

# Supplementary materials

Communication

## Bioassay-Guided Fractionation with Antimalarial and Antimicrobial Activities of *Paeonia officinalis*

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**Abstract:** Bioassay-guided fractionation technique of roots of *Paeonia officinalis* led to isolation and structure elucidation of seven known compounds, including four monoterpene glycosides: lactiflorin (1), paeoniflorin (4), galloyl paeoniflorin (5), and (Z)-(1S,5R)- $\beta$ -pinen-10-yl  $\beta$ -vicianoside (7), two phenolics; benzoic acid (2) and methyl gallate (3), and one sterol glycoside;  $\beta$ -sitosterol 3-O- $\beta$ -D-glucopyranoside (6). The different fractions and the isolated compounds were evaluated for their antimicrobial and antimalarial activities. Fraction II and III showed antifungal activity against *Candida neoformans* with IC<sub>50</sub> value of 28.11 and 74.37  $\mu$ g/mL, respectively, comparing with the standard fluconazole (IC<sub>50</sub> = 4.68  $\mu$ g/mL), and antibacterial potential against *Pseudomonas aeruginosa* (IC<sub>50</sub> = 20.27 and 24.82  $\mu$ g/mL, respectively), *Klebsiella pneumoniae* (IC<sub>50</sub> = 43.21 and 94.4  $\mu$ g/mL, respectively), comparing with the standard meropenem (IC<sub>50</sub> = 28.67 and 43.94  $\mu$ g/mL, respectively). Compounds 3 and 5 showed antimalarial activity against *Plasmodium falciparum* D6 with IC<sub>50</sub> value of 1.57 and 4.72  $\mu$ g/mL and *P. falciparum* W2 with IC<sub>50</sub> value of 0.61 and 2.91  $\mu$ g/mL, respectively, comparing with the standard chloroquine (IC<sub>50</sub> = 0.026 and 0.14  $\mu$ g/mL, respectively).

**Keywords:** *Paeonia officinalis*; phytoconstituents; antimalarial; antimicrobial.

**Lactiflorin (1):**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 8.03 (2H, d,  $J=7.2$  Hz, H-2',H-6'), 7.66 (1H, t,  $J=7.5$  Hz, H-4'), 7.54 (2H, t,  $J=7.5$  Hz, H-3',H-5), 4.92 (1H, d,  $J=4.5$  Hz, H-9), 4.80 (2H, s, H-8), 4.64 (1H, d,  $J=8.0$  Hz, H-1''), 3.87 (2H, br d,  $J=11.4$  Hz, H-6''), 3.68 (1H, m, H-5''), 3.53 (1H, td,  $J=4.45, 7.4, 9.3$  Hz, H-3''), 3.37 (1H, td,  $J=4.35, 7.4, 9.6$  Hz, H-4''), 3.23 (1H, dd,  $J=8.1, 9.3$  Hz, H-2''), 2.82 (1H, d,  $J=17.8$  Hz, H-2b), 2.78 (1H, dt,  $J=4.5, 4.5, 13.4$  Hz, H-4), 2.55 (1H, t,  $J=13.4$  Hz, H-5b), 2.51 (1H, d,  $J=17.8$  Hz, H-2a), 2.19 (1H, dd,  $J=4.7, 14.0$  Hz, 5H-a), 1.41 (3H, s, Me-10).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ): 48.2 (C-1), 86.7 (C-2), 38.7 (C-3), 81.7 (C-4), 31.6 (C-5), 104.1 (C-6), 56.8 (C-7), 63.8 (C-8), 219.3 (C-3), 16.3 (C-10), glucopyranosyl moiety; 96.4 (C-1'), 74.6 (C-2'), 80.0 (C-3'), 71.5 (C-4'), 76.0 (C-5'), 62.2 (C-6'), benzoyl moiety; 130.7 (C-1''), 130.6 (C-2'',6''), 129.9 (C-3'',5''), 134.9 (C-4''), 167.8 (C-7''). HR-ESI-MS  $m/z$  485.1431  $[\text{M} + \text{Na}]^+$  calc. 485.1424 for  $\text{C}_{23}\text{H}_{26}\text{O}_{10}\text{Na}$ .

**Benzoic acid (2)**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 8.04 (2H, d,  $J=7.5$  Hz, H-2,H-6), 7.58 (1H, t,  $J=6.9$  Hz, H-4), 7.46 (2H, t,  $J=7.5$  Hz, H-3,H-5).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ) 131.8 (C-1), 130.6 (C-2, C-6), 129.4 (C-3,C-5), 134.0 (C-4). HR-ESI-MS  $m/z$ : 121.0282  $[\text{M}-\text{H}]^-$  calc. 121.0290 for  $\text{C}_7\text{H}_5\text{O}_2$ .

**Methyl gallate (3)**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 7.08 (2H, s, H-2,H-6), 3.82 (3H, s,  $\text{OCH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ) 121.3 (C-1), 110.2 (C-2, C-6), 146.2 (C-3,C-5), 139.7 (C-4), 169.2 (C=O), 52.5 ( $\text{OCH}_3$ ). HR-ESI-MS  $m/z$ : 183.0361  $[\text{M}-\text{H}]^-$  calc. 183.0293 for  $\text{C}_8\text{H}_7\text{O}_5$ .

**Paeoniflorin (4):**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ): 1.73 (1H, d,  $J=11.8$  Hz, H-3a), 1.92 (1H, d,  $J=11.8$  Hz, H-3b), 2.55 (1H, d,  $J=6.5$  Hz, H-5), 1.78 (1H, d,  $J=10.6$  Hz, H-7a), 2.46 (1H, dd,  $J=10.6, 6.8$  Hz, H-7b), 5.43 (1H, s, H-9), 1.27 (3H, s, H-10), 4.58 (1H, d,  $J=7.8$  Hz, H-1'), 8.07 (2H, d,  $J=7.2$  Hz, H-2'',H-6''), 7.50 (2H, t,  $J=7.6$  Hz, H-3'',H-5''), 7.63 (1H, t,  $J=7.4$  Hz, H-4'').  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ): 89.4 (C-1), 87.4 (C-2), 44.6 (C-3), 106.5 (C-4), 44.0 (C-5), 71.8 (C-6), 23.5 (C-7), 61.8 (C-8), 102.4 (C-9), 19.7 (C-10), glucopyranosyl moiety; 100.2 (C-1'), 75.1 (C-2'), 78.0 (C-3'), 72.3 (C-4'), 78.1 (C-5'), 62.9 (C-6'), benzoyl moiety; 131.2 (C-1''), 130.8 (C-2'', C-6''), 129.8 (C-3'', C-5''), 134.6 (C-4''), 168.2 (C-7''). HR-ESI-MS  $m/z$ : 503.1516  $[\text{M}+\text{Na}]^+$  calc. 503.1529 for  $\text{C}_{23}\text{H}_{28}\text{O}_{11}\text{Na}$ .

**Galloyl paeoniflorin (5):**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) 1.83 (1H, d,  $J=12.5$  Hz, H-3a), 2.20 (1H, d,  $J=12.5$  Hz, H-3b), 2.60 (1H, d,  $J=6.2$  Hz, H-5), 1.96 (1H, d,  $J=10.8$  Hz, H-7a), 2.51 (1H, dd,  $J=10.8, 6.8$  Hz, H-7b), 5.45 (1H, s, H-9), 1.39 (3H, s, H-10), 4.54 (1H, d,  $J=7.6$  Hz, H-1'), 8.01 (2H, d,  $J=7.2$  Hz, H-2'',H-6''), 7.46 (2H, t,  $J=7.6$  Hz, H-3'',H-5''), 7.59 (1H, t,  $J=7.4$  Hz, H-4''), 7.12 (2H, s, H-2''',6''').  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ): 89.3 (C-1), 87.3 (C-2), 44.4 (C-3), 106.4 (C-4), 43.8 (C-5), 71.9 (C-6), 23.0 (C-7), 61.7 (C-8), 102.2 (C-9), 19.6 (C-10), glucopyranosyl moiety; 100.0 (C-1'), 74.9 (C-2'), 77.8 (C-3'), 72.1 (C-4'), 75.1 (C-5'), 64.7 (C-6'), benzoyl moiety; 131.0 (C-1''), 130.7 (C-2'', C-6''), 129.7 (C-3'', C-5''), 134.5 (C-4''), 168.2 (C-7''), galloyl moiety; 121.5 (C-1'''), 110.3 (C-2''', C-6'''), 146.6 (C-3''', C-5'''), 139.9 (C-4'''), 168.2 (C-7'''). HR-ESI-MS  $m/z$ : 655.1666  $[\text{M}+\text{Na}]^+$  calc. 655.1639 for  $\text{C}_{30}\text{H}_{32}\text{O}_{15}\text{Na}$ .

**$\beta$ -sitosterol 3-O- $\beta$ -D-glucopyranoside (6)**  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ ): 4.25 (1H, m, H-3), 5.33 (1H, br s, H-6), 0.65 (3H, s, H-18), 0.92 (3H, s, H-19), 0.97 (3H, d,  $J=6.4$  Hz, H-21), 0.88 (3H, d,  $J=7.6$  Hz, H-26), 0.86 (3H, d,  $J=7.0$  Hz, H-27), 5.01 (1H, d,  $J=7.7$  Hz, H-1').  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ ): 37.3 (C-1), 30.0 (C-2), 78.4 (C-3), 39.1 (C-4), 140.7 (C-5), 121.7 (C-6), 32 (C-7), 31.9 (C-8), 50.1 (C-9), 36.7 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.6 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 11.8 (C-18), 19.0 (C-19), 36.2 (C-20), 18.8 (C-21), 34.0 (C-22), 26.2 (C-23), 45.8 (C-24), 29.3 (C-25), 19.2 (C-26), 19.8 (C-27), 23.2 (C-28), 12.0 (C-29), glucopyranosyl moiety; 102.4 (C-1'), 75.1 (C-2'), 78.2 (C-3'), 71.4 (C-4'), 77.9 (C-5'), 62.6 (C-6').

**(Z)-(1S,5R)- $\beta$ -pinen-10-yl  $\beta$ -vicianoside (7):**  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) 3.05 (1H, t,  $J=5.5$  Hz, H-1), 1.45 (2H, m, H-3), 1.27 (2H, m, H-4), 1.94 (1H, m, H-5), 2.31 (2H, m, H-7), 1.27 (3H, s, H-8), 0.78 (3H, s, H-9), 6.21 (1H, s, H-10), 4.37 (1H, d,  $J=7.8$  Hz, H-1'), 4.04 (1H, dd,  $J=11.5, 2.2$  Hz, H-6'a), 3.76 (1H, dd,  $J=11.5, 6.6$  Hz, H-6'a), 4.34 (1H, d,  $J=6.8$  Hz, H-1''), 3.87 (1H, dd,  $J=12.4, 3.1$  Hz, H-5'').  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ): 43.4 (C-1), 123.6 (C-2), 24.8 (C-3), 20.3 (C-4), 42.1 (C-5), 43.8 (C-6), 26.7 (C-7), 22.3 (C-8), 26.6 (C-9), 137.5 (C-10), glucopyranosyl moiety; 104.2 (C-1'), 74.2 (C-2'), 77.9 (C-3'), 71.4 (C-4'), 74.7 (C-5'), 69.5 (C-6'), arabinopyranosyl moiety; 105.2 (C-1''), 72.5 (C-2''), 77.1 (C-3''), 69.7 (C-4''), 66.9 (C-5''). HR-ESI-MS  $m/z$ : 469.2040  $[\text{M}+\text{Na}]^+$  calc. 469.2050 for  $\text{C}_{21}\text{H}_{34}\text{O}_{10}\text{Na}$ .

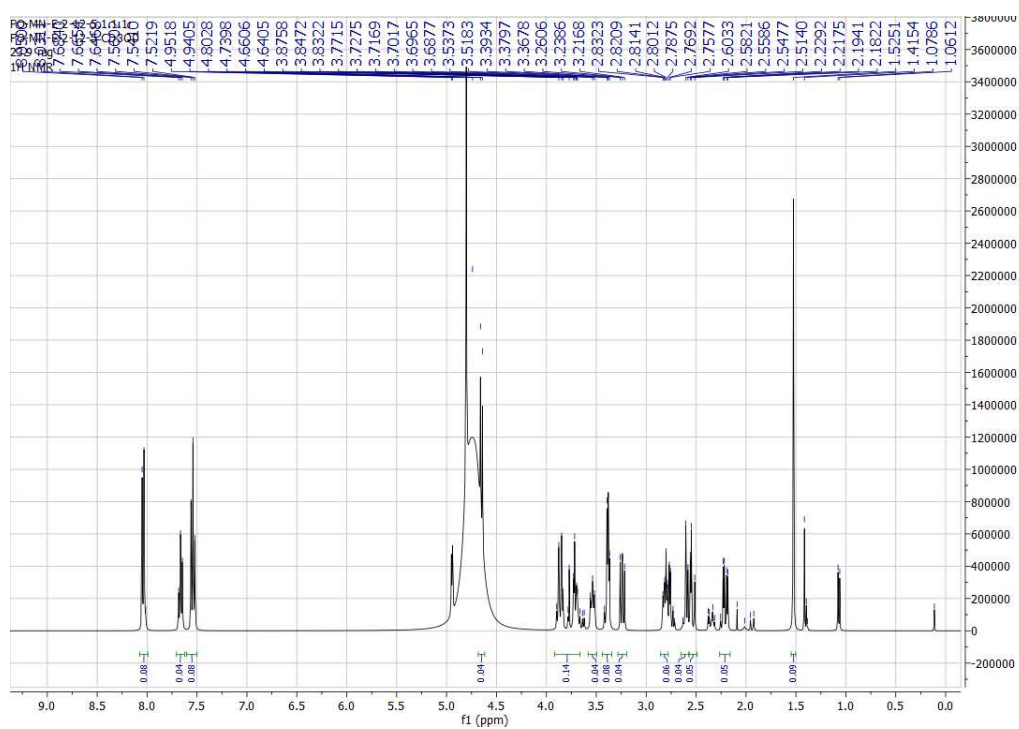


Figure S1. <sup>1</sup>H NMR spectrum of compound 1 (400 MHz, CD<sub>3</sub>OD).

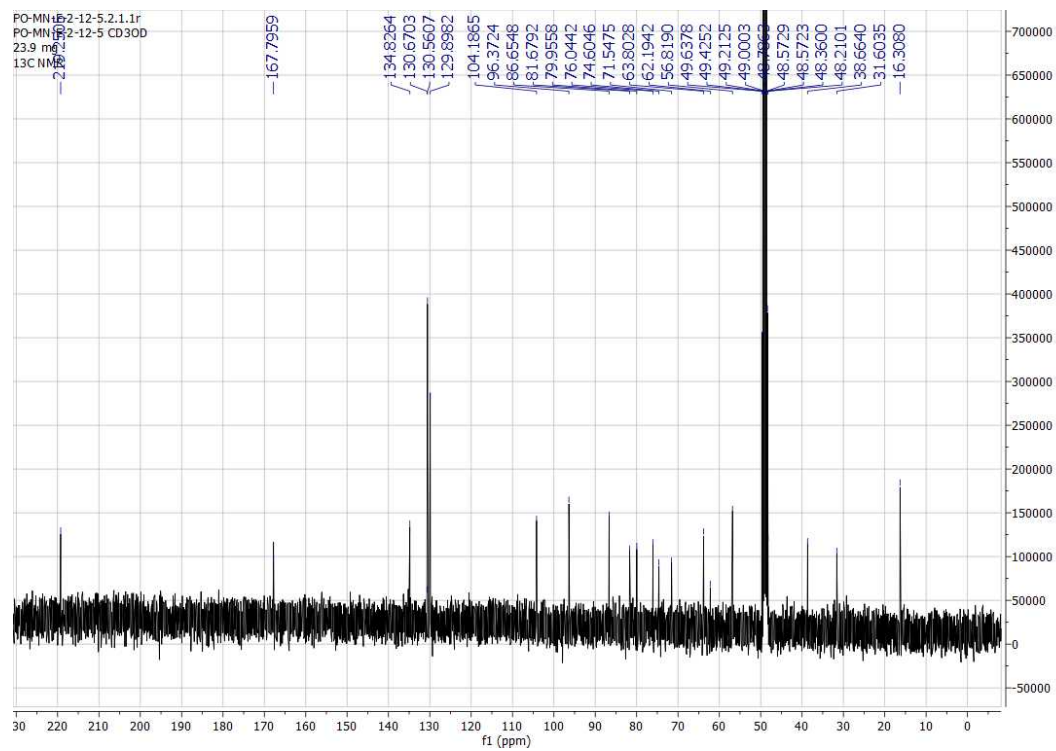
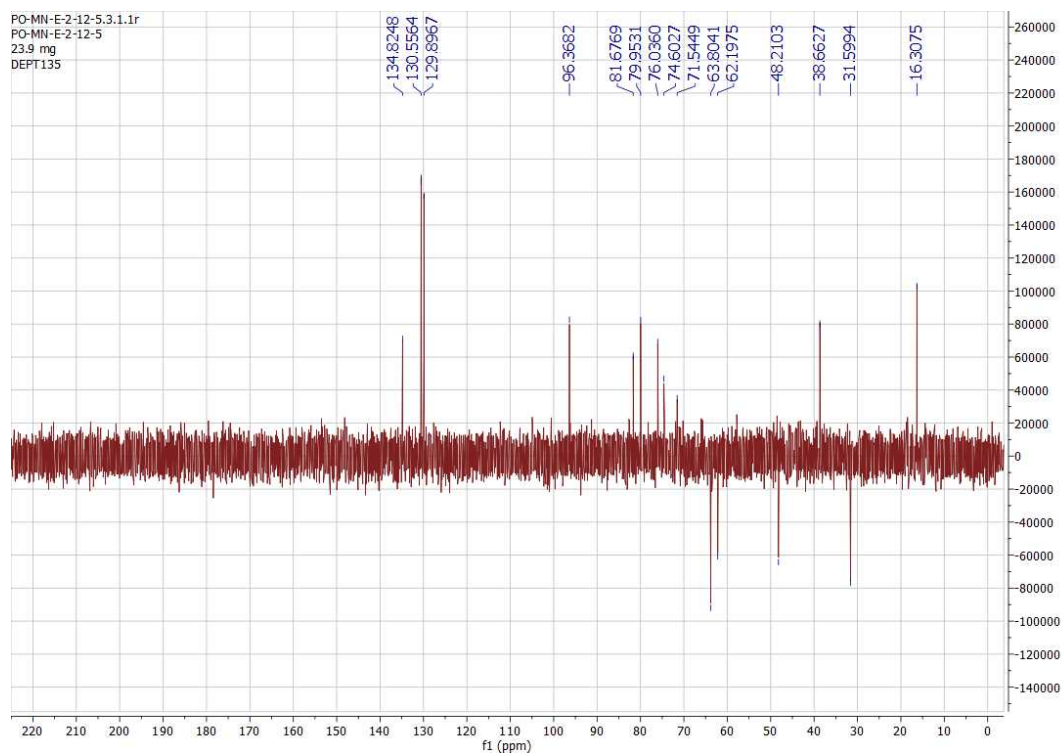
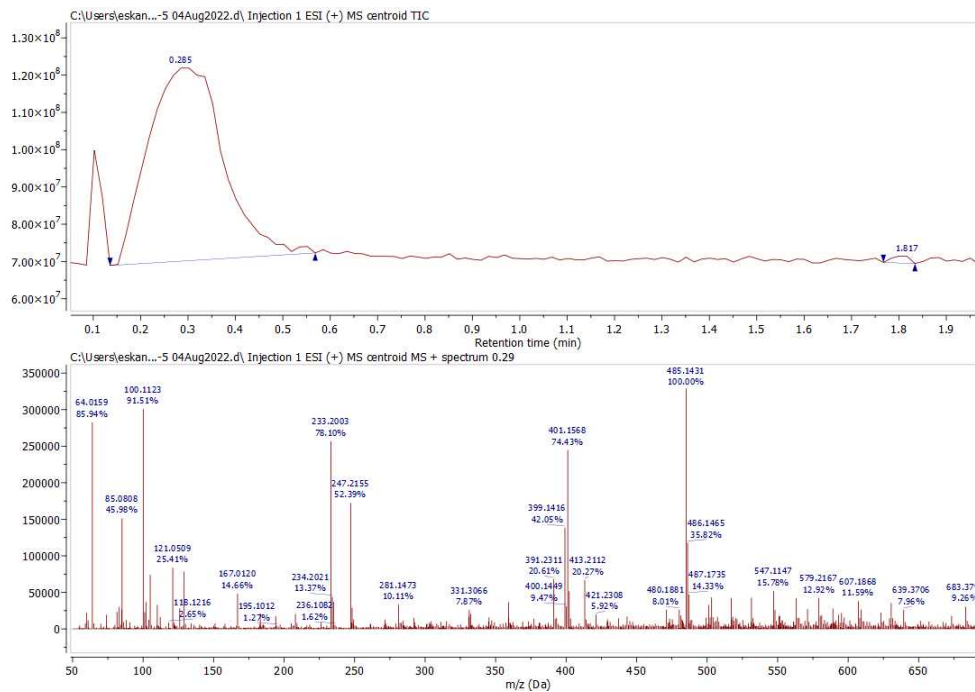


Figure S2. <sup>13</sup>C NMR spectrum of compound 1 (100 MHz, CD<sub>3</sub>OD).

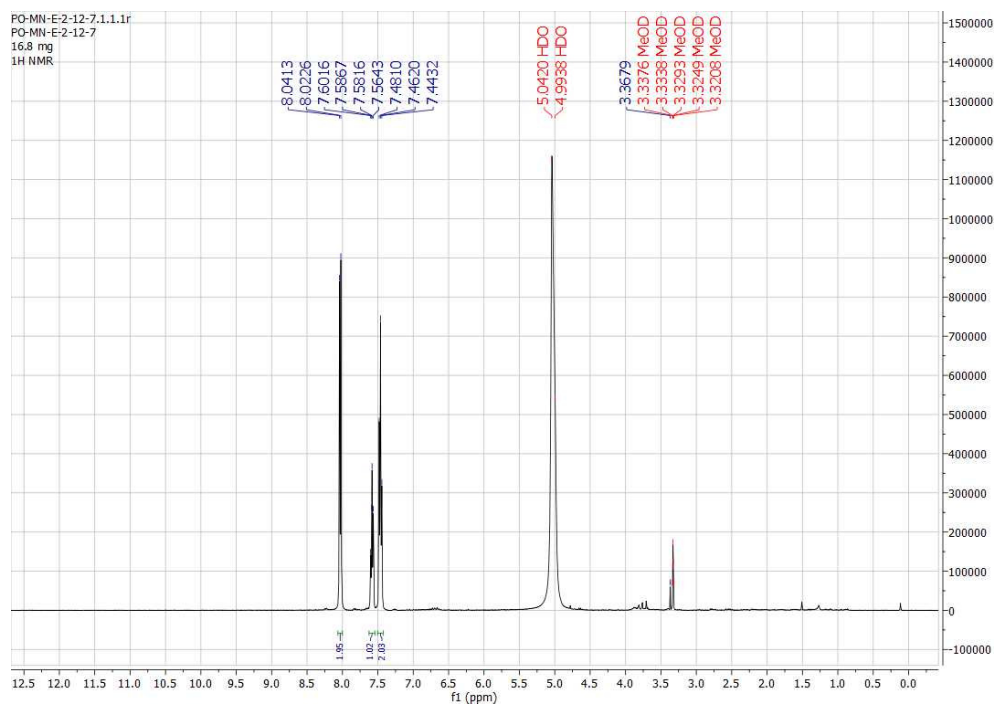


**Figure S3. DEPT NMR spectrum of compound 1 (100 MHz, CD<sub>3</sub>OD).**

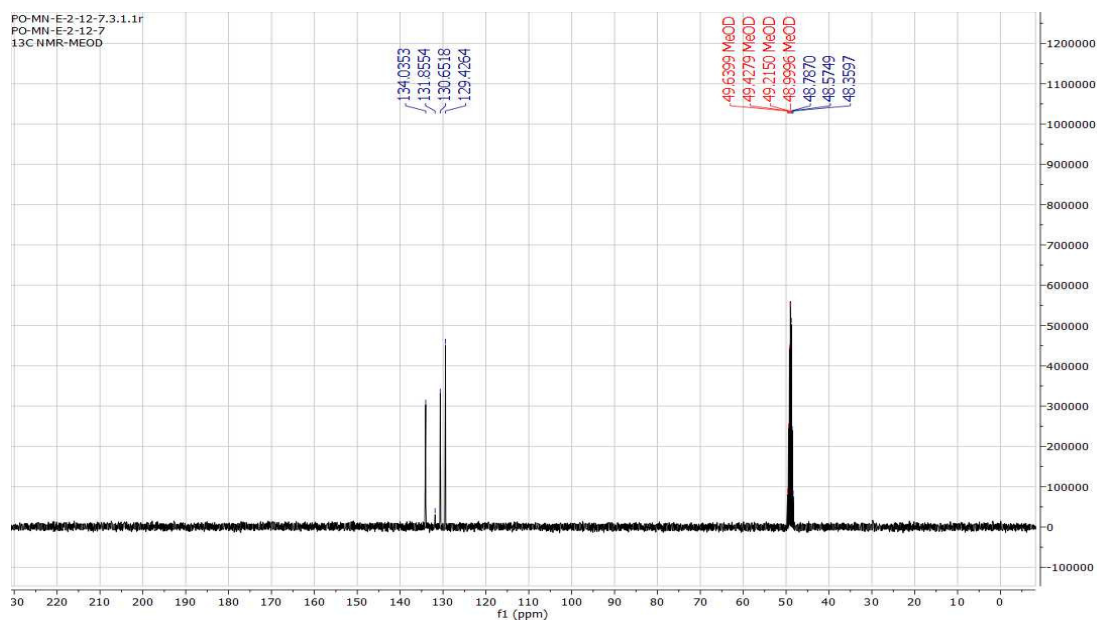
**Compound 1  $m/z$ : 485.1431 [M+Na]<sup>+</sup> calc. 485.1424 for C<sub>23</sub>H<sub>26</sub>O<sub>10</sub>Na**



**Figure S4. HR-ESI-MS spectrum of compound 1.**

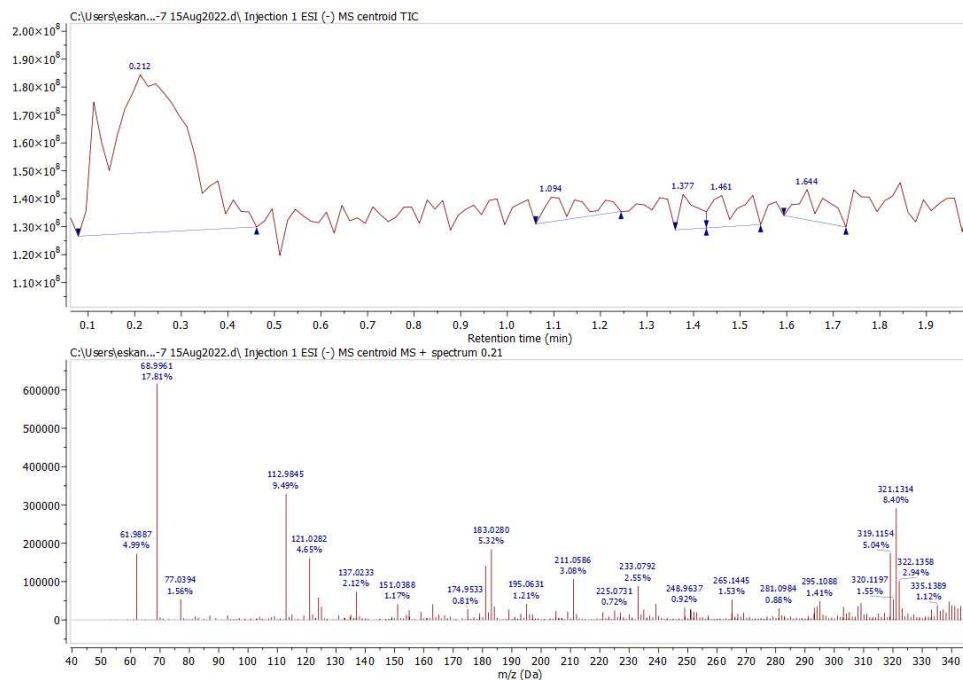


**Figure S5. <sup>1</sup>H NMR spectrum of compound 2 (400 MHz, CD<sub>3</sub>OD).**

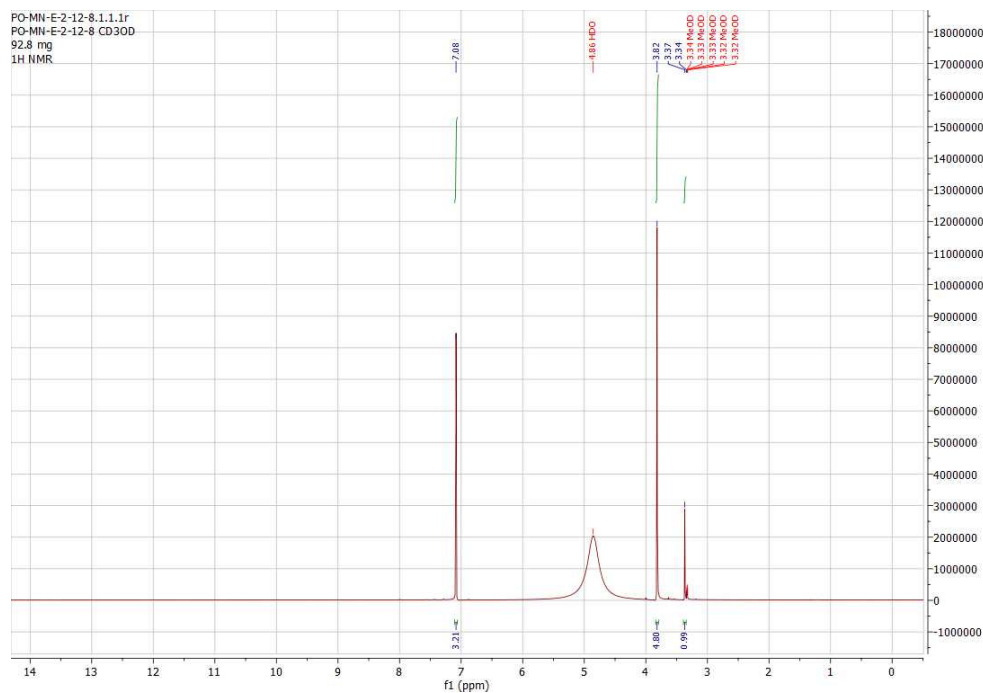


**Figure S6. <sup>13</sup>C NMR spectrum of compound 2 (100 MHz, CD<sub>3</sub>OD).**

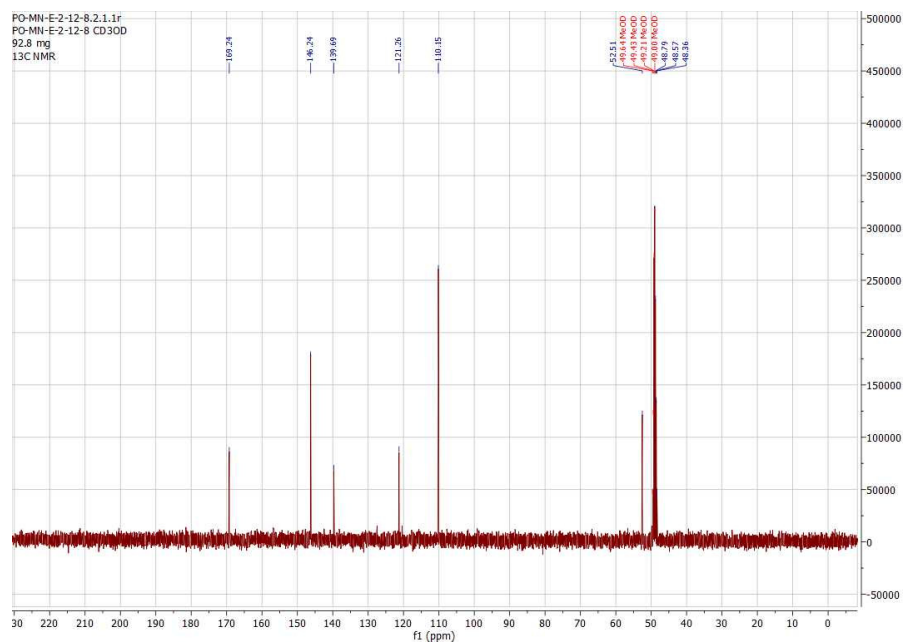
**Compound 2  $m/z$ : 121.0282  $[M-H]^-$  calc. 121.0290 for  $C_7H_5O_2$**



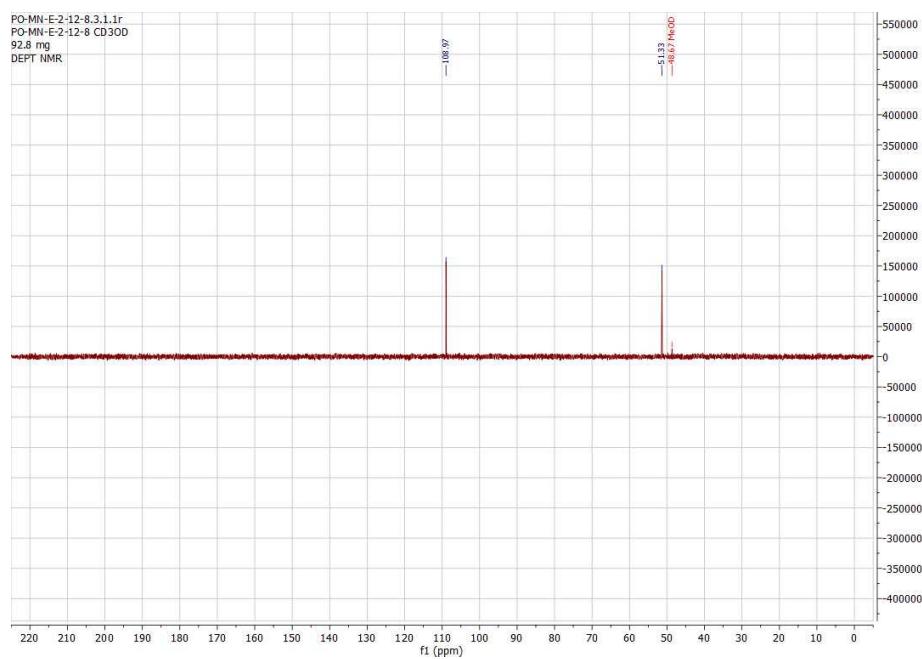
**Figure S7. HR-ESI-MS spectrum of compound 2.**



**Figure S8.  $^1H$  NMR spectrum of compound 3 (400 MHz,  $CD_3OD$ ).**

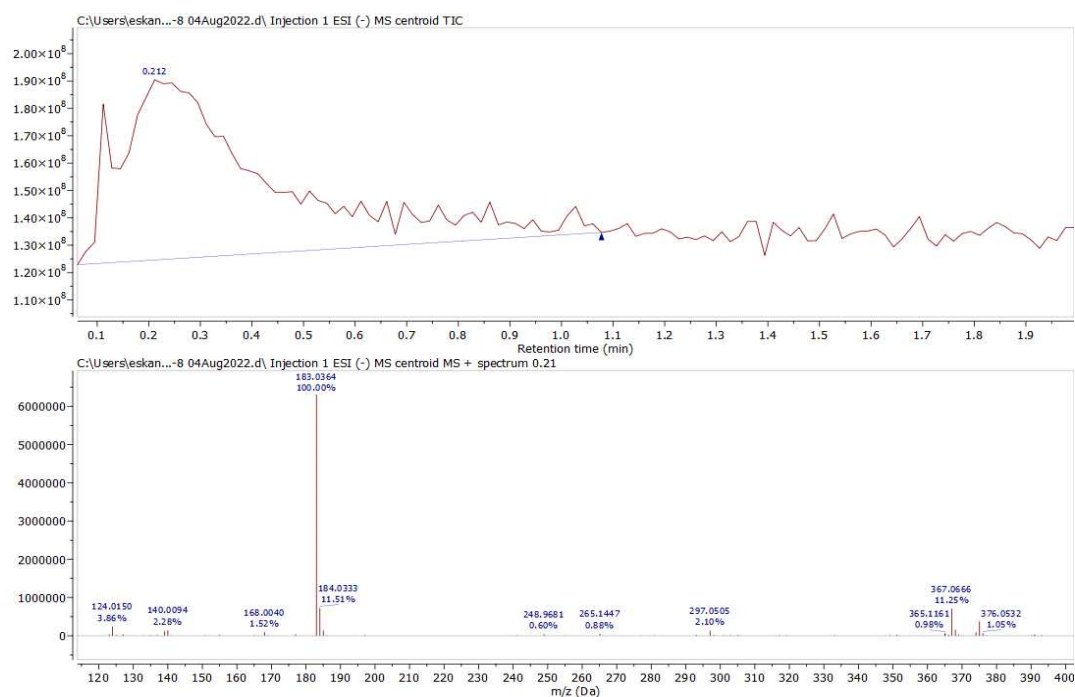


**Figure S9.  $^{13}\text{C}$  NMR spectrum of compound 3 (100 MHz,  $\text{CD}_3\text{OD}$ ).**

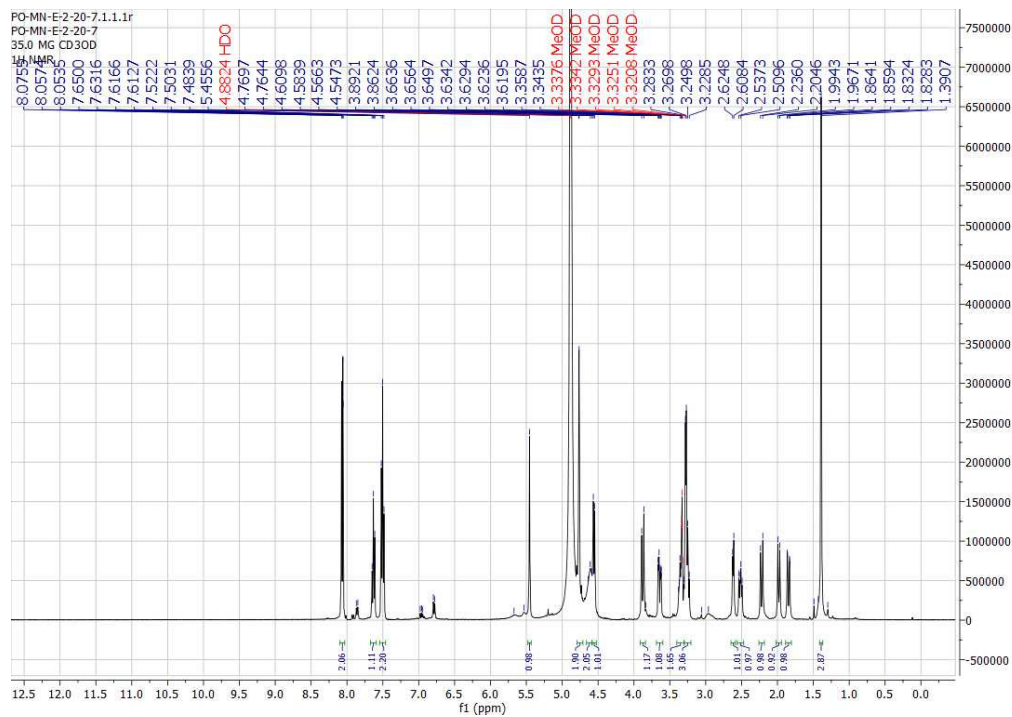


**Figure S10. DEPT NMR spectrum of compound 3 (100 MHz,  $\text{CD}_3\text{OD}$ ).**

**Compound 3  $m/z$ : 183.0361  $[M-H]^-$  calc. 183.0293 for  $C_8H_7O_5$**

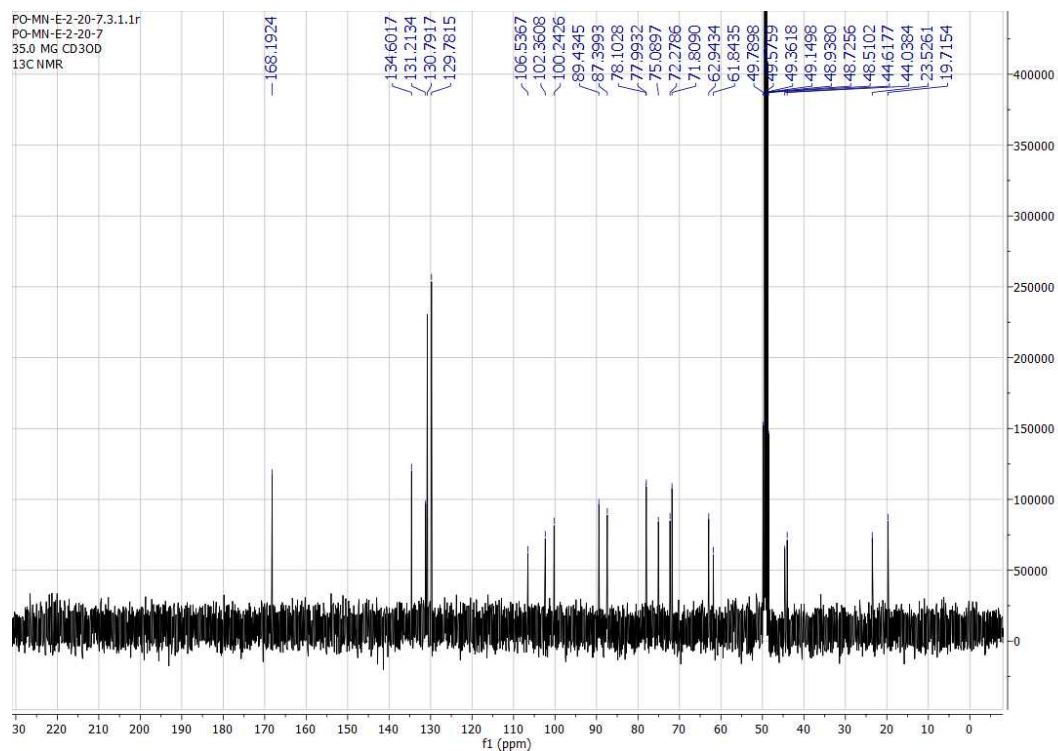


**Figure S11. HR-ESI-MS spectrum of compound 3.**

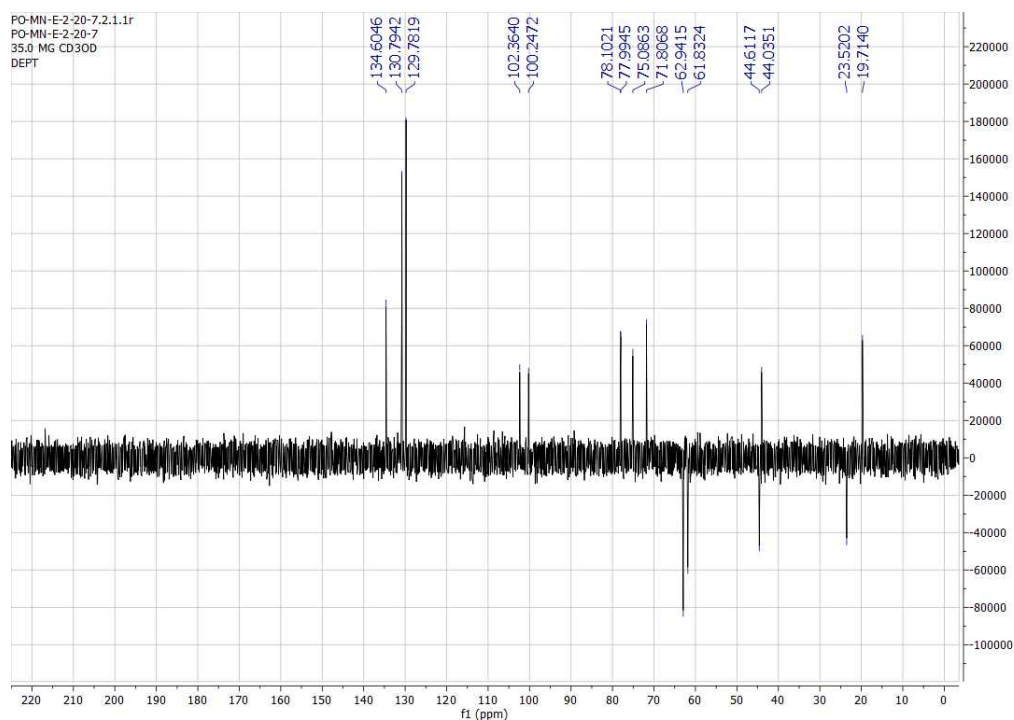


**Figure S12. <sup>1</sup>H NMR spectrum of compound 4 (400 MHz, CD<sub>3</sub>OD).**



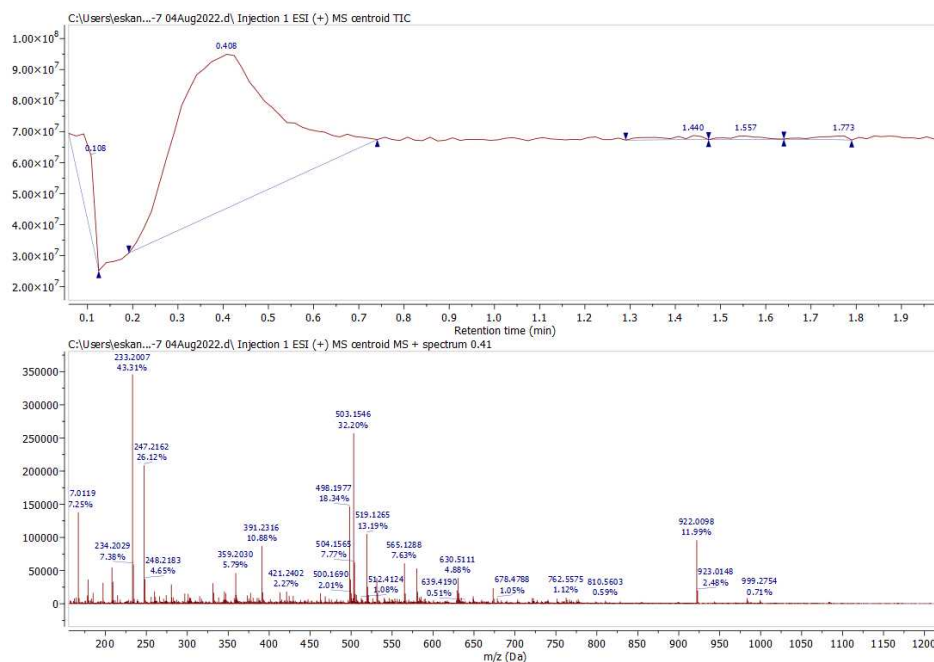


**Figure S13.  $^{13}\text{C}$  NMR spectrum of compound 4 (100 MHz,  $\text{CD}_3\text{OD}$ ).**

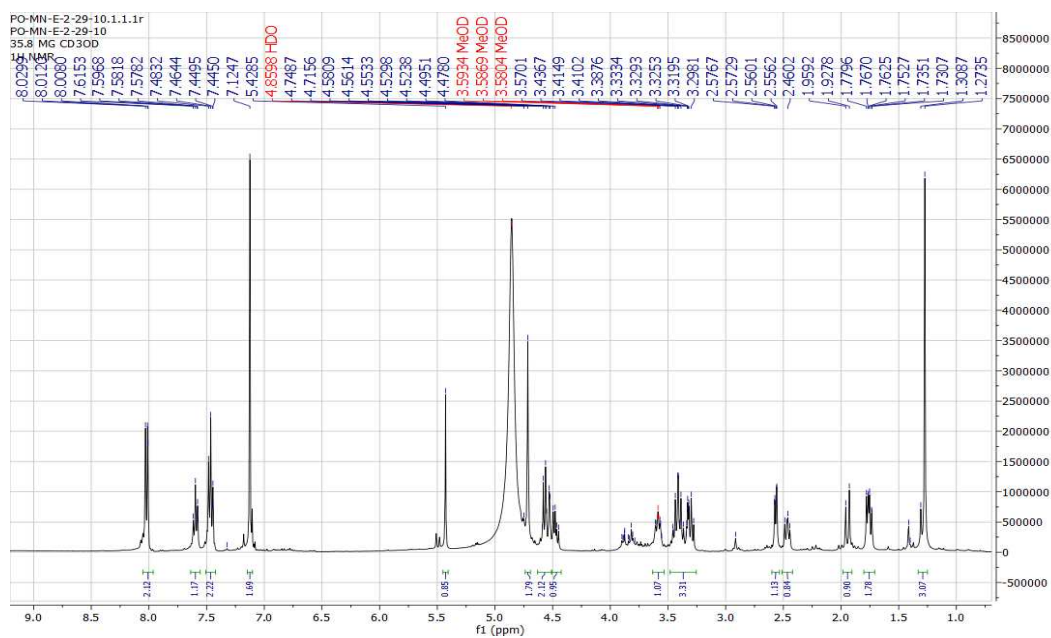


**Figure S14. DEPT NMR spectrum of compound 4 (100 MHz,  $\text{CD}_3\text{OD}$ ).**

**Compound 4  $m/z$ : 503.1516  $[M+Na]^+$  calc. 503.1529 for  $C_{23}H_{28}O_{11}Na$**



**Figure S15. HR-ESI-MS spectrum of compound 4.**



**Figure S16.  $^1H$  NMR spectrum of compound 5 (400 MHz,  $CD_3OD$ ).**

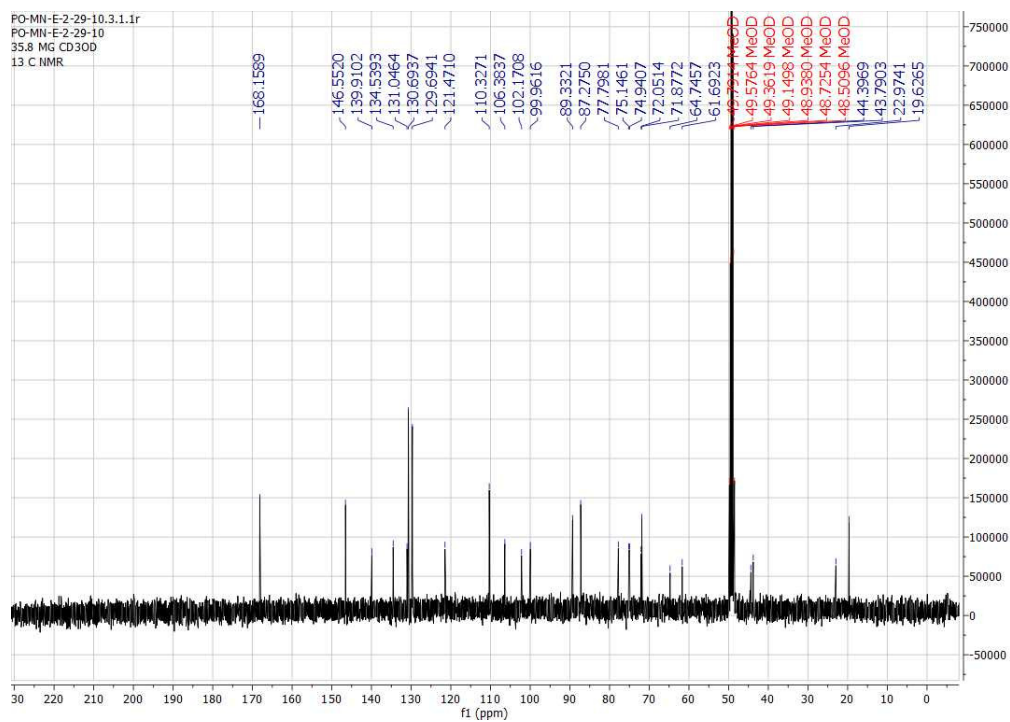


Figure S17.  $^{13}\text{C}$  NMR spectrum of compound 5 (100 MHz,  $\text{CD}_3\text{OD}$ ).

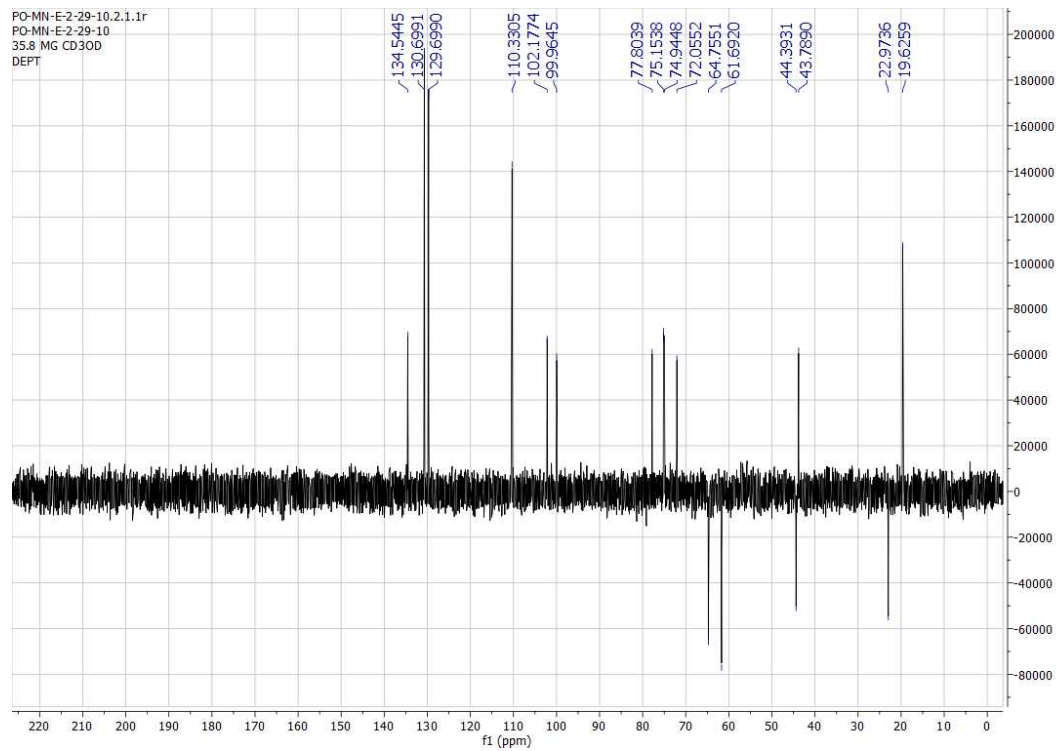
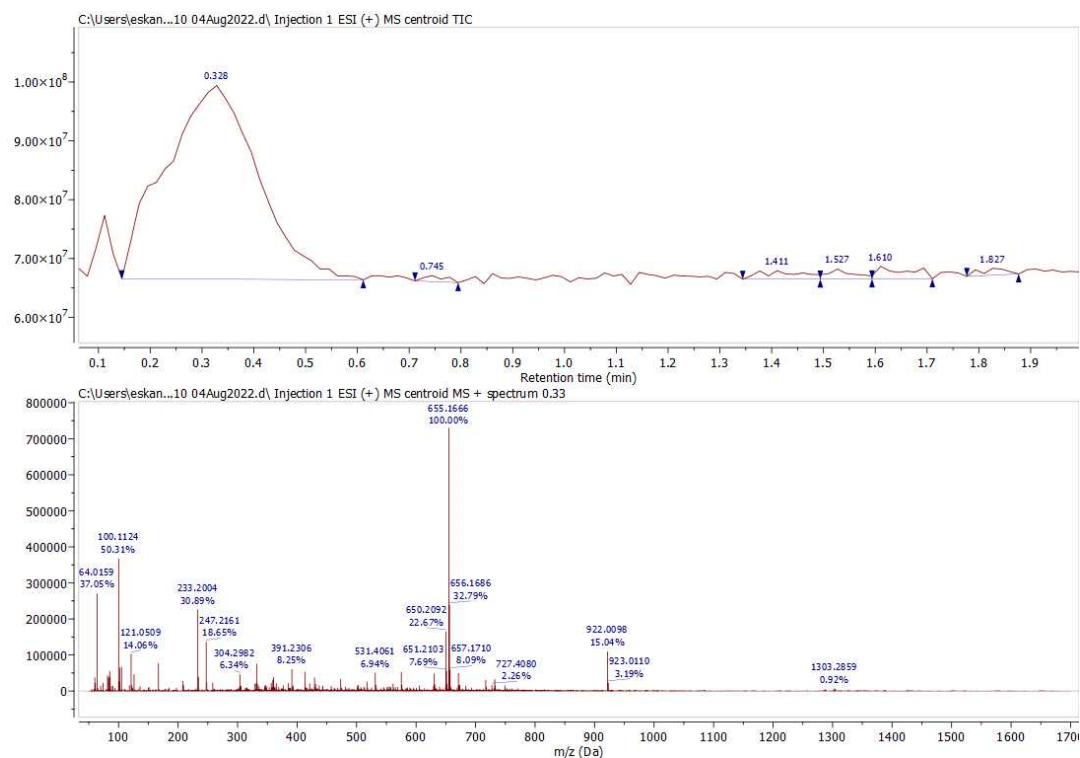
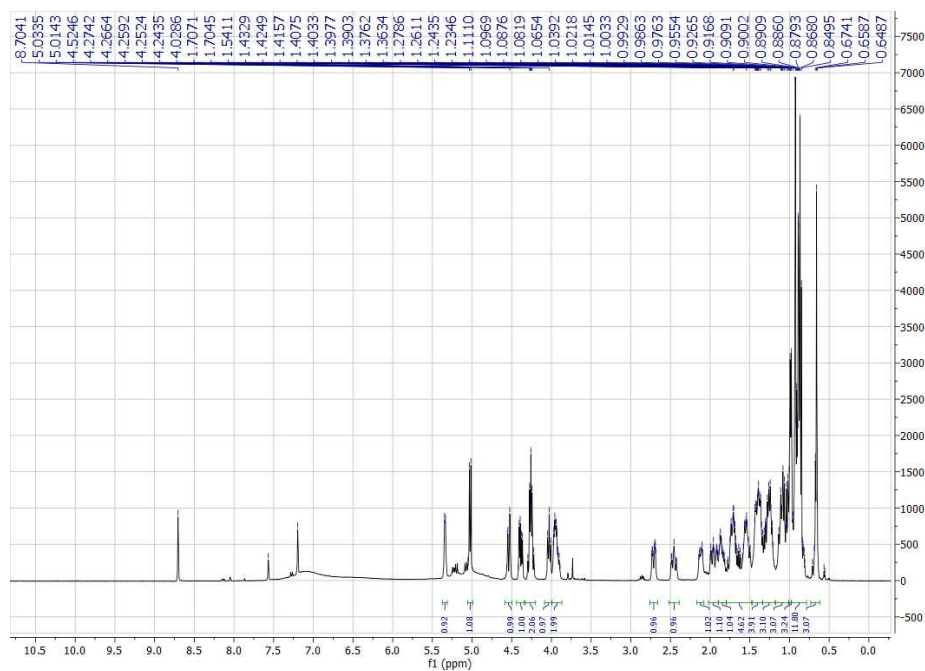


Figure S18. DEPT NMR spectrum of compound 5 (100 MHz,  $\text{CD}_3\text{OD}$ ).

**Compound 5  $m/z$ : 655.1666  $[M+Na]^+$  calc. 655.1639 for  $C_{30}H_{32}O_{15}Na$**



**Figure S19. HR-ESI-MS spectrum of compound 5.**



**Figure S20.  $^1H$  NMR spectrum of compound 6 (400 MHz,  $C_5D_5N$ ).**

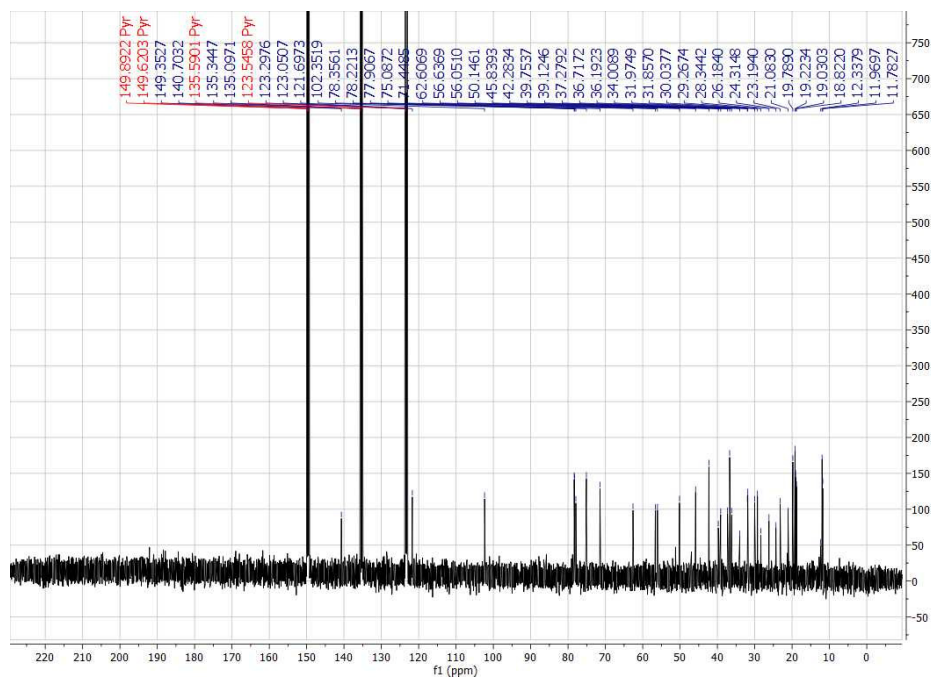


Figure S21.  $^{13}\text{C}$  NMR spectrum of compound 6 (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ ).

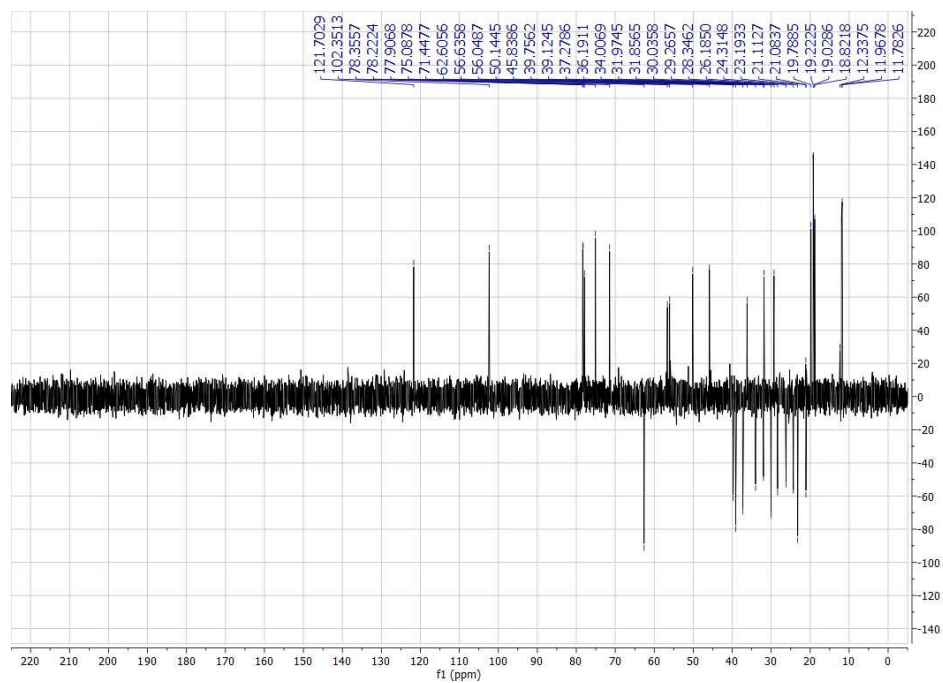
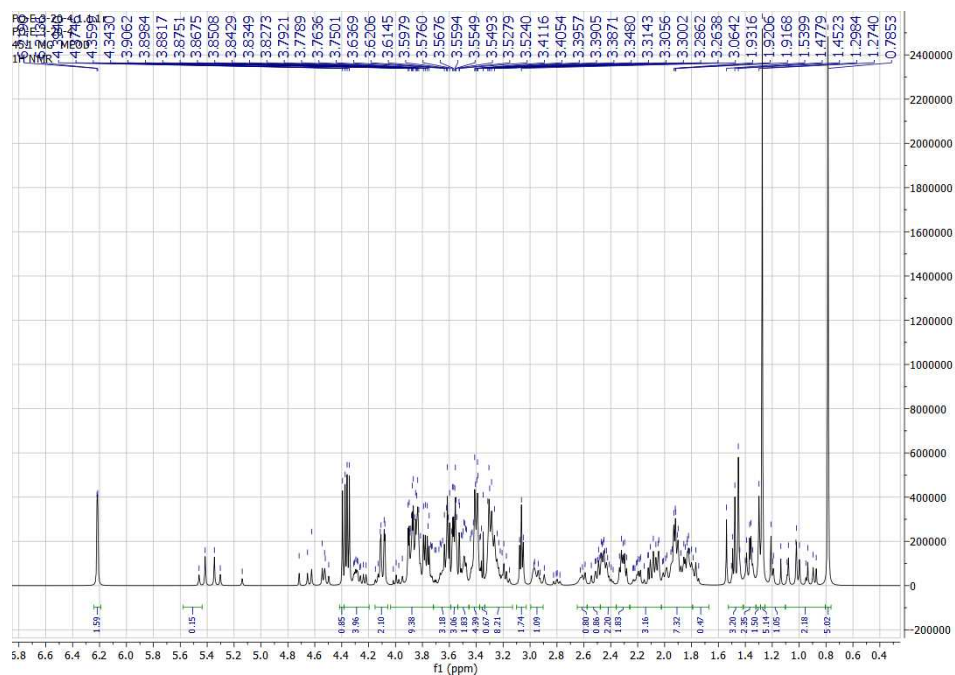
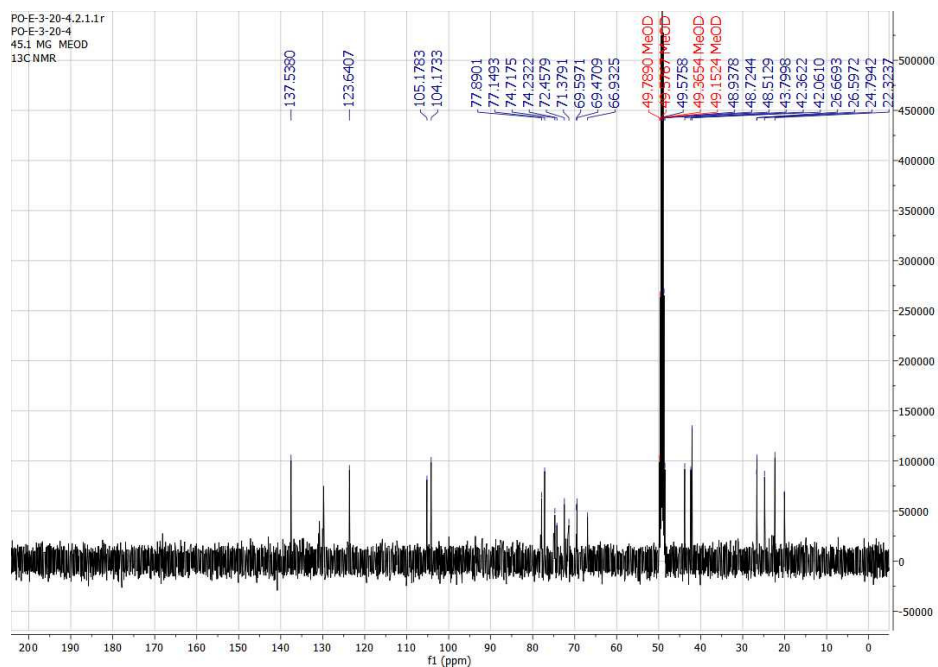


Figure S22. DEPT NMR spectrum of compound 6 (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ ).

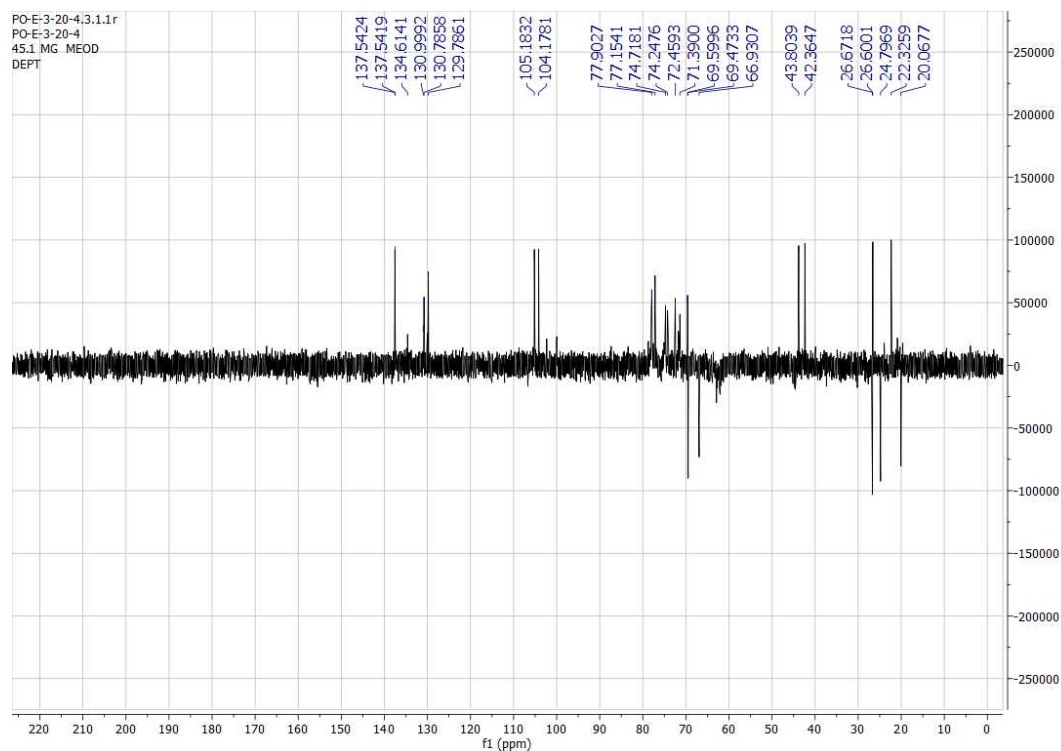


**Figure S23. <sup>1</sup>H NMR spectrum of compound 7 (400 MHz, CD<sub>3</sub>OD).**



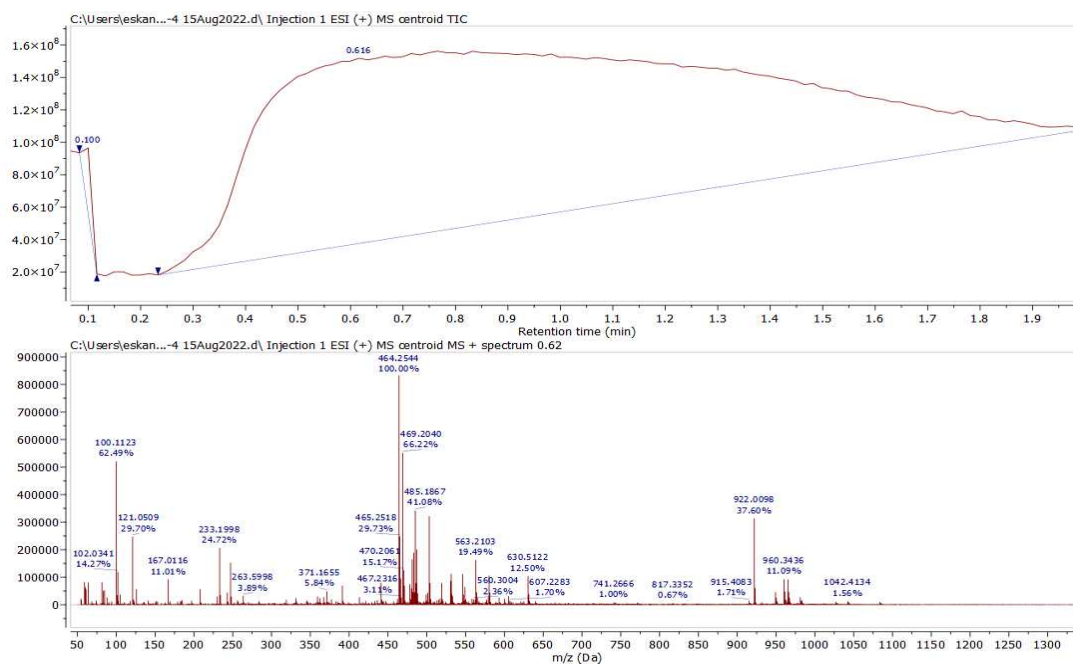
**Figure S24. <sup>13</sup>C NMR spectrum of compound 7 (100 MHz, CD<sub>3</sub>OD).**





**Figure S25. DEPT NMR spectrum of compound 7 (100 MHz, CD<sub>3</sub>OD).**

**Compound 7  $m/z$ : 469.2040 [M+Na]<sup>+</sup> calc. 469.2050 for C<sub>21</sub>H<sub>34</sub>O<sub>10</sub>Na**



**Figure S26. HR-ESI-MS spectrum of compound 7.**