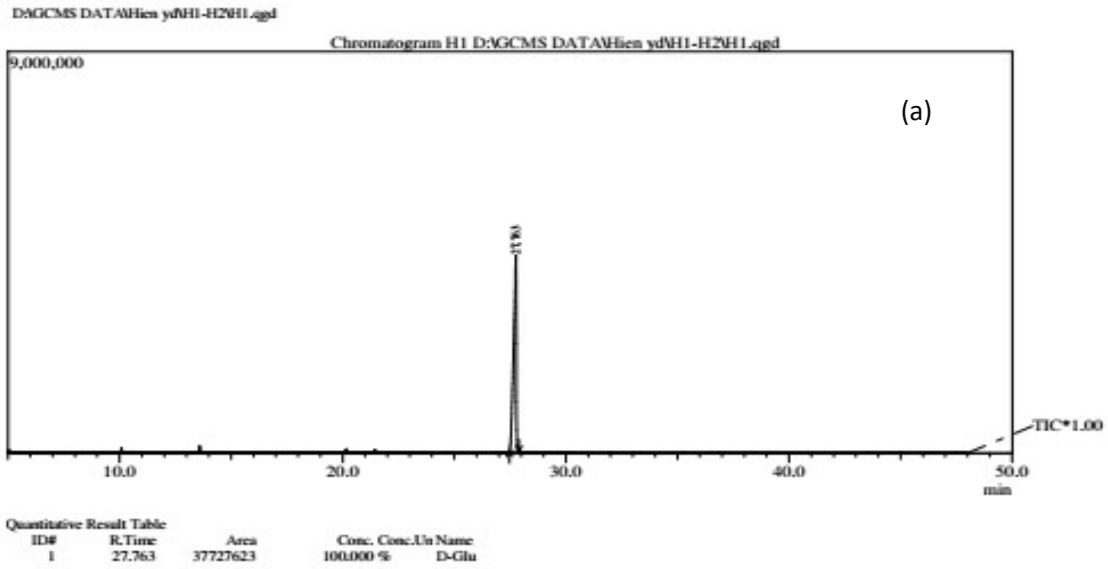


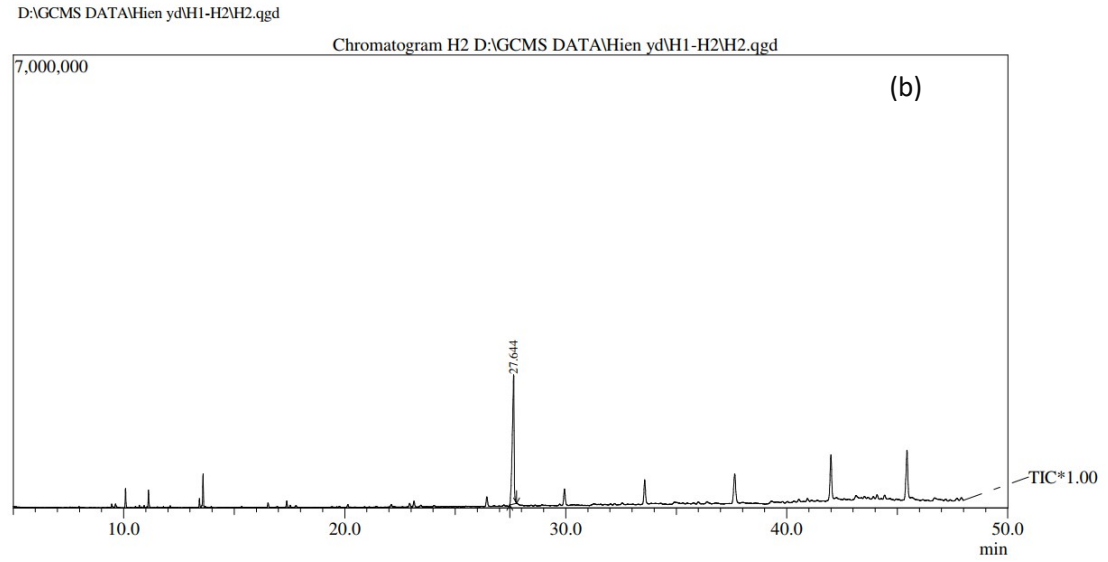
Figure S23. ^{13}C-NMR spectrum of **1** in CD_3OD.150 MHz

GC/MS chromatography



Quantitative Result Table

ID#	R.Time	Area	Conc.	Conc.Un	Name
1	27.763	37727623	100.000	%	D-Glu



Quantitative Result Table

ID#	R.Time	Area	Conc.	Conc.Un	Name
1	27.644	12531685	100.000	%	D-Glu

Figure S24. Determination of monosaccharide compositions of **1** by GC/MS
(a) D-glucose derivative (b) **1** derivative

Method:

Alditol per-acetate derivatives were prepared by the method reported by Ho DV which slightly modifying the method reported by Pettolino et al. Compound **1** (2 mg) were hydrolyzed with 4 M trifluoroacetic acid (1 mL) in a sealed tube at 105 °C for 4 h. After cooling, the reaction mixture was diluted with 4 mL water and extracted with dichloromethane (2 mL×3 times). The aqueous layer wasevaporated with methanol under reduced pressure. The residue was then dissolved in deionized water (1 mL) and incubated with sodium borohydride (33 mg) at 40 °C for 90 mins. After reduction, three drops of glacial acetic acid were slowly added to the tube to destroy any excess $NaBH_4$. The mixture was dissolved in 2 mL of methanol–glacial acetic acid (95:5, v/v) and evaporated in vacuo. This step was repeated three times to remove the boric acid completely. The obtained alditols were acetylated with 1 mL of acetic anhydride–pyridine (1:1, v/v) at 110 °C for 3 h. After cooling to room temperature, the acetylation mixture was diluted with 4 mL water and partitioned with dichloromethane (2 mL×2 times). The combined dichloromethane extract was washed with 5 mL water (three times) and then placed in a heating block (37 °C), to yield the alditol per-acetate derivatives as pale yellow oil. The resulting derivatives were analyzed by GC/MS with an Equity®-5 (Supelco) capillary column (30 m×0.25 mm i.d.). Helium was used as the carrier gas, at a flow rate of 1.2 mL/min. The column, injector, and detector were set at 80, 220, and 230 °C, respectively. The oven temperature was programmed as follows: initially, 80 °C for 2 min, increased to 180 °C at 10 °C/min and held at 180 °C for 2 min, increased to 220 °C at 2 °C/min and held at 220 °C for 5 min, further increased to 240 °C at 5 °C/min and held for 5 min at the final temperature. The monosaccharide present in 1 was confirmed to be D-glucose by comparing the retention times of the alditol per-acetate derivatives with the corresponding derivatives prepared from standard sugars (D-glucose) under the same conditions.

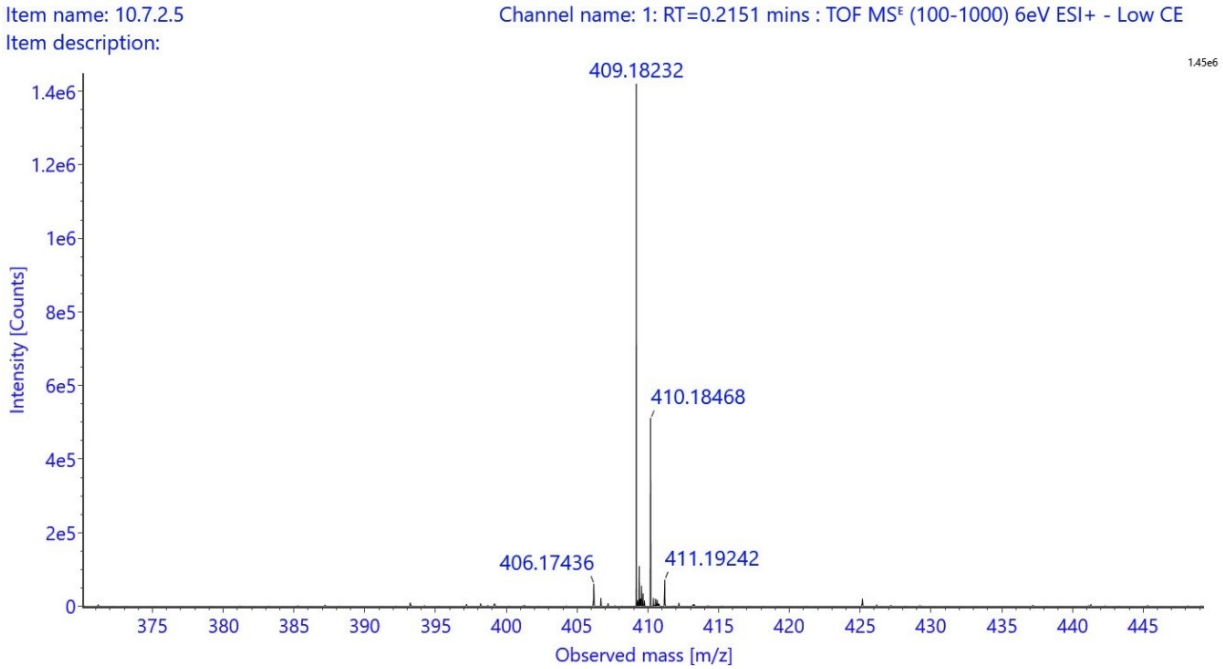


Figure S25. MS spectrum of **2**

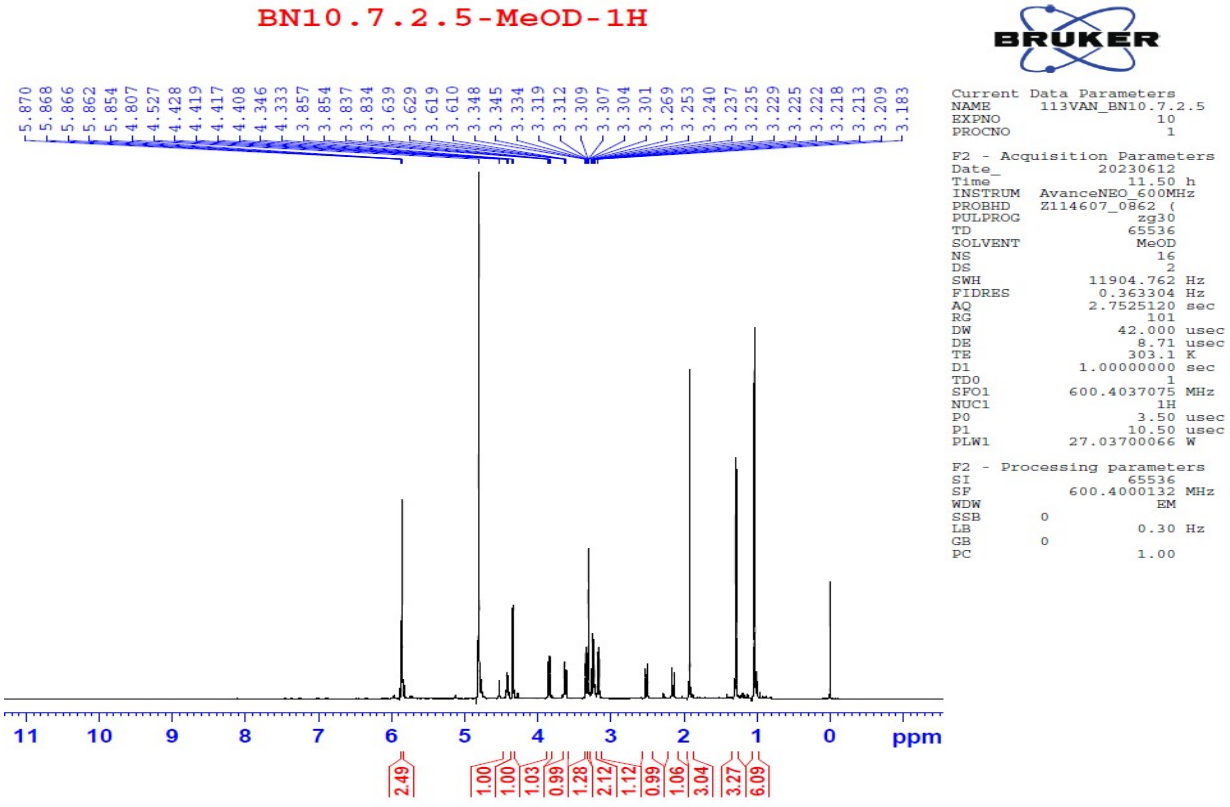


Figure S26. ^{1}H-NMR spectrum of **2** in CD_3OD. 600 MHz

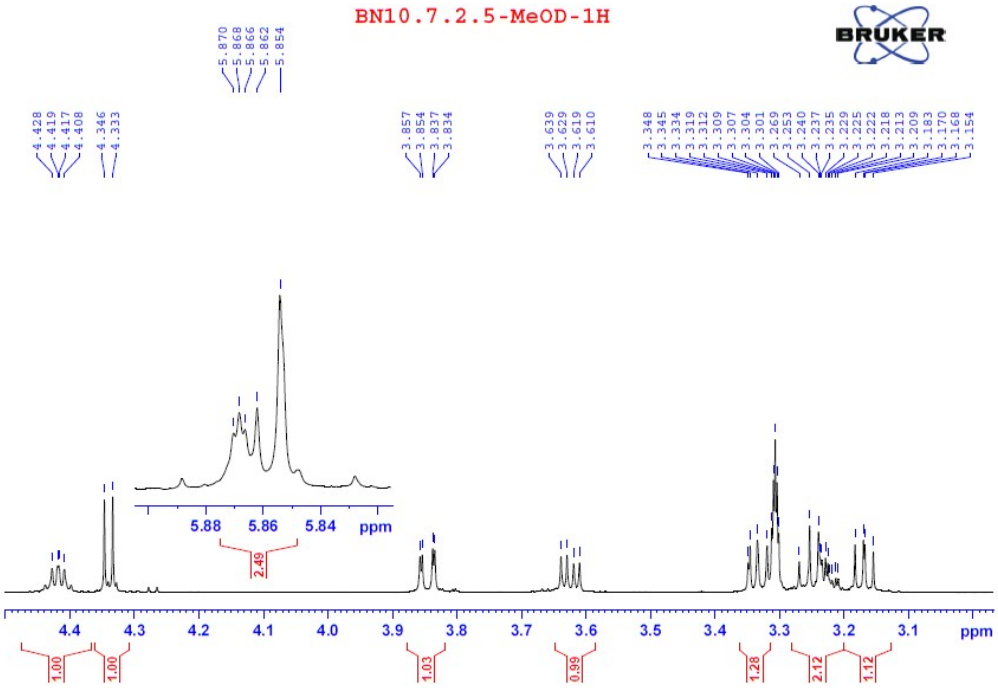


Figure S27. ^{1}H-NMR spectrum of **2** from 3.1 – 4.4 and 5.82 – 5.90 ppm region

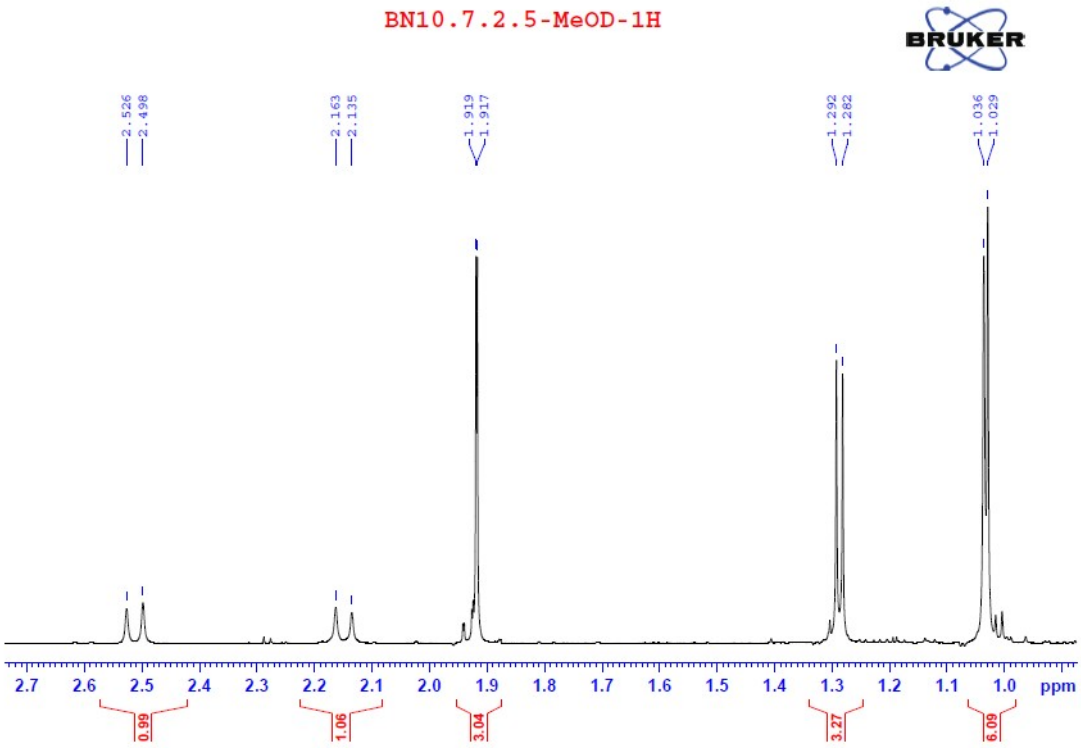


Figure S28. ^{1}H-NMR spectrum of **2** from 1.0 to 2.7 ppm region

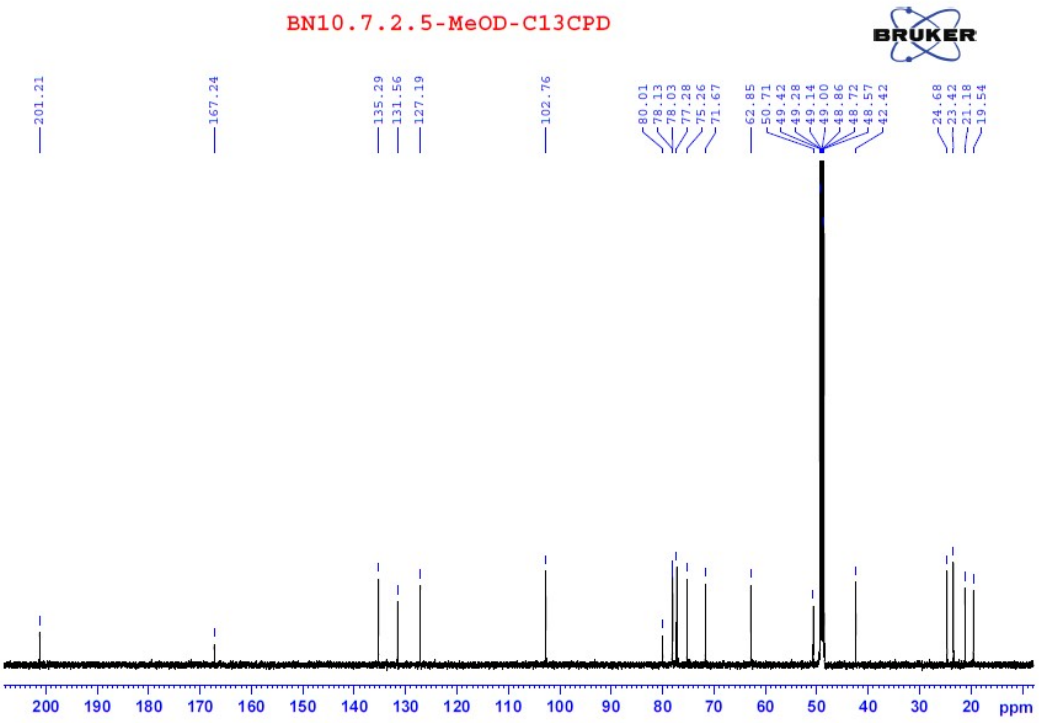


Figure S29. ^{13}C -NMR spectrum of **2** in CD_3OD. 150 MHz

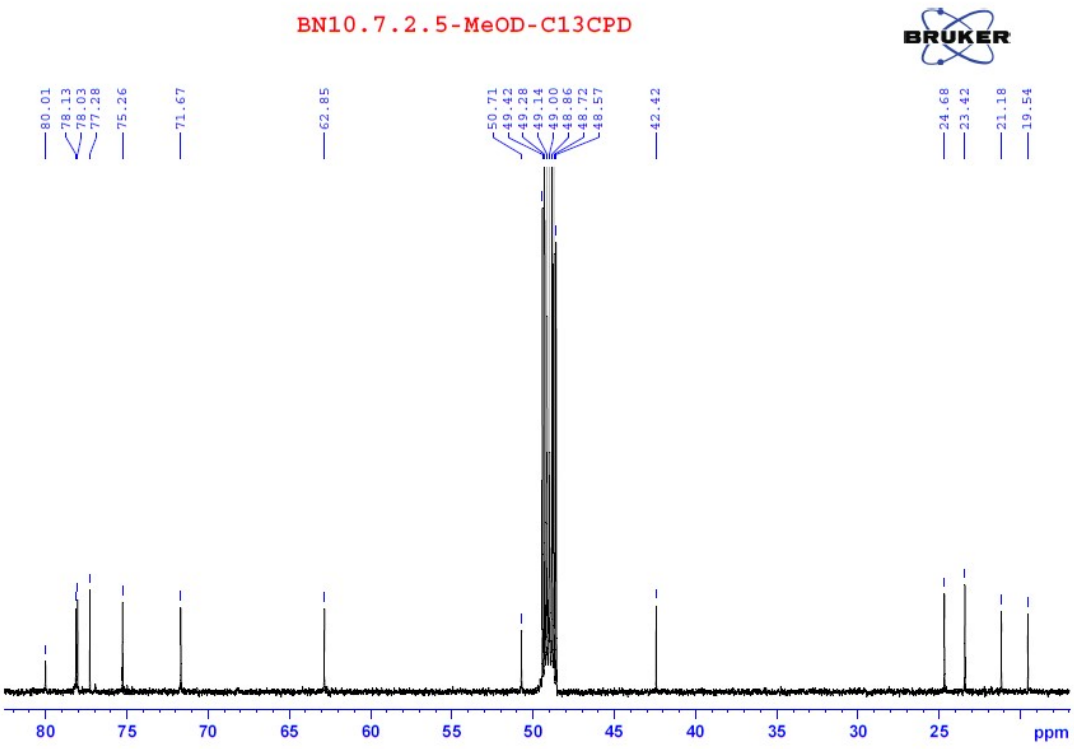


Figure S30. ^{13}C -NMR spectrum of **2** fro 19.5 to 80 ppm region

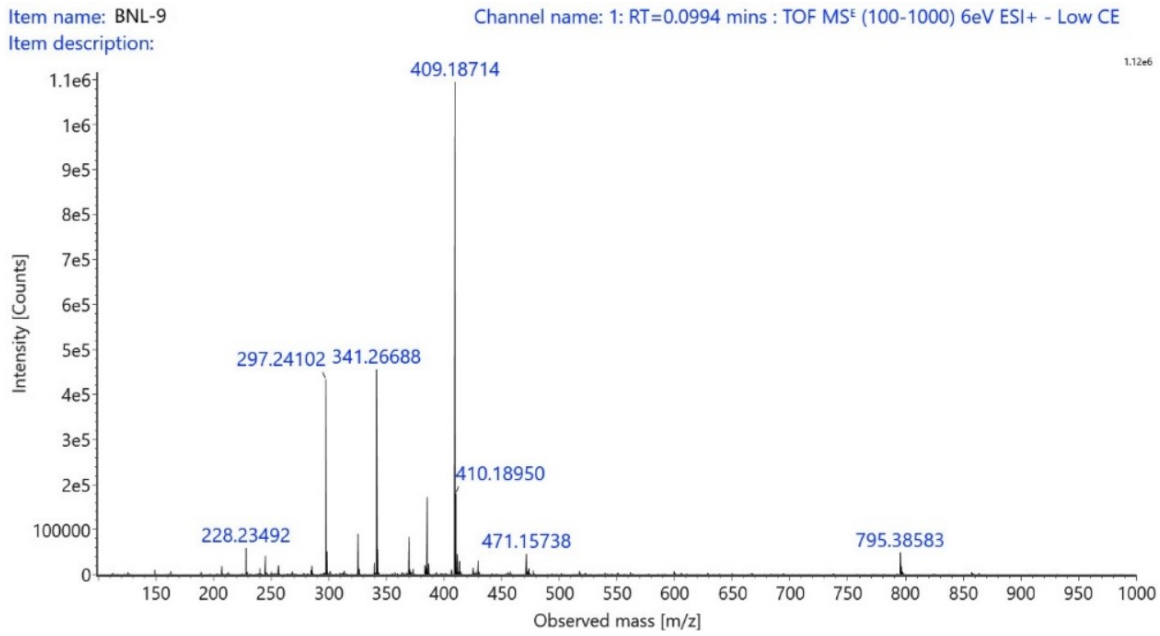


Figure S31. MS spectrum of **3**

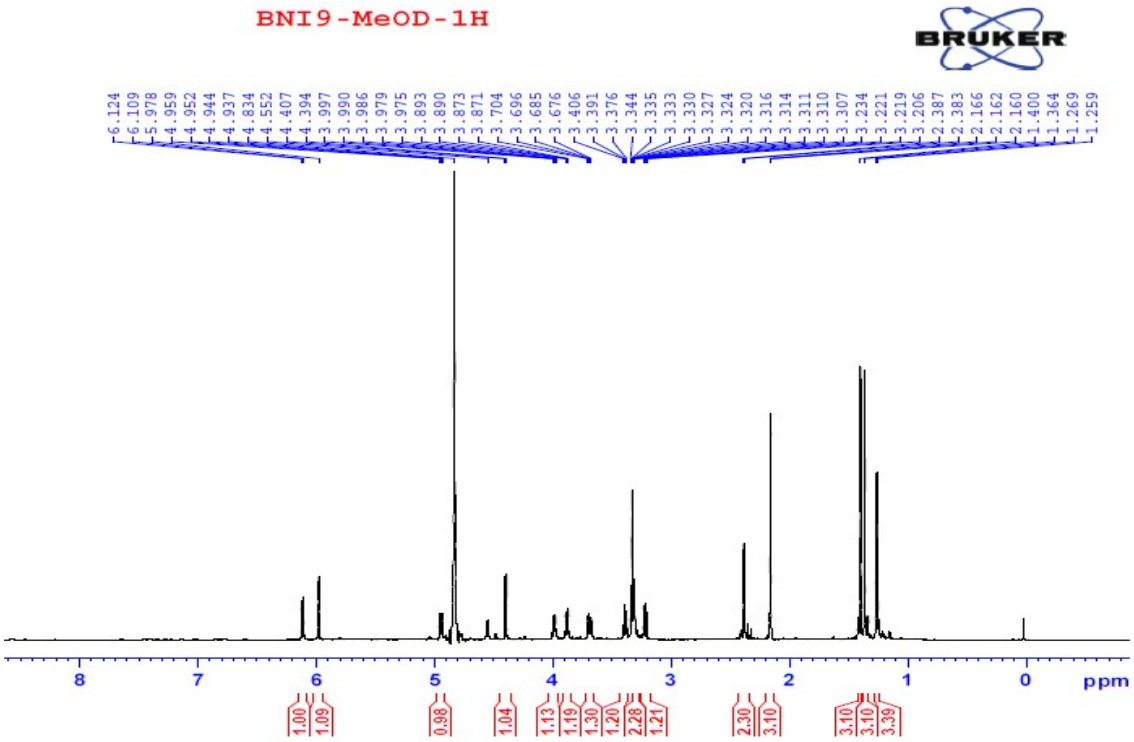


Figure S32. ^{1}H-NMR spectrum of **3** in CD_3OD

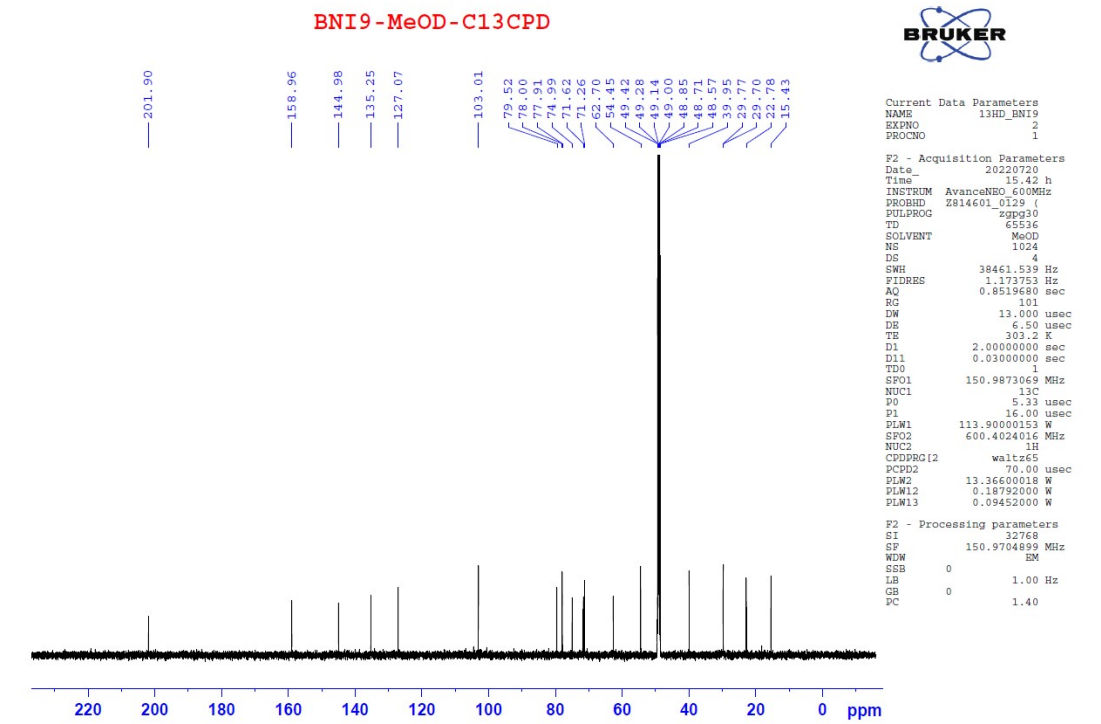


Figure S33. ^{13}C-NMR spectrum of **3** in CD_3OD

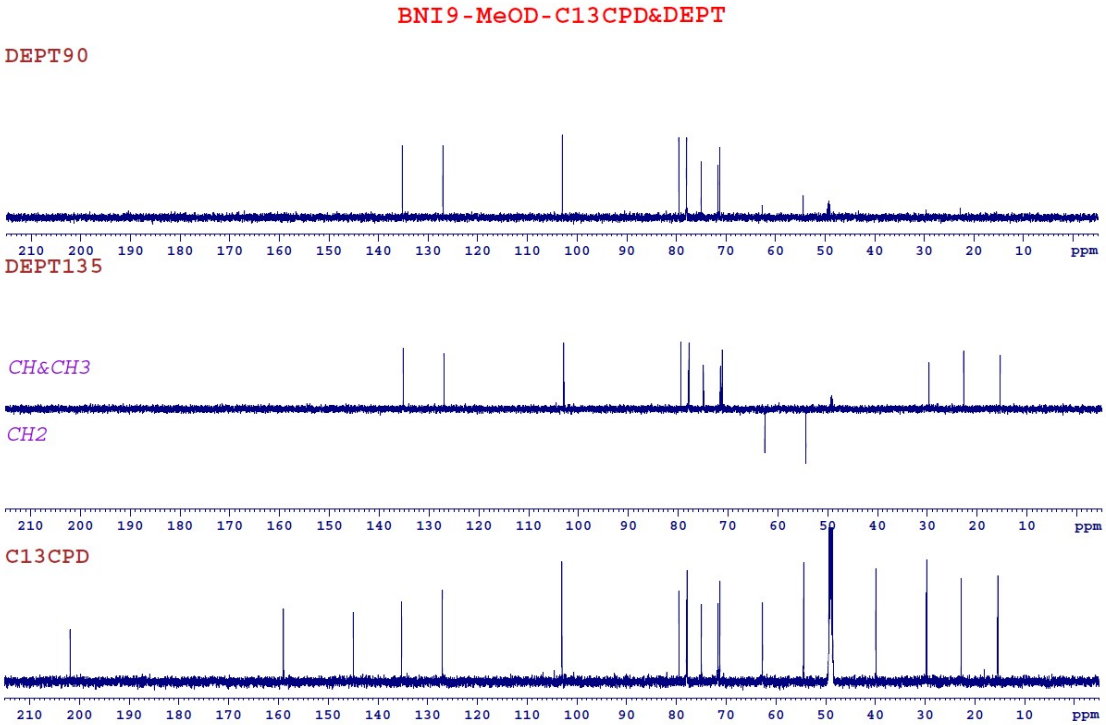


Figure S34. DEPT spectrum of **3**

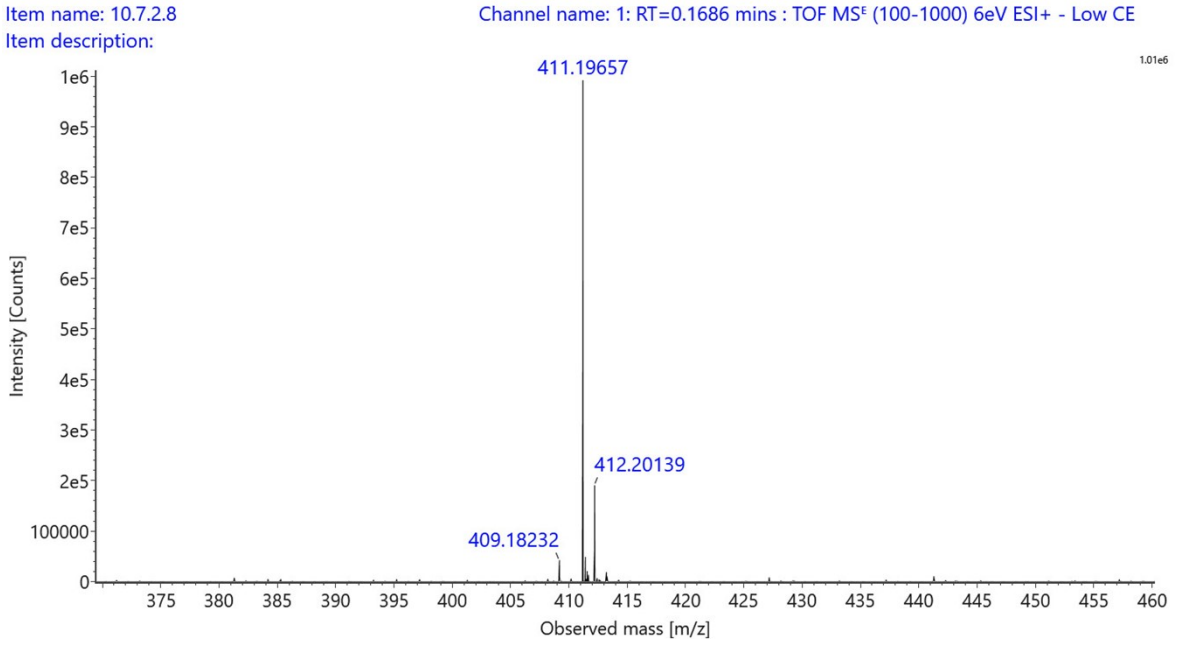


Figure S35. MS spectrum of **4**

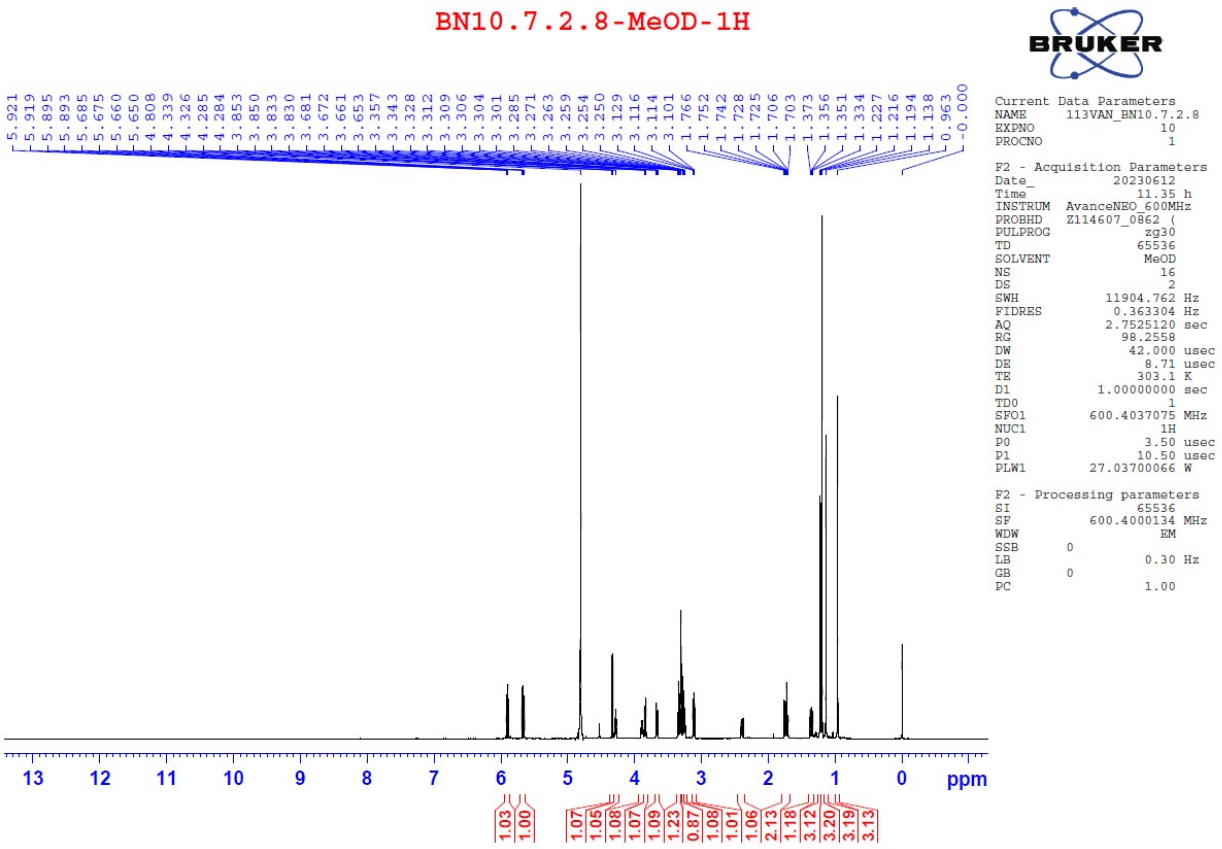


Figure S36. ^{1}H-NMR spectrum of **4** in CD_3OD

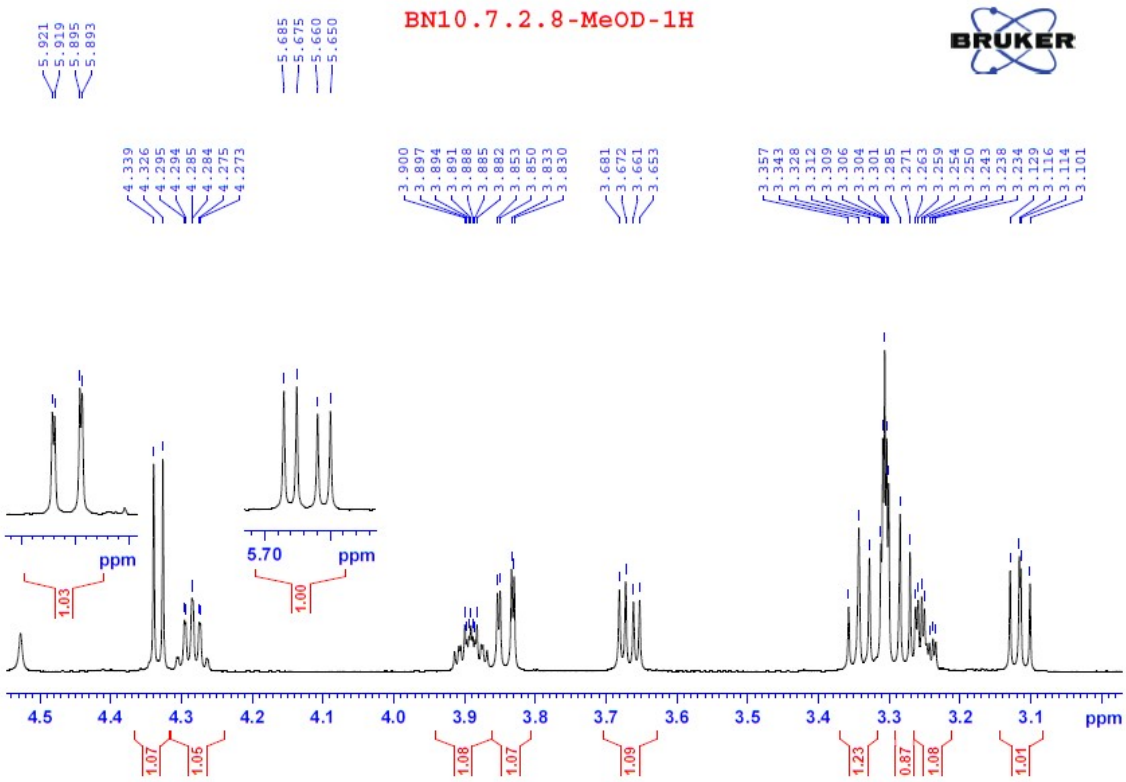


Figure S37. ^{1}H-NMR spectrum of **4** from 3.0–4.5 and 5.6–6.0 ppm region

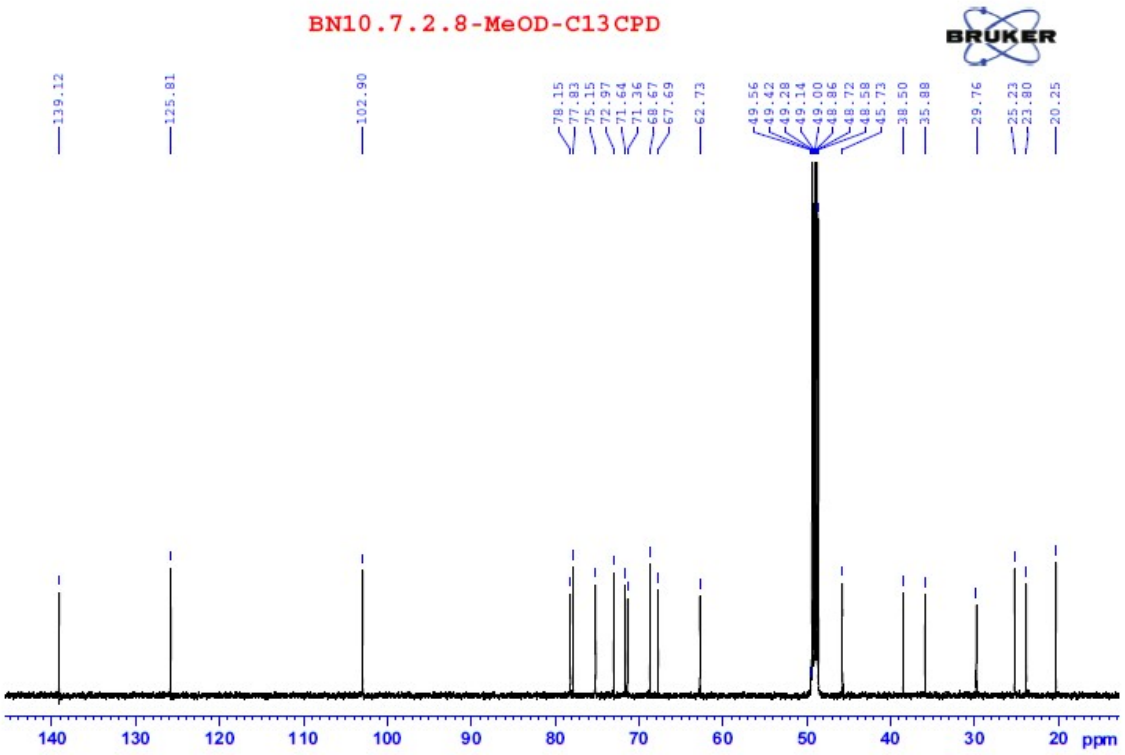


Figure S38. ^{13}C-NMR spectrum of **4** in CD_3OD

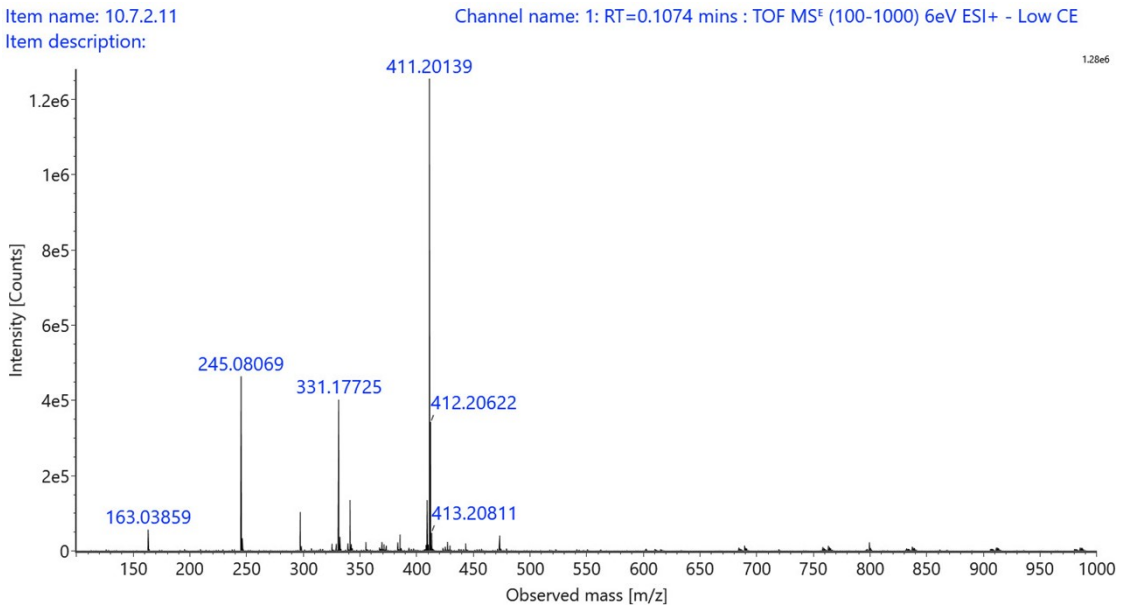


Figure S39. MS spectrum of **5**

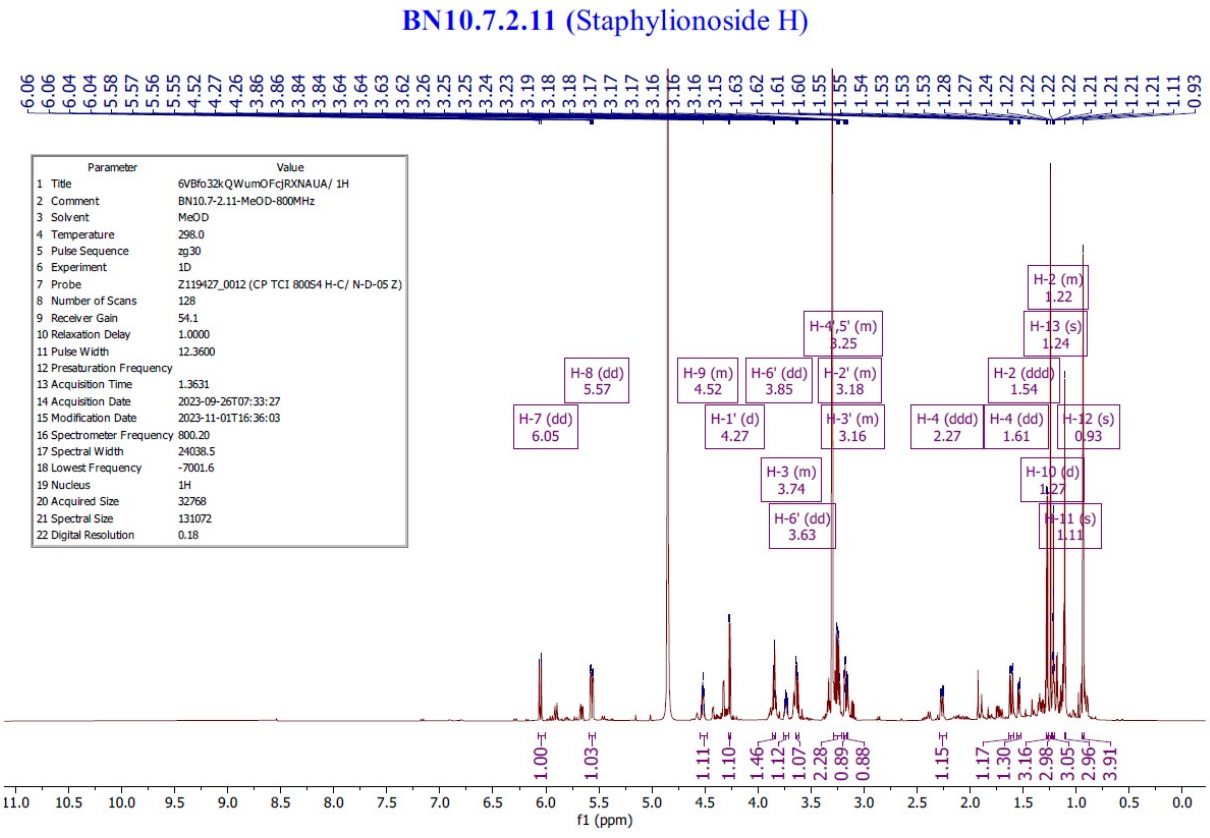


Figure S40. ^{1}H-NMR spectrum of **5** in CD_3OD

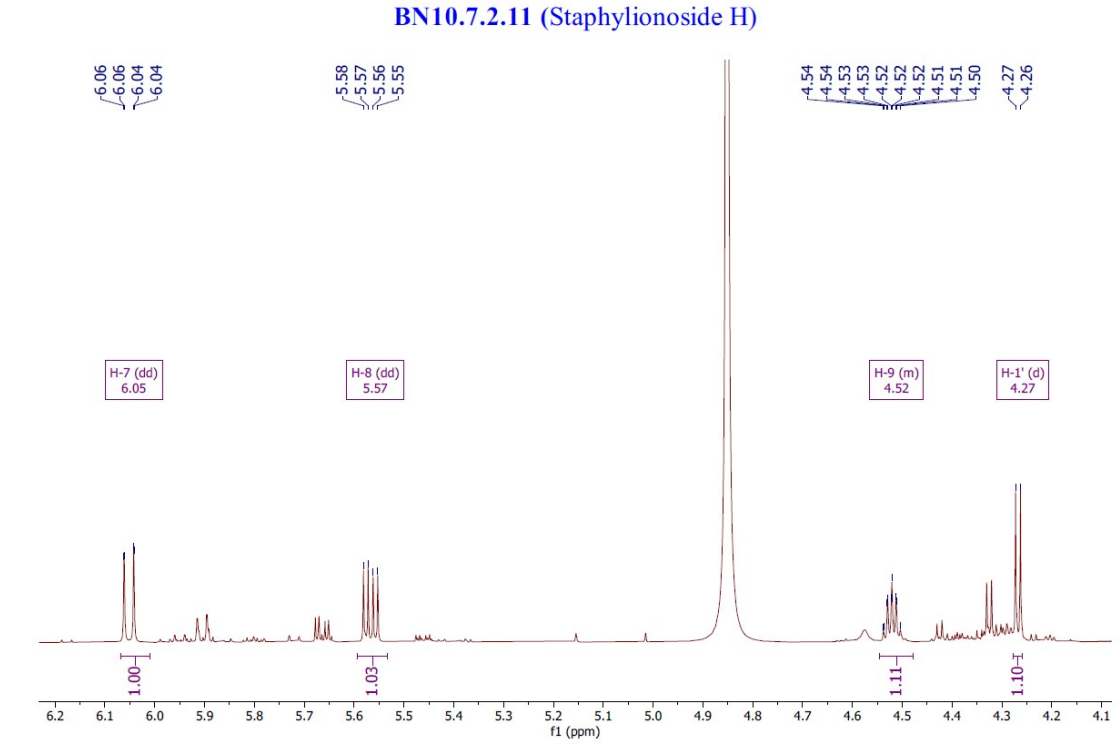


Figure S41. ^{1}H-NMR spectrum of **5** from 4.1 to 6.2 ppm region

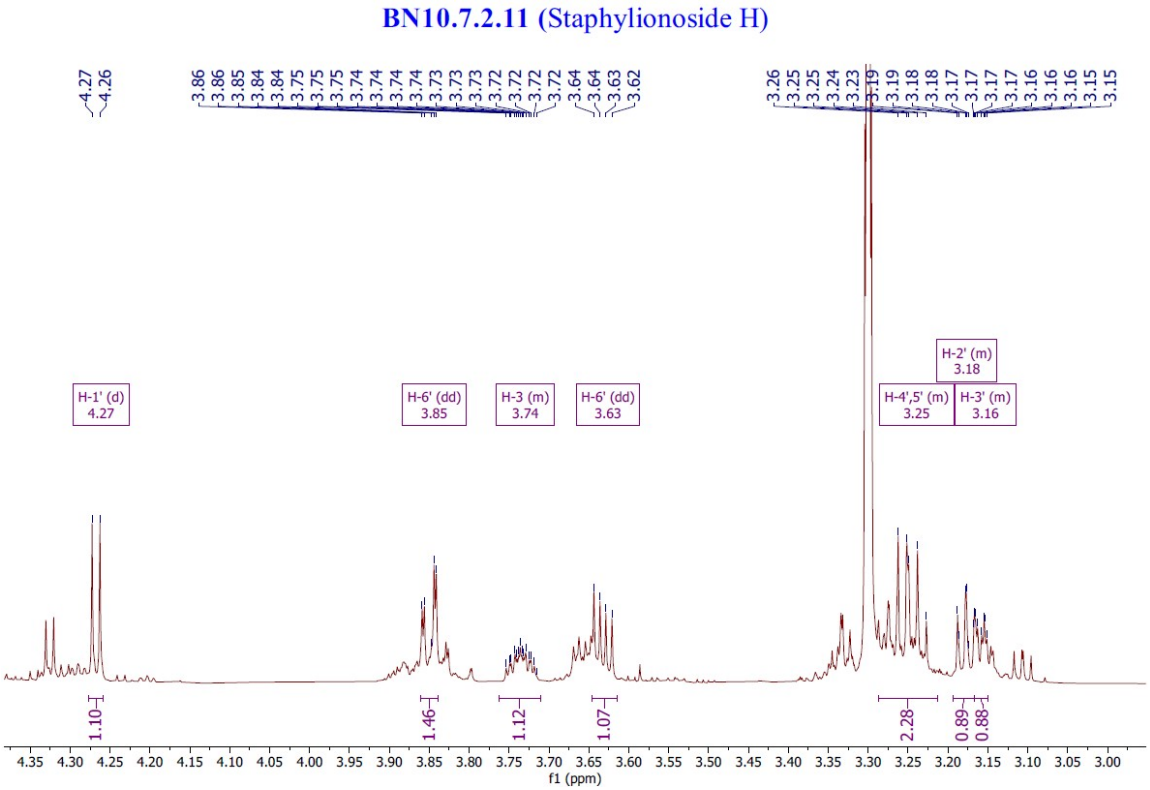


Figure S42. ^{1}H-NMR spectrum of **5** from 3.0 to 4.35 ppm region

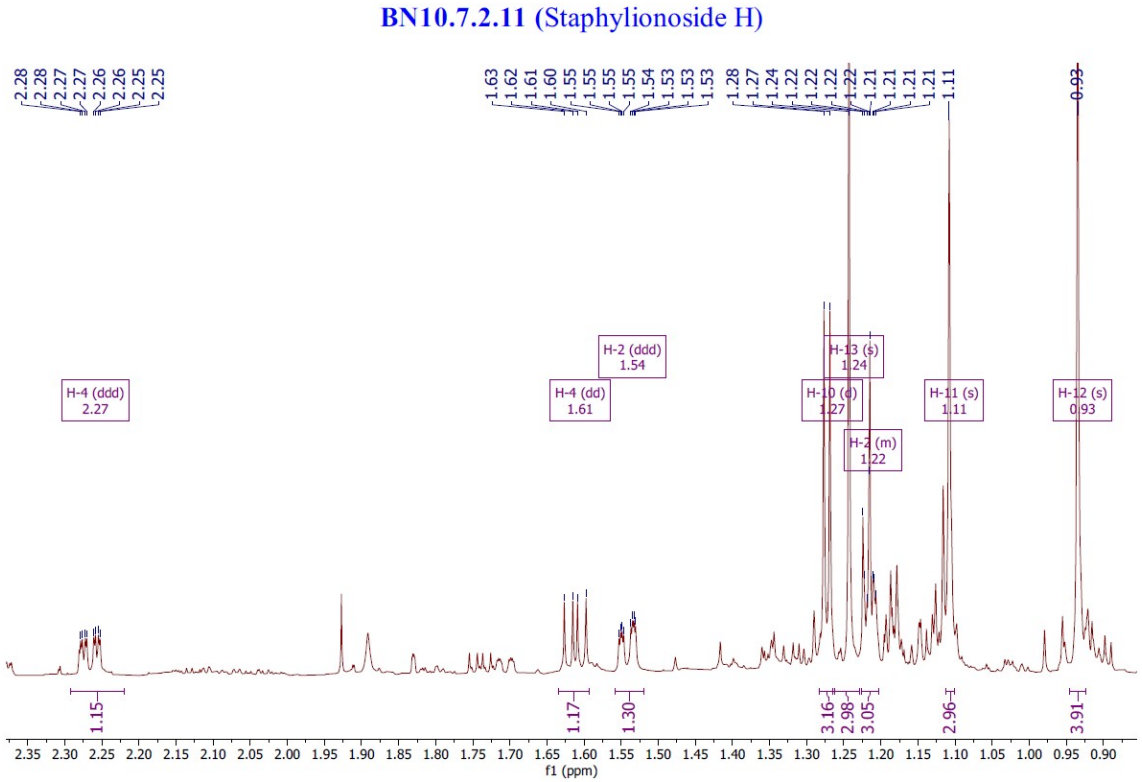


Figure S43. ^{1}H-NMR spectrum of **5** from 0.9 to 2.35 ppm region

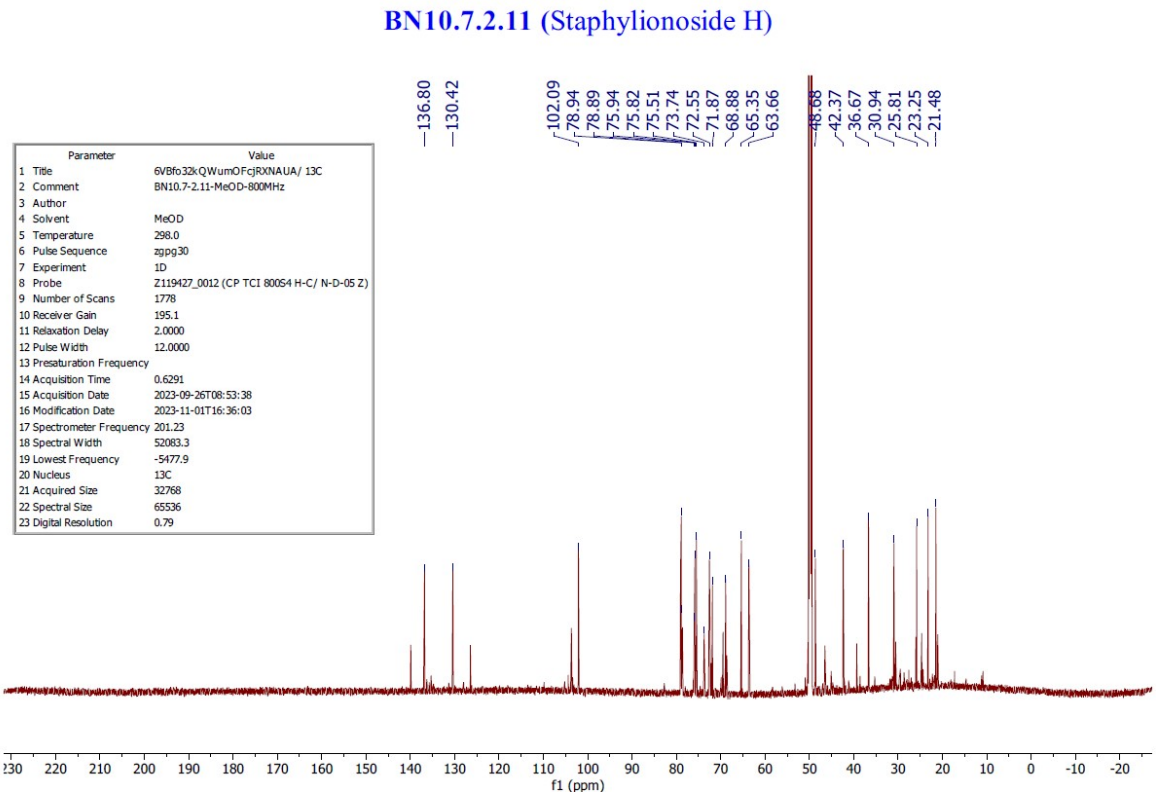


Figure S44. ^{13}C-NMR spectrum of **5**

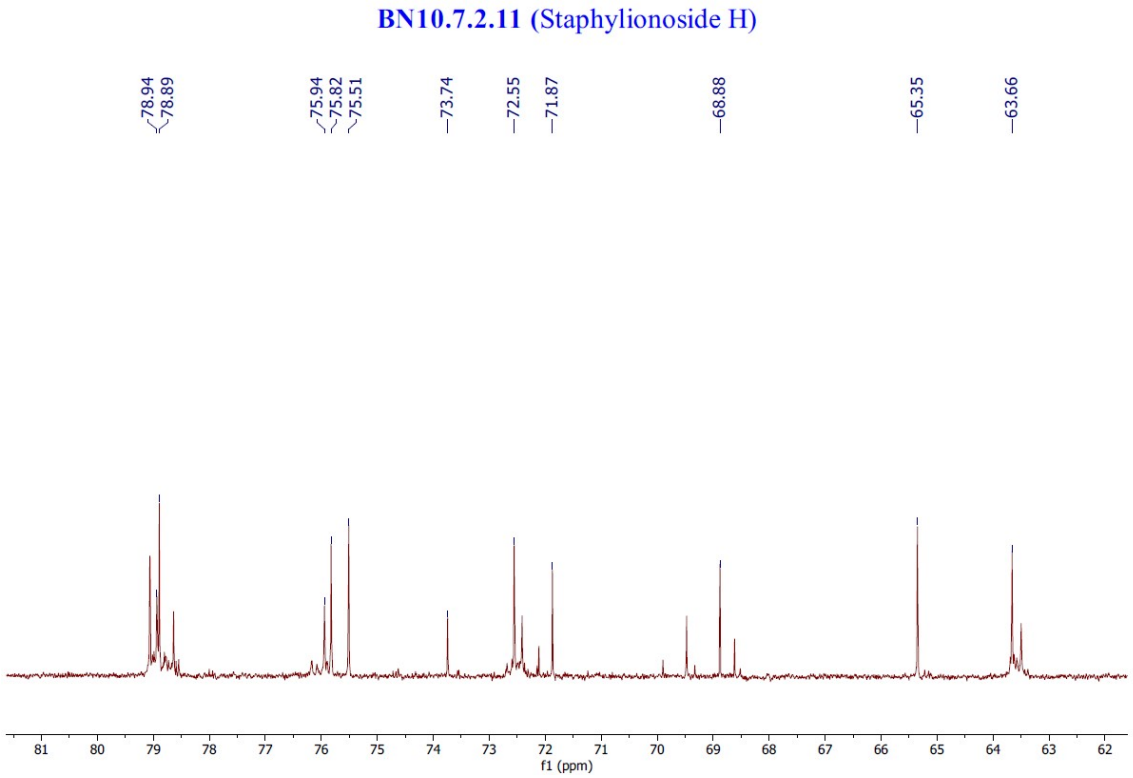


Figure S45. ^{13}C-NMR spectrum of **5** from 62 to 81 ppm region

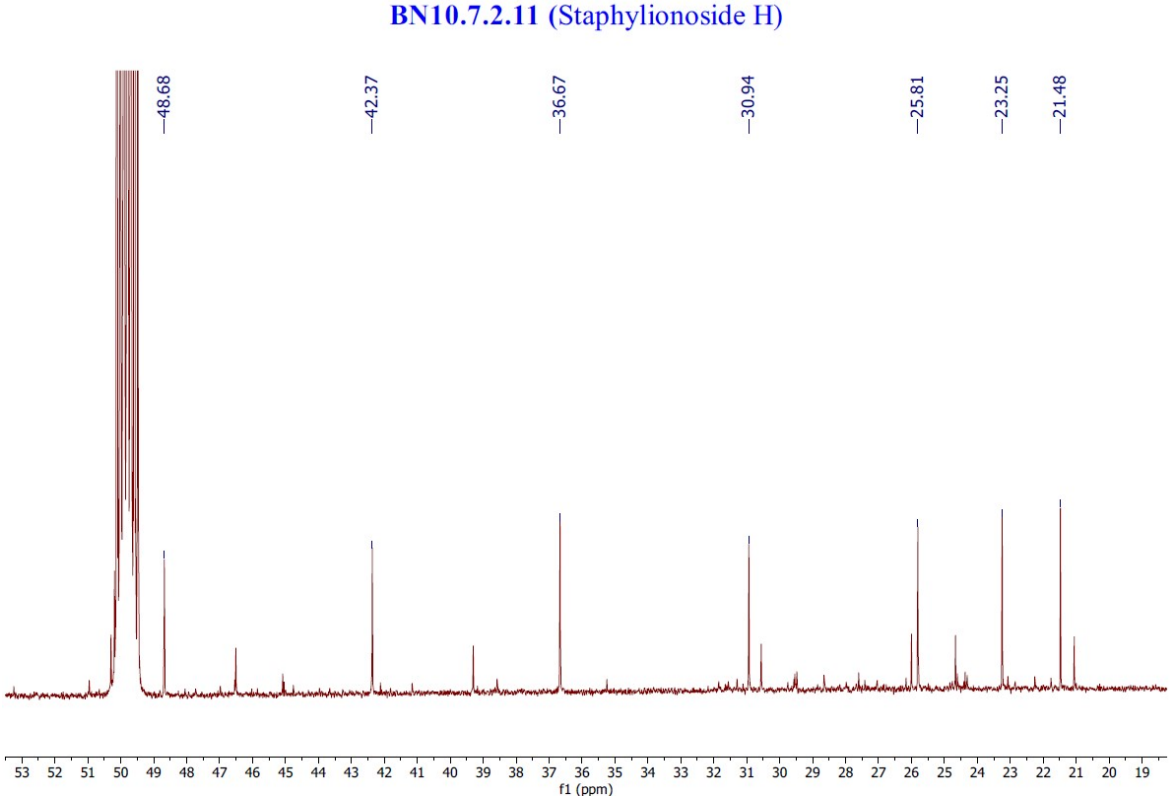


Figure S46. ^{13}C-NMR spectrum of **5** from 19 to 53 ppm region

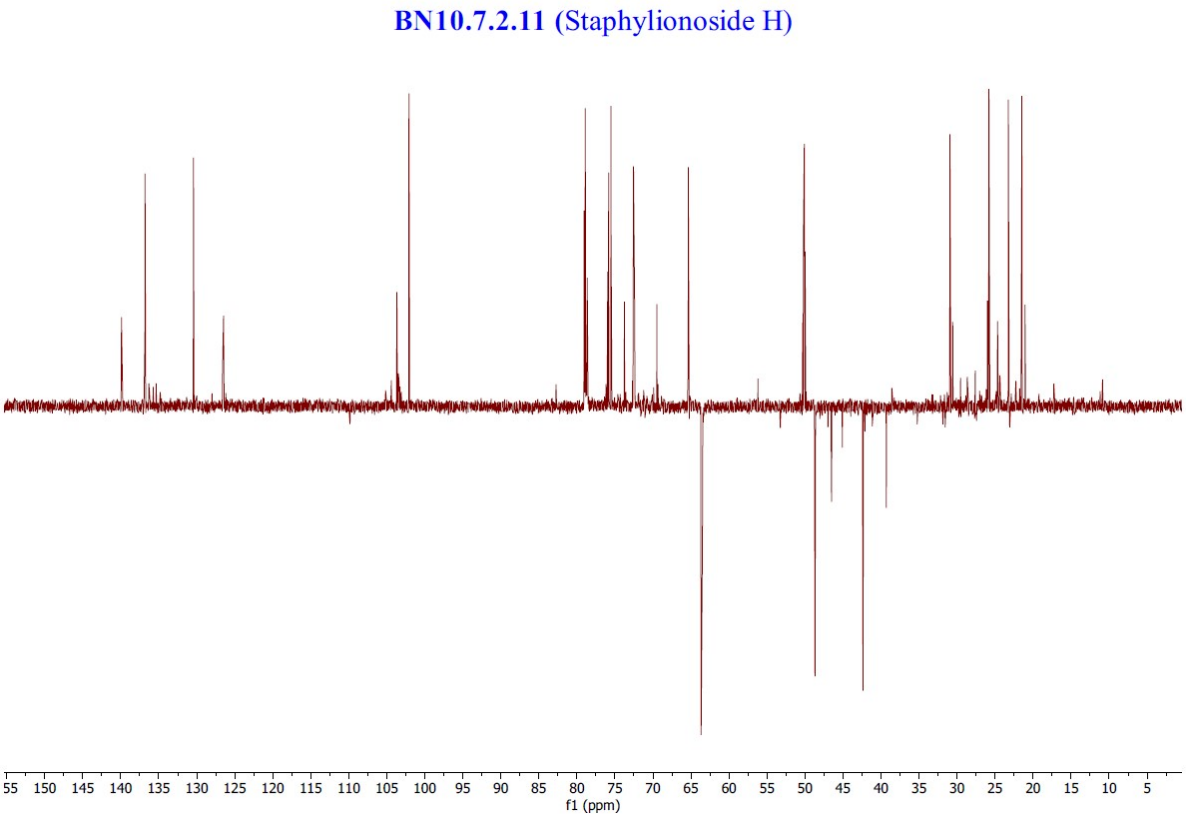


Figure S47. DEPT spectrum of **5**

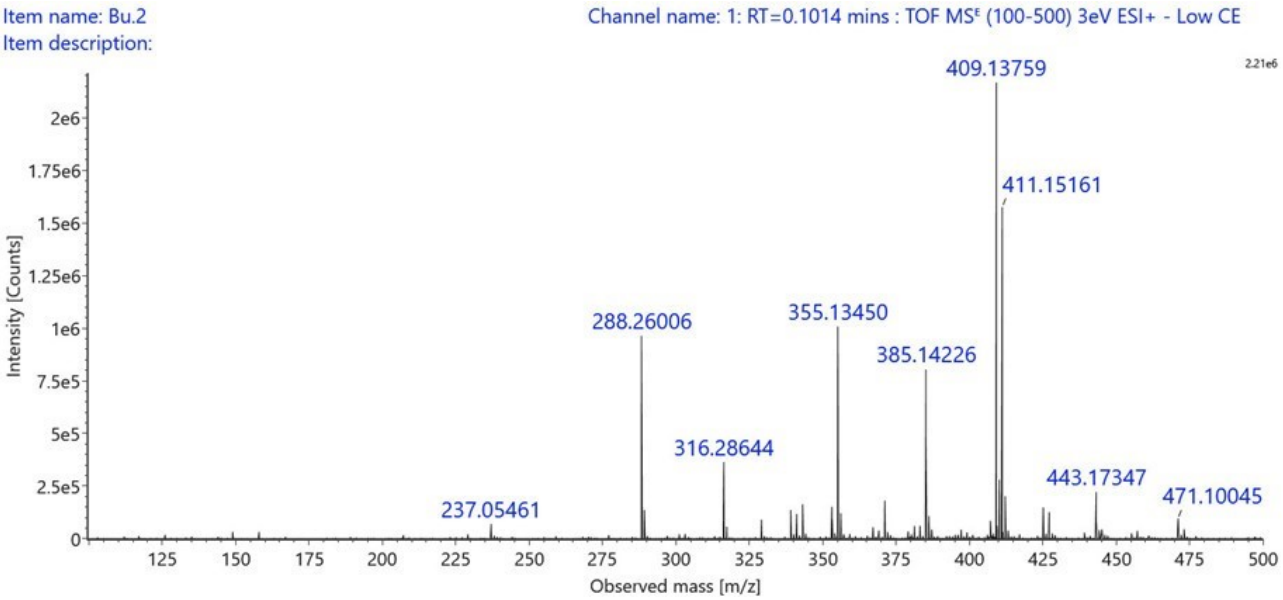


Figure S48. MS spectrum of **6**

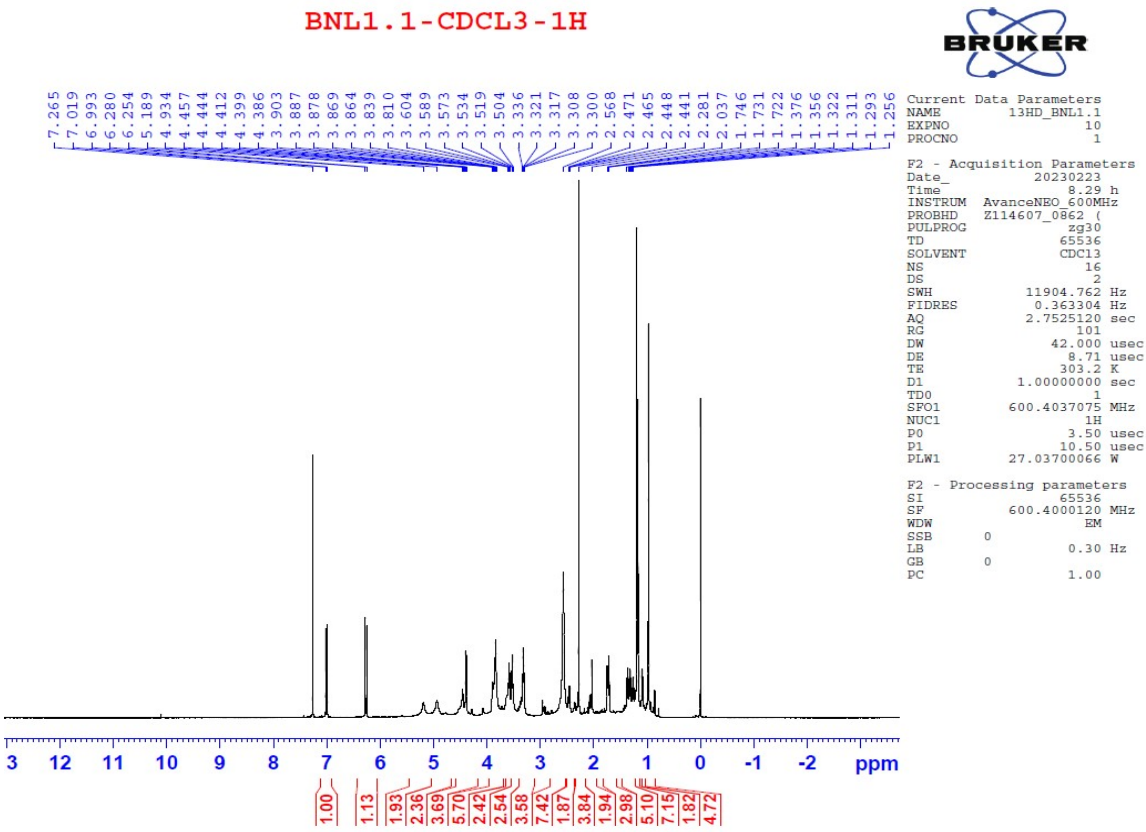


Figure S49. ^{1}H-NMR spectrum of **6** in $CDCl_3$

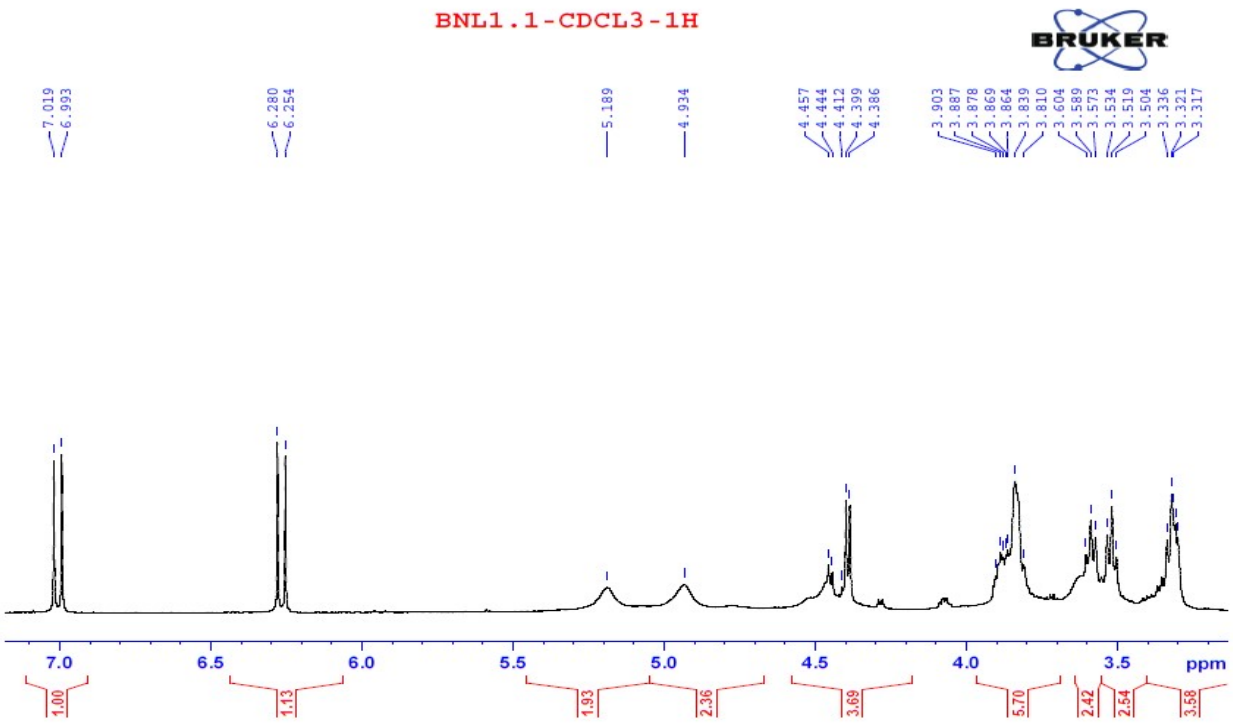


Figure S50. ^{1}H-NMR spectrum of **6** from 3.0 to 7.0 ppm region

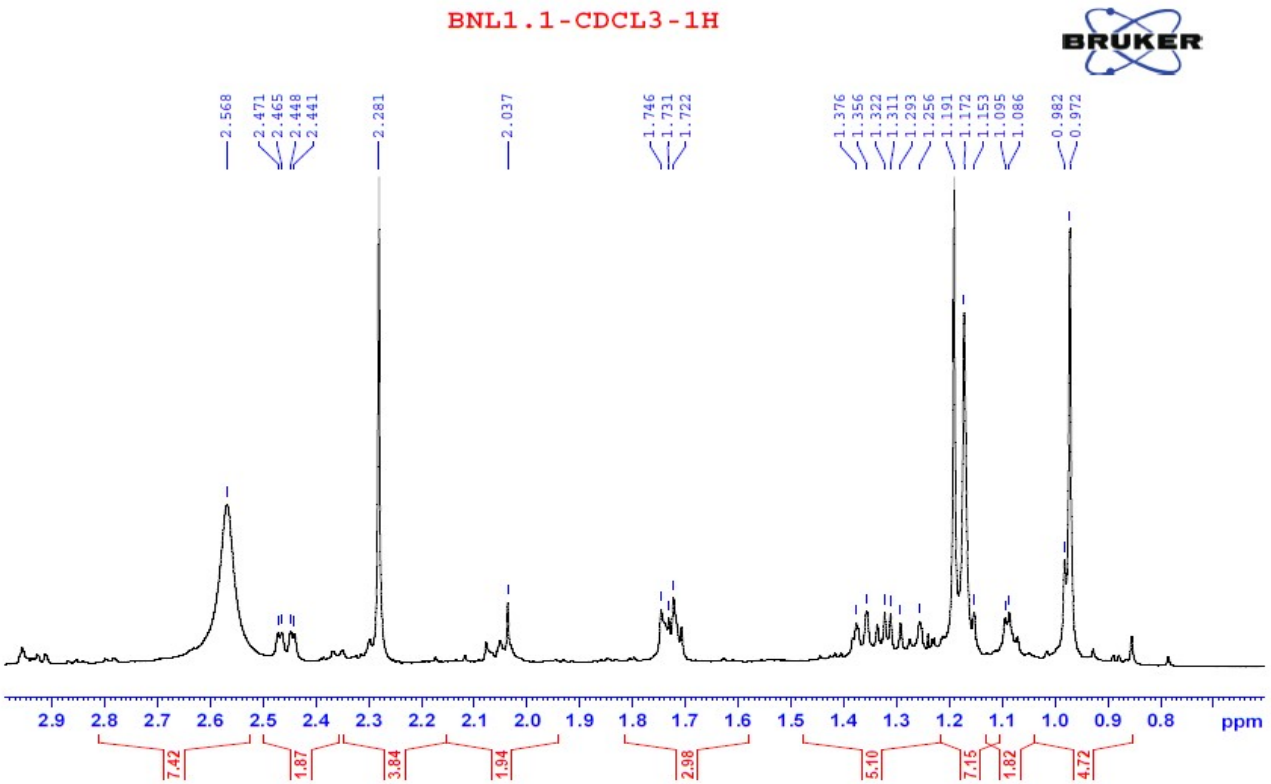


Figure S51. ^{1}H-NMR spectrum of **6** from 0.8 to 2.9 ppm region

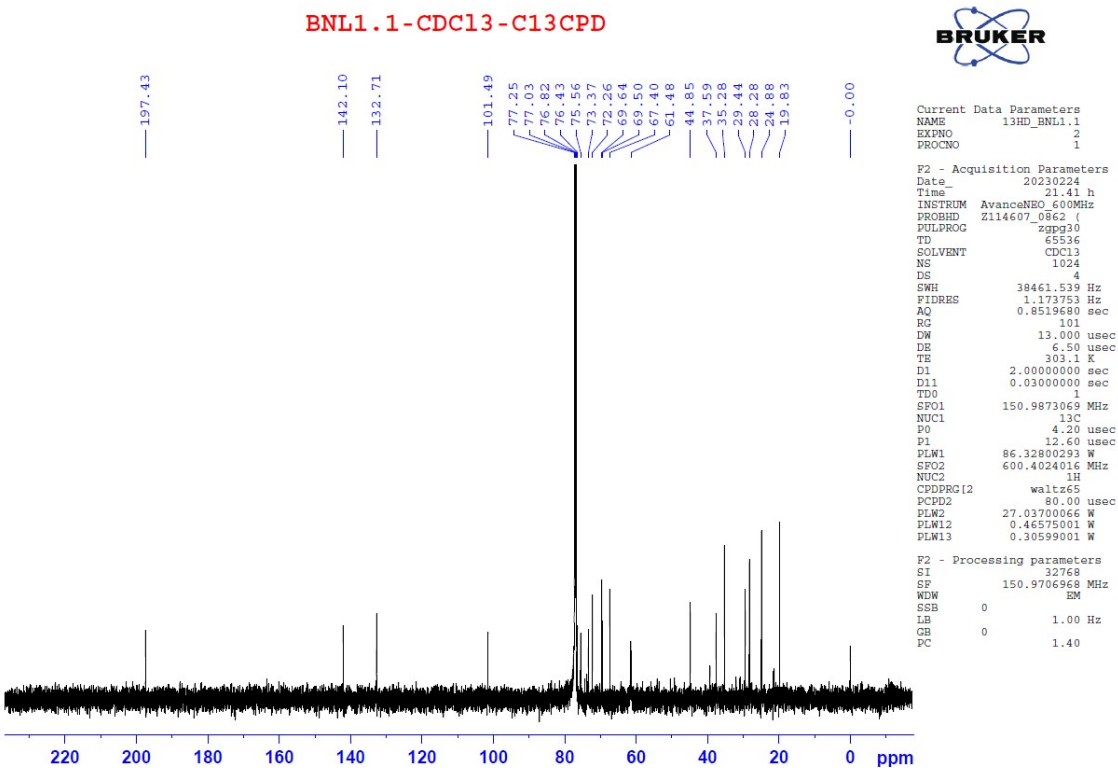


Figure S52. ^{13}C-NMR spectrum of **6** in $CDCl_3$

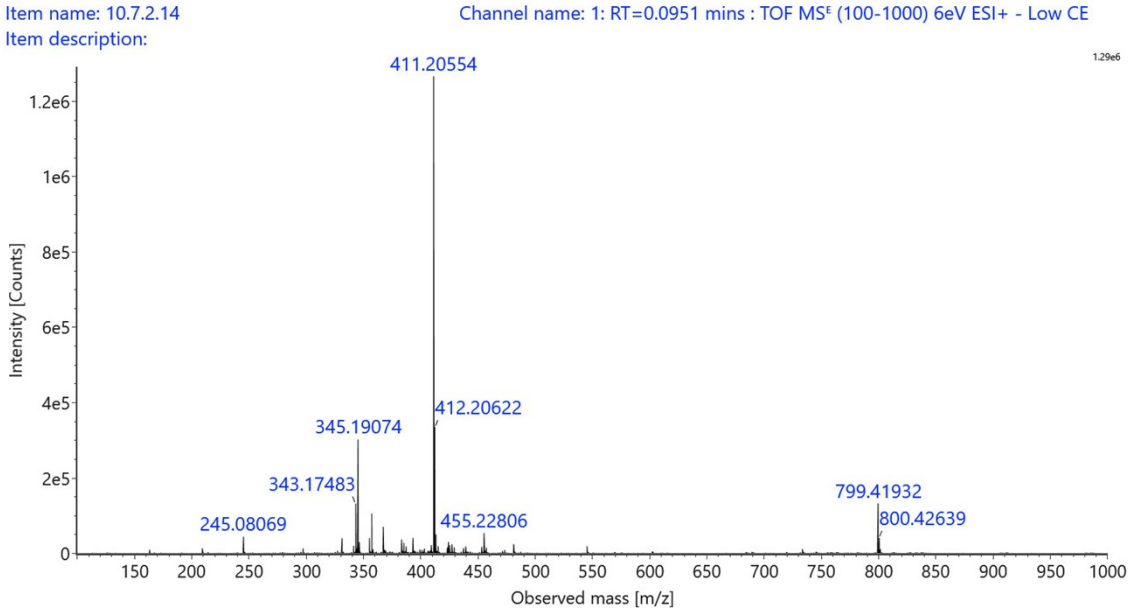


Figure S53. MS spectrum of **7**

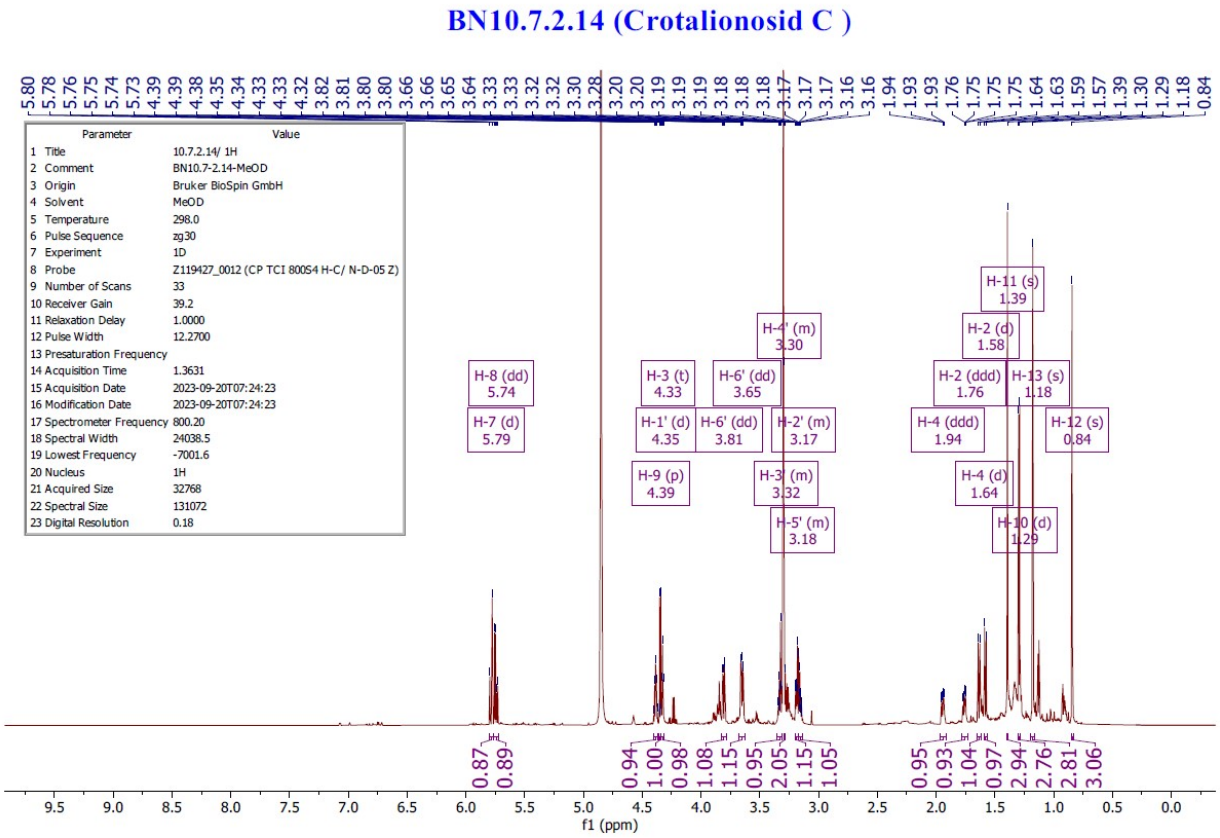


Figure S54. ^{1}H-NMR spectrum of **7** in CD_3OD

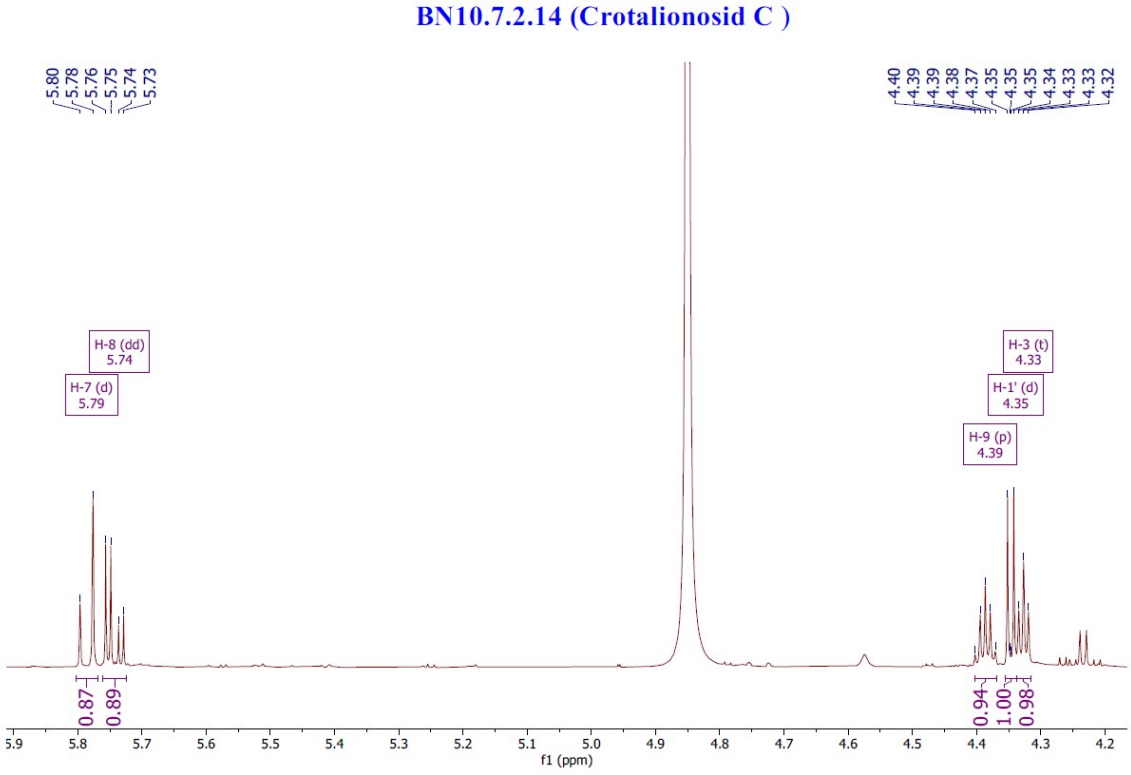


Figure S55. . ^{1}H-NMR spectrum of **7** from 4.2 to 5.9 ppm region

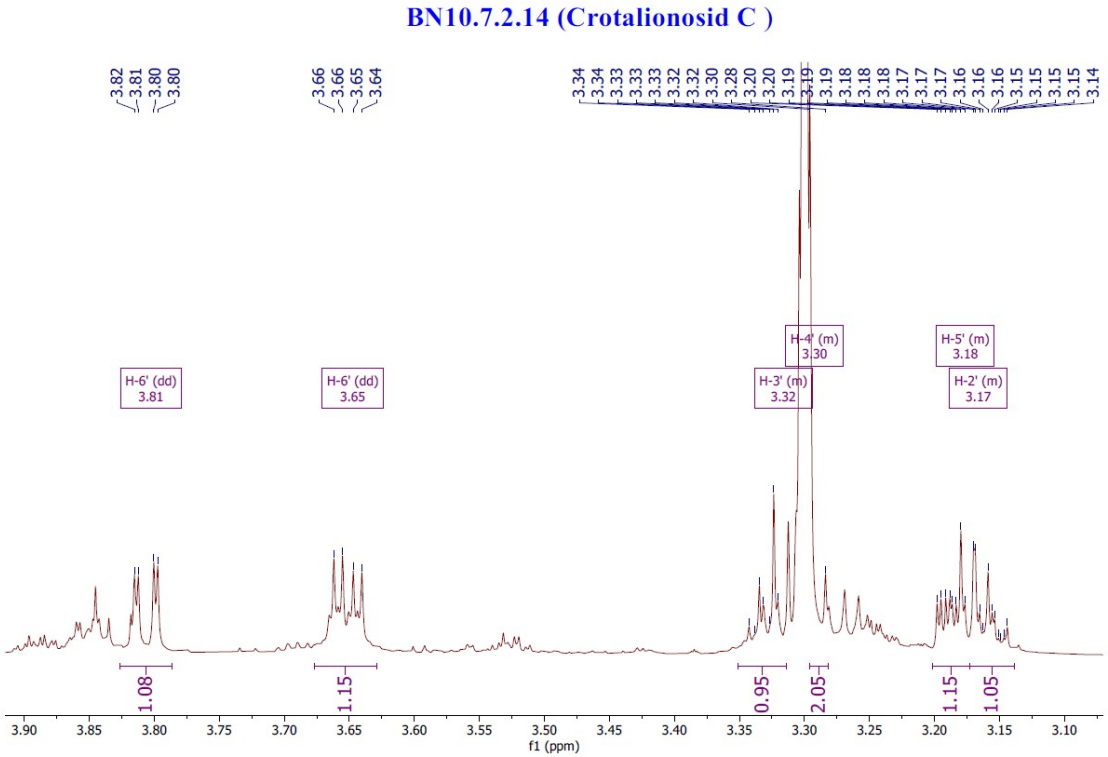


Figure S56. ^{1}H-NMR spectrum of **7** from 3.1 to 3.9 ppm region

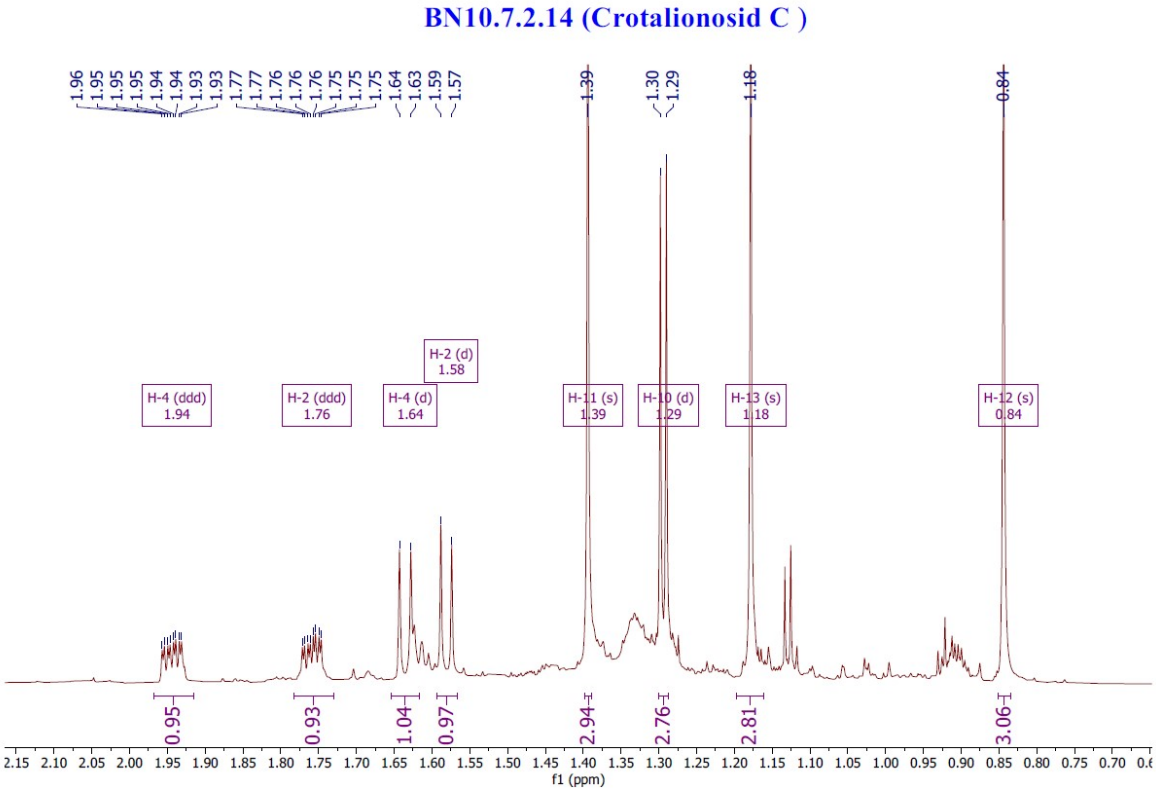


Figure S57. ^{1}H-NMR spectrum of **7** from 0.7 to 2.15 ppm region

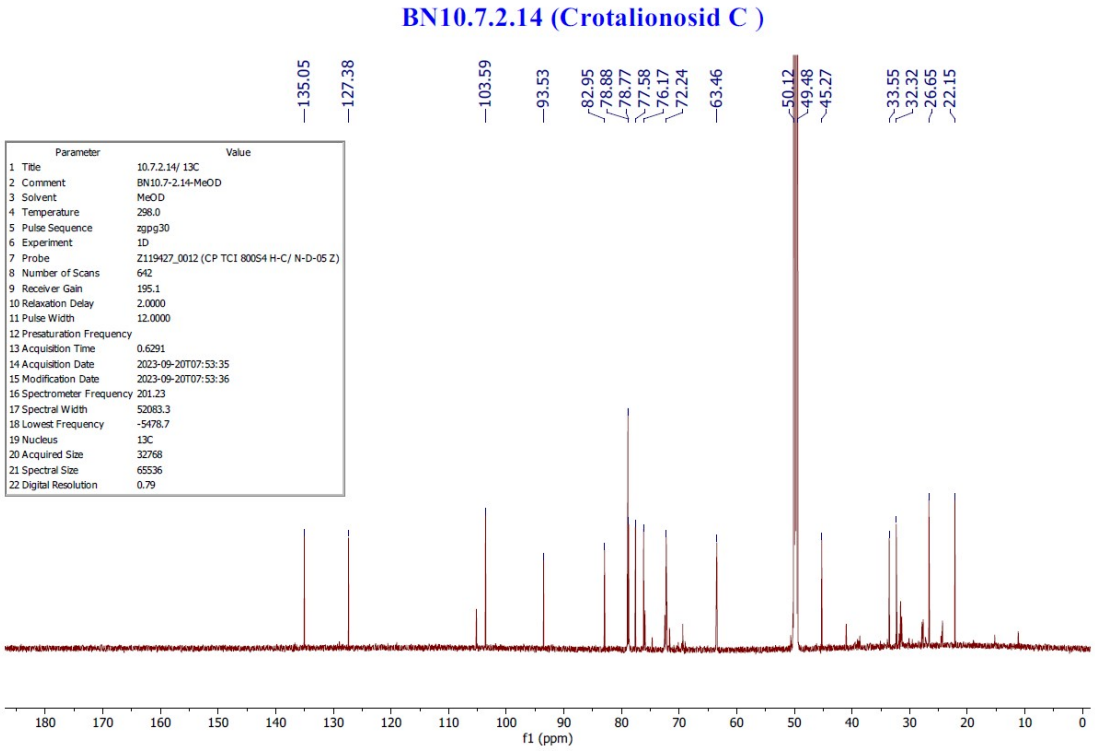


Figure S58. ^{13}C-NMR spectrum of **7** in CD_3OD

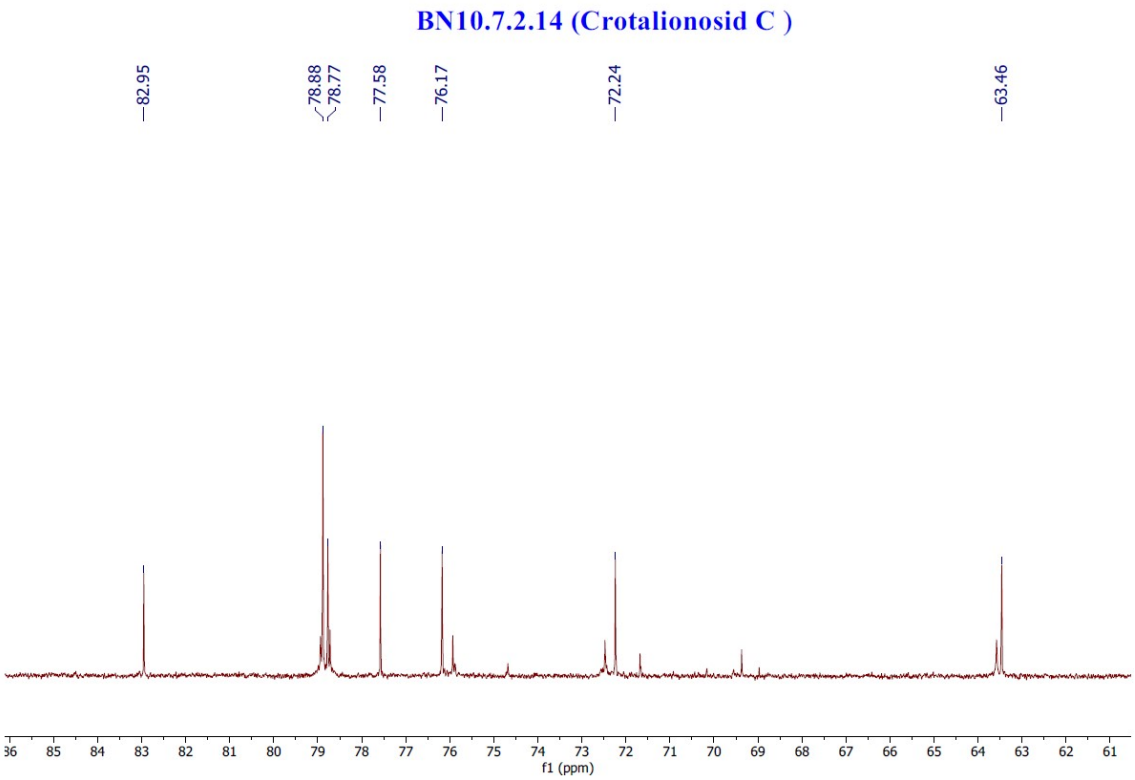


Figure S59. ^{13}C-NMR spectrum of **7** from 61 to 86 ppm region

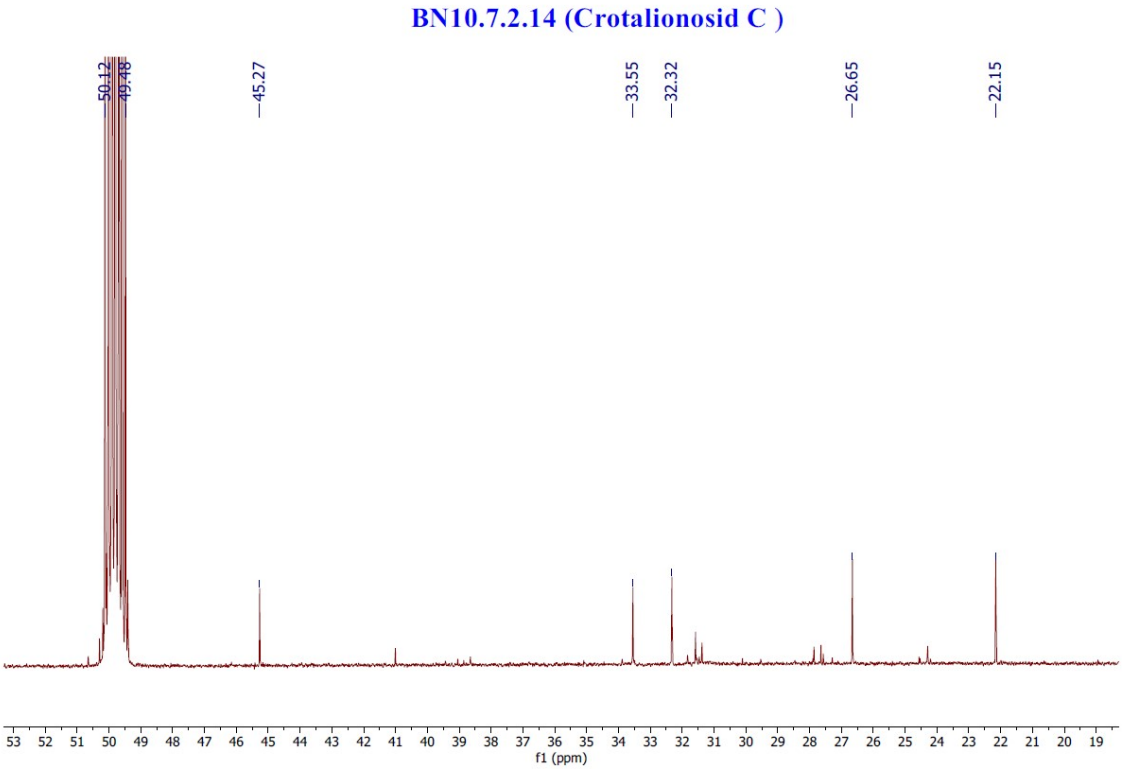


Figure S60. ^{13}C-NMR spectrum of **7** from 19 to 53 ppm region

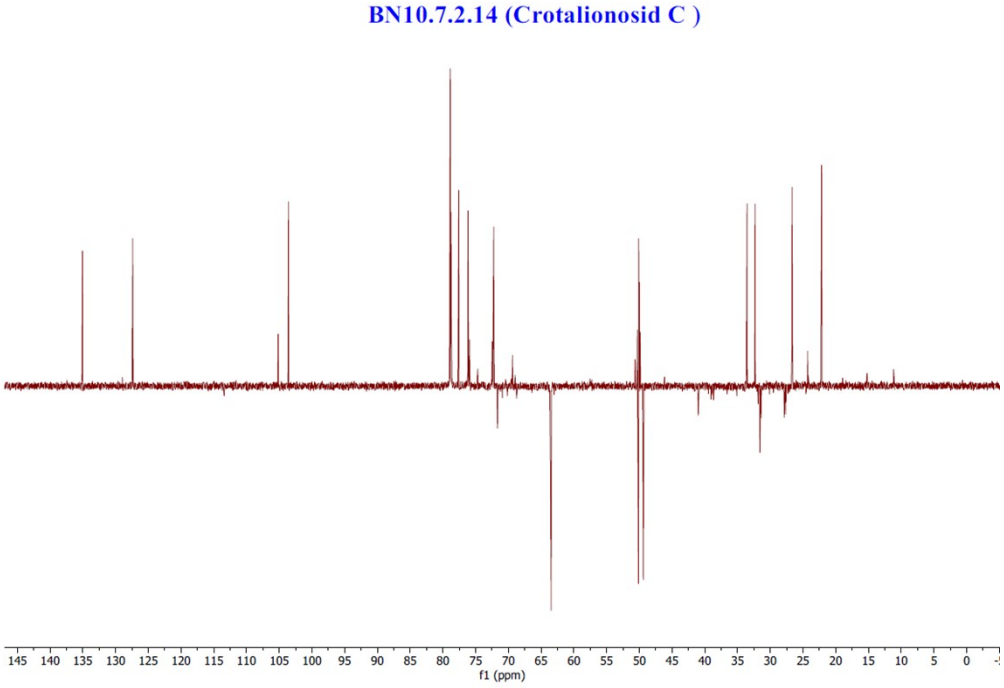


Figure S61. DEPT spectrum of **7**

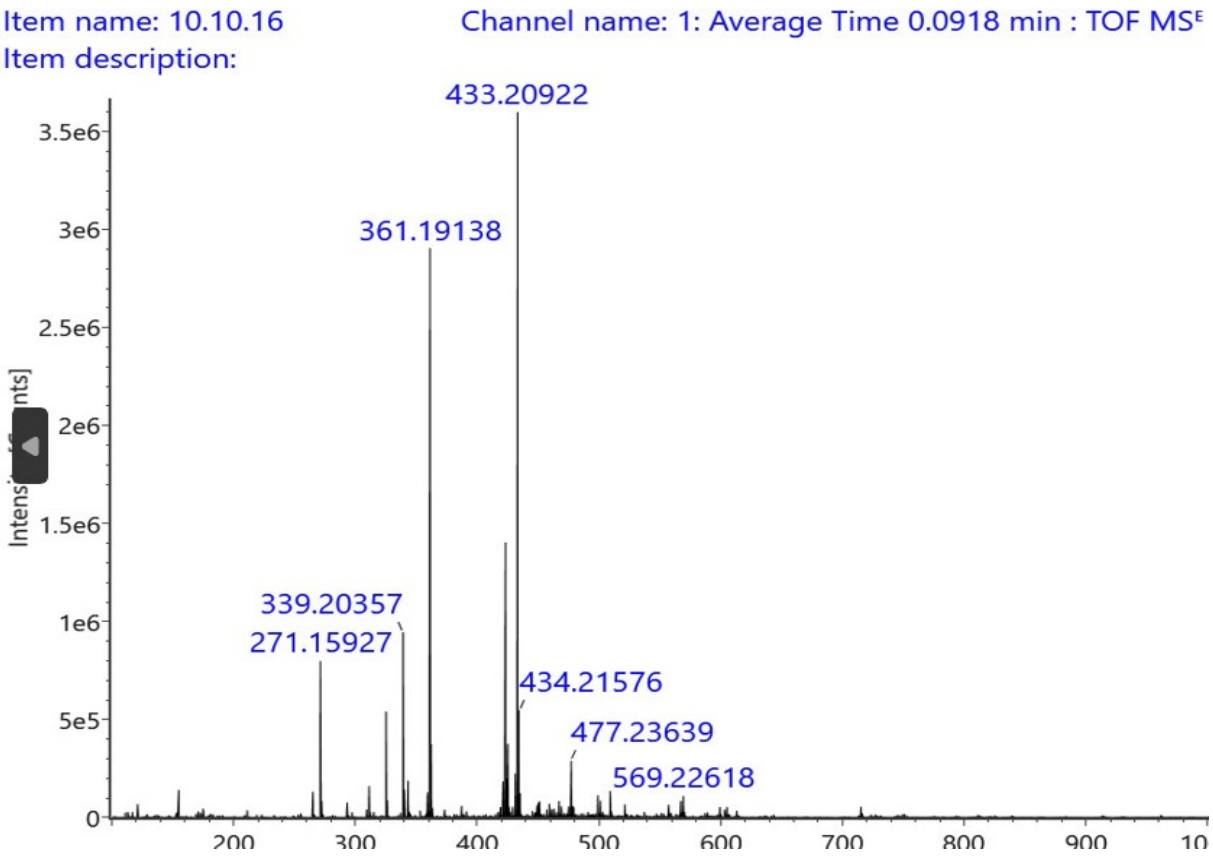


Figure S62. MS spectrum of **8**

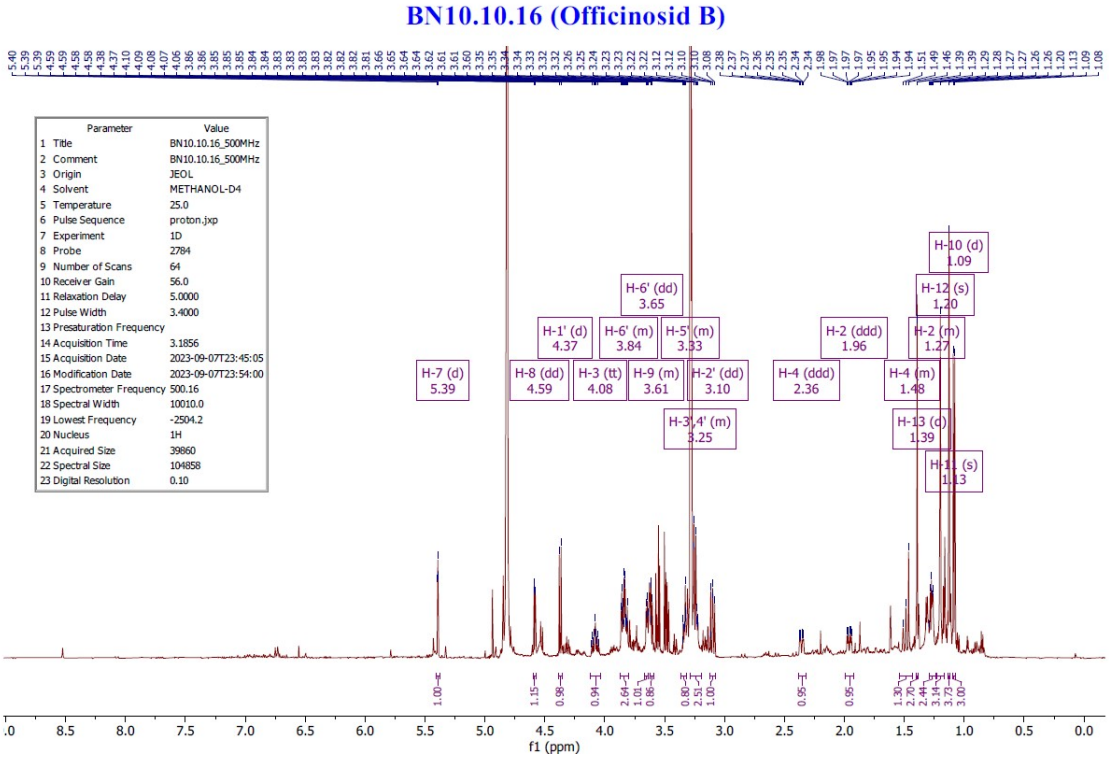


Figure S63. ^{1}H-NMR spectrum of **8** in CD_3OD

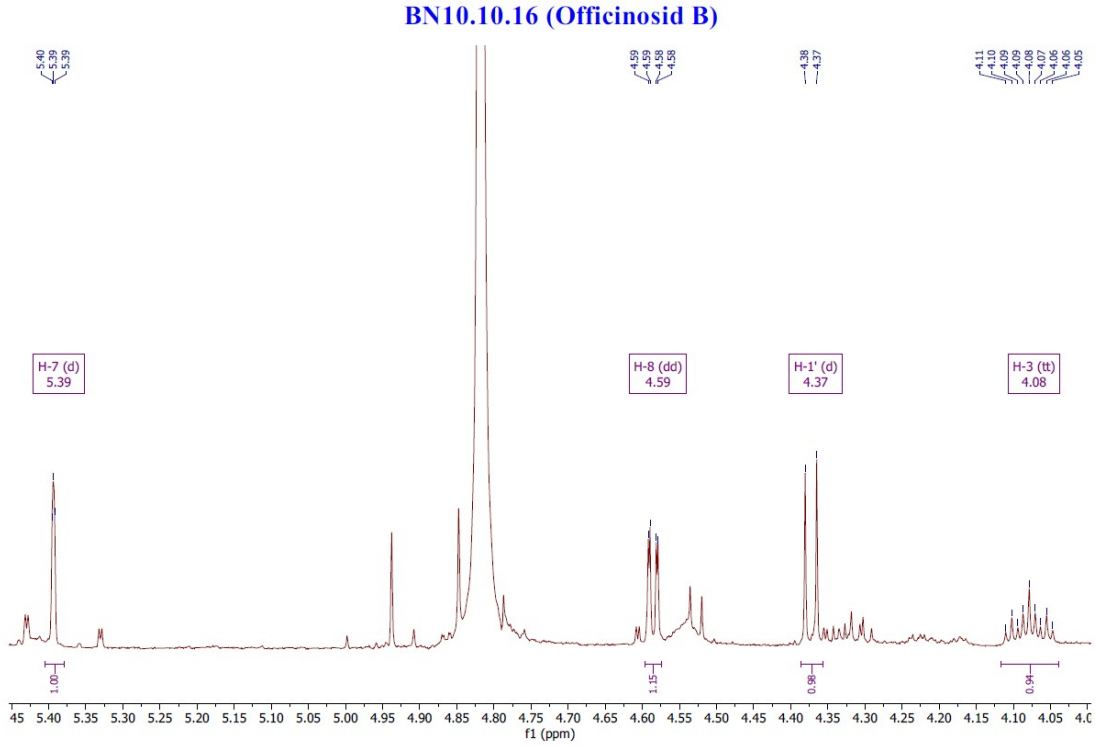


Figure S64. ^{1}H-NMR spectrum of **8** from 4.0 to 5.4 ppm region

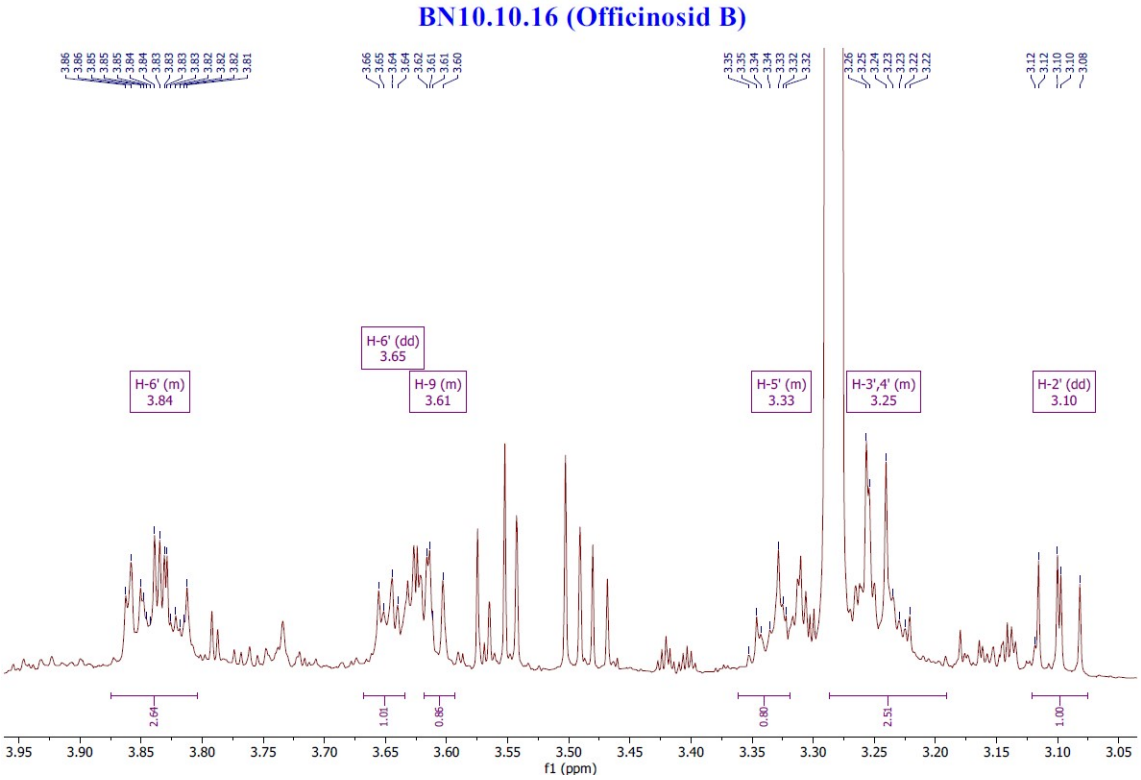


Figure S65. ^{1}H-NMR spectrum of **8** from 3.05 to 3.95 ppm region

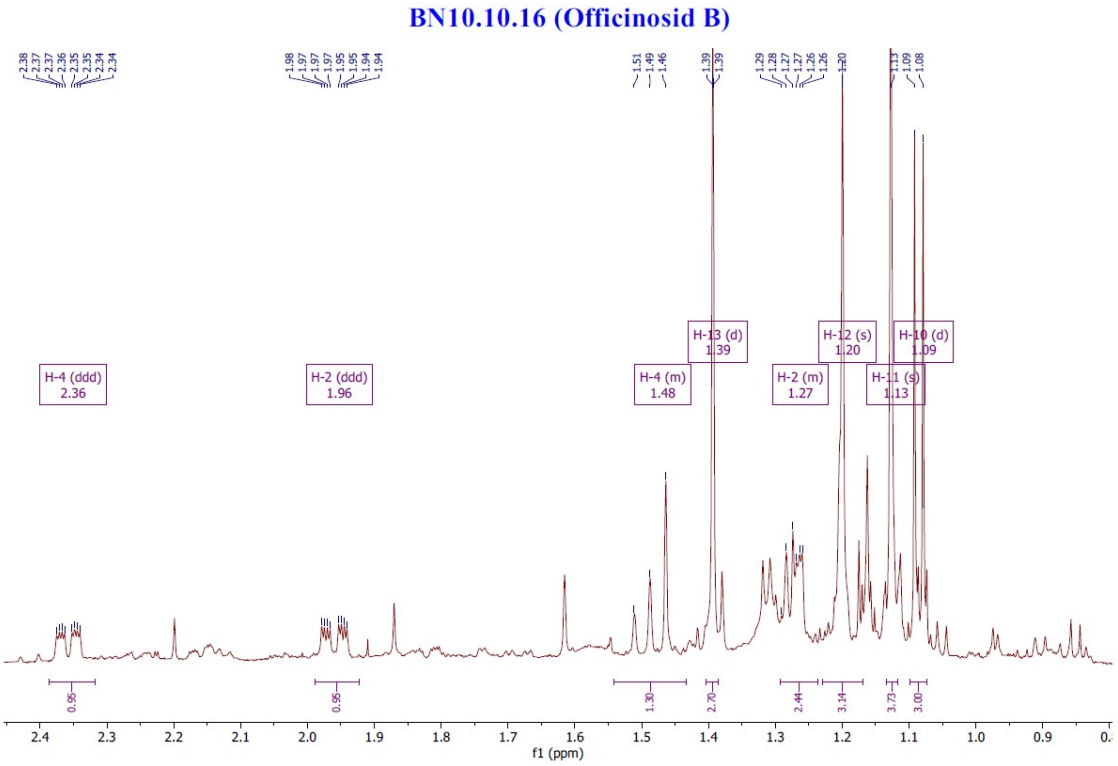


Figure S66. ^{1}H-NMR spectrum of **8** from 0.9 to 2.4 ppm region

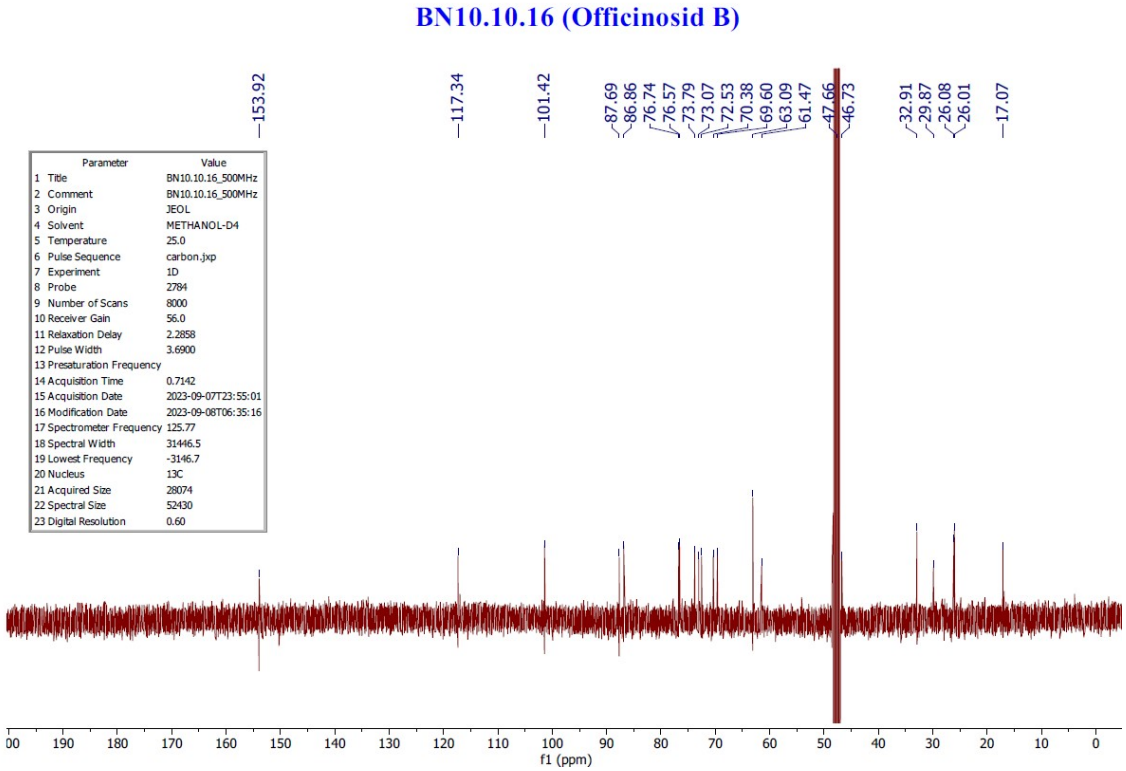


Figure S67. ^{13}C-NMR spectrum of **8** in CD_3OD

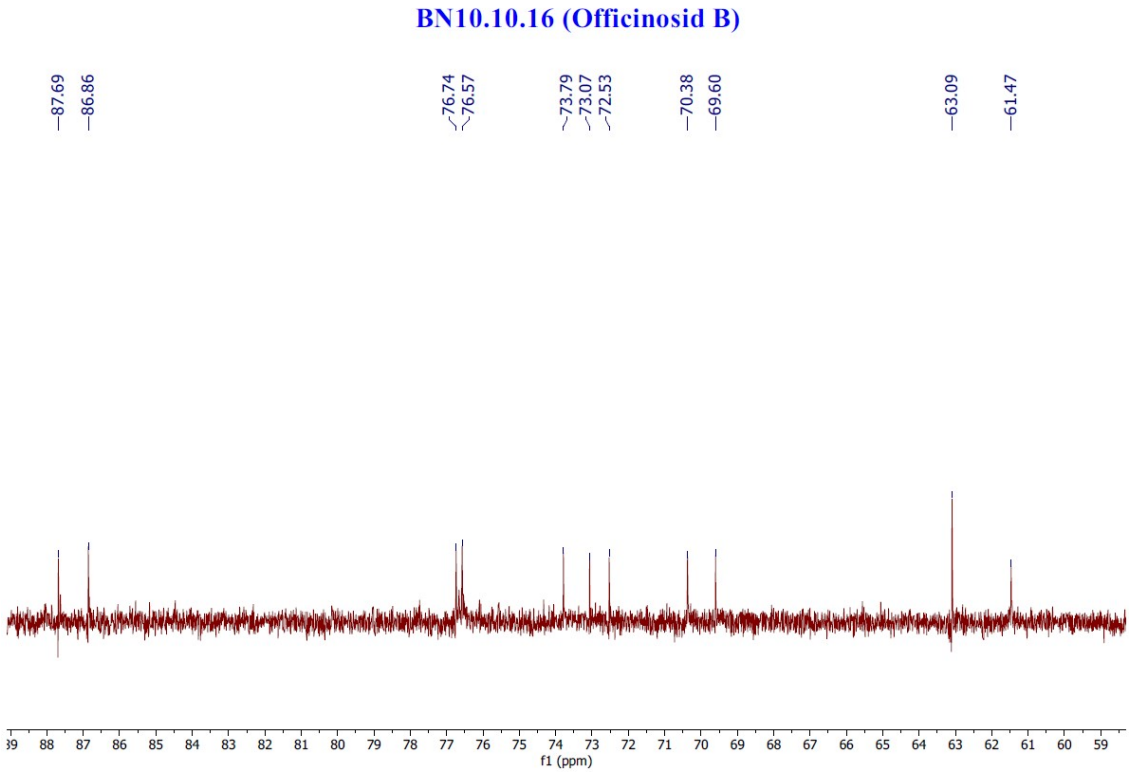


Figure S68. ^{13}C-NMR spectrum of **8** from 59 to 88 ppm region

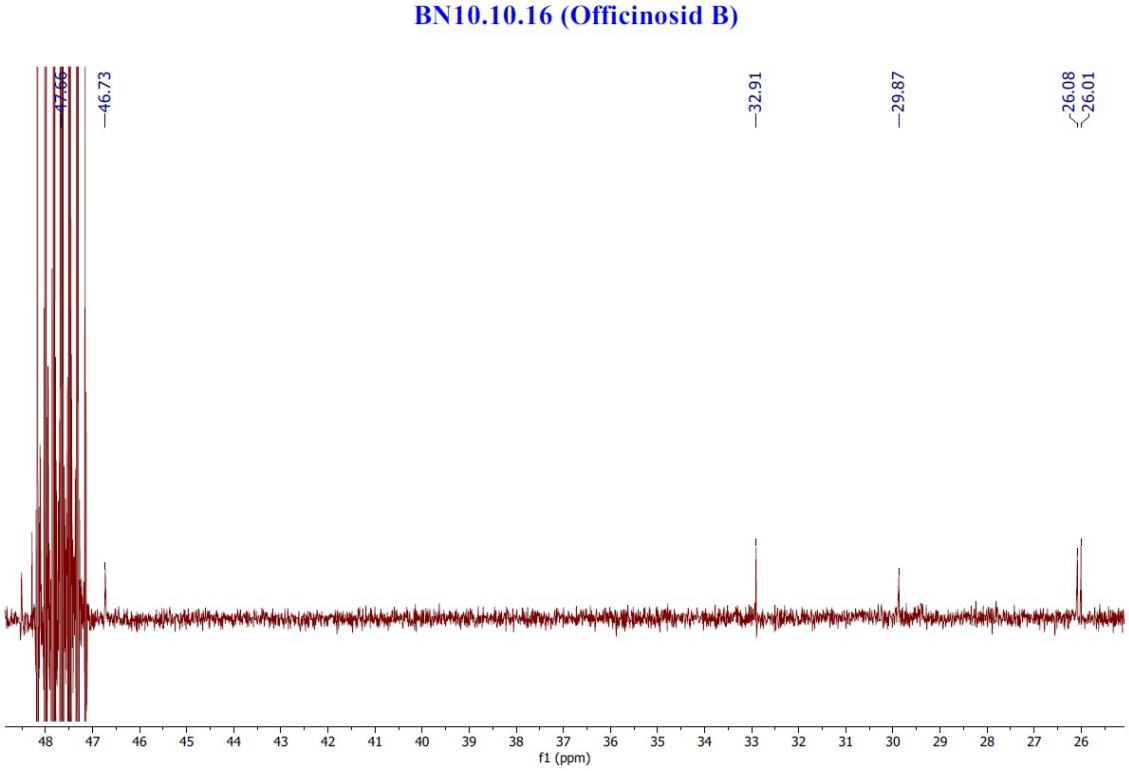


Figure S69. ^{13}C-NMR spectrum of **8** from 26 –to 48 ppm region

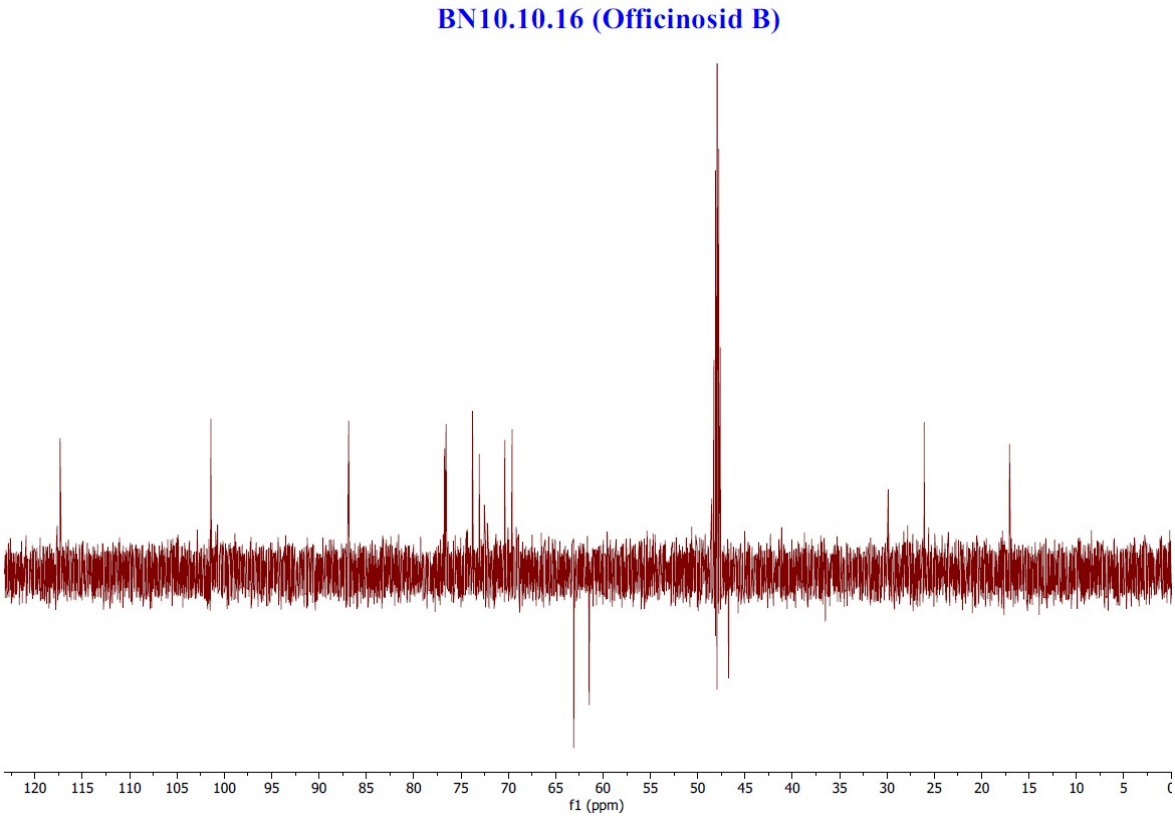


Figure S70. DEPT spectrum of **8**

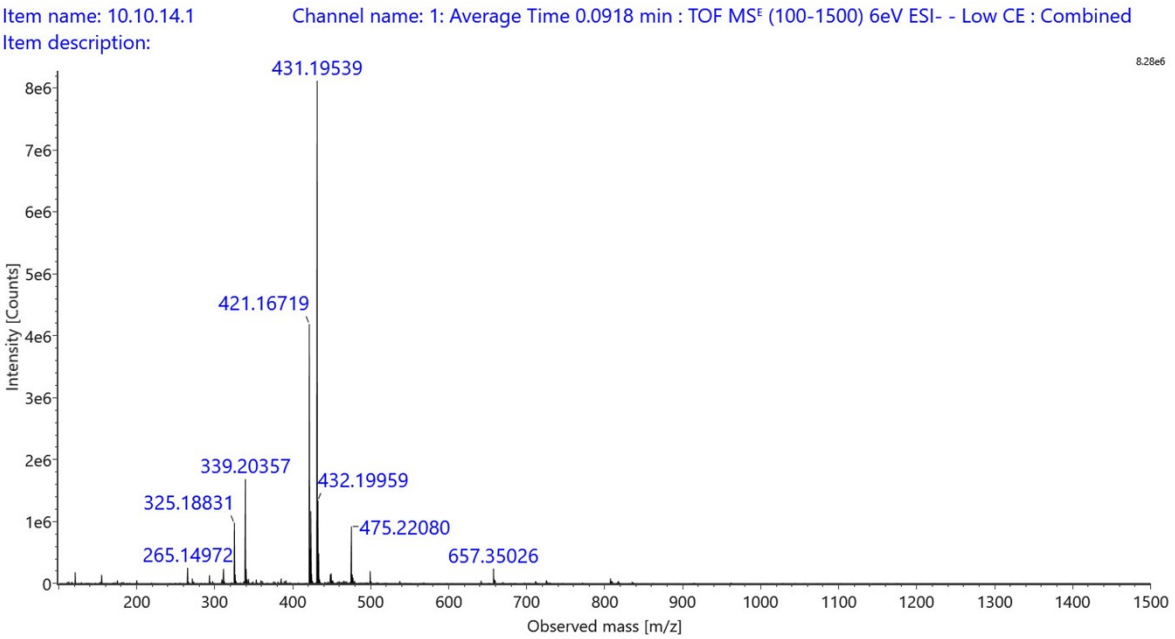


Figure S71. MS spectrum of **9**

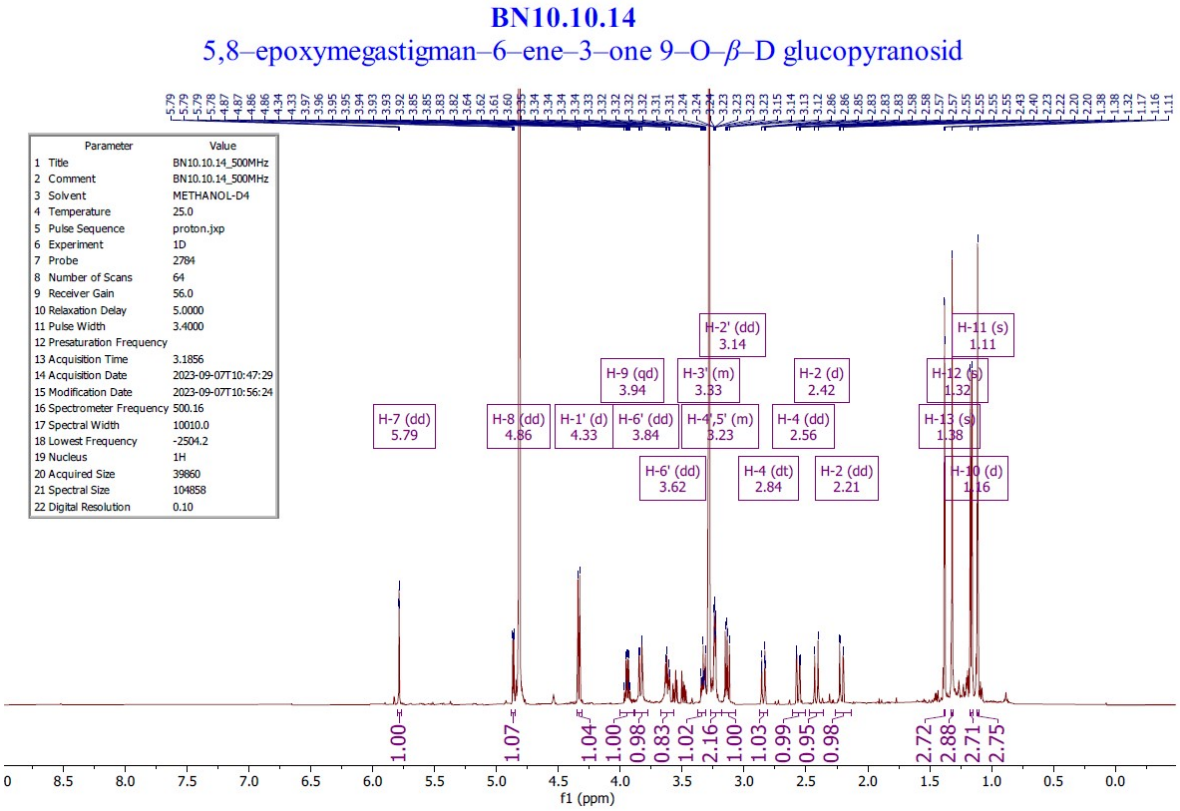


Figure S72. ^{1}H-NMR spectrum of **9** in CD_3OD

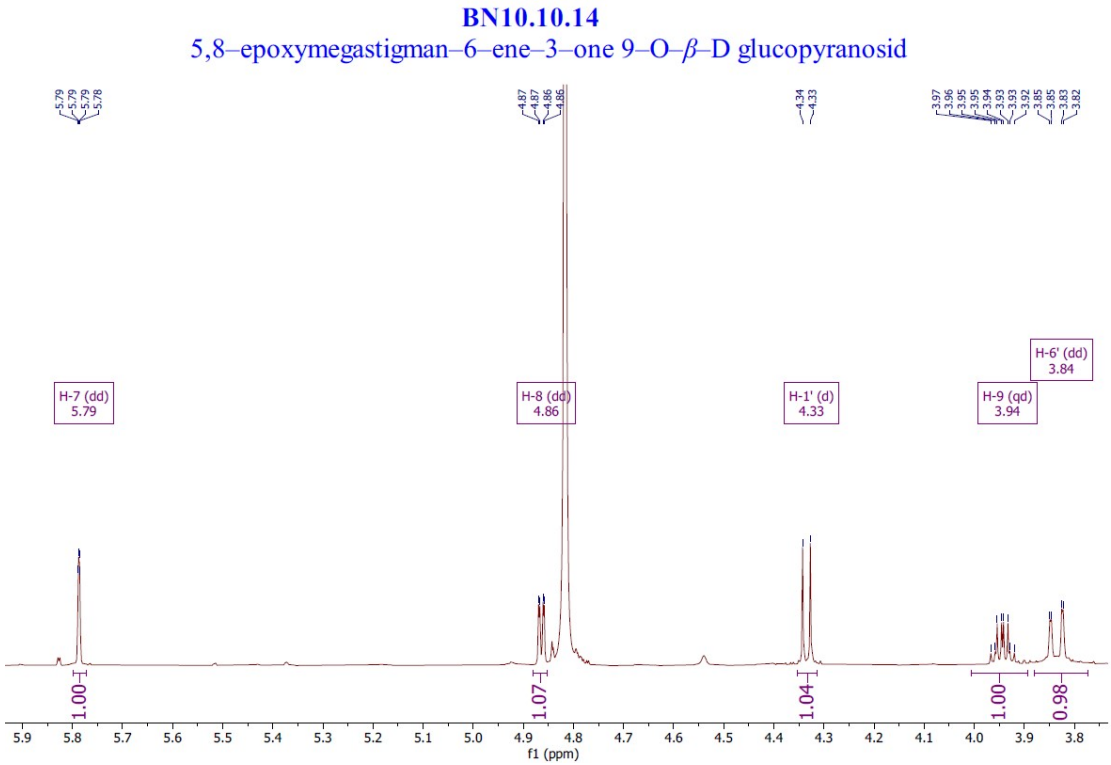


Figure S73. ^{1}H-NMR spectrum of **9** from 3.8 to 5.9 ppm region

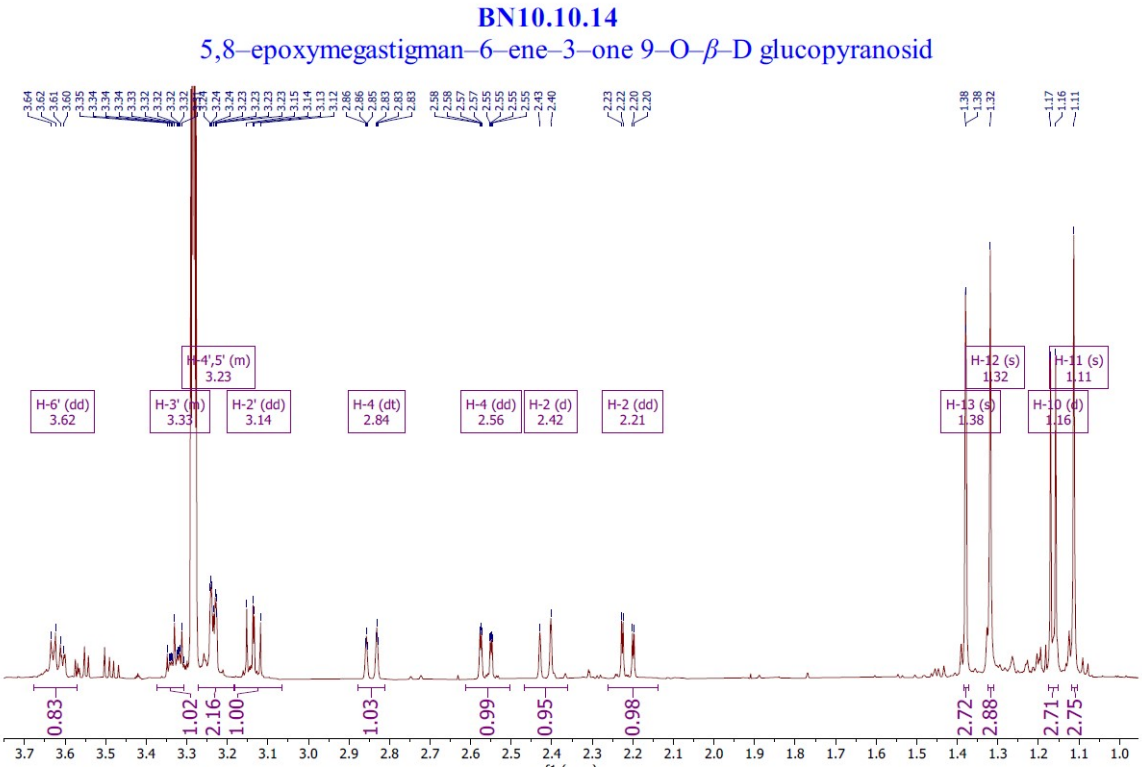


Figure S74. ^{1}H-NMR spectrum of **9** from 1.0 to 3.7 ppm region

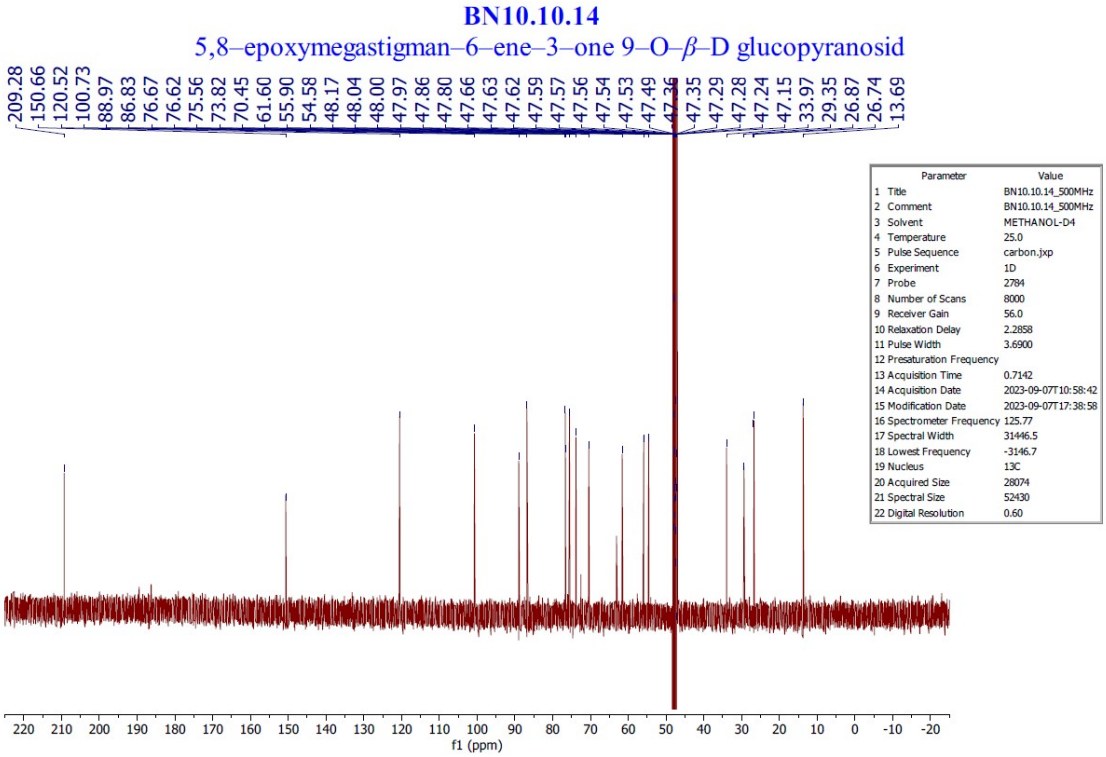


Figure S75. ^{13}C-NR spectrum of **9** in CD_3OD

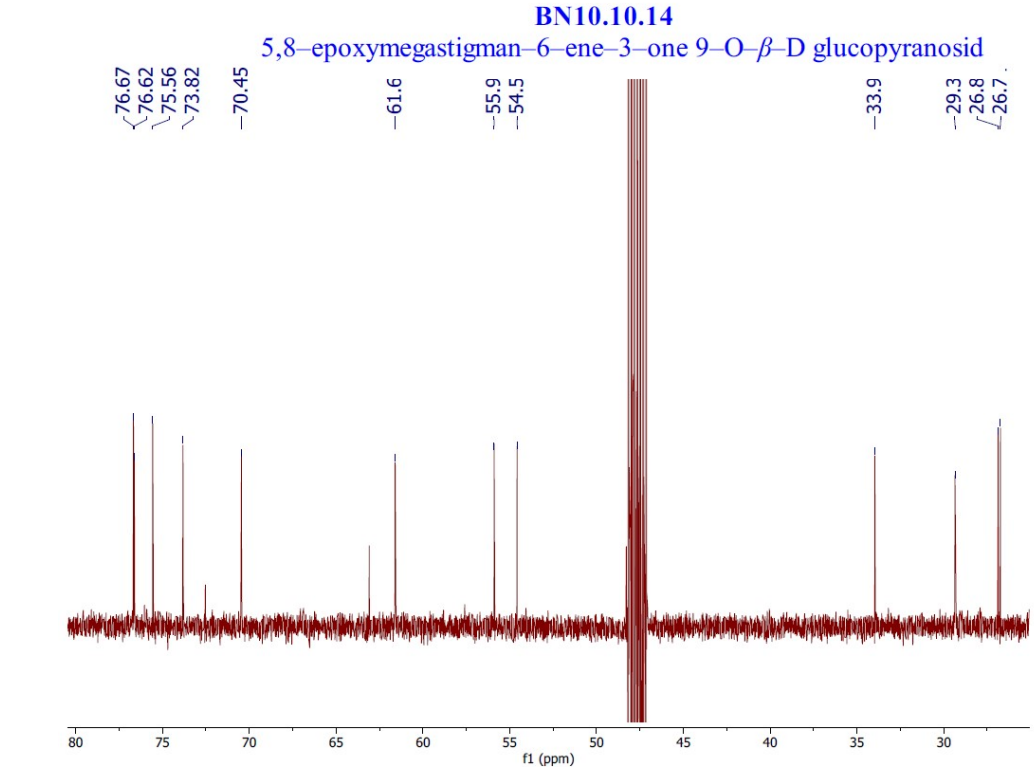


Figure S76. ^{13}C-NMR spectrum of **9** from 20 to 80 ppm region

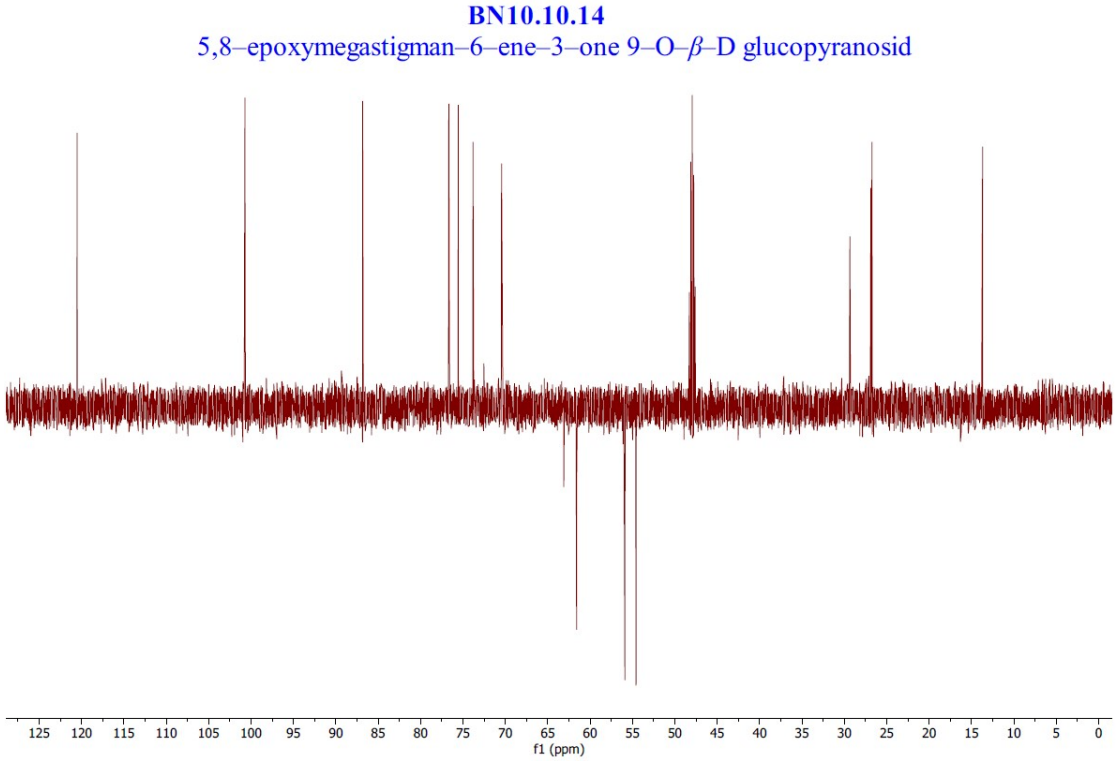


Figure S77. DEPT spectrum of **9**

Table S1. Effects of megastigmane compounds on pro-inflammatory cytokine (IL-1*β*)

No	IL-1*β* Concentration (pg/mL)						
	3	**2**	**6**	**1**	**Hydroxytyrosol 4–*β*–D–glucoside**	**DMSO**	**LPS**
1	86.01	66.29	67.10	93.33	91.62	170.75	**490.47**
2	65.96	65.75	58.91	80.65	101.90	139.71	**475.22**
3	75.76	83.92	61.57	96.45	88.96	142.68	**451.49**
4	58.06	68.80	74.89	85.35	91.75	157.16	**523.01**
5	74.67	72.04	54.86	94.68	96.01	155.08	**389.03**
Mean	**72.09**	**71.36**	**63.47**	**90.09**	**94.05**	**153.08**	**465.84**
SE	**4.73**	**3.33**	**3.48**	**3.03**	**2.26**	**5.57**	**22.44**

Table S2. Effects of megastigmane compounds on anti-inflammatory cytokine (IL-10)

No	IL–10 Concentration (pg/mL)						
	3	**2**	**6**	**1**	**Hydroxytyrosol 4–*β*–D–glucoside**	**DMSO**	**LPS**
1	279.26	198.76	237.82	268.63	176.19	150.38	**100.63**
2	297.14	176.27	263.26	184.14	164.97	133.85	**122.60**
3	219.63	185.47	313.10	258.87	166.77	123.62	**112.50**
4	260.10	189.79	297.60	197.65	165.90	145.51	**93.96**
5	246.12	204.29	225.70	181.38	162.10	132.50	**97.40**
Mean	**260.45**	**190.92**	**267.49**	**218.14**	**167.19**	**137.17**	**105.42**
SE	**16.15**	**5.84**	**19.43**	**18.14**	**1.01**	**4.49**	**6.68**