

## SUPPLEMENTARY MATERIAL

**Antimicrobial, cytotoxic and  $\alpha$ -glucosidase inhibitory activities of constituents isolated from**

***Homotrigona apicalis* propolis - *In vitro* and molecular docking studies**

Diep Thi Lan Phuong<sup>1,\*</sup>, Nguyen Van Phuong<sup>2</sup>, Nguyen Le Tuan<sup>1</sup>, Nguyen Thanh Cong<sup>2,3</sup>, Nguyen Thu Hang<sup>2</sup>, Le Nguyen Thanh<sup>4,5,\*</sup>, Vu Thi Hue<sup>5</sup>, Nguyen Quoc Vuong<sup>4,5</sup>, Nguyen Thi Thu Ha<sup>6</sup>,  
Milena Popova<sup>7</sup>, Boryana Trusheva<sup>7</sup>, Vassya Bankova<sup>7</sup>

<sup>1</sup> *Faculty of Natural Sciences, Quy Nhon University, Binh Dinh, Vietnam*

<sup>2</sup> *Hanoi University of Pharmacy, Hanoi, Vietnam*

<sup>3</sup> *Department of Pharmacy, Dai Nam University, Hanoi, Vietnam*

<sup>4</sup> *Graduate University of Science and Technology, Vietnam Academy of Science and Technology  
(VAST), Hanoi, Vietnam*

<sup>5</sup> *Institute of Marine Biochemistry, VAST, Hanoi, Vietnam*

<sup>6</sup> *Institute of Chemistry, VAST, Hanoi, Vietnam*

<sup>7</sup> *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences,  
Acad. G. Bonchev str., bl.9, Sofia, Bulgaria*

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\*Correspondence: [diepthilanthuong@gnu.edu.vn](mailto:diepthilanthuong@gnu.edu.vn) (Diep Thi Lan Phuong)

[lethanh@imbc.vast.vn](mailto:lethanh@imbc.vast.vn) (Le Nguyen Thanh)

## ABSTRACT

The chemical investigation of *Homotrigona apicalis* propolis collected in Binh Dinh province, Vietnam lead to the isolation of nine compounds, including four sesquiterpenes: spathulenol (**1**), 1 $\alpha$ H,5 $\beta$ H-aromandendrane-4 $\beta$ ,10 $\alpha$ -diol (**2**), 1 $\beta$ , 6 $\alpha$ -Dihydroxy-4(15)-eudesmene (**3**) and 1 $\beta$ H,5 $\beta$ H-aromandendrane-4 $\alpha$ ,10 $\beta$ -diol (**4**), three triterpenes: acetyl oleanolic acid (**5**), 3 $\alpha$ -hydroxytirucalla-8,24-dien-21-oic acid (**6**) and ursolic acid (**7**), two xanthenes: cochinchinone A (**8**) and  $\alpha$ -mangostin (**9**). Sesquiterpens **1–4** and triterpene **6** were isolated for the first time from stingless bee propolis. In the antibacterial activity evaluation, the EtOH extract only showed moderate activity on *S. aureus* but isolated compounds **7–9** showed good antibacterial activity with IC<sub>50</sub> values of 0.56 to 17.33  $\mu$ g/mL. The EtOH extract displayed selective cytotoxicity against A-549 cancer cell line with IC<sub>50</sub> values of 22.82 $\pm$ 0.86  $\mu$ g/mL, while xanthenes **8** and **9** exhibited good activity against KB, HepG-2 and A-549 cancer cell lines with IC<sub>50</sub> values range from 7.55  $\pm$  0.25 to 29.27  $\pm$  2.07  $\mu$ g/mL. Cytotoxic effects of xanthone **8** and **9** are proposed by the inhibition of the EGFR and HER2 pathways using molecular docking study. Compounds **5**, **8** and **9** showed potential  $\alpha$ -glucosidase inhibitory activities, that was further confirmed by computational studies.

**Keywords:** *Homotrigona apicalis*; sesquiterpenes; triterpenes; xanthenes;  $\alpha$ -glucosidase; cytotoxicity

*Physical and Spectroscopic Data of Compounds*

**Spathulenol (1)** Colorless oil;  $[\alpha]_D^{25} + 50.3$  (*c* 0.1, CHCl<sub>3</sub>); ESI-MS *m/z* 219 [M-H]<sup>+</sup>. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ (ppm): 4.69 (1H, br s, H-15a), 4.66 (1H, br s, H-15b), 2.42 (1H, dd, *J* = 6.6 Hz, 13.2 Hz, H-9b), 2.20 (1H, m, H-1), 2.05 (1H, m, H-9a), 1.97 (1H, m, H-8b), 1.92 (1H, m, H-2b), 1.78 (1H, m, H-3b), 1.64 (1H, m, H-2a), 1.54 (1H, m, H-3a), 1.31 (1H, t, *J* = 10.8 Hz, H-5), 1.28 (3H, s, H-14), 1.05 (3H, s, H-12), 1.04 (3H, s, H-13), 1.01 (H-8a), 0.71 (1H, m, H-7), 0.47 (1H, dd, *J* = 9.6, 11.4 Hz, H-6).

<sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ (ppm): 153.5 (C-10), 106.3 (C-15), 81.0 (C-4), 54.4 (C-5), 53.4 (C-1), 41.7 (C-3), 38.9 (C-9), 29.9 (C-6), 28.7 (C-12), 27.5 (C-7), 26.7 (C-2), 26.1 (C-14), 24.8 (C-8), 20.3 (C-11), 16.3 (C-13).

**1αH,5βH-aromandendrane-4β,10α-diol (2)** Colorless oil, ESI-MS *m/z* 237 [M-H]<sup>+</sup>. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ (ppm): 1.86 (1H, m, H-1), 1.82 (1H, m, H-8b), 1.76 (1H, dd, *J* = 6.6 Hz, 13.2 Hz, H-9b), 1.69-1.58 (4H, m, H-2, H-3), 1.72 (1H, t, *J* = 13.2 Hz, H-9a), 1.28 (3H, s, H-14), 1.18 (1H, t, *J* = 10.8 Hz, H-5), 1.17 (3H, s, H-15), 1.06 (6H, s, H-12, H-13), 0.88 (1H, m, H-8a), 0.64 (1H, m, H-7), 0.43 (1H, dd, *J* = 9.6 Hz, 10.8 Hz, H-6). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ (ppm): 80.4 (C-4), 75.0 (C-10), 56.4 (C-1), 48.5 (C-5), 44.5 (C-9), 41.2 (C-3), 28.7 (C-13), 28.3 (C-6), 26.6 (C-7), 24.5 (C-14), 23.8 (C-2), 20.3 (C-15), 20.2 (C-8), 19.6 (C-11), 16.3 (C-12).

**1β, 6α-Dihydroxy-4(15)-eudesmene (3)** Colorless oil, ESI-MS *m/z* 237 [M-H]<sup>+</sup>. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ (ppm): 5.02 (1H, d, *J* = 1.2 Hz, H-15a), 4.74 (1H, d, *J* = 1.2 Hz, H-15b), 3.71 (1H, t, *J* = 10.2 Hz, H-6), 3.42 (1H, dd, *J* = 4.8, 11.4 Hz, H-1), 2.33 (1H, ddd, *J* = 2.4, 4.8, 13.2 Hz, H-3a), 2.24 (1H, td, *J* = 2.4, 7.2 Hz, H-11), 2.07 (1H, td, *J* = 2.4, 4.8 Hz, H-3b), 1.92 (1H, td, *J* = 3.0, 12.0 Hz, H-9a), 1.87 (1H, m, H-2a), 1.74 (1H, d, *J* = 10.2 Hz, H-5), 1.55 (2H, m, H-2b, H-8a), 1.29 (1H, m, H-7), 1.24 (1H, m, H-8b), 1.18 (1H, m, H-9b), 0.96 (3H, d, *J* = 7.2 Hz, H-12), 0.87 (3H, d, *J* = 7.2 Hz, H-13), 0.71 (3H, s, H-14). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ (ppm): 146.2 (C-4), 107.8 (C-15), 79.0 (C-1), 67.0 (C-6), 55.9 (C-5), 49.3 (C-7), 41.7 (C-10), 36.3 (C-9), 35.1 (C-3), 31.9 (C-2), 26.0 (C-11), 21.1 (C-12), 18.2 (C-8), 16.2 (C-13), 11.6 (C-14).

**1βH,5βH-aromandendrane-4α,10β-diol (4)** Colorless oil, ESI-MS *m/z* 237 [M-H]<sup>+</sup>. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) δ (ppm): 2.48 (1H, m, H-1), 1.86 (1H, m, H-2), 1.34 (3H, s, H-15), 1.20 (3H, s, H-14), 1.04 and 1.03 (each 3H, s, H-12 and H-13), 0.88 (1H, m, H-8a), 0.62 (1H, m, H-7). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ (ppm): 82.1 (C-4), 74.3 (C-10), 54.1 (C-1), 47.8 (C-5), 38.0 (C-9), 37.5 (C-3),

32.1 (C-15), 28.8 (C-7), 28.6 (C-13), 25.6 (C-14), 25.4 (C-6), 25.2 (C-2), 18.8 (C-8), 18.7 (C-11), 16.2 (C-12).

**Acetyl oleanolic acid (5):** White amorphous powder; ESI-MS  $m/z$  499  $[M+H]^+$ .

$^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 5.25 (1H, t,  $J = 3.6$  Hz, H-12), 4.49 (1H, m, H-3 $\alpha$ ), 2.04 (3H, s, CH<sub>3</sub>-31), 1.06 (3H, s, CH<sub>3</sub>-27), 0.95 (3H, s, CH<sub>3</sub>-26), 0.94 (3H, d,  $J = 7.2$  Hz, CH<sub>3</sub>-29), 0.86 (3H, s, CH<sub>3</sub>-23), 0.85 (3H, d,  $J = 6.6$  Hz, CH<sub>3</sub>-30), 0.84 (3H, s, CH<sub>3</sub>-24), 0.78 (3H, s, CH<sub>3</sub>-25).  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 182.1 (C-28), 171.0 (C-31), 138.1 (C-13), 125.7 (C-12), 80.9 (C-3), 55.3 (C-5), 52.7 (C-18), 47.9 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.0 (C-20), 38.8 (C-19), 38.3 (C-1), 37.7 (C-4), 37.7 (C-22), 37.3 (C-10), 32.9 (C-7), 30.6 (C-21), 28.1 (C-23), 28.0 (C-15), 24.1 (C-16), 23.6 (C-27), 23.5 (C-11), 23.3 (C-2), 21.3 (C-30), 21.4 (C-32), 18.2 (C-6), 17.1 (C-29), 17.0 (C-26), 15.5 (C-25).

**3 $\alpha$ -Hydroxytirucalla-8,24-dien-21-oic acid (6)** White amorphous powder; ESI-MS  $m/z$  457  $[M+H]^+$ .  $^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 5.09 (1H, t,  $J = 7.2$  Hz, H-24), 3.45 (1H, t,  $J = 2.4$  Hz, H-3), 2.29 (1H, m, H-20), 1.67 (3H, s, H-27), 1.58 (3H, s, H-26), 0.96 (3H, s, H-28), 0.88 (3H, s, H-30), 0.86 (3H, s, H-29), 0.83 (3H, s, H-18).  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ ): 180.1 (C-21), 134.3 (C-9), 132.9 (C-8), 132.1 (C-25), 123.6 (C-24), 76.1 (C-3), 49.6 (C-14), 47.3 (C-20), 46.9 (C-17), 44.8 (C-5), 43.8 (C-13), 37.6 (C-4), 37.2 (C-10), 32.5 (C-1), 29.8 (C-22), 29.3 (C-15), 28.9 (C-12), 27.1 (C-16), 26.9 (C-7), 25.9 (C-2), 25.7 (C-23), 25.6 (C-27), 24.5 (C-30), 22.2 (C-29), 21.5 (C-11), 19.8 (C-19), 18.7 (C-6), 17.6 (C-26), 15.8 (C-18).

**Ursolic acid (7):** White amorphous powder, ESI-MS  $m/z$  457  $[M+H]^+$ .  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3 + \text{CD}_3\text{OD}$ )  $\delta$  (ppm): 5.16 (1H, t,  $J = 7.5$  Hz, H-12), 3.12 (1H, m, H-3), 1.01 (3H, s, H-27), 0.90 (3H, s, H-23), 0.86 (3H, d,  $J = 6.5$  Hz, H-29), 0.85 (3H, s, H-24), 0.74 (3H, s, H-26), 0.70 (3H, s, H-25), 0.80 (3H, d,  $J = 6.5$  Hz, H-30).  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3 + \text{CD}_3\text{OD}$ ): 180.5 (C-28), 138.0 (C-13), 125.4 (C-12), 78.8 (C-3), 55.1 (C-5), 52.7 (C-18), 47.7 (C-17), 47.5 (C-9), 41.9 (C-14), 39.3 (C-8), 39.0 (C-4), 38.8 (C-19), 38.6 (C-20), 38.5 (C-1), 36.8 (C-10), 36.7 (C-22), 32.9 (C-7), 30.6 (C-21), 27.9 (C-23), 27.9 (C-2), 26.7 (C-15), 24.1 (C-16), 23.4 (C-27), 23.1 (C-11), 21.0 (C-30), 18.2 (C-6), 16.9 (C-29), 16.8 (C-26), 15.47 (C-25), 15.2 (C-24).

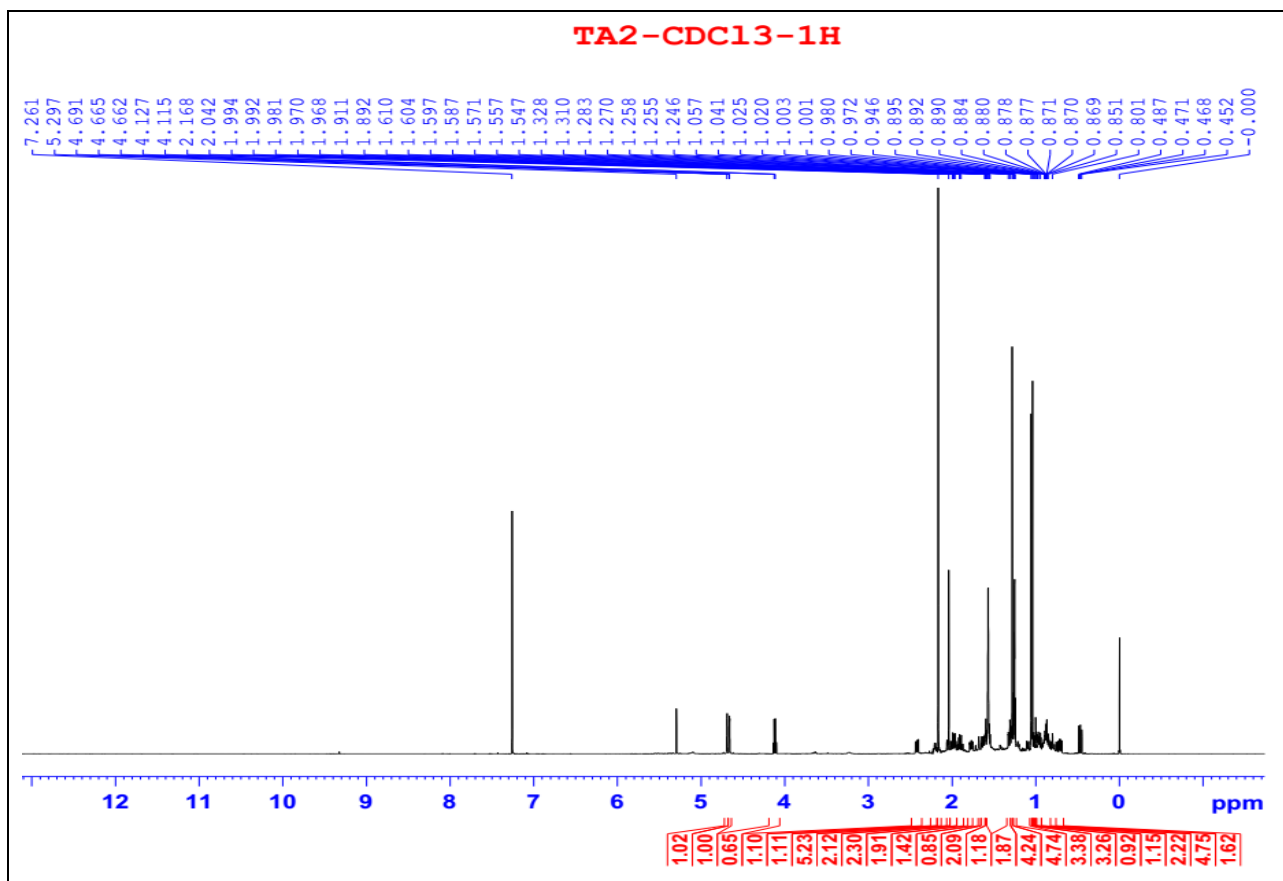
**Cochinchinone A (8):** yellow solid. ESI-MS  $[M+H]^+$  449.  $^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 13.12 (1H, s, 1-OH), 7.50 (1H, d,  $J = 3.0$  Hz, H-8), 7.36 (1H, d,  $J = 8.4$  Hz, H-5), 7.25 (1H, dd,  $J = 3.0$ ; 8.4 Hz, H-6), 6.45 (1H, s, OH), 5.49 (1H, s, OH), 5.28 (2H, m, H-2', H-2''), 5.05 (1H, t,  $J = 7.2$  Hz, H-6''), 3.56 (2H, d,  $J = 7.2$  Hz, H-1''), 3.46 (2H, d,  $J = 6.6$  Hz, H-1'), 2.09 (2H, m, H-5''), 2.05 (2H, m, H-4''), 1.88 (3H, s, H-9''), 1.85 (3H, s, H-4'), 1.76 (3H, s, H-5'), 1.63 (3H, s, H-8''), 1.57 (3H, s, H-10'').  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ ): 180.1 (C-21), 134.3 (C-9), 132.9 (C-8), 132.1 (C-25), 123.6 (C-24), 76.1 (C-3), 49.6 (C-14), 47.3 (C-20), 46.9 (C-17), 44.8 (C-5), 43.8 (C-13), 37.6 (C-4),

37.2 (C-10), 32.5 (C-1), 29.8 (C-22), 29.3 (C-15), 28.9 (C-12), 27.1 (C-16), 26.9 (C-7), 25.9 (C-2), 25.7 (C-23), 25.6 (C-27), 24.5 (C-30), 22.2 (C-29), 21.5 (C-11), 19.8 (C-19), 18.7 (C-6), 17.6 (C-26), 15.8 (C-18).

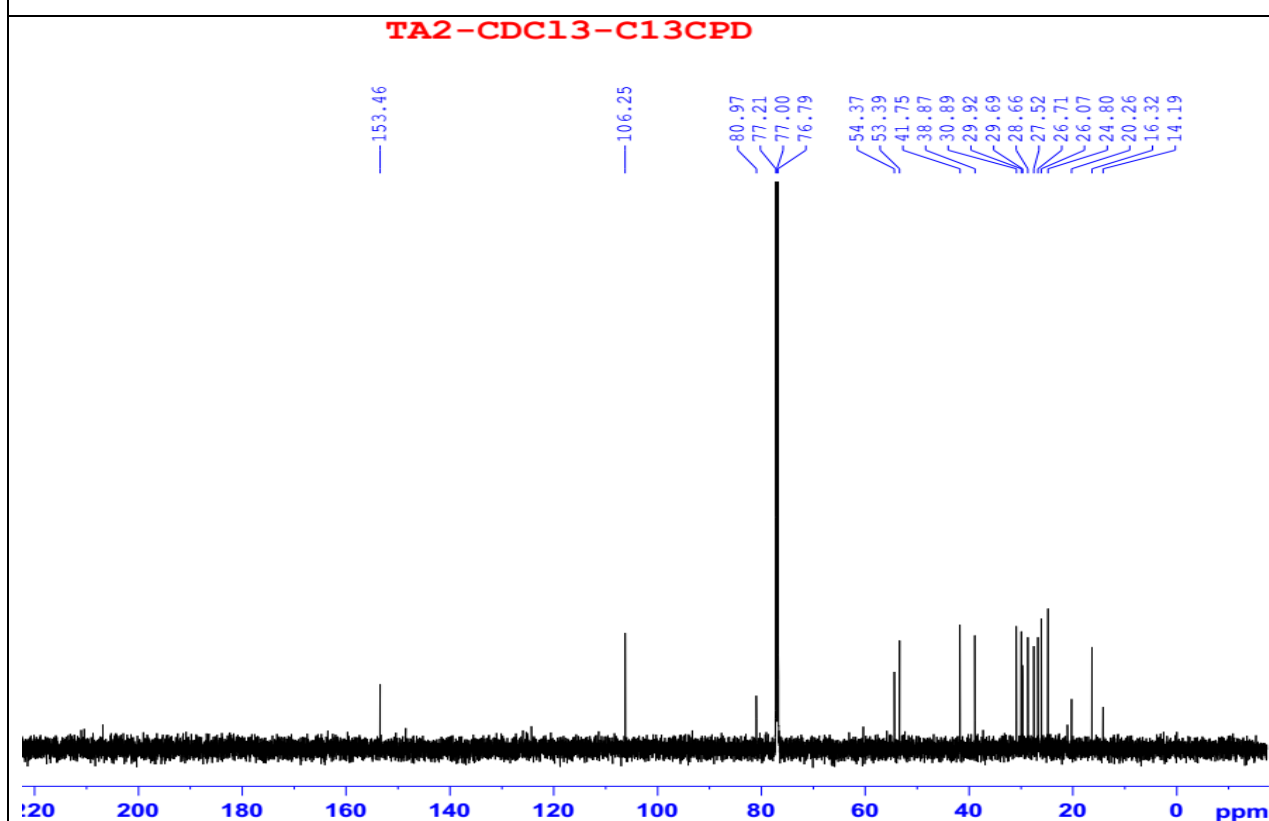
**$\alpha$ -mangostin (9):** yellow solid. ESI-MS  $[M+H]^+$  411.  **$^1\text{H-NMR}$**  (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  (ppm): 6.70 (1H, s, H-5); 6.25 (1H, s, H-4); 5.25 (2H, m, H-2', H-2''); 4.09 (2H, d,  $J = 6.5\text{Hz}$ , H-1''); 3.77 (3H, s, 7-OMe), 3.30 (2H, d,  $J = 7.5\text{ Hz}$ , H-1'); 1.84 (3H, s, H-4''); 1.79 (3H, s, H-5''); 1.69 (3H, s, H-4'); 1.67 (3H, s, H-5').  **$^{13}\text{C-NMR}$**  (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  (ppm): 183.1 (C-9); 163.6 (C-1); 161.5 (C-3); 157.9 (C-4a); 156.7 (C-4b); 156.1 (C-6); 144.8 (C-7); 138.4 (C-8); 131.8 (C-3''), 131.6 (C-3'), 125.1 (C-2''); 123.8 (C-2'); 112.1 (C-8a); 111.4 (C-2); 103.7 (C-9a); 102.7 (C-5), 93.1 (C-4), 61.3 (7-OMe), 27.1 (C-1''); 25.9 (C-4'); 25.9 (C-4''); 22.2 (C-1'); 18.3 (C-5''); 17.9 (C-5').

**Table S1.** Docking score of isolated compounds with  $\alpha$ -glucosidase

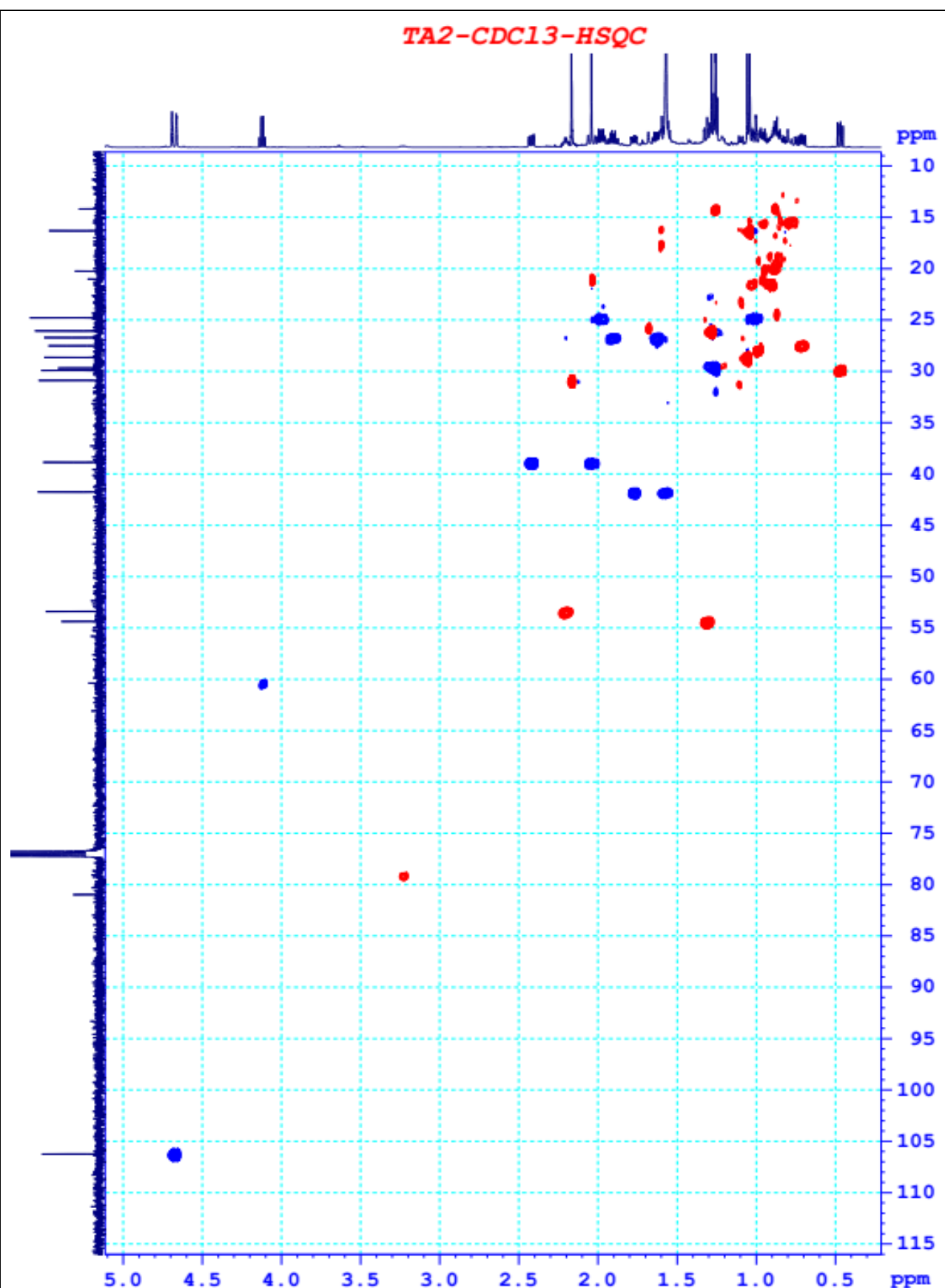
No.	Compound	IC <sub>50</sub> (μg/mL)	Docking score (kcal/mol)
1	<b>1</b>	>256	-6.6
2	<b>2</b>	>256	-7.0
3	<b>3</b>	>256	-7.0
4	<b>4</b>	>256	-7.0
5	<b>5</b>	8.44±0.23	-8.0
6	<b>6</b>	>256	-7.2
7	<b>7</b>	25.34 ± 0.54	-6.1
8	<b>8</b>	9.07±0.65	-9.3
9	<b>9</b>	1.90±0.10	-9.9
<b>10</b>	<b>Acarbose</b>	<b>134.56±3.02</b>	<b>-7.6</b>



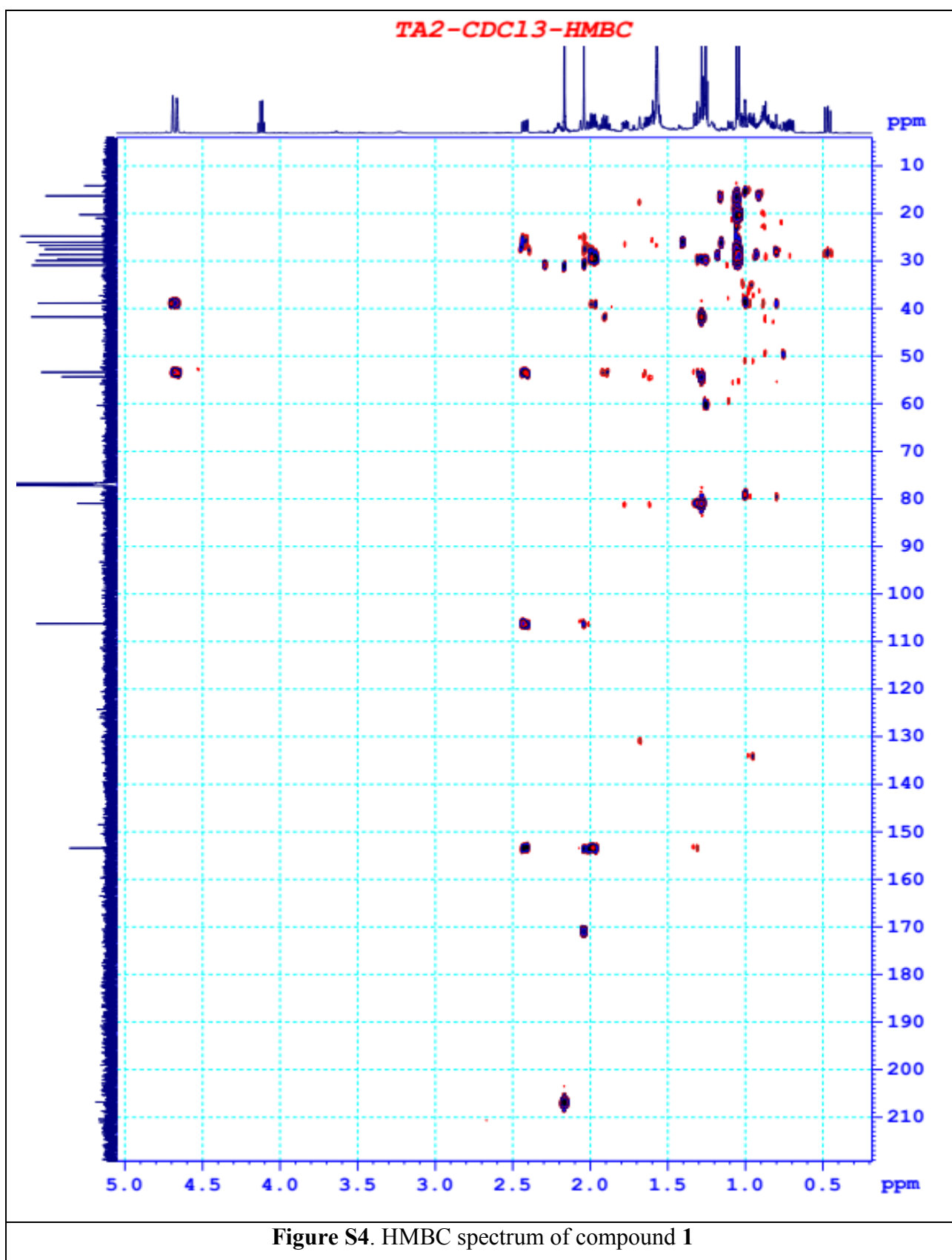
**Figure S1.**  $^1\text{H}$ -NMR spectrum of compound **1**



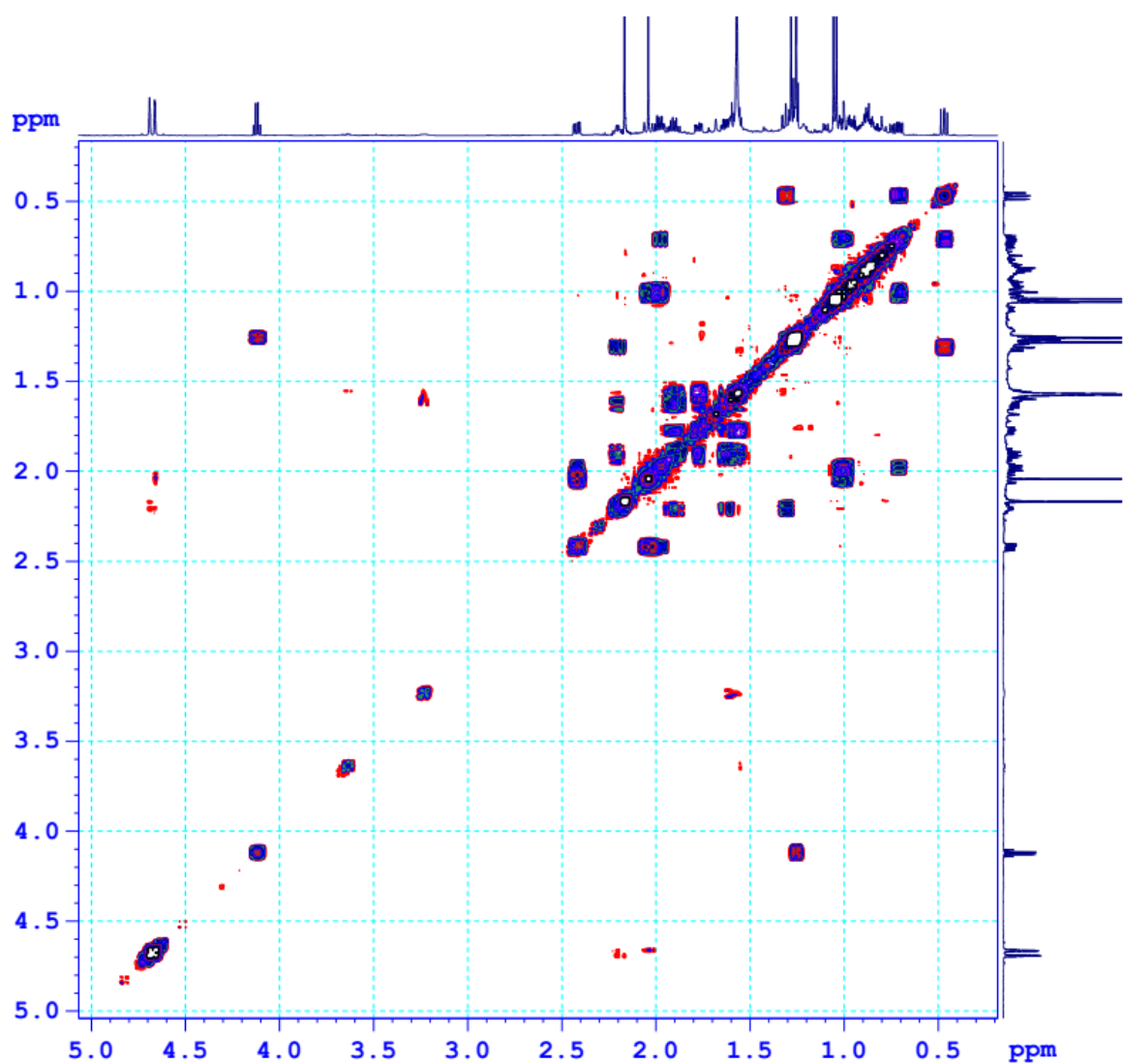
**Figure S2.**  $^{13}\text{C}$ -NMR spectrum of compound **1**



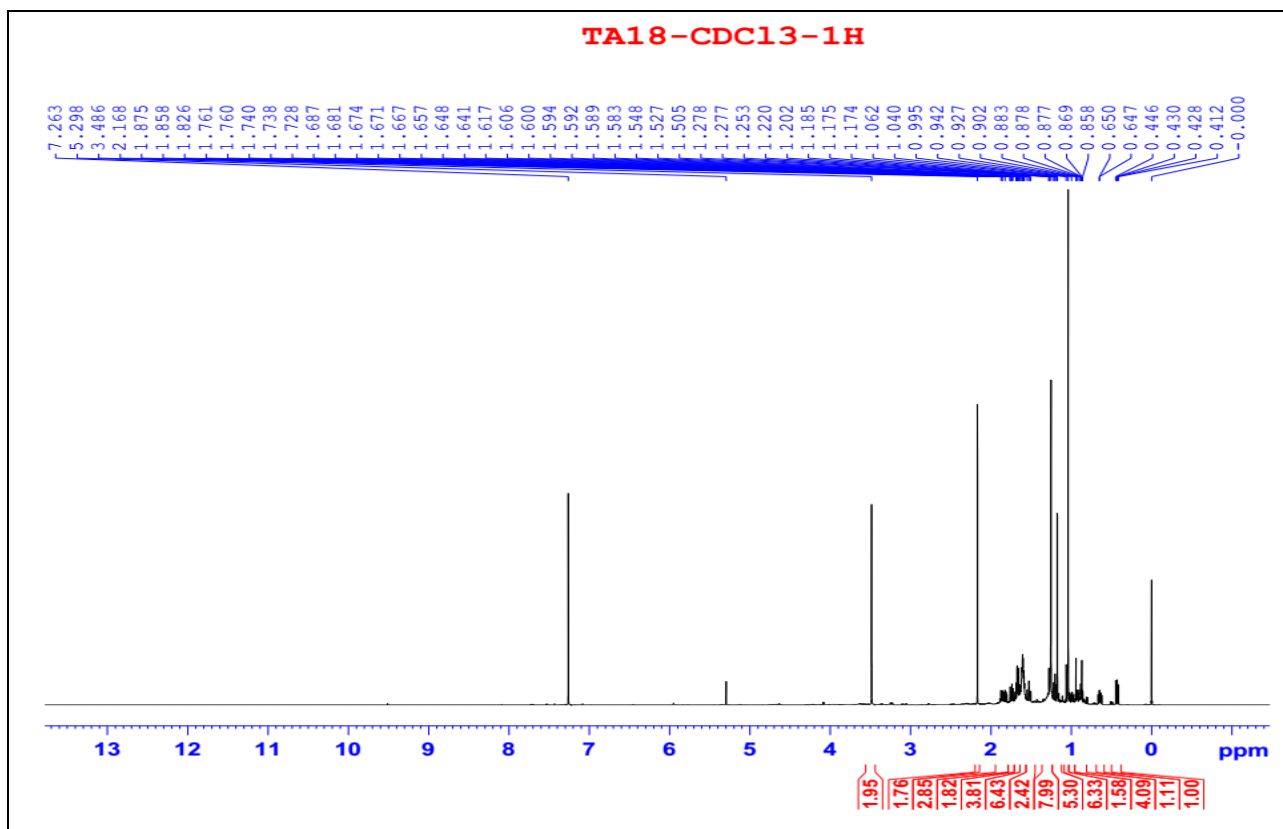
**Figure S3.** HSQC spectrum of compound **1**



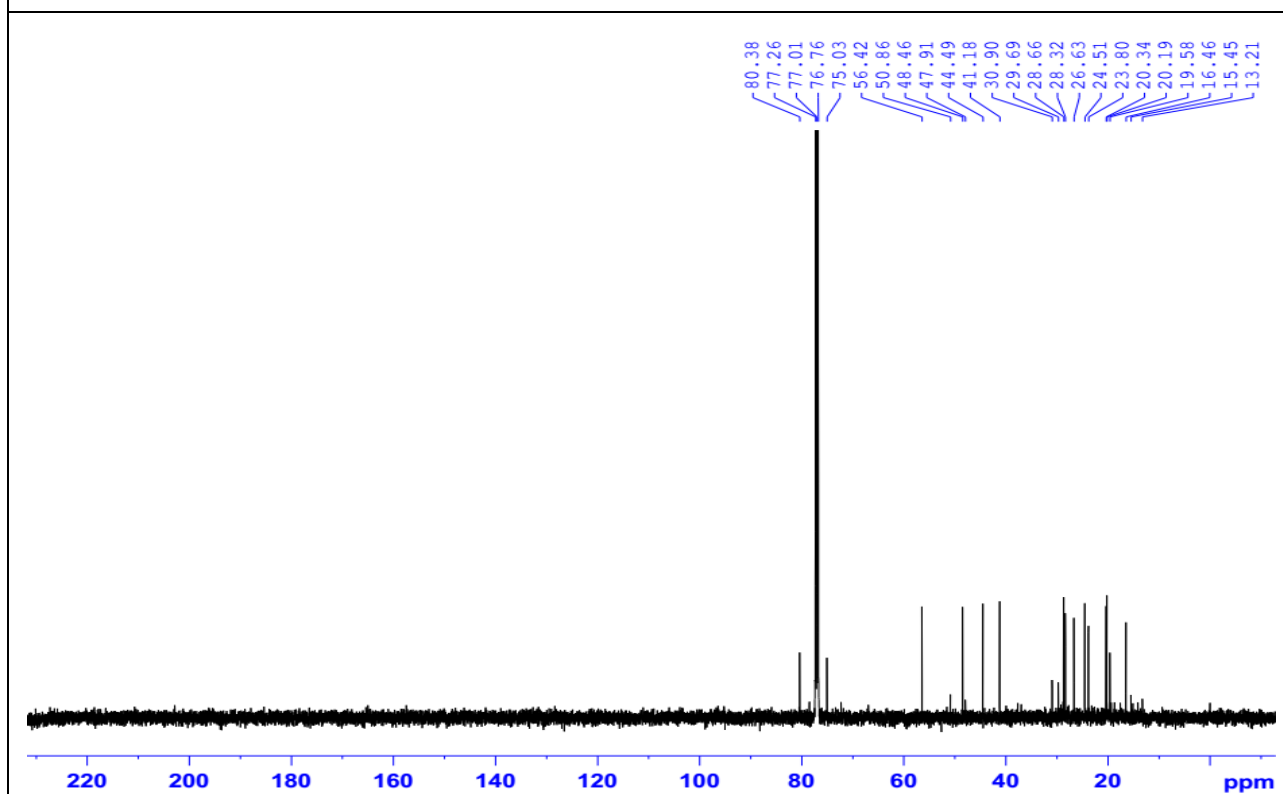
*TA2-CDC13-COSYGP*



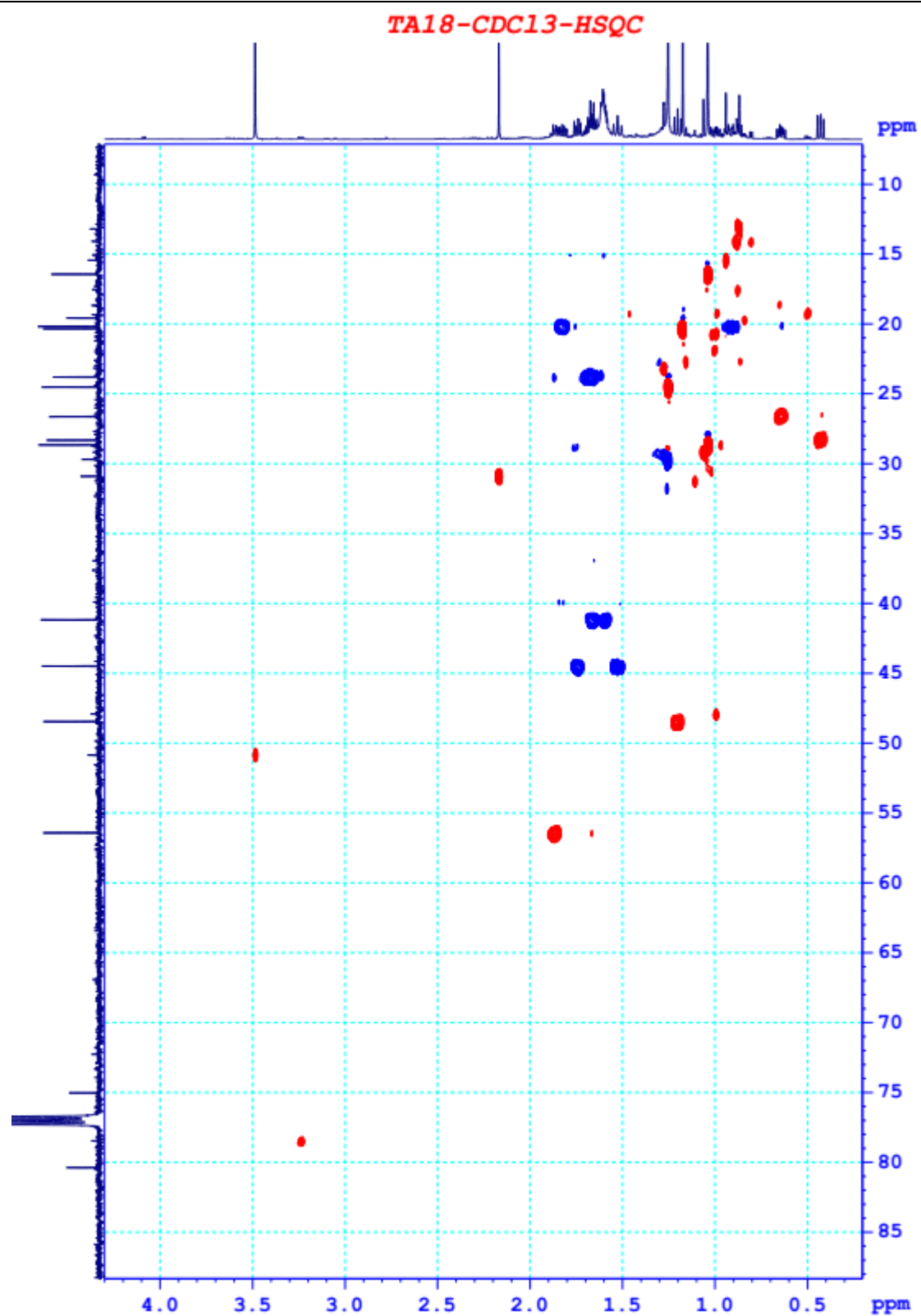
**Figure S5.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 1



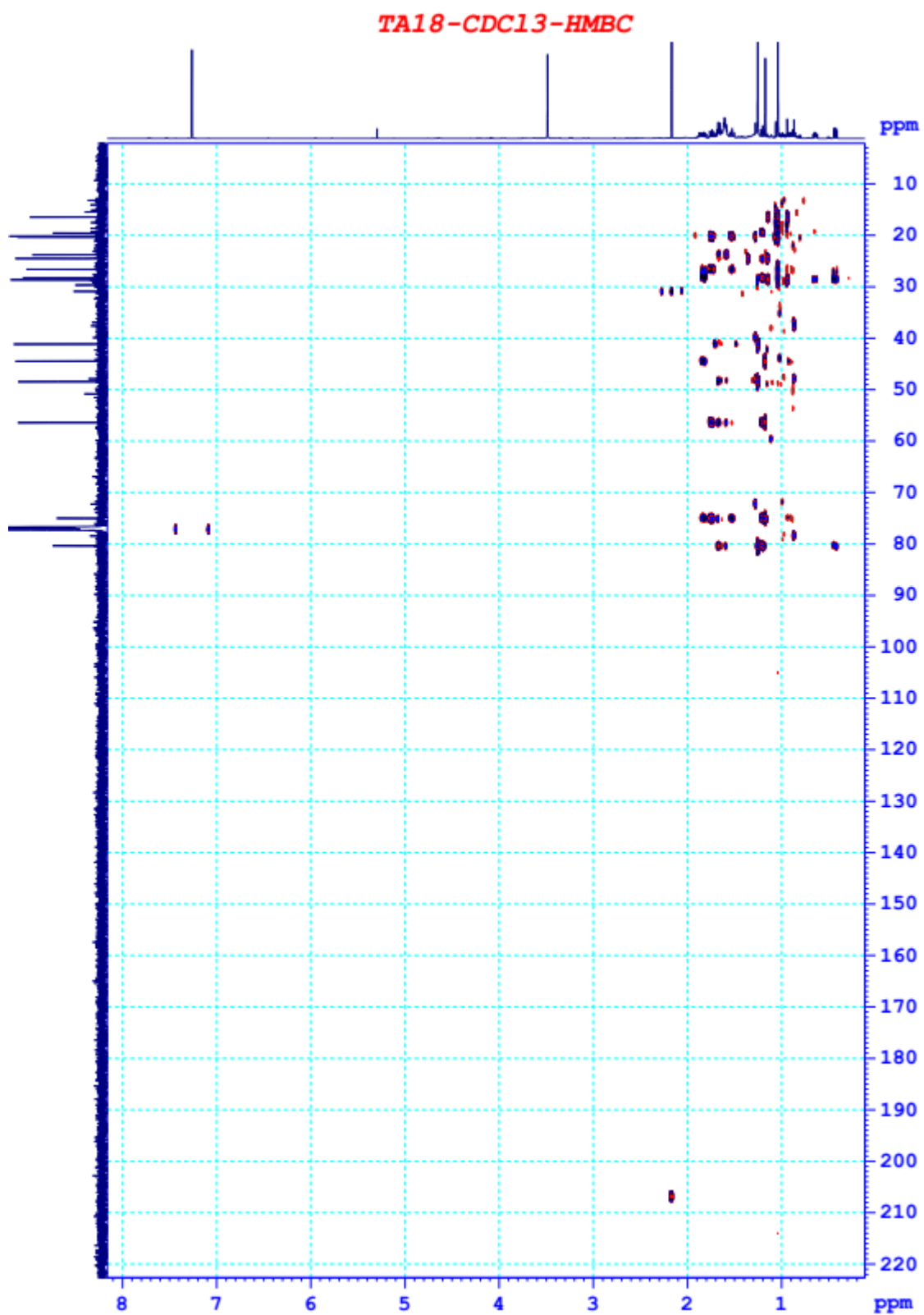
**Figure S6.**  $^1\text{H}$ -NMR spectrum of compound **2**



**Figure S7.**  $^{13}\text{C}$ -NMR spectrum of compound **2**



**Figure S8.** HSQC spectrum of compound **2**



**Figure S9.** HMBC spectrum of compound **2**

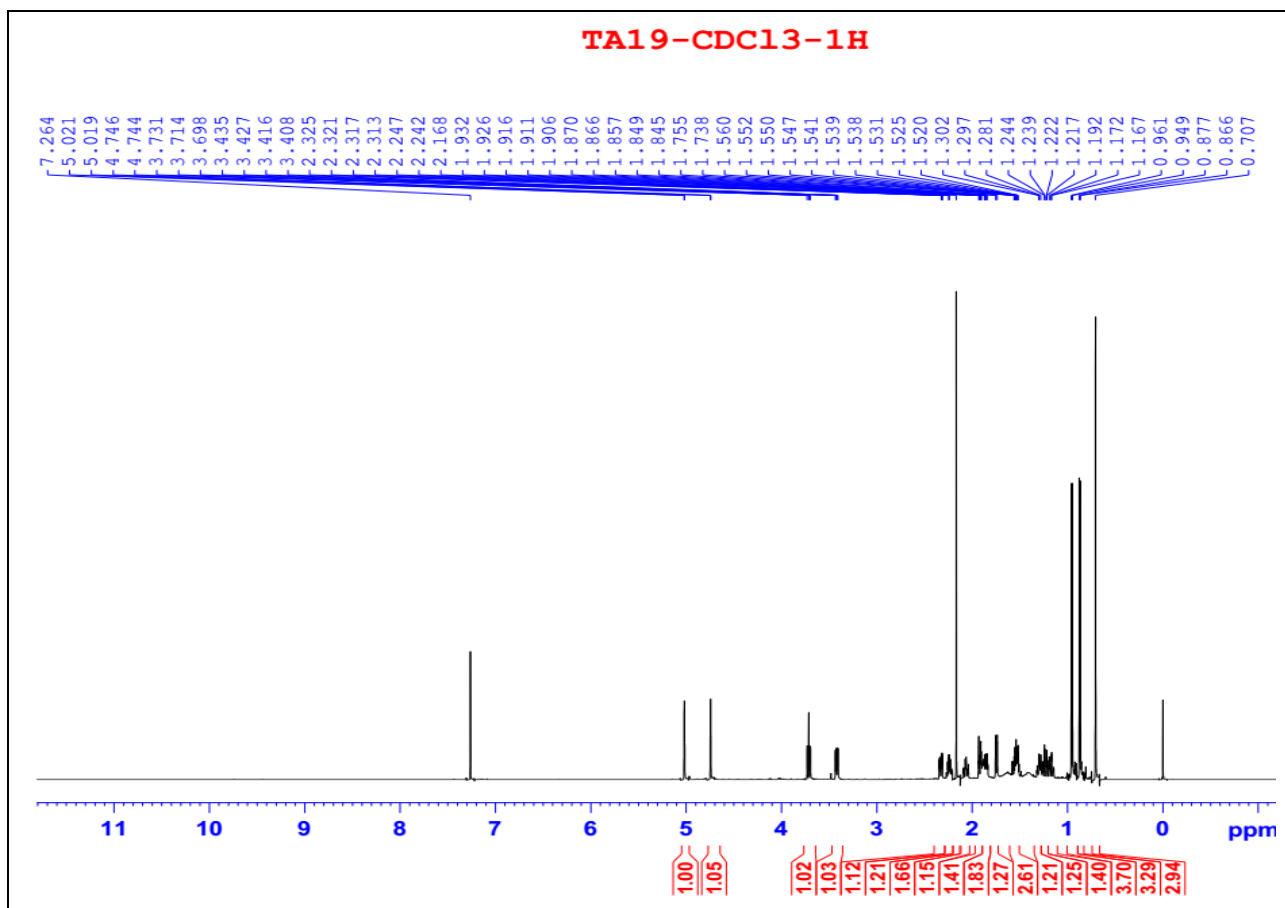


Figure S10.  $^1\text{H}$ -NMR spectrum of compound **3**

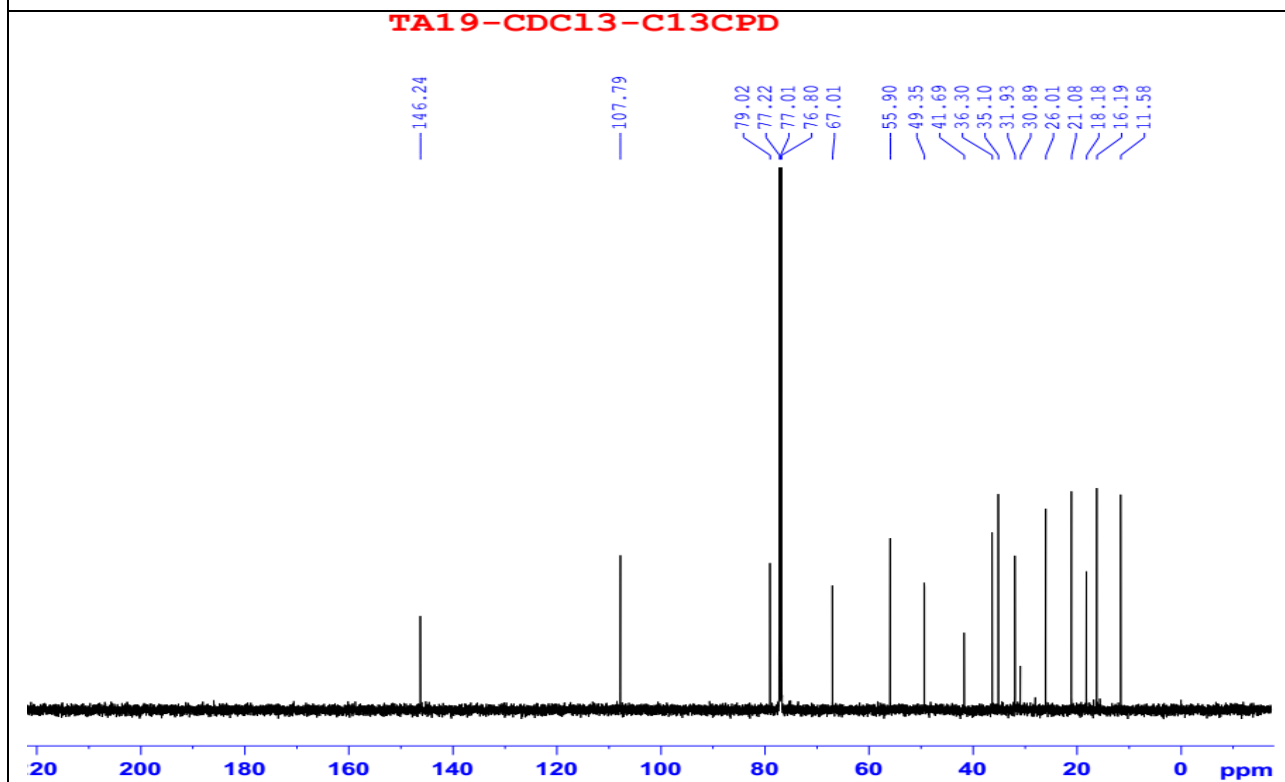
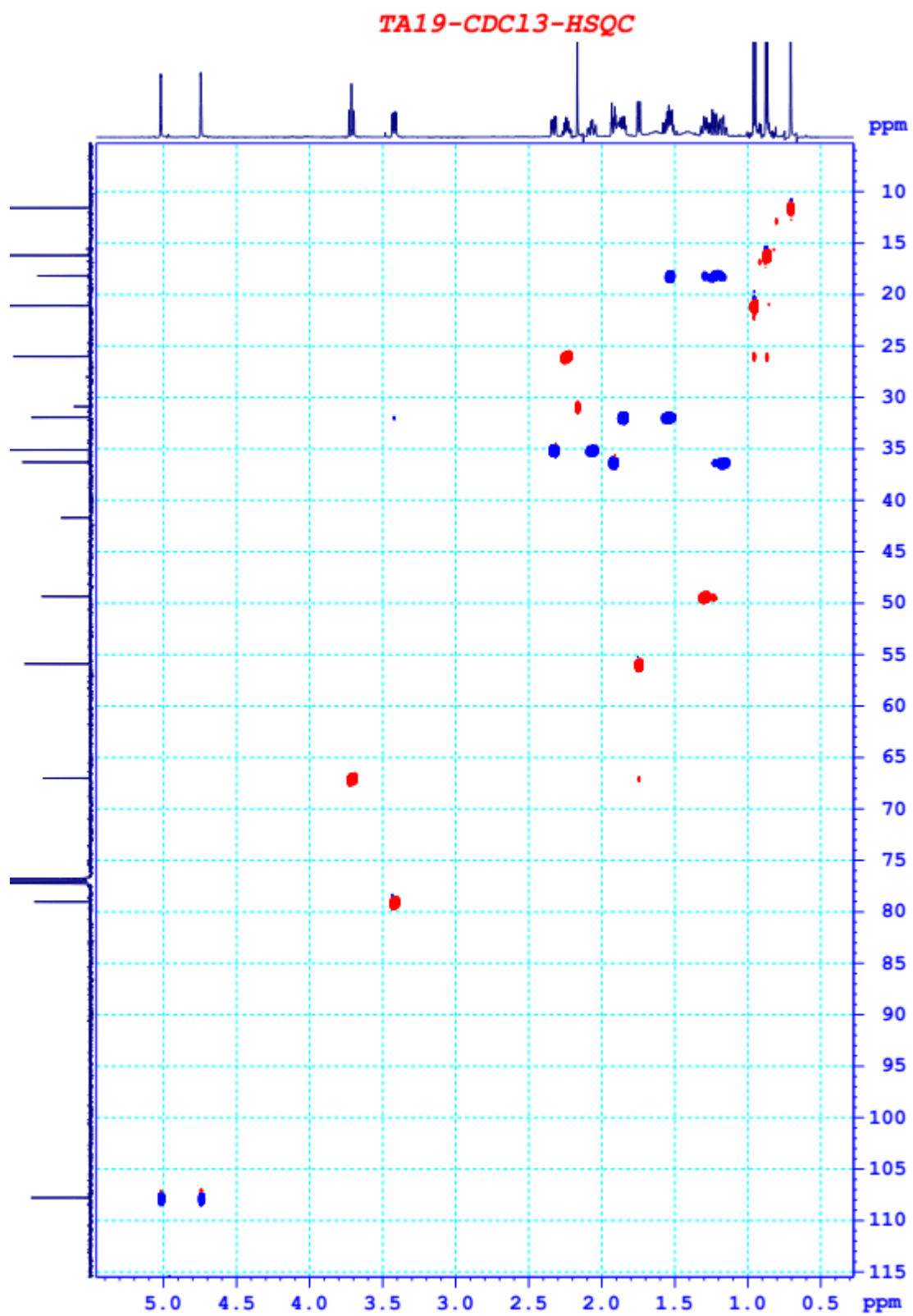
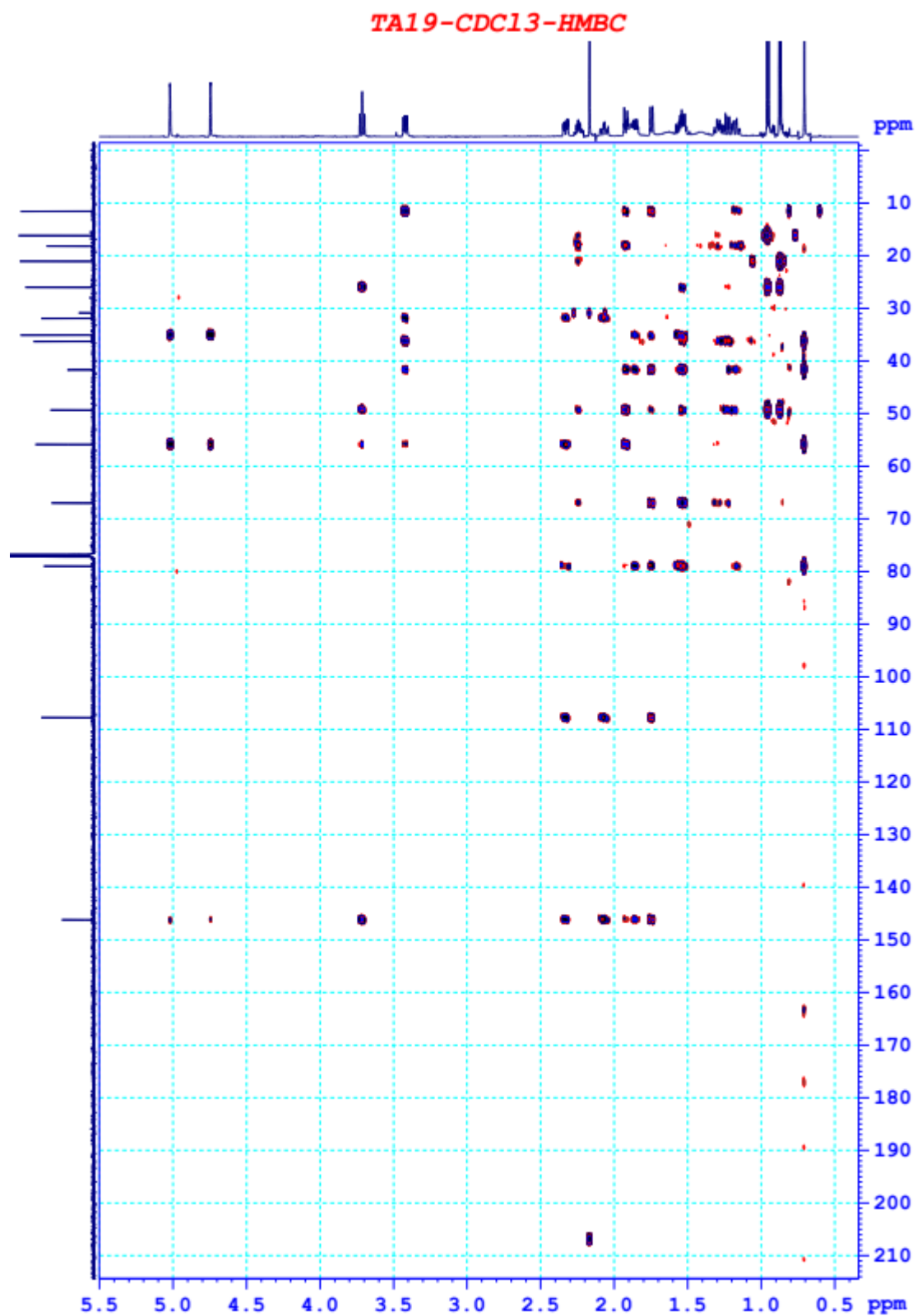


Figure S11.  $^{13}\text{C}$ -NMR spectrum of compound **3**



**Figure S12.** HSQC spectrum of compound **3**



**Figure S13.** HMBC spectrum of compound **2**



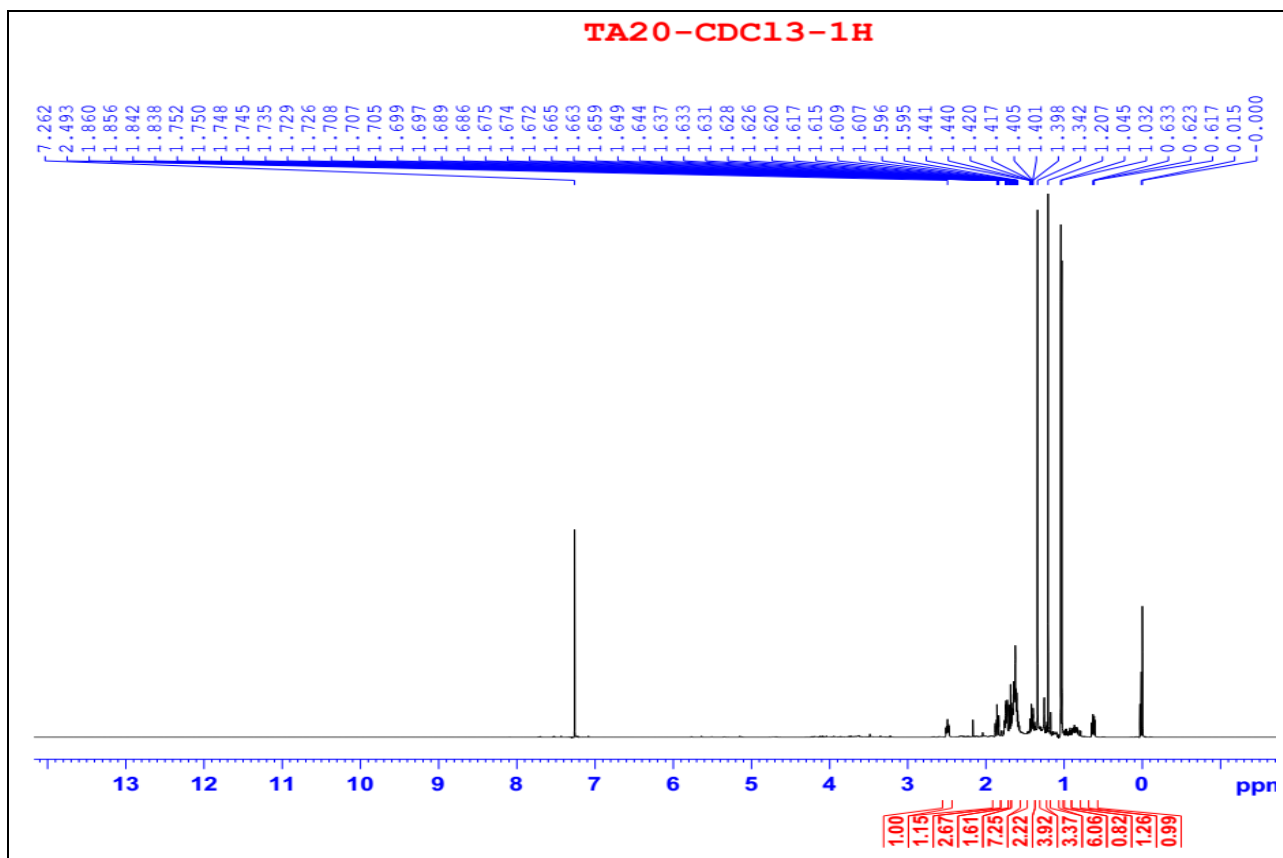


Figure S15.  $^1\text{H}$ -NMR spectrum of compound 4

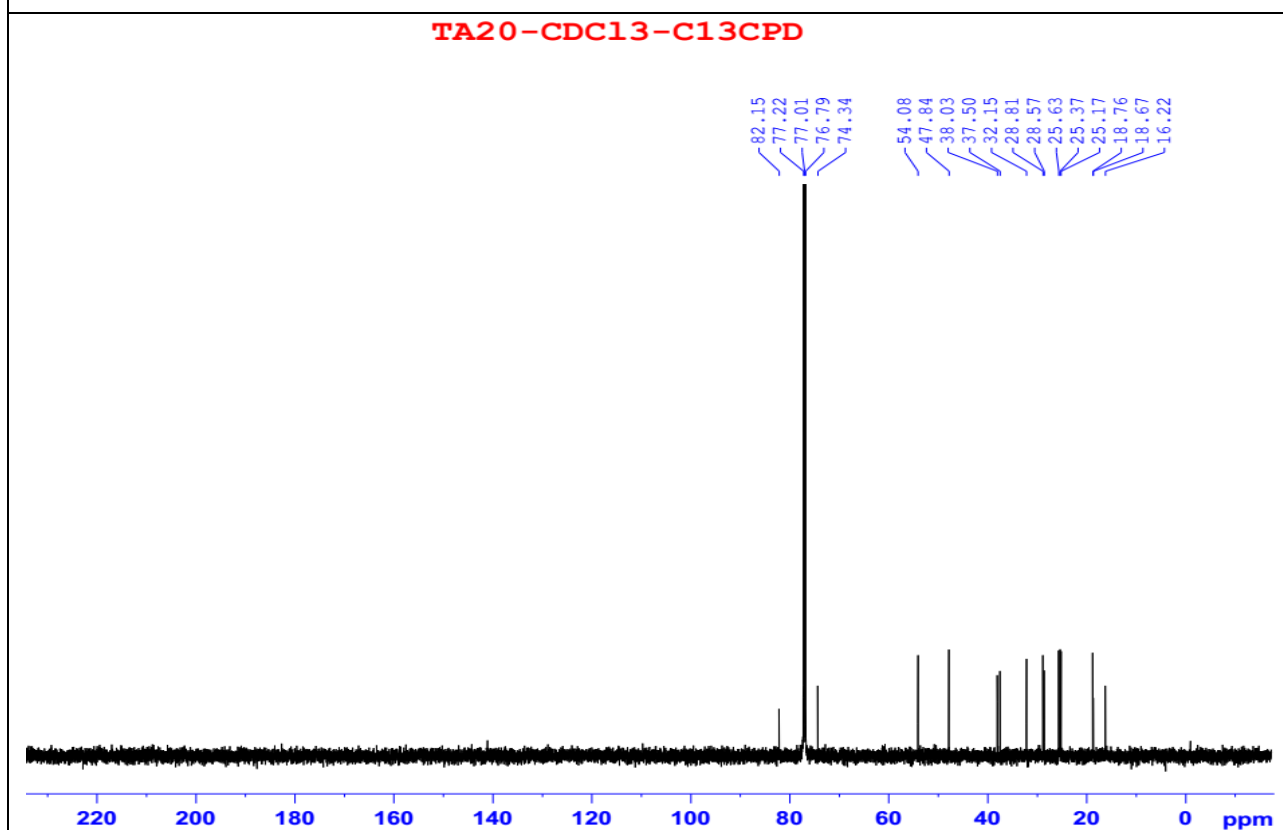
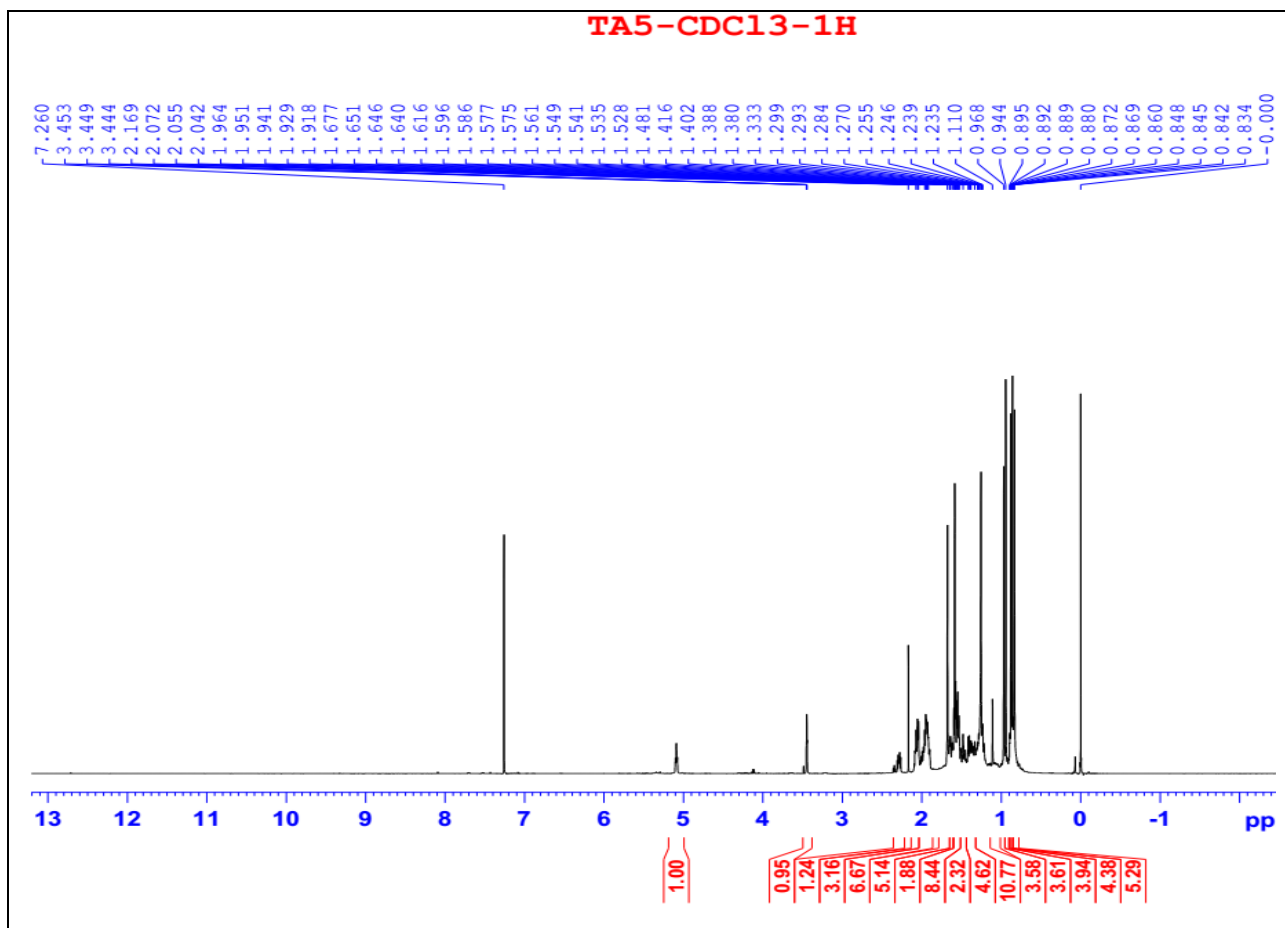
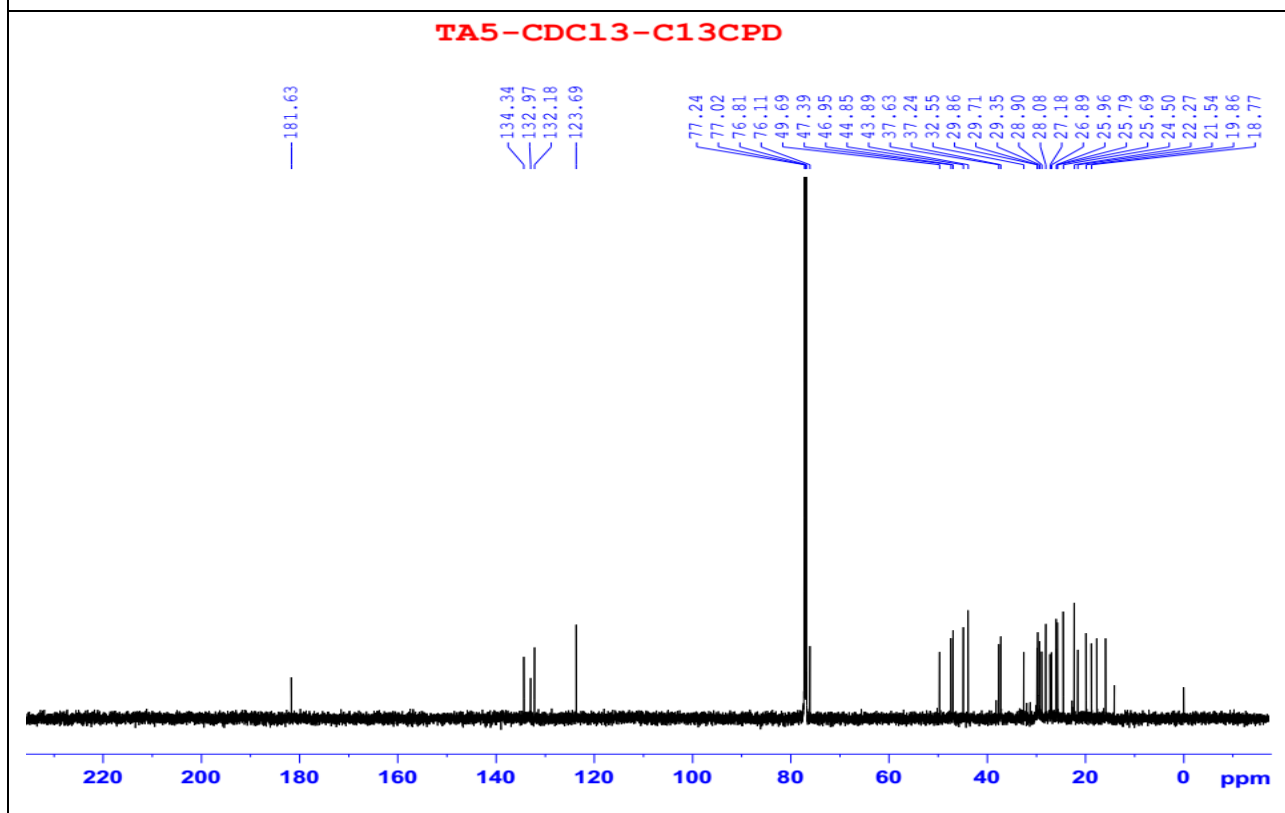


Figure S16.  $^{13}\text{C}$ -NMR spectrum of compound 4





**Figure S19.**  $^1\text{H}$ -NMR spectrum of compound **6**



**Figure S20.**  $^{13}\text{C}$ -NMR spectrum of compound **6**

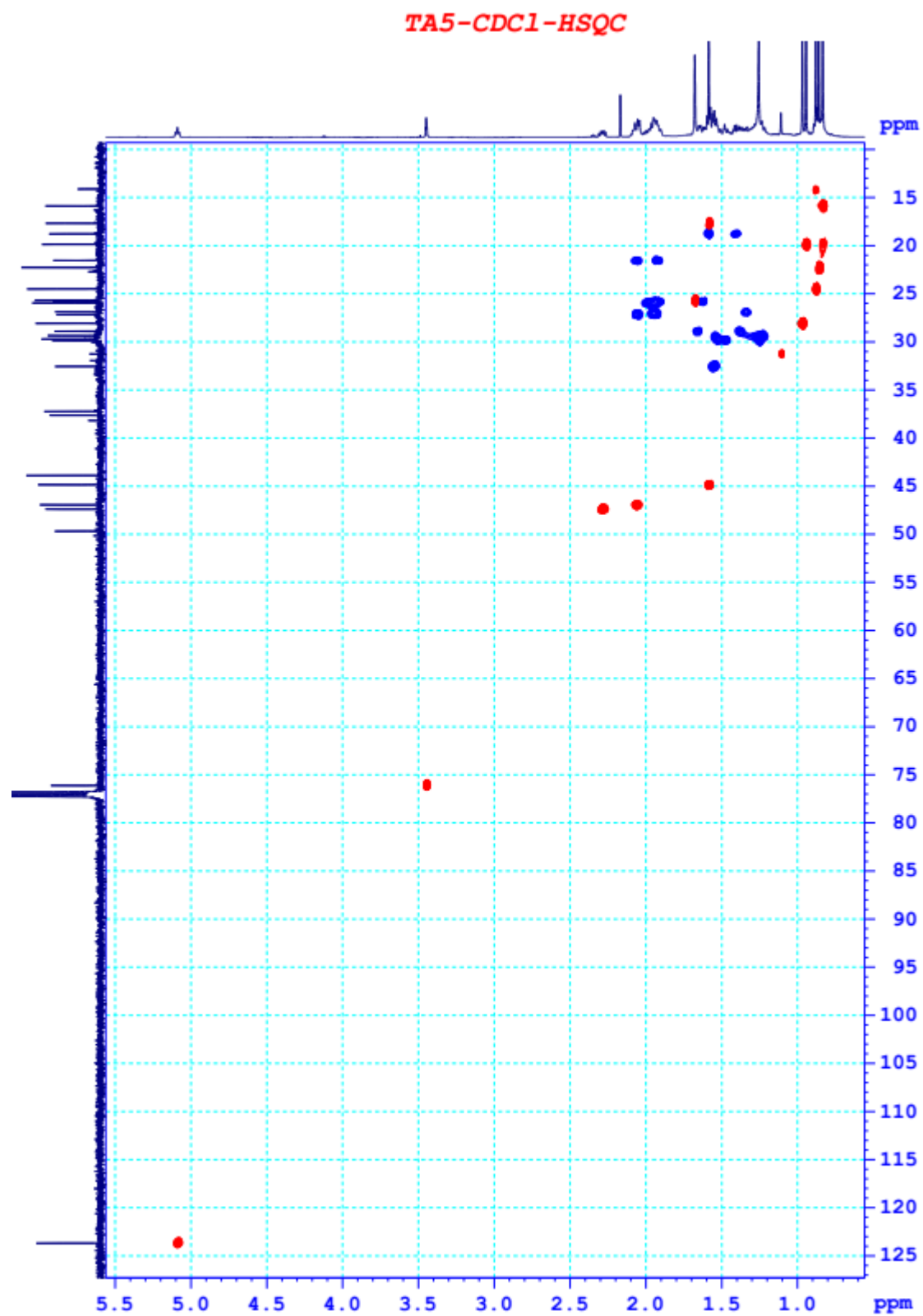
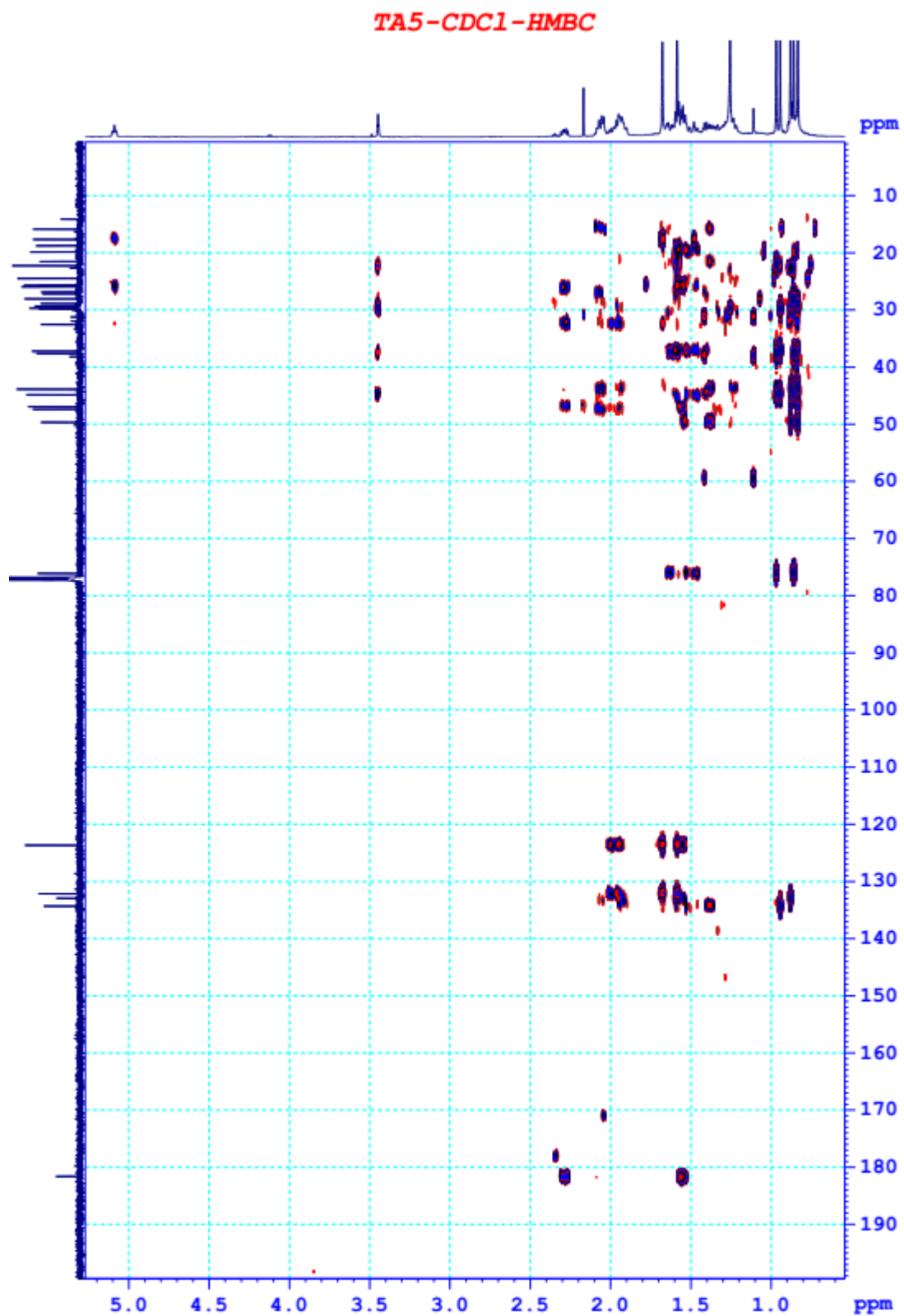


Figure S21. HSQC spectrum of compound **6**



**Figure S22.** HMBC spectrum of compound 6

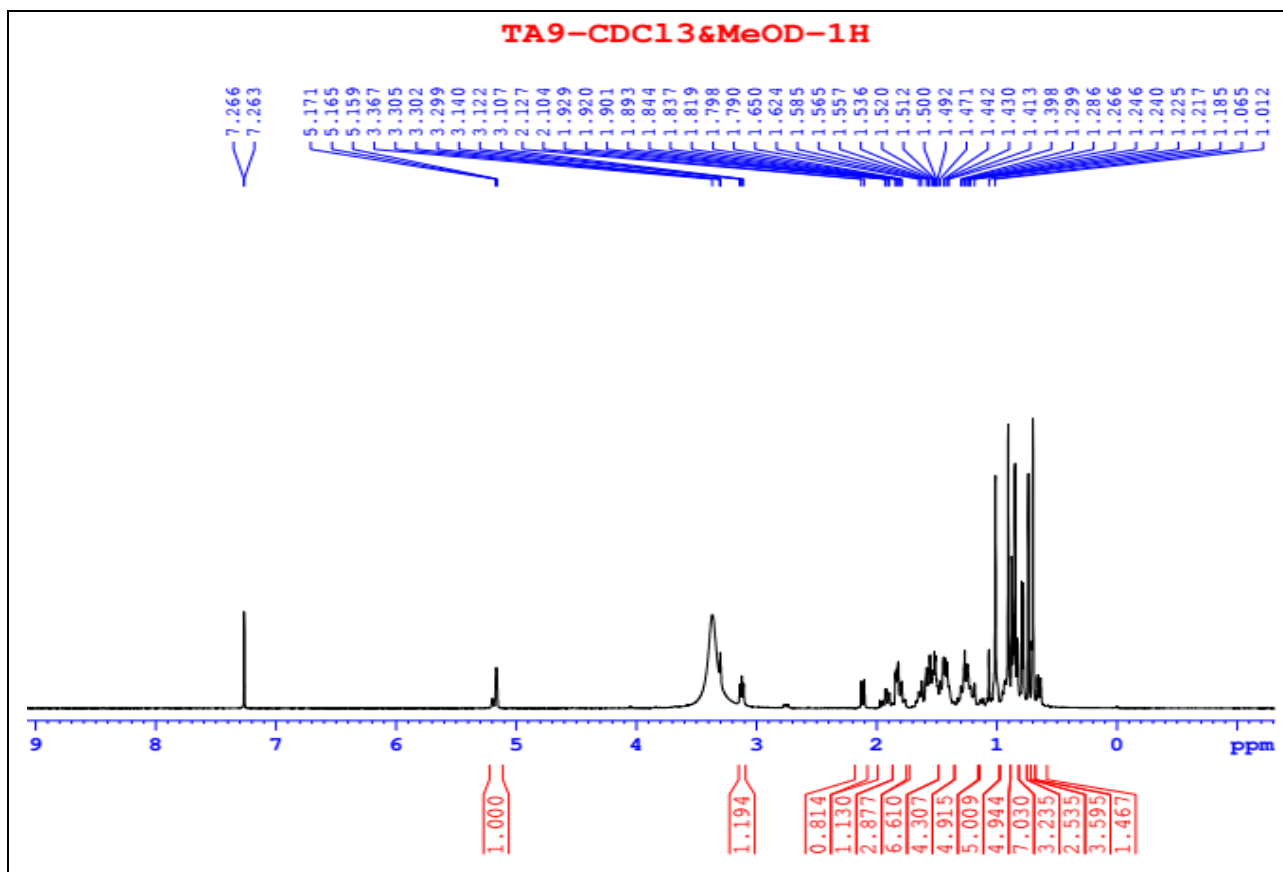


Figure S23.  $^1\text{H}$ -NMR spectrum of compound 7

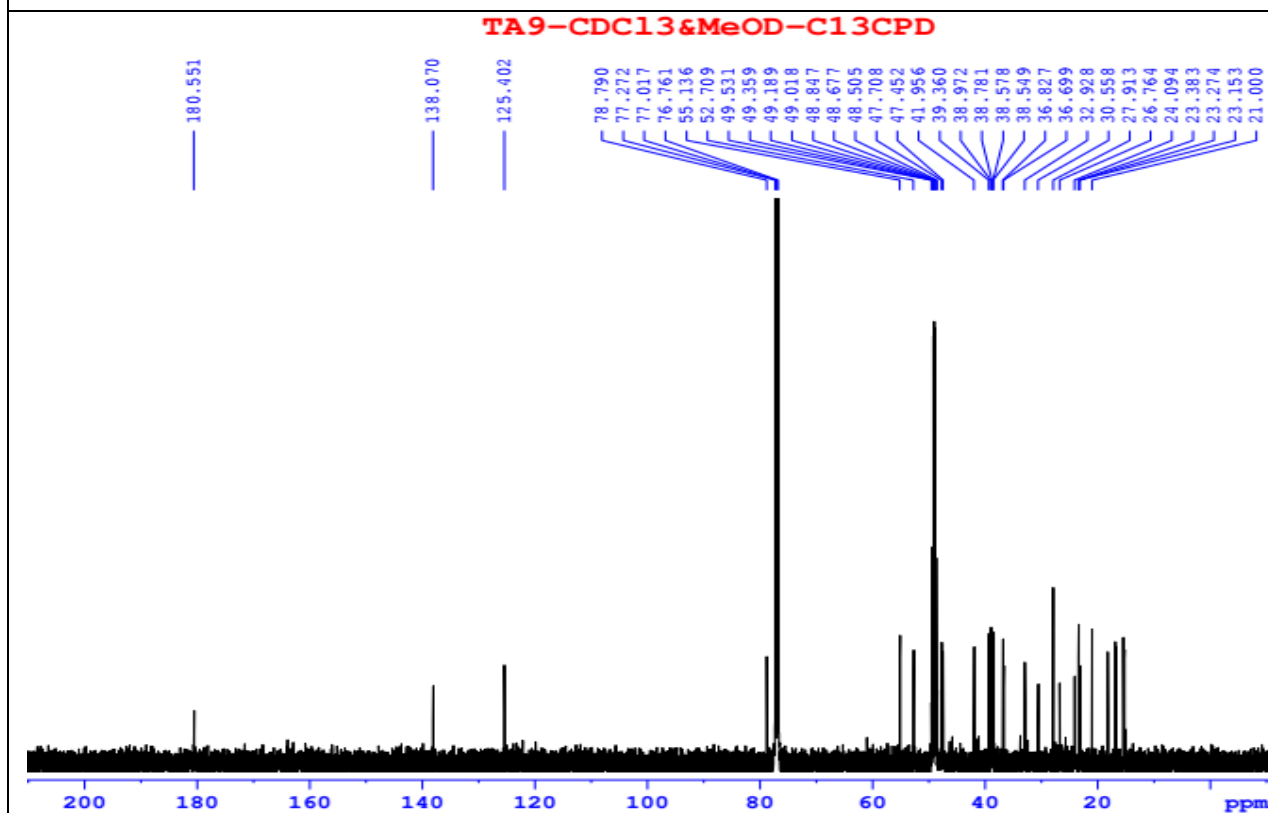


Figure S24.  $^{13}\text{C}$ -NMR spectrum of compound 7

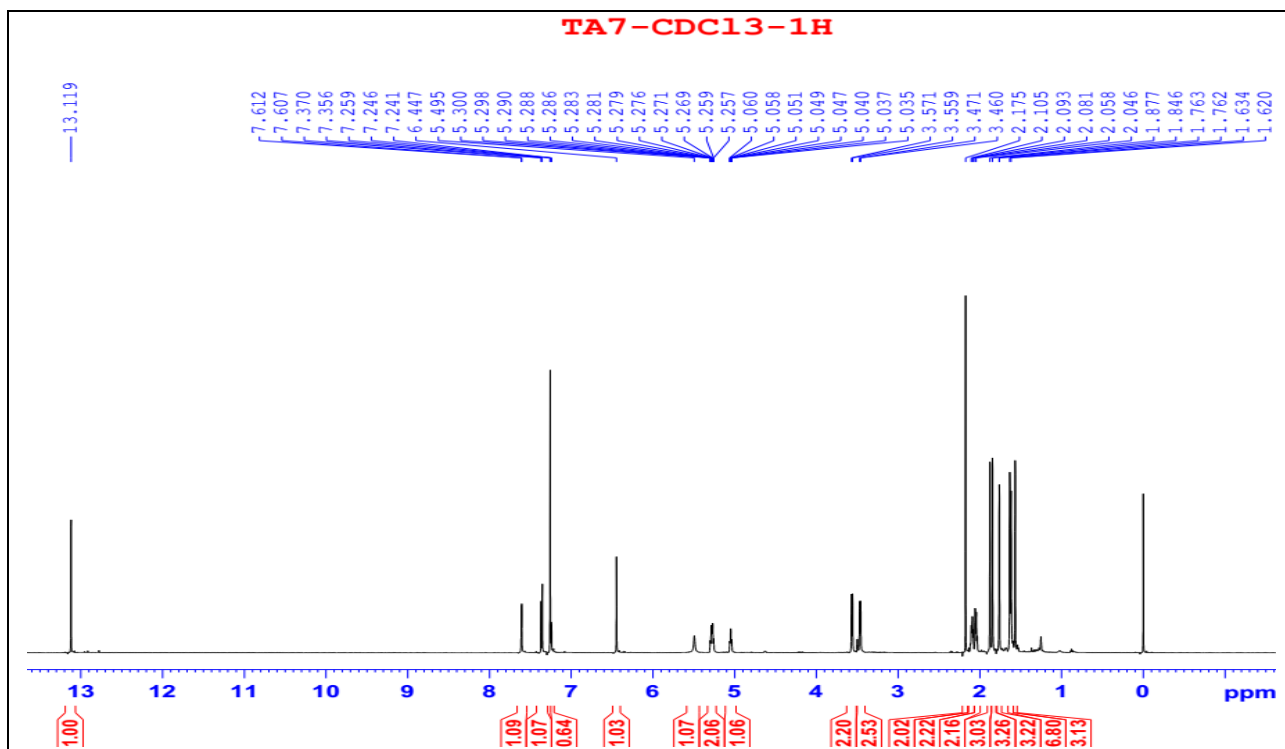


Figure S25.  $^1\text{H}$ -NMR spectrum of compound **8**

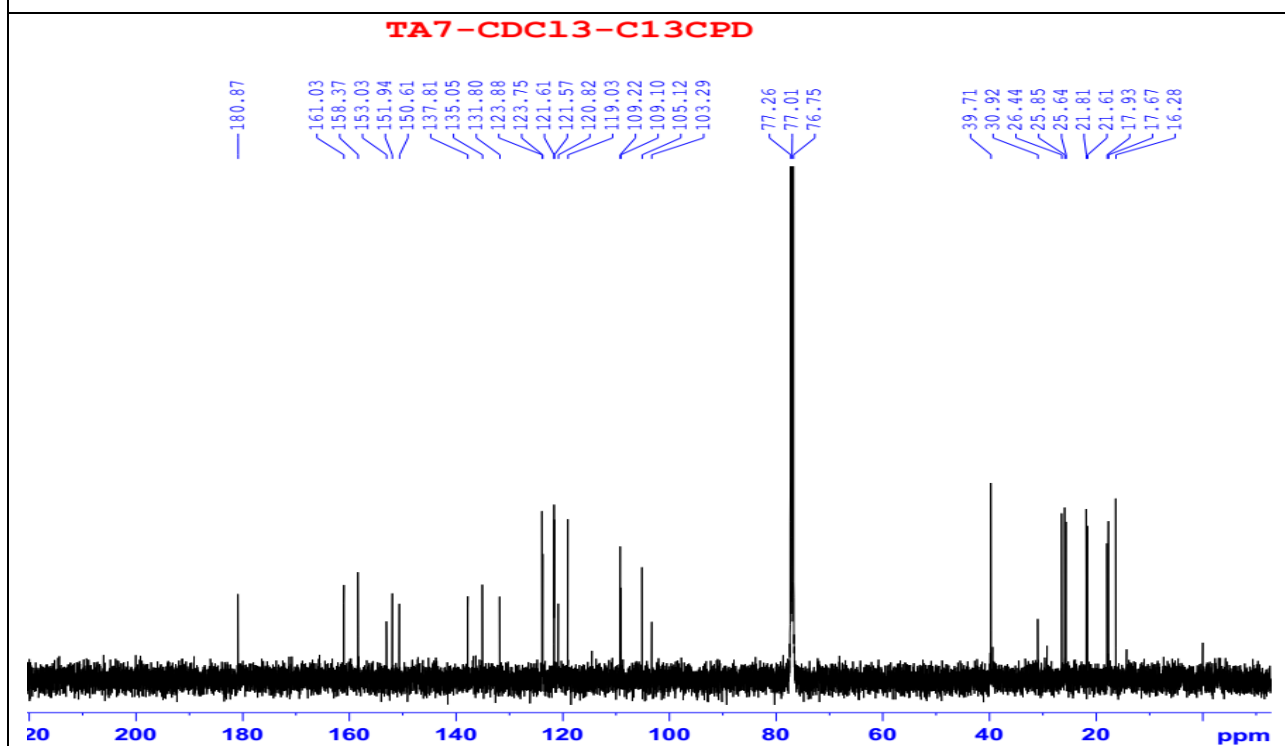
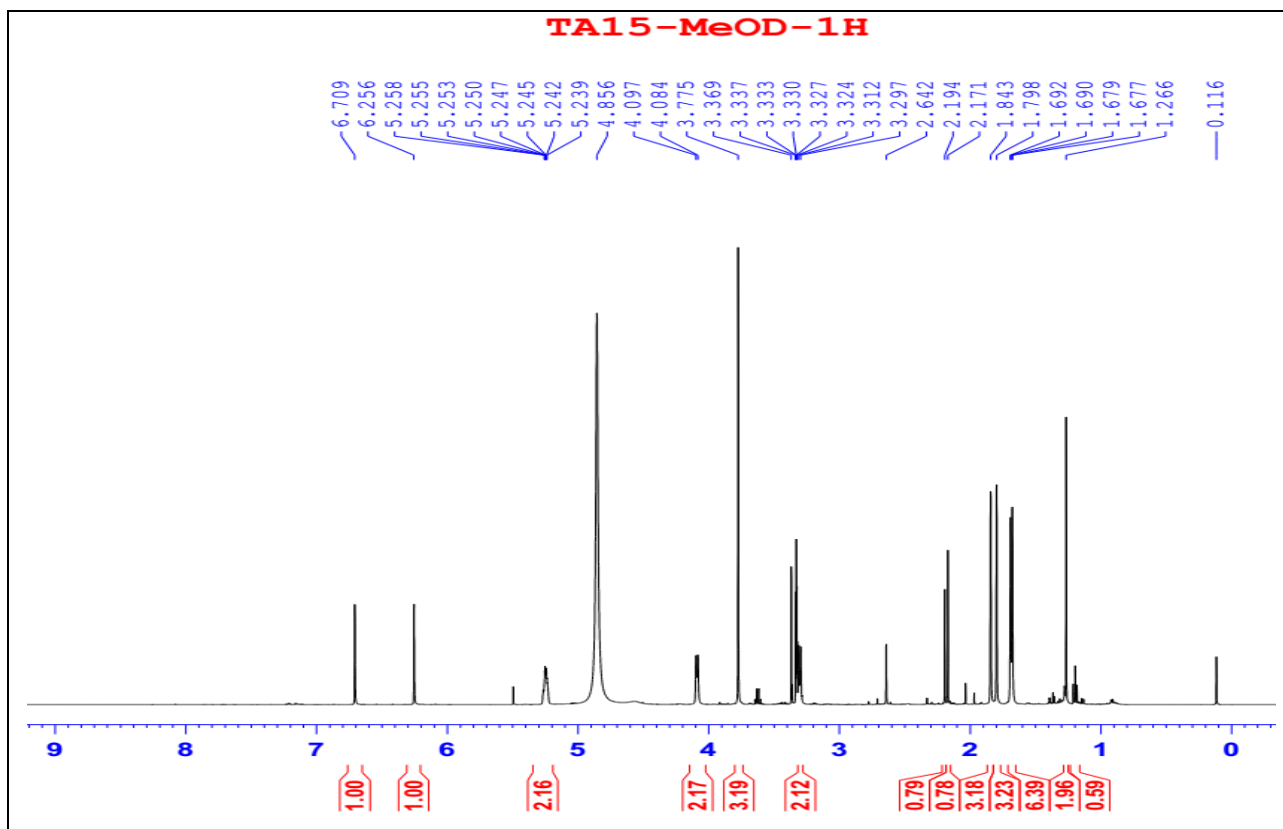
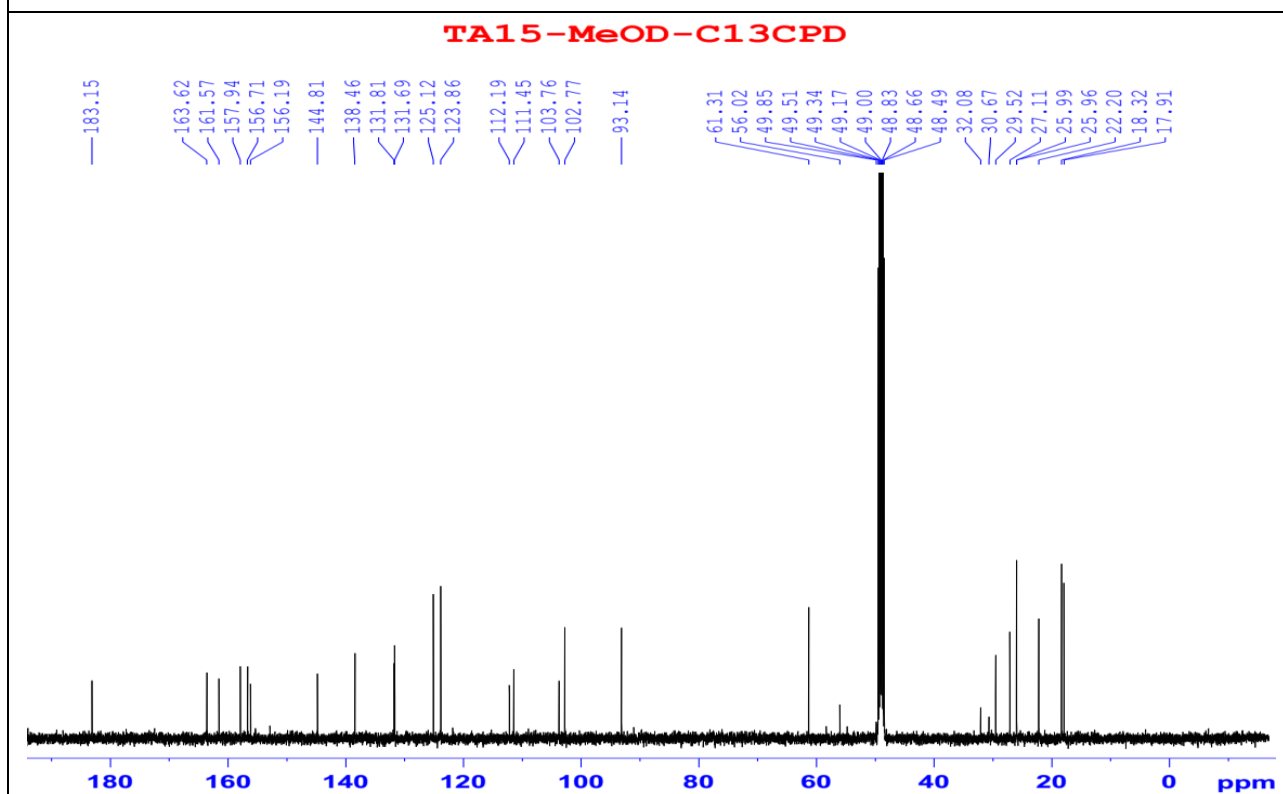


Figure S26.  $^{13}\text{C}$ -NMR spectrum of compound **8**



**Figure S27.**  $^1\text{H}$ -NMR spectrum of compound **9**



**Figure S28.**  $^{13}\text{C}$ -NMR spectrum of compound **9**