

Structure activity relationship study of Mezzettiasides natural products and their four new disaccharide analogues for anticancer/antibacterial activity

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Supporting Information

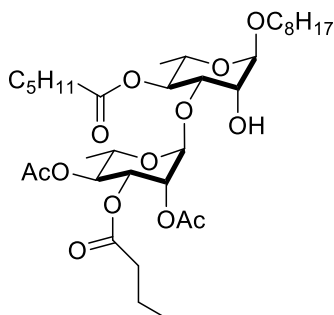
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## Section A: General Information

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a 400, 500 or 600 MHz spectrometer. Chemical shifts were reported relative to internal tetramethylsilane ( $\delta$  0.00 ppm) or  $\text{CDCl}_3$  ( $\delta$  7.26 ppm) or  $\text{CD}_3\text{OD}$  ( $\delta$  4.90 ppm) for  $^1\text{H}$  NMR and  $\text{CDCl}_3$  ( $\delta$  77.23 ppm) or  $\text{CD}_3\text{OD}$  ( $\delta$  49.0 ppm) for  $^{13}\text{C}$  NMR. Infrared (IR) spectra were obtained on a FT-IR spectrometer. Optical rotations were measured with a digital polarimeter in the solvent specified. Flash column chromatography was performed on 60-200 or 230-400 mesh silica gel. Analytical thin-layer chromatography was performed with precoated glass backed plates and visualized by quenching of fluorescence and by charring after treatment with *p*-anisaldehyde or potassium permanganate stain.  $R_f$  values were obtained by elution in the stated solvent ratios. Acetonitrile, diethyl ether, tetrahydrofuran, methylene dichloride and triethylamine were dried by passing through activated alumina column with argon gas pressure. Commercial reagents were used without purification unless otherwise noted. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon/nitrogen using oven- or flame-dried glassware and standard syringe/septa techniques.

## Section B: Experimental Procedures

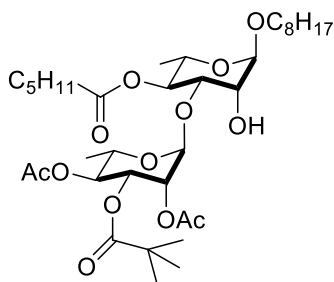
### 1-Octyloxy-2,4-*O*-diacetyl-3-*O*-*n*-butyryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-*O*-hexanoyl- $\alpha$ -L-rhamnopyranoside (**15**):



To a solution of disaccharide alcohol **13** (11 mg, 0.016 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.16 mL) at 0 °C was added butanoic acid (3.0  $\mu\text{L}$ , 0.032 mmol) followed by addition of *N,N'*-dicyclohexylcarbodiimide (7.4 mg, 0.036 mmol) and DMAP (0.3 mg, 0.002 mmol). Reaction was continued to stir at 0 °C for 3h. After 3h, the reaction mixture was diluted with EtOAc and washed with saturated solution of  $\text{NaHCO}_3$  (5 mL). The organic layers were concentrated under reduced pressure. To the crude product (10 mg) dissolved in THF was added thiourea (6.1 mg, 0.08 mmol),  $\text{NaHCO}_3$  (3.9 mg, 0.047 mmol) and *n*- $\text{Bu}_4\text{NI}$  (2.5 mg, 0.007 mmol) and reaction was continued to reflux at 50-65 °C for 2 h. Reaction completed, diluted with EtOAc and washed with  $\text{NH}_4\text{Cl}$ . The crude product was further purified using silica gel flash chromatography eluting with 18-20% EtOAc/hexanes to give product **15** ( 7.6 mg, 0.011 mmol, 84%):  $R_f$  (30% EtOAc/hexane) = 0.4;  $[\alpha]_D^{25} = -44$  ( $C = 0.67$ ,  $\text{CH}_2\text{Cl}_2$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3290, 2888, 2012, 1914, 1722, 1290, 1238, 1160, 1011, 998, 765;  $^1\text{H}$  NMR (400MHz,  $\text{CDCl}_3$ )  $\delta$  5.26 (d,  $J = 9.6$  Hz, 1H), 5.11 (br, 1H), 5.09 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.06 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.91 (s, 1H), 4.77 (s, 1H), 4.03 (dd,  $J = 8.8, 3.6$  Hz, 1H), 3.98 (m, 2H), 3.77 (dq,  $J = 8.0, 7.2$  Hz, 1H), 3.66 (ddd,  $J = 8.0, 6.4, 6.4$  Hz, 1H), 3.41 (ddd,  $J = 8.8, 6.4, 6.4$  Hz, 1H), 2.43 (m, 1H), 2.35 (m, 1H), 2.22 (dd,  $J = 7.6, 6.4$  Hz, 2H), 2.13 (s, 3H), 2.03 (s, 3H), 1.64 (m, 6H), 1.30 (m, 14H), 1.22 (d,  $J = 6.0$  Hz, 3H), 1.19 (d,  $J = 6.0$  Hz, 3H) 0.92-0.87 (m, 9H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.2, 172.3, 170.2, 170.1, 99.3, 98.9, 78.2, 71.9, 71.2, 71.0, 70.2, 68.6, 68.2, 67.4,

66.6, 36.1, 34.3, 32.0, 31.5, 29.6, 29.6, 29.5, 26.3, 24.7, 22.9, 22.5, 21.1, 21.0, 18.5, 17.7, 17.6, 14.3, 14.1, 13.7; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>34</sub>H<sub>58</sub>O<sub>13</sub> + Na]<sup>+</sup>: 697.3770 Found: 697.3759.

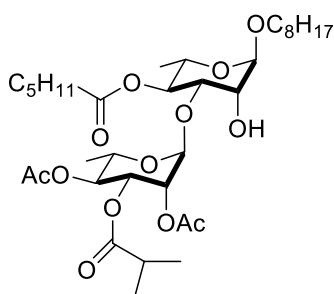
**1-Octyloxy-2,4-O-diacetyl-3-O-pivaloyl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-hexanoyl- $\alpha$ -L-rhamnopyranoside (16):**



To a solution of disaccharide alcohol **13** (10 mg, 0.015 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.15 mL) at 0 °C was added pivaloyl chloride (3.1  $\mu$ L, 0.03 mmol) followed by addition of pyridine (3  $\mu$ L, 0.04 mmol) and DMAP (0.4 mg, 20 mol%). Reaction was continued to stir at 0 °C for 5 h. Monitored by TLC, reaction completed, diluted with EtOAc and washed with dil. HCl (2 mL, 1N). Finally washed with saturated NaHCO<sub>3</sub> solution and the combined organic layers were concentrated under reduced pressure. The crude product (8.0 mg) was dissolved in THF, added thiourea (4.8 mg, 0.063 mmol), NaHCO<sub>3</sub> (3.1 mg, 0.04 mmol) and *n*-Bu<sub>4</sub>NI (1.9 mg, 0.005 mmol). The reaction was continued to reflux for 3 h. Reaction completed, diluted with EtOAc and washed with NH<sub>4</sub>Cl. The crude product was further purified using silica gel flash chromatography eluting with 18-20% EtOAc/hexanes to give the desired product **16** (6 mg, 0.009 mmol, 83%): *R<sub>f</sub>* (30% EtOAc/hexane) = 0.35; [ $\alpha$ ]<sub>D</sub><sup>25</sup> = – 20 (*C* = 0.19, CH<sub>2</sub>Cl<sub>2</sub>); IR (thin film, cm<sup>-1</sup>) 3121, 2916, 2167, 2102, 1903, 1743, 1650, 1322, 1219, 1024, 1003, 765; <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  5.19-5.13 (m, 2H), 5.14 (m, *J* = 9.6 Hz, 2H), 4.90 (s, 1H), 4.78 (s, 1H), 4.04 (d, *J* = 8.8 Hz, 1H), 4.01-3.98 (m, 2H), 3.77 (dq, *J* = 8.8, 6.4 Hz, 1H), 3.68 (ddd, *J* = 8.8, 6.4, 6.4 Hz, 1H), 3.43 (ddd, *J* = 8.8, 6.8, 6.8 Hz, 1H), 2.43 (m, 1H), 2.33 (m, 1H), 2.21 (s, 1H), 2.12 (s, 3H), 2.02 (s, 3H), 1.66-1.55 (m, 4H), 1.30 (m, 14H), 1.23 (d, *J* = 6.4 Hz, 3H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.11 (m, 8H),

0.88 (m, 6H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  176.9, 173.2, 170.1, 170.0, 99.3, 98.9, 78.2, 71.8, 71.0, 70.9, 69.9, 68.7, 68.1, 67.3, 66.5, 38.9, 34.3, 32.0, 31.5, 29.5 (2C), 29.5, 27.0 (2C), 26.3, 24.7, 22.9, 22.5, 21.0 (2C), 17.7, 17.6, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z):  $[\text{M} + \text{Na}]^+$  calcd for  $[\text{C}_{35}\text{H}_{60}\text{O}_{13}\text{C} + \text{Na}]^+$ : 711.3926, Found: 711.3909.

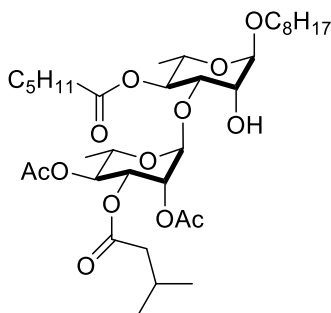
**1-Octyloxy-2,4-O-diacetyl-3-O-isobutyryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-hexanoyl- $\alpha$ -L-rhamnopyranoside (17):**



To a stirred solution of disaccharide alcohol **13** (10 mg, 0.015 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.1 mL) at 0 °C was added isobutyryl chloride (3.1  $\mu\text{L}$ , 0.029 mmol) followed by addition of pyridine (3  $\mu\text{L}$ , 0.037 mmol) and 4-Dimethylaminopyridine (0.2 mg, 10 mol%). Reaction was continued to stir at 0 °C for 3 h. Monitored by TLC, reaction completed, diluted with EtOAc and washed with dil HCl (2 mL, 1N). Finally washed with saturated  $\text{NaHCO}_3$  solution and the combined organic layers were concentrated under reduced pressure to obtain the crude product. The crude product (9 mg) was directly used to carry out the chloroacetate deprotection. To the crude product dissolved in THF (0.1 mL) was added thiourea (4.9 mg, 0.064 mmol),  $\text{NaHCO}_3$  (3.2 mg, 0.04 mmol) and *n*- $\text{Bu}_4\text{NI}$  (1.9 mg, 0.005 mmol). The reaction was continued to reflux for 2 h. Reaction completed then diluted with EtOAc and washed with  $\text{NH}_4\text{Cl}$ . The crude product was further purified using silica gel flash chromatography eluting with 15-18% EtOAc/hexanes to give the desired product **17** (6.1 mg, 0.009 mmol, 85%):  $R_f$  (30% EtOAc/hexane) = 0.35;  $[\alpha]_D^{25} = -26$  ( $C = 0.24$ ,  $\text{CH}_2\text{Cl}_2$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3119, 2920, 2029, 1996, 1902, 1747, 1372, 1222, 1163, 1025, 1004, 765, 635;  $^1\text{H}$

NMR (400MHz, CDCl<sub>3</sub>)  $\delta$  5.24 (dd,  $J = 10.4, 3.6$  Hz, 1H), 5.16 (m, 1H), 5.09 (m,  $J = 9.2, 9.2$  Hz, 2H), 4.91 (s, 1H), 4.78 (s, 1H), 4.05 (d,  $J = 9.6$  Hz, 1H), 3.98 (m, 2H), 3.77 (dq,  $J = 8.8, 6.4$  Hz, 1H), 3.66 (ddd,  $J = 8.4, 6.8, 6.8$  Hz, 1H), 3.41 (ddd,  $J = 8.8, 7.2, 7.2$  Hz, 1H), 2.46 (m, 1H), 2.35 (m, 1H), 2.22 (br, 1H), 2.13 (s, 3H), 2.03 (s, 3H), 1.64-1.57 (m, 4H), 1.30 (m, 14H), 1.23 (d,  $J = 6.0$  Hz, 3H), 1.19 (d,  $J = 6.0$  Hz, 3H), 1.09 (m, 6H), 0.88 (m, 6H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  175.6, 173.2, 170.2, 170.1, 99.3, 99.0, 78.2, 71.9, 71.1, 71.0, 70.1, 68.6, 68.2, 67.3, 66.6, 34.3, 34.1, 32.0, 31.5, 29.6, 29.6, 29.5, 26.3, 24.7, 22.9, 22.5, 21.1, 21.0, 19.0, 18.8, 17.7, 17.6, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>34</sub>H<sub>58</sub>O<sub>13</sub> + Na]<sup>+</sup>: 697.3770, Found: 697.3765.

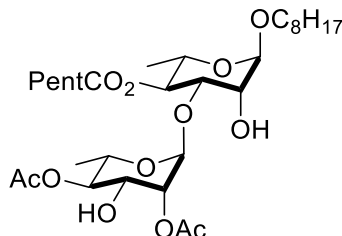
**1-Octyloxy-2,4-O-diacetyl-3-O-isovaleryl- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-hexanoyl- $\alpha$ -L-rhamnopyranoside (18):**



To a solution of alcohol **13** (11 mg, 0.016 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.16 mL) at 0 °C was added 3-Methylbutanoic acid or isovaleric acid (4.4  $\mu$ L, 0.040 mmol) followed by addition of DCC (9.3 mg, 0.045 mmol) and DMAP (0.16 mg, 20 mol%). Reaction continued to stir at 0 °C for 2 h. Monitored by TLC, reaction completed, diluted with EtOAc and washed with saturated solution of NaHCO<sub>3</sub> (5 mL). The organic layers were concentrated under reduced pressure. The crude product (9 mg) was directly used for deprotection of chloroacetyl group. To the crude product dissolved in THF was added thiourea (6.0 mg, 0.08 mmol), NaHCO<sub>3</sub> (3.8 mg, 0.05 mmol) and *n*-Bu<sub>4</sub>Ni (2.4 mg, 0.0065 mmol), reaction was continued to reflux at 60-65 °C for 3 h. Reaction completed, diluted with EtOAc and washed with NH<sub>4</sub>Cl. The crude product was further purified using silica gel flash chromatography eluting with 18-20%

EtOAc/hexanes to give the desired product **18** (7.2 mg, 0.01 mmol, 80%):  $R_f$  (30% EtOAc/hexane) = 0.4;  $[\alpha]_D^{25} = -38$  ( $c = 0.66$ ,  $\text{CH}_2\text{Cl}_2$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3250, 2926, 1899, 1748, 1610, 1447, 1322, 1024, 765, 635;  $^1\text{H}$  NMR (500MHz,  $\text{CDCl}_3$ )  $\delta$  5.26 (d,  $J = 9.6, 3.5$  Hz, 1H), 5.11 (br, 1H), 5.07 (m, 2H), 4.91 (s, 1H), 4.77 (s, 1H), 4.04 (dq,  $J = 10.0, 6.5$  Hz, 1H), 3.98 (m, 2H), 3.74 (dq,  $J = 10.0, 6.5$  Hz, 1H), 3.66 (ddd,  $J = 9.5, 6.0, 6.0$  Hz, 1H), 3.41 (ddd,  $J = 9.0, 6.0, 6.0$  Hz, 1H), 2.43 (m, 1H), 2.35 (m, 1H), 2.24 (d,  $J = 3.5$  Hz, 1H), 2.15 (s, 3H), 2.03 (s, 3H), 1.61 (m, 4H), 1.29 (m, 14H), 1.22-1.17 (m, 8H), 0.91-0.88 (m, 12H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.2, 171.8, 170.2, 170.1, 99.3, 98.9, 78.2, 71.9, 71.1, 71.0, 70.2, 68.5, 68.2, 67.4, 66.6, 43.3, 34.3, 32.0, 31.5, 29.9, 29.6, 29.6, 29.5, 26.3, 25.8, 24.7, 22.9, 22.5, 22.4, 21.1, 21.0, 17.7, 17.7, 14.3, 14.1; HRMS–MALDI–TOF (CCA) ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $[\text{C}_{35}\text{H}_{60}\text{O}_{13}\text{C} + \text{Na}]^+$ : 711.3926, Found: 711.3965.

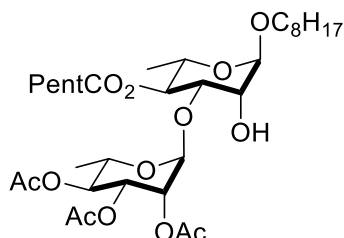
#### Mezzettiaside-10 (10):



Data:  $R_f$ (50% EtOAc/hexane) = 0.3;  $[\alpha]_D^{25} = -48.1$  ( $c = 0.10$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3515, 2922, 1740, 1378, 1325, 1266, 845, 829, 721;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.07 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.94 (s, 1H), 4.91 (d,  $J = 1.6$  Hz, 1H), 4.88 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.76 (s, 1H), 4.04 (dd,  $J = 9.6, 3.6$  Hz, 1H), 3.96 (m, 3H), 3.77 (dq,  $J = 9.6, 6.0$  Hz, 1H), 3.66 (ddd,  $J = 10.4, 6.8, 6.8$  Hz, 1H), 3.41 (ddd,  $J = 10.4, 6.4, 6.4$  Hz, 1H), 2.44 (m, 1H), 2.36 (m, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 1.65 (m, 4H), 1.32-1.27 (m, 14H), 1.21 (d,  $J = 6.0$  Hz, 3H), 1.18 (d,  $J = 6.0$  Hz, 3H), 0.93 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.5, 171.6, 170.5, 99.4, 99.2, 78.5, 74.5, 73.0, 72.0, 71.2, 68.3, 68.2, 67.0, 66.4, 34.3, 32.0, 31.5, 29.6, 29.5, 29.4, 26.3, 24.8, 22.9,

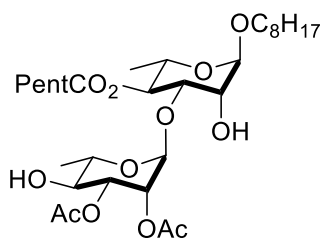
22.5, 21.20, 21.17, 17.6, 17.6, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>30</sub>H<sub>52</sub>O<sub>12</sub> + Na]<sup>+</sup>: 627.3351, Found: 627.3378.

**Mezzettiaside-11 (11):**



Data:  $R_f$  (40% EtOAc/hexane) = 0.4;  $[\alpha]_D^{25} = -54.2$  ( $c = 0.1$ , CHCl<sub>3</sub>); IR (thin film, cm<sup>-1</sup>) 3490, 2952, 2927, 1752, 1371, 1258, 1226, 1137, 1077, 764, 750; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.26 (dd,  $J = 10.4, 2.6$  Hz, 1H), 5.10 (dd,  $J = 3.6, 1.6$  Hz, 1H), 5.08 (dd,  $J = 10.4, 10.4$  Hz, 1H), 5.05 (dd,  $J = 10.4, 10.4$  Hz, 1H), 4.91 (s, 1H), 4.77 (s, 1H), 4.06 (dd,  $J = 9.6, 2.6$  Hz, 1H), 3.96 (m, 2H), 3.78 (dq,  $J = 9.6, 6.4$  Hz, 1H), 3.65 (ddd,  $J = 10.4, 7.2, 7.2$  Hz, 1H), 3.42 (ddd,  $J = 10.4, 7.2, 7.2$  Hz, 1H), 2.43 (m, 1H), 2.33 (m, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.65 (m, 4H), 1.30-1.25 (m, 14H), 1.22 (d,  $J = 6.0$  Hz, 3H), 1.19 (d,  $J = 5.6$  Hz, 3H), 0.88 (m, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  173.1, 170.3, 170.1, 169.8, 99.3, 98.9, 78.3, 71.9, 71.2, 71.0, 70.2, 68.9, 68.2, 67.4, 66.6, 34.3, 32.0, 31.5, 29.59, 29.54, 29.45, 26.3, 24.7, 22.9, 22.5, 21.1, 21.0, 20.9, 17.7, 17.6, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>32</sub>H<sub>54</sub>O<sub>13</sub> + Na]<sup>+</sup>: 669.3457, Found: 669.3454

**Mezzettiaside-9 (9):**

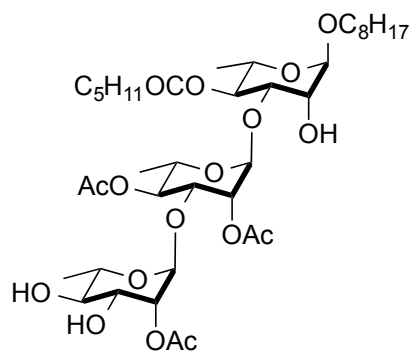


Data:  $R_f$  (50% EtOAc/hexane) = 0.4;  $[\alpha]_D^{25} = -47$  ( $c = 1.0$ , CHCl<sub>3</sub>); IR (thin film, cm<sup>-1</sup>) 3477, 2925, 2859, 1745, 1377, 1275, 1260, 1232, 1138, 1076, 1051, 764, 750;



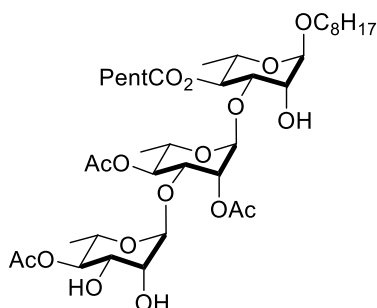
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.15 (dd,  $J = 10.0, 1.2$  Hz, 1H), 5.10 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.02 (dd,  $J = 2.8, 1.2$  Hz, 1H), 4.87 (s, 1H), 4.78 (s, 1H), 3.98 (m, 1H), 3.93 (dd,  $J = 10.4, 2.8$  Hz, 1H), 3.90 (dq,  $J = 9.6, 6.4$  Hz, 1H), 3.77 (dq,  $J = 9.6, 6.4$  Hz, 1H), 3.67 (m, 2H), 3.43 (ddd,  $J = 8.8, 6.8, 6.8$  Hz, 1H), 2.45 (m, 1H), 2.34 (m, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 1.62 (m, 4H), 1.37-1.25 (m, 17H), 1.18 (d,  $J = 6.0$  Hz, 3H), 0.90 (m, 6H);  $^{13}\text{C}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  173.3, 171.6, 170.1, 99.4, 99.1, 78.1, 72.1, 72.0, 71.6, 71.0, 70.5, 69.7, 68.1, 66.5, 34.3, 32.0, 31.5, 29.6, 29.6, 29.5, 26.3, 24.8, 22.9, 22.5, 21.1, 21.1, 17.8, 17.7, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z):  $[\text{M} + \text{Na}]^+$  calcd for  $[\text{C}_{30}\text{H}_{52}\text{O}_{12} + \text{Na}]^+$ : 627.3351, Found: 627.3387.

**Mezzettiaside-8 (8):**



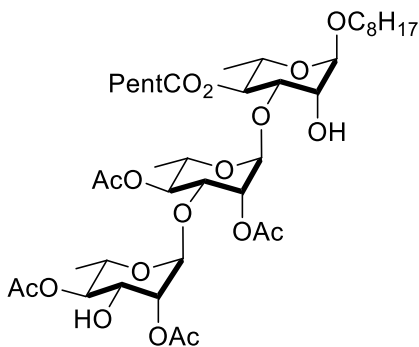
Data:  $R_f$  (100% EtOAc/hexane) = 0.5;  $[\alpha]_D^{25} = -40$  ( $c = 0.1, \text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3454, 2925, 2854, 1741, 1459, 1376, 1264, 1236, 1076, 1046, 736, 704;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.06 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.04 (dd,  $J = 10.0, 10.0$  Hz, 1H), 4.99 (dd,  $J = 3.2, 2.4$  Hz, 1H), 4.88 (s, 3H), 4.76 (s, 1H), 4.06 (dd,  $J = 10.4, 3.6$  Hz, 1H), 3.94 (m, 3H), 3.80 (m, 2H), 3.66-3.60 (2H), 3.45 (m, 2H), 2.42 (m, 1H), 2.34 (1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 1.62 (m, 4H), 1.29 (m, 24H), 1.19 (m, 6H), 0.88 (brs, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.3, 170.8, 170.7, 170.5, 99.5, 99.4 (2C), 78.9, 74.9, 73.4, 72.5, 72.4, 71.9, 71.9, 71.2, 69.9, 69.1, 68.2, 67.5, 66.4, 34.3, 32.0, 31.5, 29.9, 29.9, 29.5, 29.4, 26.3, 24.7, 22.9, 22.5, 21.2, 21.0, 17.6, 17.4, 14.3, 14.1; HRMS (ESI) calcd for  $[\text{C}_{38}\text{H}_{64}\text{O}_{17} + \text{H}]^+$ : 793.4222, Found: 793.4253.

#### Mezzettiaside-4 (4):



Data:  $R_f$  (70% EtOAc/hexane) = 0.3;  $[\alpha]_D^{25} = -56.9$  ( $c = 0.46$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3414, 3231, 2928, 2856, 1742, 1457, 1375, 1231, 1137, 1076, 1045, 980;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 5.06 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.03 (s, 1H), 5.02 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.97 (dd,  $J = 3.6, 1.2$  Hz, 1H), 4.85 (s, 1H), 4.79 (s, 1H), 4.78 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.18 (dd,  $J = 10.4, 3.6$  Hz, 1H), 3.98 (m, 2H), 3.90 (dd,  $J = 9.6, 3.6$  Hz, 1H), 3.85 (d,  $J = 2.0$  Hz, 1H), 3.79 (dq,  $J = 9.6, 6.2$  Hz, 1H), 3.73 (dd,  $J = 9.6, 3.2$  Hz, 1H), 3.70 (dq,  $J = 9.6, 6.2$  Hz, 1H), 3.65 (ddd,  $J = 9.6, 6.2, 6.2$  Hz, 1H), 3.42 (ddd,  $J = 9.6, 6.2, 6.2$  Hz, 1H), 2.44-2.35 (m, 2H), 2.12 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 1.63 (m, 4H), 1.31 (m, 14H), 1.24 (m, 6H), 1.14 (d,  $J = 6.8$  Hz, 3H), 0.89 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  174.1, 172.3, 170.5, 170.3, 101.8, 99.7, 99.5, 79.4, 75.3, 75.2, 72.5, 72.3, 72.0, 71.2, 71.1, 69.9, 68.2, 67.4, 66.7, 66.4, 34.3, 32.0, 31.5, 29.6, 29.5, 29.4, 26.3, 24.7, 22.9, 22.5, 21.3, 21.2, 21.1, 17.62, 17.63, 17.3, 14.3, 14.1; HRMS (ESI) calcd for  $[\text{C}_{38}\text{H}_{64}\text{O}_{17} + \text{H}]^+$ : 793.4222, Found: 793.4226.

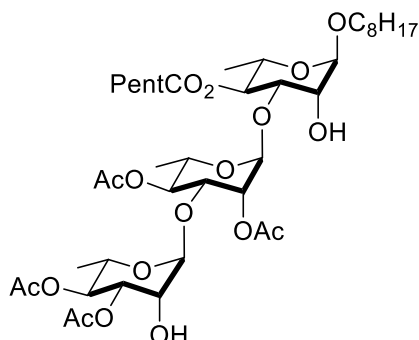
#### Mezzettiaside-2 (2):



Data:  $R_f$  (70% EtOAc/hexane) = 0.65;  $[\alpha]_D^{25} = -30.2$  ( $c = 0.25$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3453, 2956, 2927, 2859, 1745, 1462, 1452, 1378, 1234, 1137, 1076, 986,

832, 764;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.08 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.06 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.97 (dd,  $J = 3.2, 1.6$  Hz, 1H), 4.93 (s, 1H), 4.87 (dd,  $J = 3.6, 1.6$  Hz, 1H), 4.86 (s, 1H), 4.83 (dd,  $J = 10.4, 9.6$  Hz, 1H), 4.75 (s, 1H), 4.06 (dd,  $J = 9.6, 3.2$  Hz, 1H), 3.93 (m, 2H), 3.91 (dd,  $J = 8.0, 3.6$  Hz, 1H), 3.87 (dd,  $J = 10.4, 3.6$  Hz, 1H), 3.78 (dq,  $J = 9.6, 6.4$  Hz, 2H), 3.67 (ddd,  $J = 9.2, 6.4, 6.4$  Hz, 1H), 3.42 (ddd,  $J = 9.2, 6.4, 6.4$  Hz, 1H), 2.41 (m, 1H), 2.36 (m, 1H), 2.15 (s, 6H), 2.14 (brs, 3H), 2.13 (s, 3H), 1.64 (m, 4H), 1.32 (m, 23H), 0.88 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.2, 171.8, 170.6, 170.5, 170.4, 99.4, 99.2, 78.9, 74.9, 74.5, 73.0, 72.3, 71.9, 71.8, 71.2, 68.4, 68.2, 67.6, 66.9, 66.4, 34.3, 32.0, 31.5, 29.6, 29.5, 29.4, 26.3, 24.7, 22.9, 22.5, 21.2, 21.2, 21.0, 17.6, 17.3, 14.3, 14.1; HRMS (ESI) calcd for  $[\text{C}_{40}\text{H}_{66}\text{O}_{18} + \text{H}]^+$ : 835.4327, Found: 835.4338.

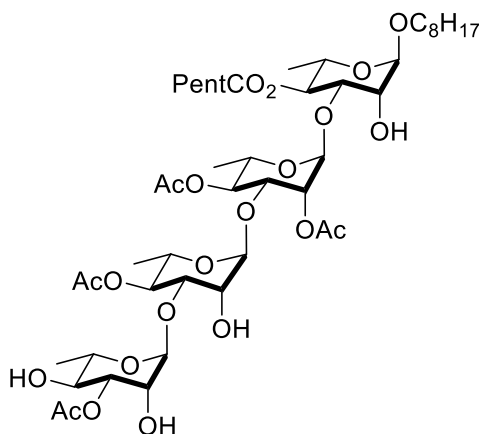
### Mezzettiaside-3 (3):



Data:  $R_f$  (70% EtOAc/hexane) = 0.6;  $[\alpha]_D^{25} = -56.7$  ( $c = 0.44$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3492, 3278, 2925, 2855, 1740, 1372, 1165, 1136, 1074, 1044, 987;  $^1\text{H}$  NMR (500 MHz  $\text{CDCl}_3$ )  $\delta$  5.08 (m, 5H), 4.92 (d,  $J = 2.0$  Hz, 1H), 4.89 (d,  $J = 2.0$  Hz, 1H), 4.76 (d,  $J = 1.5$  Hz, 1H), 4.12 (dd,  $J = 10.0, 3.5$  Hz, 1H), 3.96 (m, 3H), 3.92 (dd,  $J = 10.0, 3.0$  Hz, 1H), 3.83 (dq,  $J = 10.0, 6.5$  Hz, 2H), 3.67 (ddd,  $J = 9.5, 6.5, 3.0$  Hz, 1H), 3.43 (ddd,  $J = 10.0, 6.5, 3.0$  Hz, 1H), 2.45 (m, 1H), 2.35 (m, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.65 (m, 4H), 1.32 (m, 17H), 1.20 (d,  $J = 6.0$  Hz, 3H), 1.19 (d,  $J = 6.0$  Hz, 3H), 0.88 (br, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.4, 170.5, 170.4, 170.2, 170.1, 101.1, 99.4, 79.1, 74.7, 72.6, 71.9, 71.6, 71.55, 71.2, 71.2, 69.7, 68.2, 67.4, 67.3, 66.4, 34.3, 32.0, 31.5, 29.5, 29.4, 26.3, 24.7, 22.9, 22.5, 21.2,

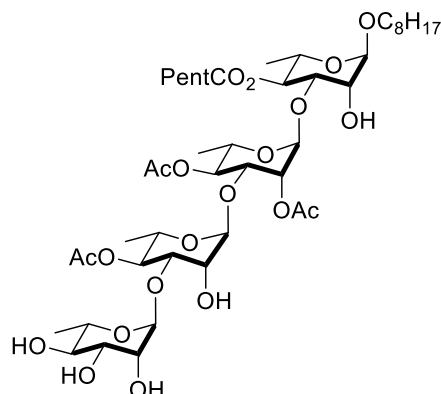
21.1, 21.1, 17.6, 17.4, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>40</sub>H<sub>66</sub>O<sub>18</sub> + Na]<sup>+</sup>; 857.4141, Found, 857.4164.

**Mezzattiaside-5 (5):**



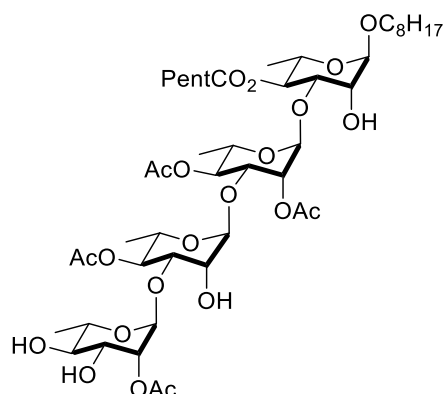
Data:  $R_f$ (100% EtOAc) = 0.5;  $[\alpha]_D^{25} = -69.3$  ( $c = 0.35$ , CHCl<sub>3</sub>); IR (thin film, cm<sup>-1</sup>) 3455, 3338, 2958, 1741, 1264, 1044, 733, 704; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.12 (br, 1H), 5.07 (dd,  $J = 9.6, 9.6$  Hz, 2H), 5.02 (dd,  $J = 10.4, 10.4$  Hz, 1H), 5.01 (dd,  $J = 9.6, 2.4$  Hz, 1H), 5.00 (d,  $J = 1.5$  Hz, 1H), 4.88 (s, 1H), 4.84 (s, 1H), 4.81 (s, 1H), 4.24 (d,  $J = 9.6, 3.2$  Hz, 1H), 3.96 (brm, 3H), 3.90 (m, 3H), 3.86 (dd,  $J = 9.6, 2.4$  Hz, 1H), 3.80 (dq,  $J = 9.6, 6.4$  Hz, 1H), 3.72 (dq,  $J = 9.6, 6.4$  Hz, 1H), 3.66 (ddd,  $J = 9.6, 6.4, 6.4$  Hz, 1H), 3.57 (q,  $J = 9.6$  Hz, 1H), 3.43 (ddd,  $J = 9.6, 6.4, 6.4$  Hz, 1H), 3.14 (br, 2H), 2.47 (m, 1H), 2.39 (m, 1H), 2.16 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.63 (m, 4H), 1.36 (m, 6H), 1.29 (m, 12H), 1.19 (m, 6H), 1.10 (d,  $J = 6.0$  Hz, 3H), 0.88 (br, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  174.3, 172.0, 170.6, 170.3, 101.8, 100.9, 100.0, 99.4, 79.8, 75.9, 74.4, 72.6, 72.3, 72.2, 72.2, 71.6, 71.2, 71.0, 70.1, 69.6, 68.2, 67.3, 67.2, 66.3, 34.3, 32.0, 31.5, 29.6, 29.54, 29.45, 26.3, 24.7, 22.9, 22.5, 21.4, 21.3, 21.1, 21.0, 17.8, 17.7, 17.6, 17.3, 14.3, 14.1; HRMS–MALDI–TOF (CCA) (m/z): [M + Na]<sup>+</sup> calcd for [C<sub>46</sub>H<sub>76</sub>O<sub>22</sub> + Na]<sup>+</sup>: 1003.4720, Found: 1003.4719.

**Mezzattiaside-7 (7):**



Data:  $R_f$ (50% EtOAc/hexane) = 0.60;  $[\alpha]_D^{25} = -55.6$  ( $c = 0.47$ , CHCl<sub>3</sub>); IR (thin film, cm<sup>-1</sup>) 3435, 2927, 1741, 1450, 1375, 1264, 1045, 896, 732, 703; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD + C<sub>6</sub>D<sub>6</sub> (6:1))  $\delta$  5.33 (dd,  $J = 9.5, 9.5$  Hz, 1H), 5.25 (dd,  $J = 3.5, 2.0$  Hz, 1H), 5.23 (dd,  $J = 10.5, 9.5$  Hz, 1H), 5.23 (dd,  $J = 10.5, 9.5$  Hz, 1H), 4.99 (s, 1H), 4.98 (d,  $J = 1.5$  Hz, 1H), 4.96 (d,  $J = 1.0$  Hz, 1H), 4.85 (d,  $J = 1.0$  Hz, 1H), 4.44 (dd,  $J = 9.5, 3.5$  Hz, 1H), 4.27 (m, 1H), 4.08 (m, 1H), 4.07 (m, 1H), 4.00 (m, 1H), 3.97 (dq,  $J = 9.5, 6.5$  Hz, 1H), 3.94-3.91 (m, 4H), 3.90 (m, 1H), 3.81 (ddd,  $J = 9.5, 7.0, 3.0$  Hz, 1H), 3.59 (dd,  $J = 9.5, 9.5$  Hz, 1H), 3.53 (ddd,  $J = 9.5, 6.5, 3.5$  Hz, 1H), 2.65 (m, 1H), 2.52 (m, 1H), 2.20 (s, 3H), 2.19 (s, 3H), 2.16 (s, 3H), 1.77 (m, 4H), 1.45 (m, 17H), 1.31 (m, 6H), 1.28 (d,  $J = 6.0$  Hz, 3H), 1.02 (m, 6H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD + C<sub>6</sub>D<sub>6</sub> (6:1))  $\delta$  173.9, 171.1, 170.9, 170.8, 103.3, 102.8, 100.7, 100.2, 78.9, 76.9, 75.4, 73.2, 73.2, 73.1, 72.8, 72.4, 71.6, 71.4, 71.3, 69.6, 68.1, 68.0, 67.4, 67.2, 34.3, 32.2, 31.7, 29.7, 29.7, 26.6, 24.9, 23.0, 22.7, 20.2, 20.2, 20.2, 17.3, 17.2, 17.1, 17.0, 13.8, 13.7; HRMS–MALDI-TOF (CCA) (m/z):  $[M + Na]^+$  calcd for  $[C_{44}H_{74}O_{21} + Na]^+$ : 961.4615, Found: 961.4645.

**Mezzattiaside-6 (6):**



Data:  $R_f$  (5% MeOH/  $\text{CH}_2\text{Cl}_2$ ) = 0.5;  $[\alpha]_D^{25} = -37.6$  ( $c = 0.22$ ,  $\text{CHCl}_3$ ); IR (thin film,  $\text{cm}^{-1}$ ) 3435, 2929, 2854, 1740, 1374, 1265, 1230, 1136, 1075, 1045, 733, 703;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.05 (dd,  $J = 9.6, 9.6$  Hz, 1H), 5.03 (dd,  $J = 9.6, 9.6$  Hz, 1H), 4.97 (br, 1H), 4.93 (dd,  $J = 10.4, 8.8$  Hz, 1H), 4.92 (br, 1H), 4.86 (brs, 1H), 4.82 (br, 1H), 4.77 (brs, 2H), 4.18 (d,  $J = 9.6$  Hz, 1H), 4.14 (d,  $J = 8.8$  Hz, 1H), 3.94 (m, 2H), 3.91 (d,  $J = 10.4$  Hz, 1H), 3.77 (m, 3H) 3.69-3.61 (m, 3H), 3.53 (dd,  $J = 9.6, 8.8$  Hz, 1H), 3.42 (ddd,  $J = 9.6, 6.4, 6.4$  Hz, 1H), 2.47 (m, 1H), 2.37 (m, 1H), 2.14 (s, 6H), 2.12 (s, 3H), 2.08 (s, 3H), 1.63 (m, 4H), 1.30 (m, 17H), 1.19 (s, 6H), 1.12 (d,  $J = 6.0$  Hz, 3H), 0.89 (m, 6H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  173.7, 171.7, 171.4, 170.6, 170.0, 101.9, 99.85, 99.64, 99.4, 79.2, 78.4, 75.2, 73.2, 72.7, 72.6, 72.3, 72.1, 72.0, 71.3, 71.1, 69.04, 69.0, 68.2, 67.4, 67.3, 66.4, 34.3, 32.0, 31.5, 29.6, 29.5, 29.4, 26.3, 24.7, 22.9, 22.5, 21.4, 21.3, 21.1, 21.0, 17.7, 17.6, 17.5, 17.3, 14.3, 14.1; HRMS–MALDI-TOF (CCA) (m/z):  $[\text{M} + \text{Na}]^+$  calcd for  $[\text{C}_{46}\text{H}_{76}\text{O}_{22} + \text{Na}]^+$ : 1003.4720, Found: 1003.4716.

## Section C: Biological Procedure

### Antibacterial Activity MIC Assays<sup>1</sup>

The five strains: *E. coli* (*imp*-4213, *zab4292::Tn5*)<sup>2</sup>, methicillin-sensitive *Staphylococcus aureus* (MSSA, HG003), methicillin-resistant *Staphylococcus aureus* (MRSA, MW2), *E. faecium* and *E. faecalis* bacterium were obtained from Prof. Kim Lewis, Department of Biology, Northeastern University. The *B. subtilis* strain (JH642, *trpC2 pheA1*)<sup>3</sup> was obtained from Prof. Alan D. Grossman, Department of Biology, Massachusetts Institute of Technology.

*Preparation of inoculum:* The Gram-(+) *B. subtilis* was cultured in liquid Luria Broth (LB) and Gram-(+)-like *imp* bacteria was cultured in liquid LB containing Kanamycin at 50 µg/mL. *S. aureus* were cultured in liquid Mueller Hinton Broth(MHB), *E. faecium* and *E. faecalis* were cultured in liquid Brain Heart Infusion(BHI).

Strains were cultured overnight in liquid medium and further diluted 10-fold into 5 mL fresh liquid medium. Cultures were incubated with shaking at 37 °C for 45 min to 2 h to obtain the cell population (approx. 10<sup>8</sup> CFU/mL) with desired optimal optical density (OD<sub>600</sub>). The cultures were further diluted approximately 1000-fold (10<sup>5</sup> CFU/mL) into fresh liquid LB/MHB/BHI medium and kept at room temperature until needed.

*Preparation of Broth Macrodilution:* The target compound stock solutions were prepared at 25 mM in dimethyl sulfoxide (DMSO). Working solutions were prepared

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<sup>1</sup> The assay was performed by the broth dilution method described by the National Committee for Clinical Laboratory Standard Methods (M7-A6, 2003)

<sup>2</sup> Sampson, B. A.; Misr, R.; Benson, S. A. *Genetics*, **1989**, *122*, 491–501.

<sup>3</sup> Perego, M.; Spiegelman, G. B.; Hoch, J. A. *Molecular Microbiology*, **1988**, *2*, 689-699

in liquid medium with the tested compounds at 4X, where X is the final concentration desired. To each well of a 96- well microtiter plate was dispensed 100  $\mu$ L of fresh liquid LB. A 100  $\mu$ L aliquot of each tested compound was added to the first well of a row, which was then serially diluted 1:2 to give a series of seven concentrations. Then 100  $\mu$ L of the already prepared culture solution was added to each well. Optical densities were recorded on Biotek synergy HT plate reader at 600 nm absorbance. After 18 – 24 hours, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration at which no visible growth of bacteria was observed.

#### **MTT Colorimetric Assays<sup>4</sup>**

The human lung epithelial cell line NCI-H460 was obtained from the American Type Culture Collection (ATCC, Manassas,VA).

The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum and 2 mM L-glutamine and 100 units/mL penicillin/streptomycin. Cell cultures were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. Cells were passaged at preconfluent densities using a solution containing 0.25% trypsin and 0.5 mM EDTA (Invitrogen). Cells were seeded at a density of 5,000 cell/well in a 96 well plate for 24 hours. Drugs were pre-dissolved in DMSO. The resulting solutions were diluted with RPMI Serum Free Media (SFM) culture by 100 times, and added to the wells in increasing concentration by 10-fold dilution, with untreated wells as controls. Control experiments showed that 0.1% DMSO had no effect on cell growth (non-cytotoxicity), each dose need to ensure DMSO concentration less than 0.1% after apply to each well of microtiter plate. After treatment, cells were incubated for a period of 48 h, and subjected to a solution of 3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT,

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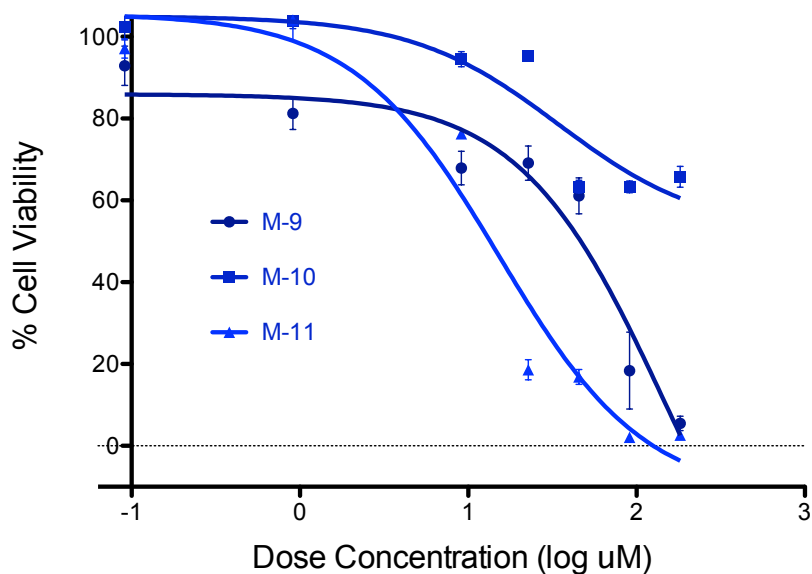
<sup>4</sup> (a) Mosmann, T. J. *Immunological Methods*, **1983**, *65*, 55-63. (b) Chanvorachote, P.; Nimmannit, U.; Stehlik, C.; Wang, L.; Jiang, B.-H.; Ongpipatanakul, B.; Rojanasakul, Y. *Cancer Res.*, **2006**, *66*, 6353-6360



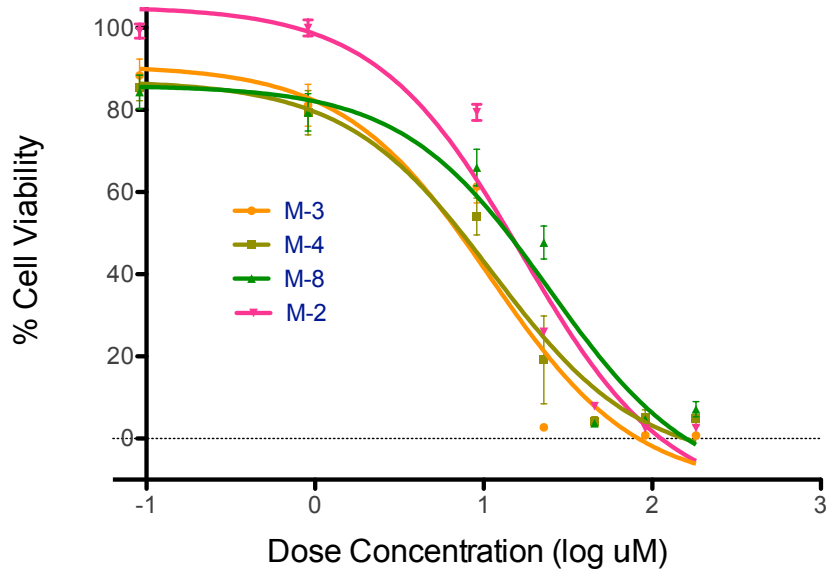
5mg/mL), 10  $\mu$ L/well solution in deionized water. After incubation at 37 °C for 4 h, the culture medium was removed, and the cells were suspended in DMSO. Followed by solubilizing the resulting formazan salt with DMSO for at least 15 min.

The viability was measured by a Gen5 Fluorescence (Biotek synergy HT) plate reader at 570 nm. The dose-dependent experiment was performed in 2 replicate wells of each compound for a single concentration with at least three experimental runs (N = 6). IC<sub>50</sub> values (calculated as the dose necessary to cause a 50% reduction in cell viability compared to untreated control cells) were analyzed by using GraphPad Prism. GraphPad Software, San Diego California USA.

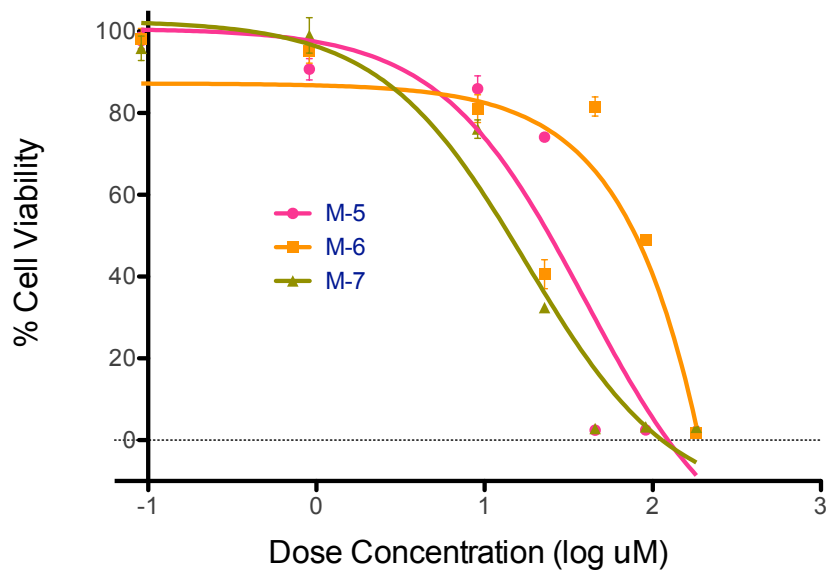
### Cytotoxicity of Mezzettiasides 9-11 against H460



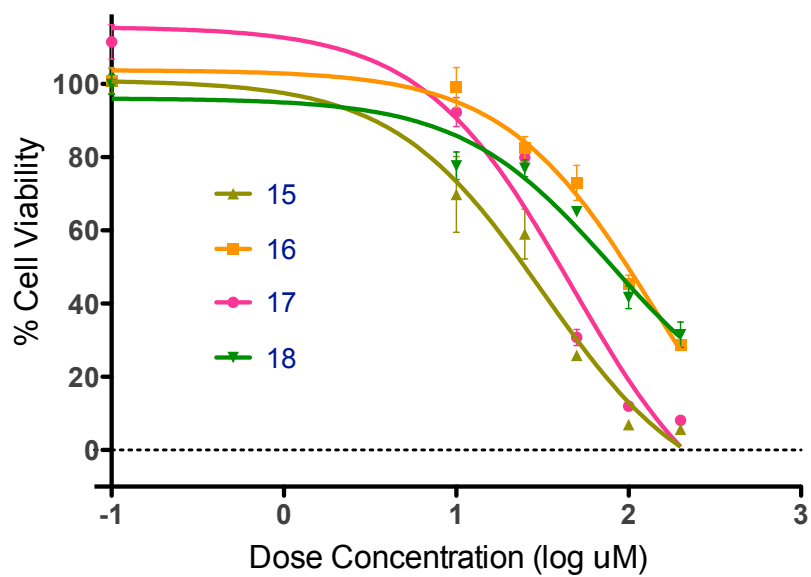
### Cytotoxicity of Mezzettiasides 2-4 & 8 against H460

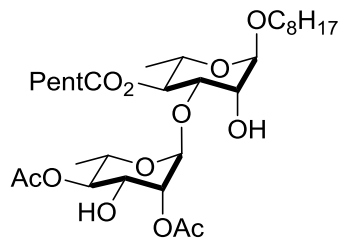


### Cytotoxicity of Mezzettiasides 5-7 against H460

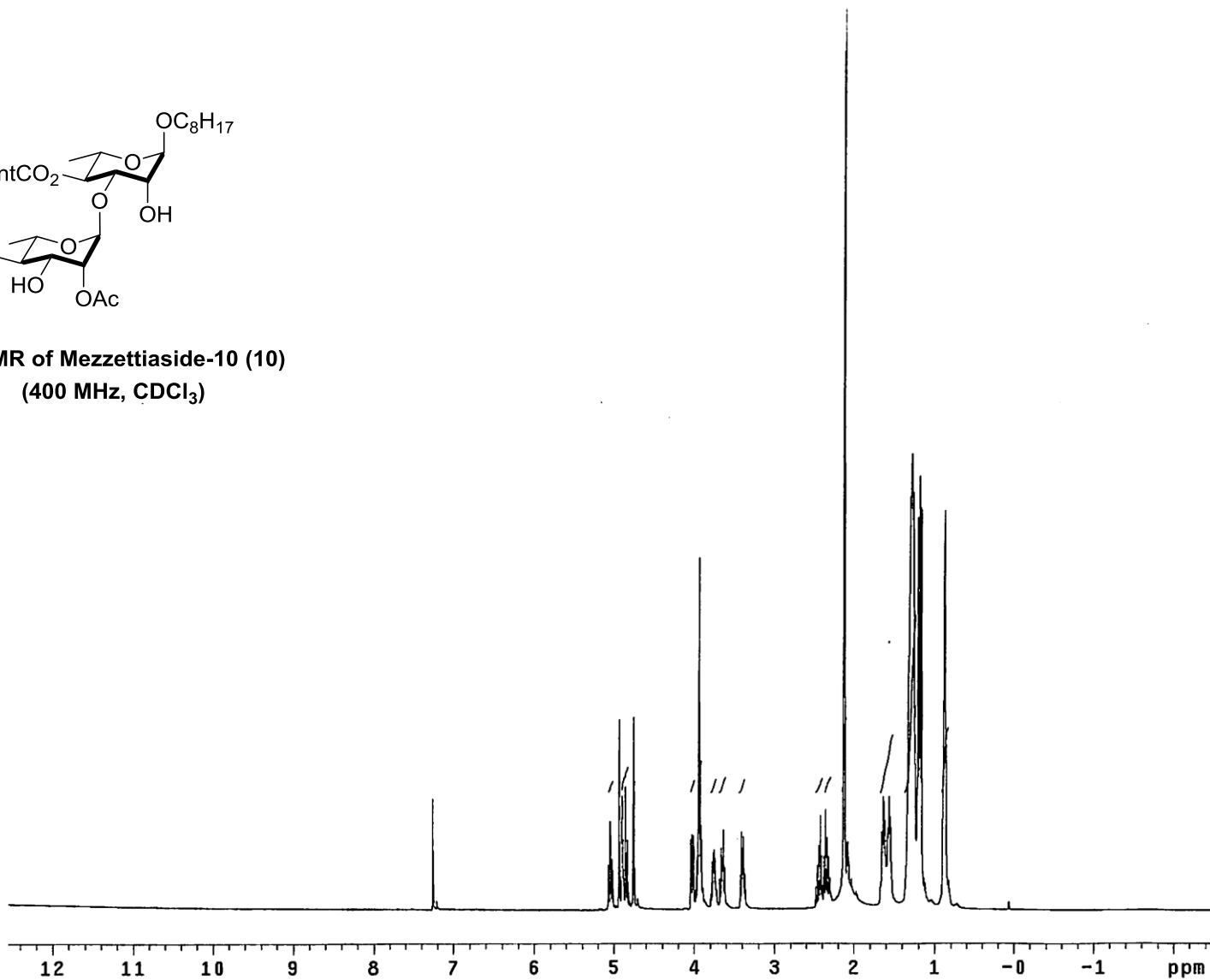


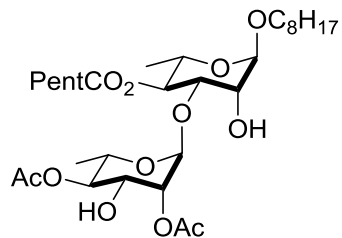
### Cytotoxicity of Mezzettiasides analogues 15-18 against H460



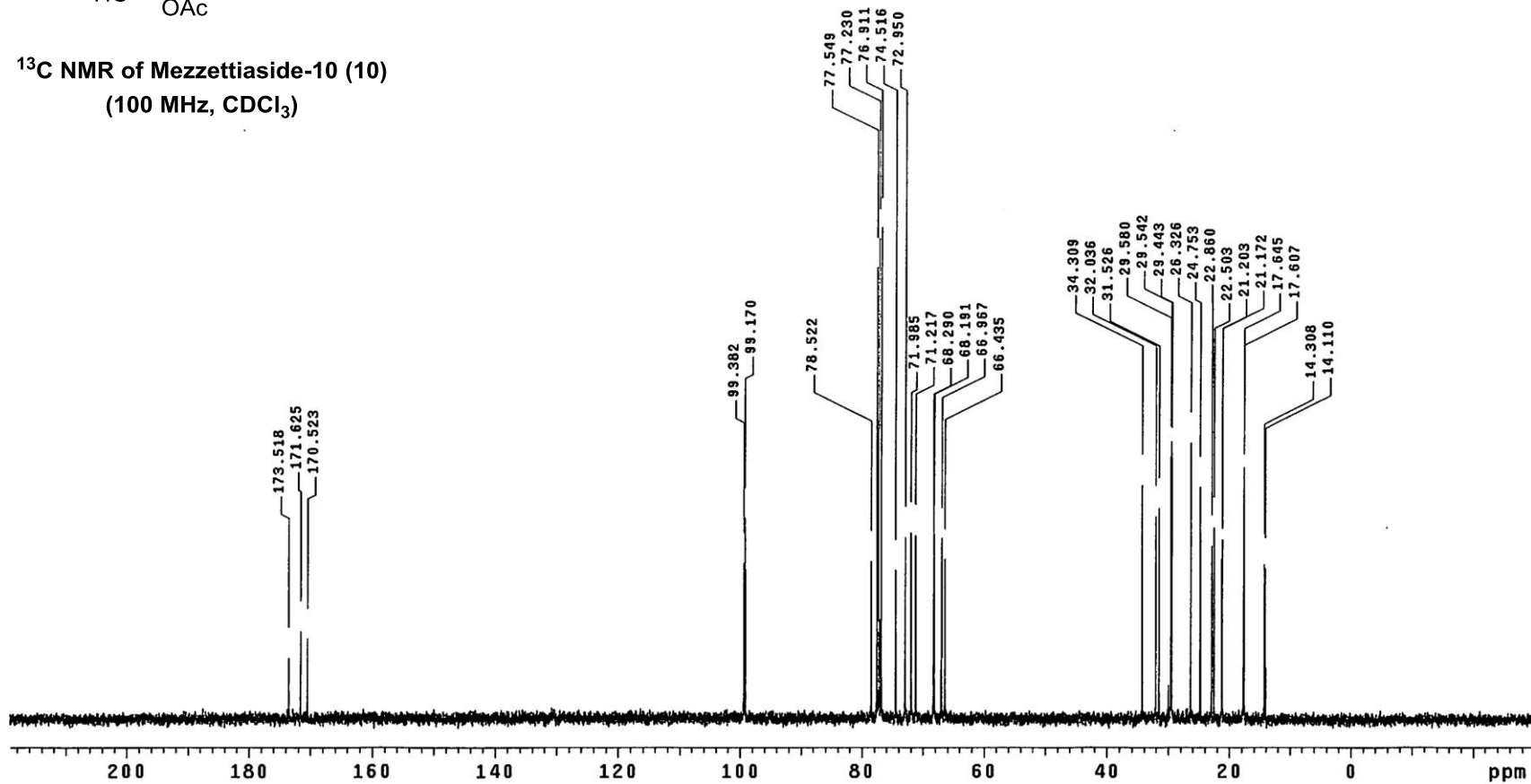


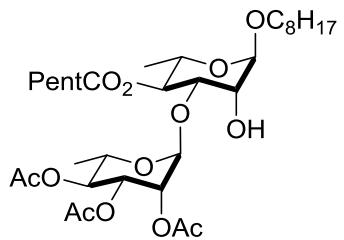
**<sup>1</sup>H NMR of Mezzettiaside-10 (10)**  
**(400 MHz, CDCl<sub>3</sub>)**



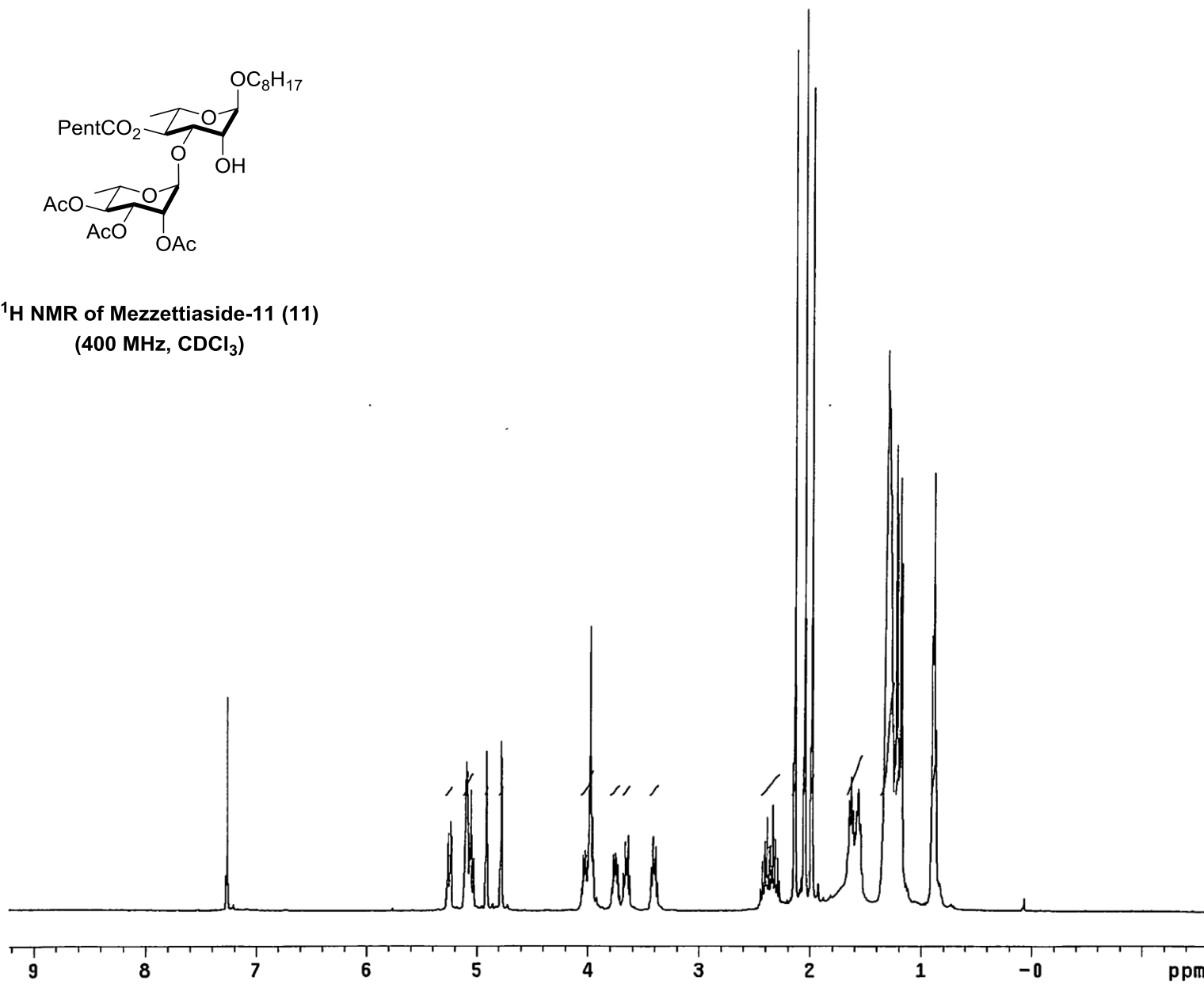


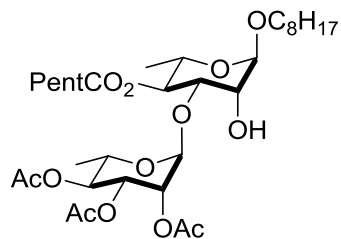
<sup>13</sup>C NMR of Mezzettiaside-10 (10)  
(100 MHz, CDCl<sub>3</sub>)



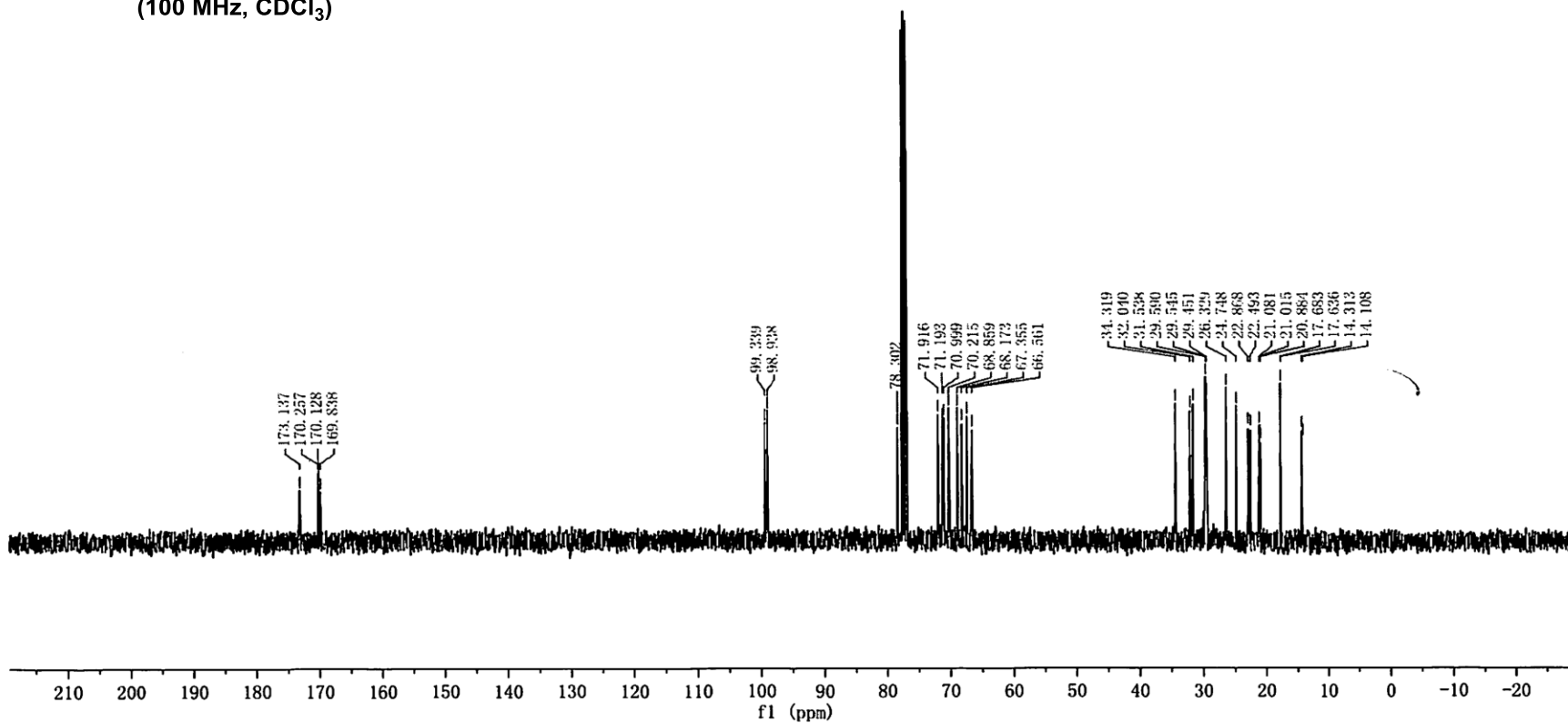


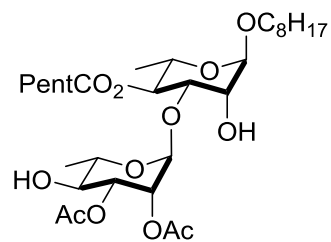
**<sup>1</sup>H NMR of Mezzettiaside-11 (11)**  
(400 MHz, CDCl<sub>3</sub>)



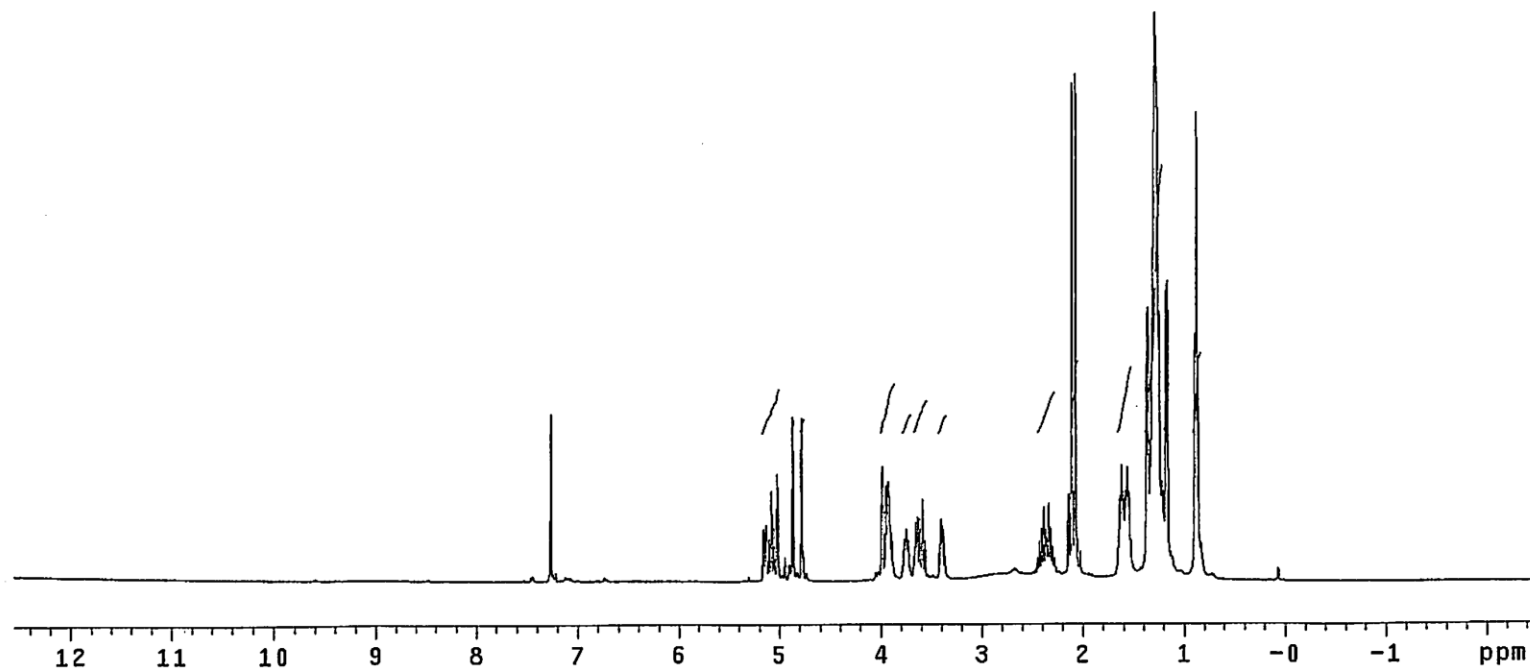


**<sup>13</sup>C NMR of Mezzettiaside-11 (11)**  
 (100 MHz, CDCl<sub>3</sub>)

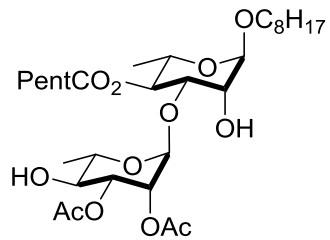




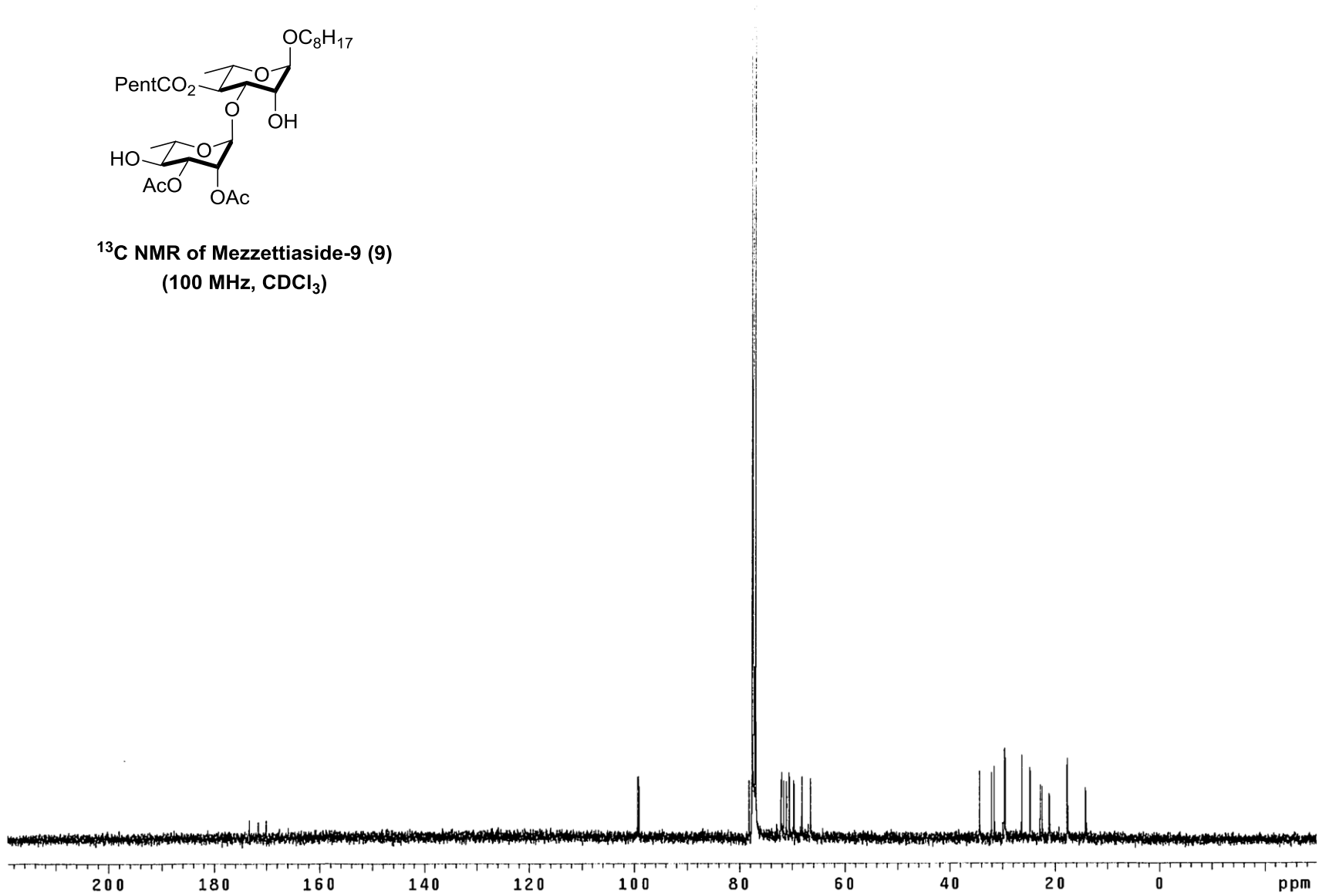
**<sup>1</sup>H NMR of Mezzettiaside-9 (9)**  
**(400 MHz, CDCl<sub>3</sub>)**

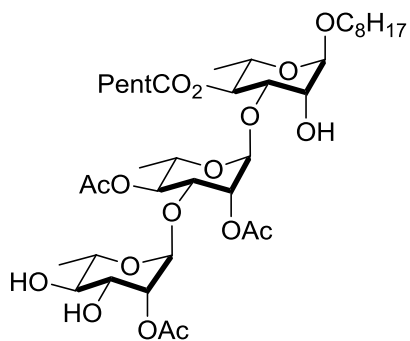




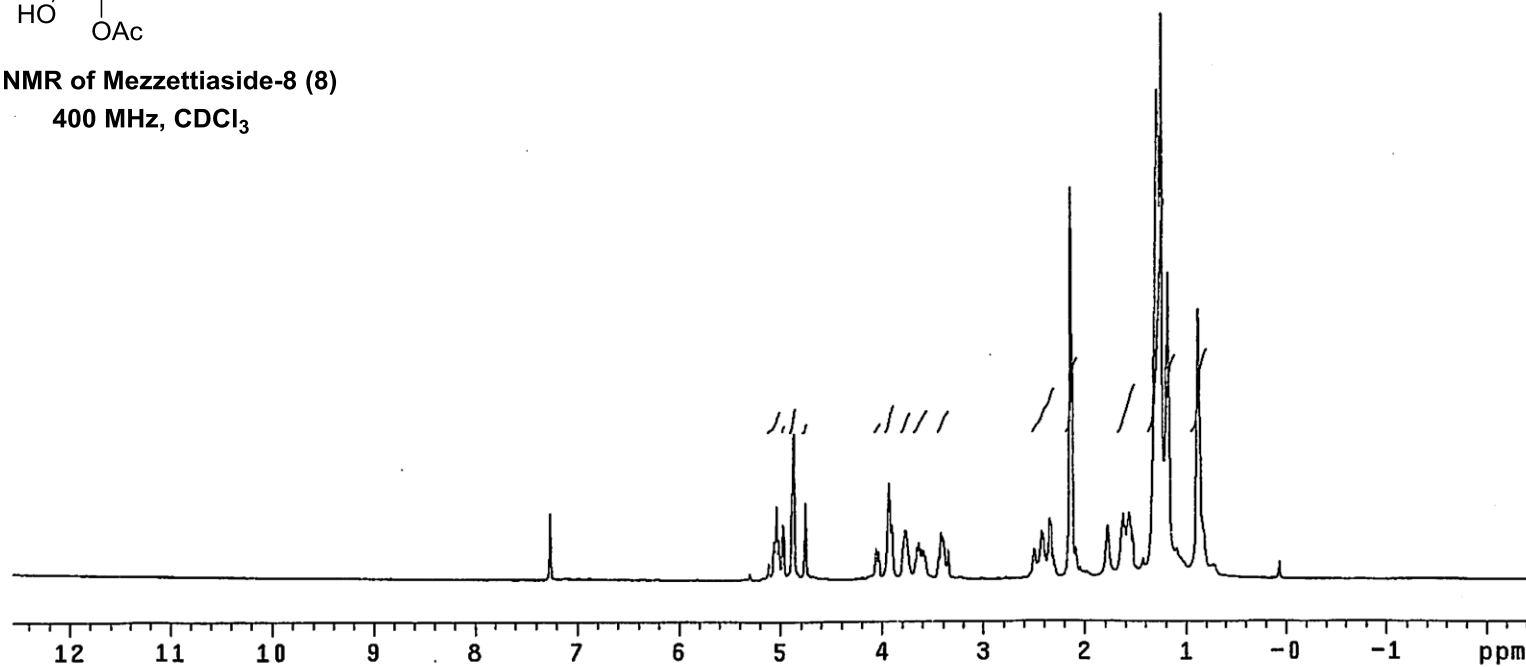


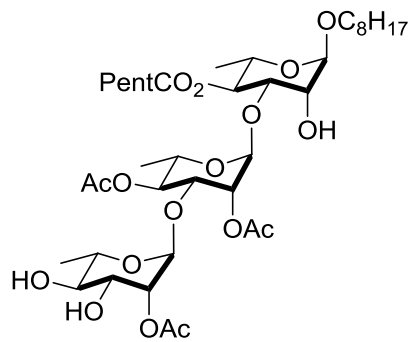
**<sup>13</sup>C NMR of Mezzettiaside-9 (9)**  
**(100 MHz, CDCl<sub>3</sub>)**



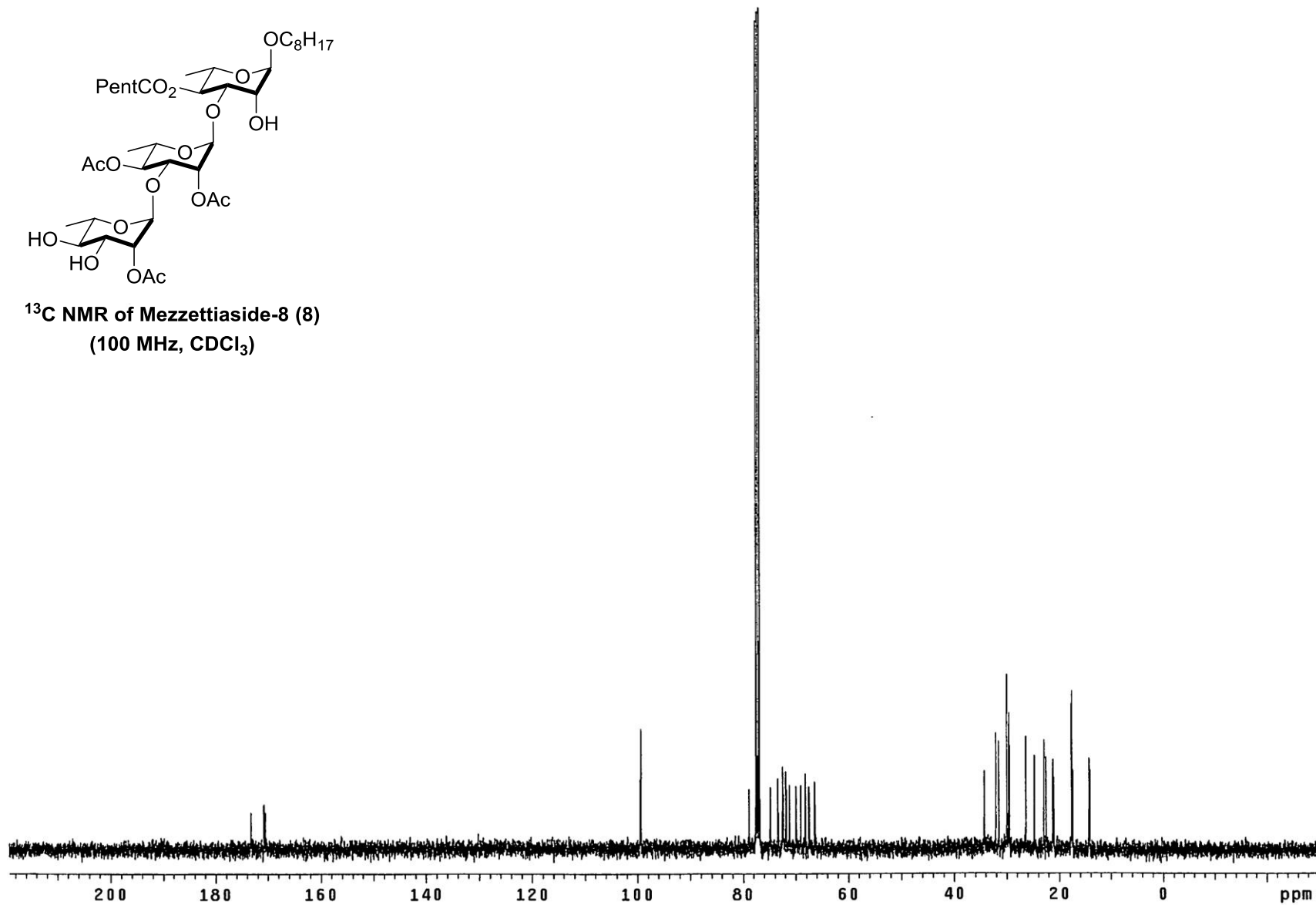


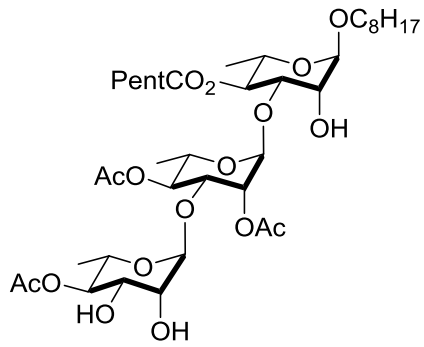
**<sup>1</sup>H NMR of Mezzettiaside-8 (8)**  
400 MHz, CDCl<sub>3</sub>



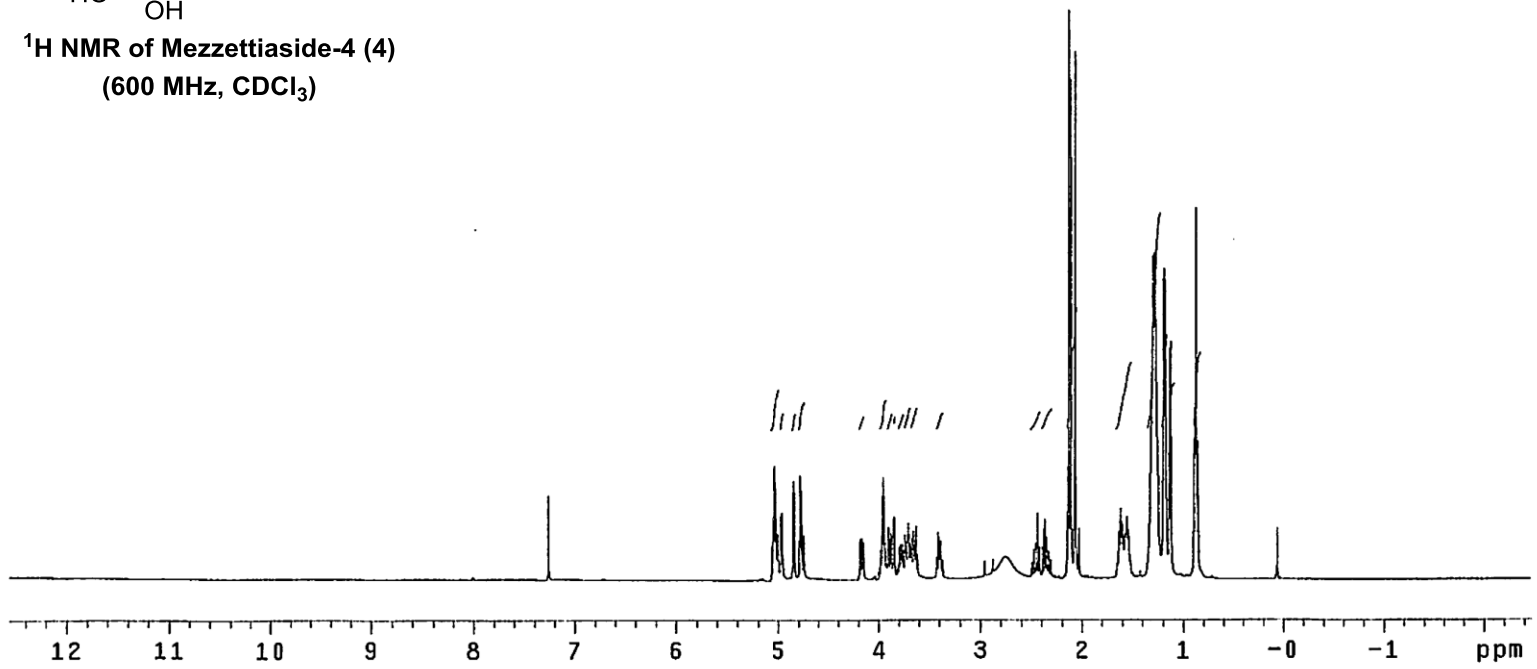


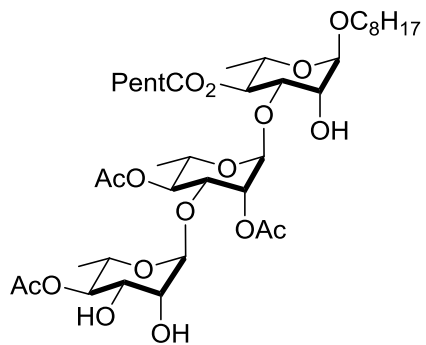
**<sup>13</sup>C NMR of Mezzettiaside-8 (8)**  
**(100 MHz, CDCl<sub>3</sub>)**



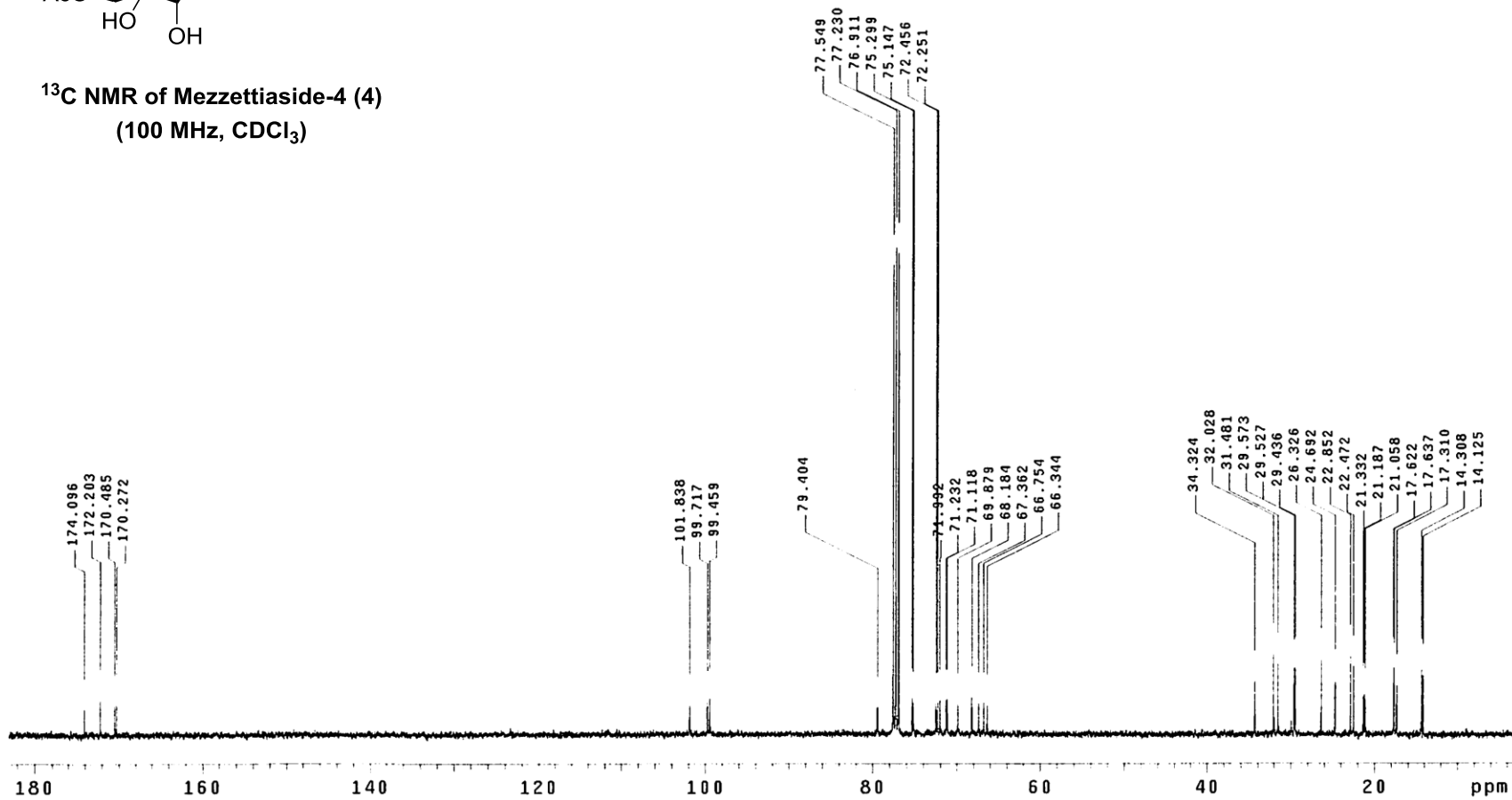


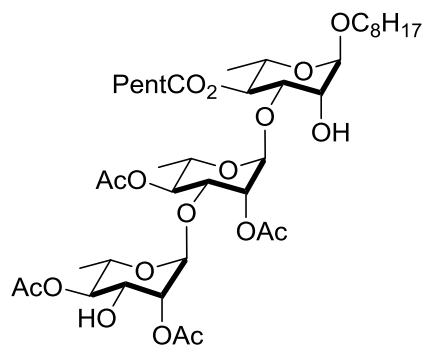
**<sup>1</sup>H NMR of Mezzettiaside-4 (4)**  
**(600 MHz, CDCl<sub>3</sub>)**



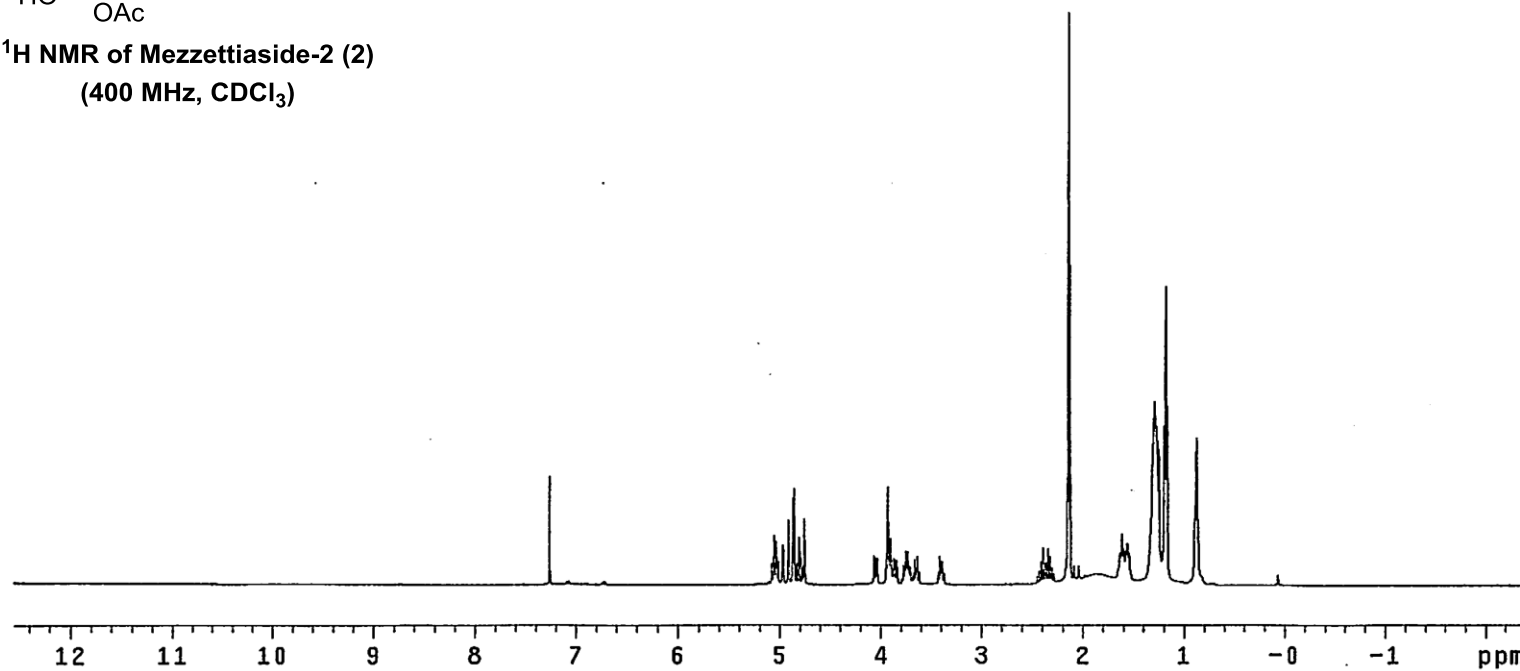


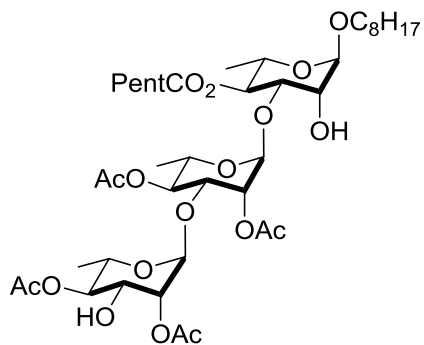
**<sup>13</sup>C NMR of Mezzettiaside-4 (4)**  
 (100 MHz, CDCl<sub>3</sub>)



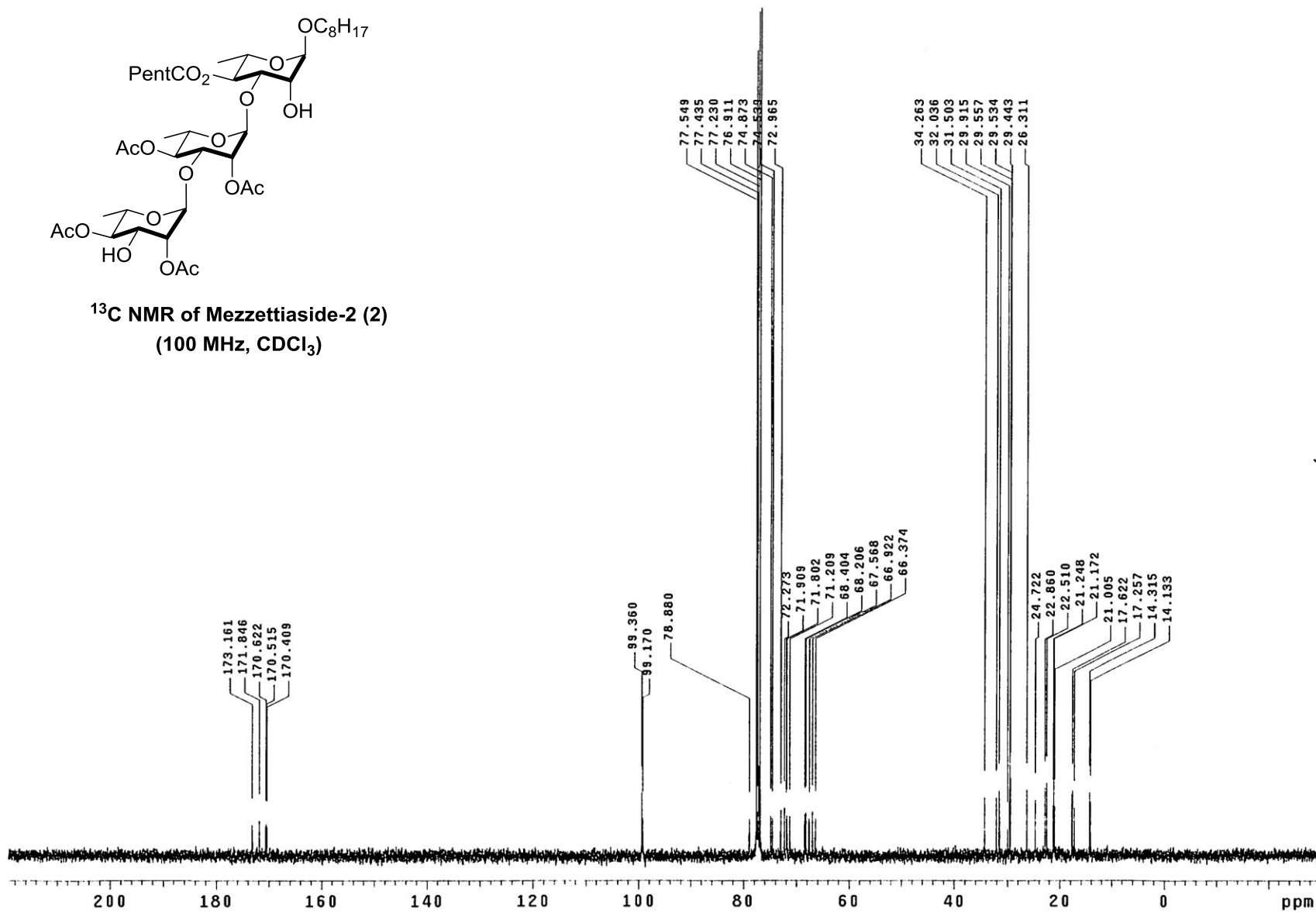


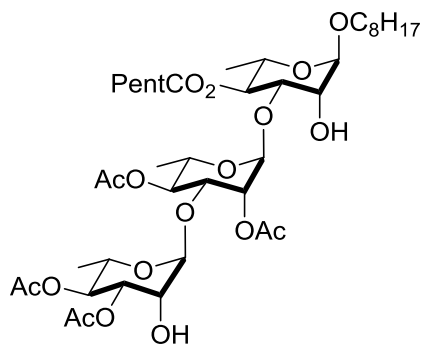
**<sup>1</sup>H NMR of Mezzettiaside-2 (2)**  
(400 MHz, CDCl<sub>3</sub>)



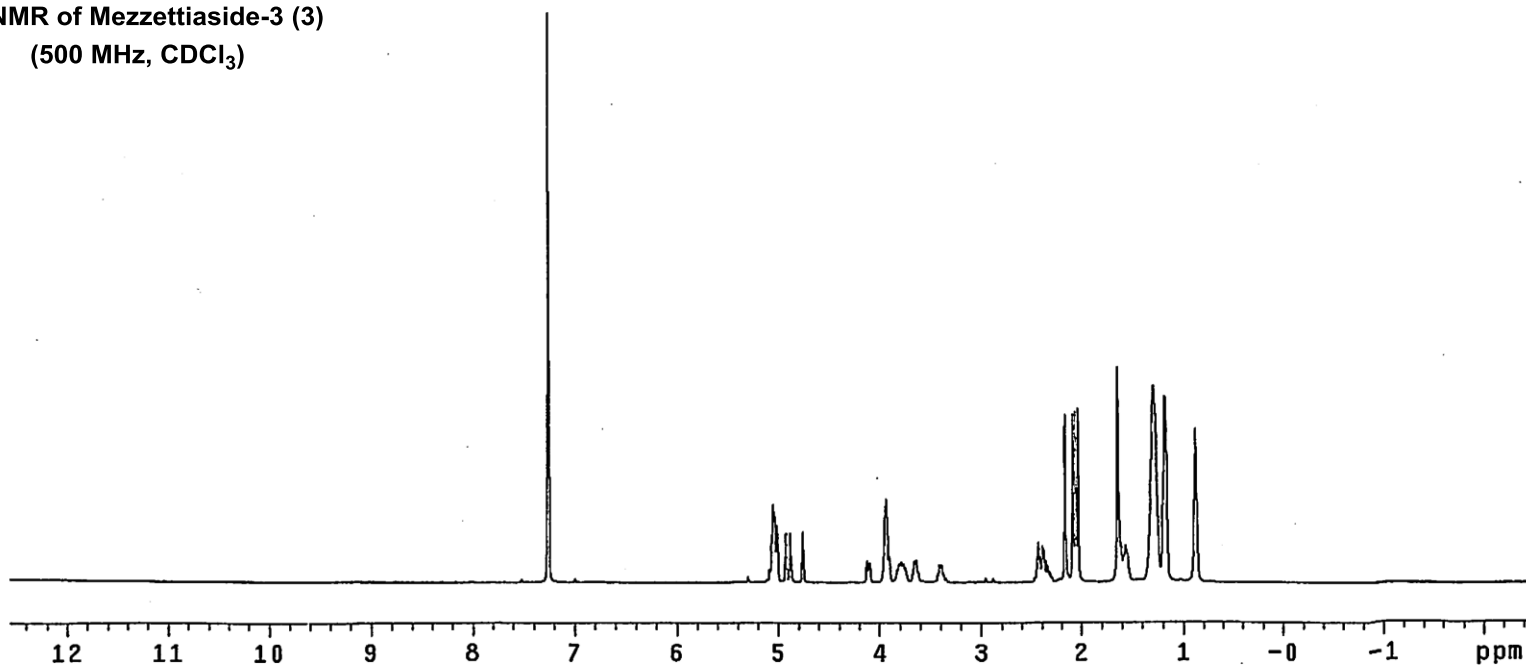


**<sup>13</sup>C NMR of Mezzettiaside-2 (2)**  
 (100 MHz, CDCl<sub>3</sub>)

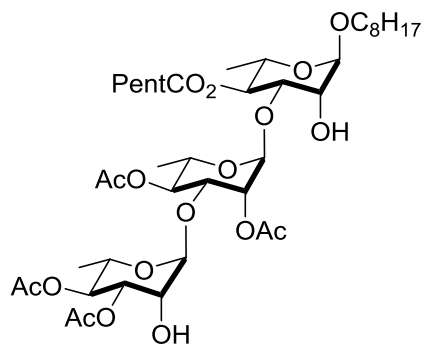




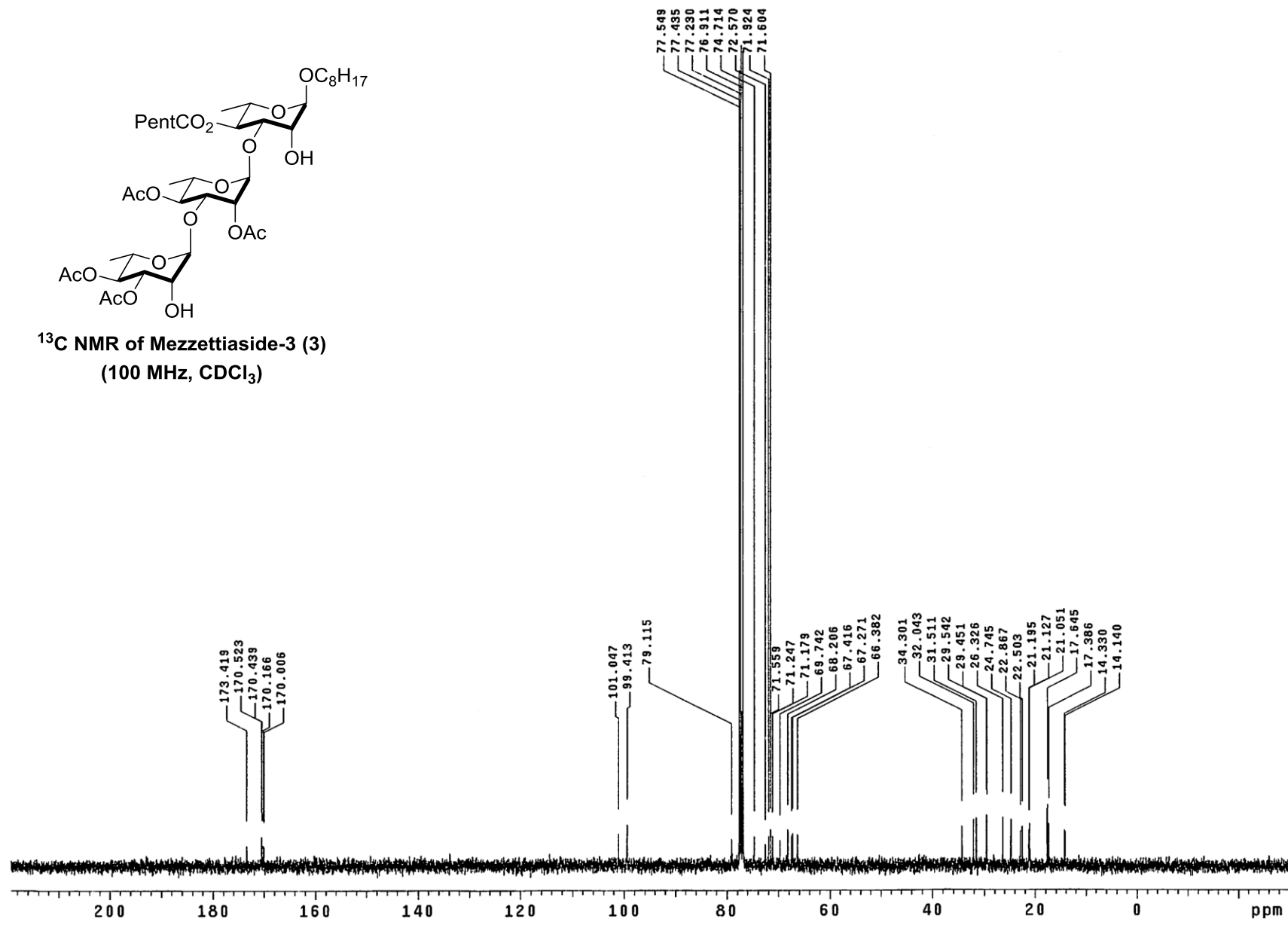
**<sup>1</sup>H NMR of Mezzettiaside-3 (3)**  
(500 MHz, CDCl<sub>3</sub>)

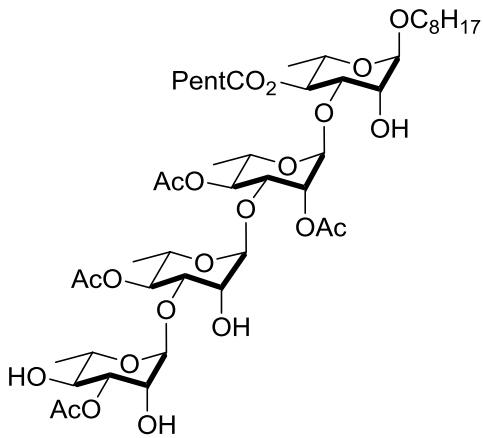




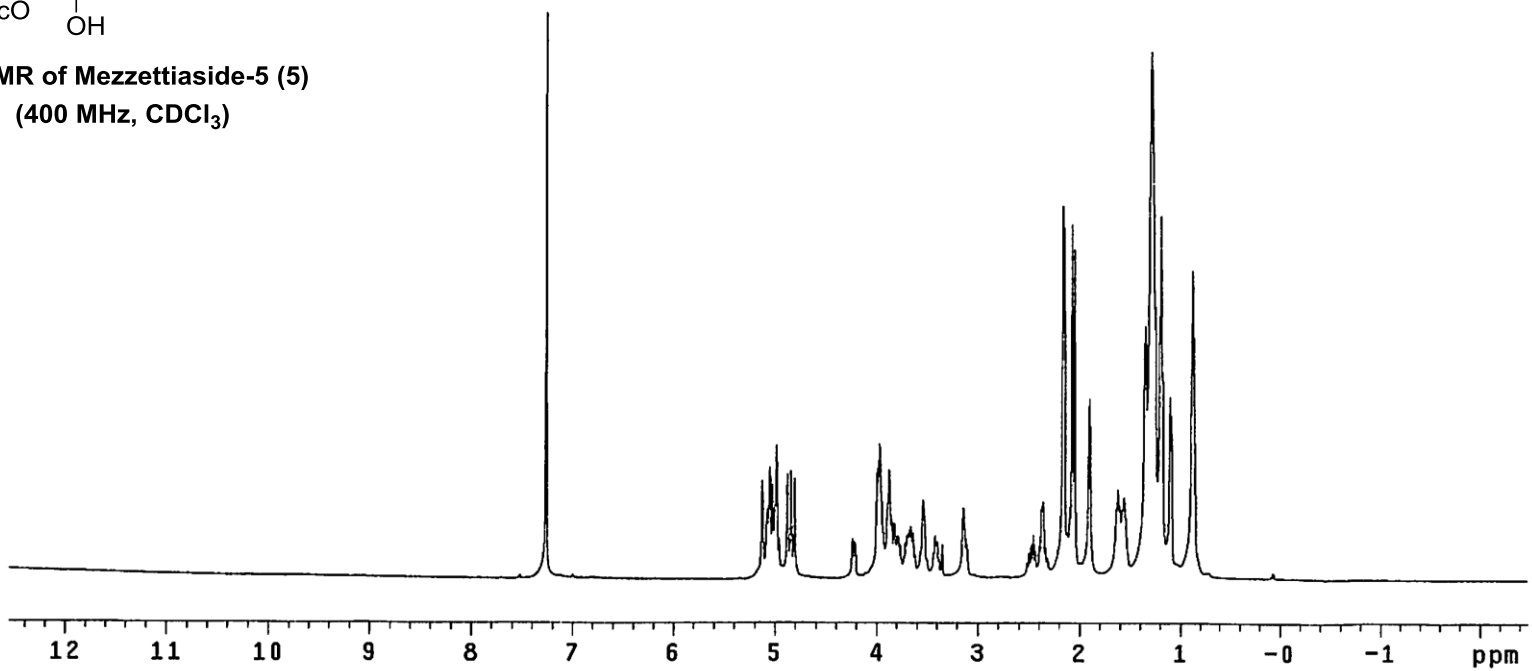


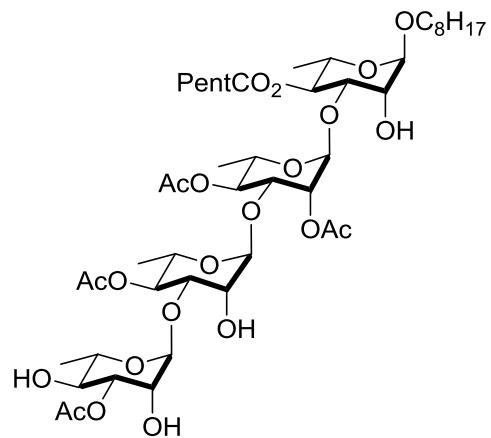
**<sup>13</sup>C NMR of Mezzettiaside-3 (3)**  
(100 MHz, CDCl<sub>3</sub>)



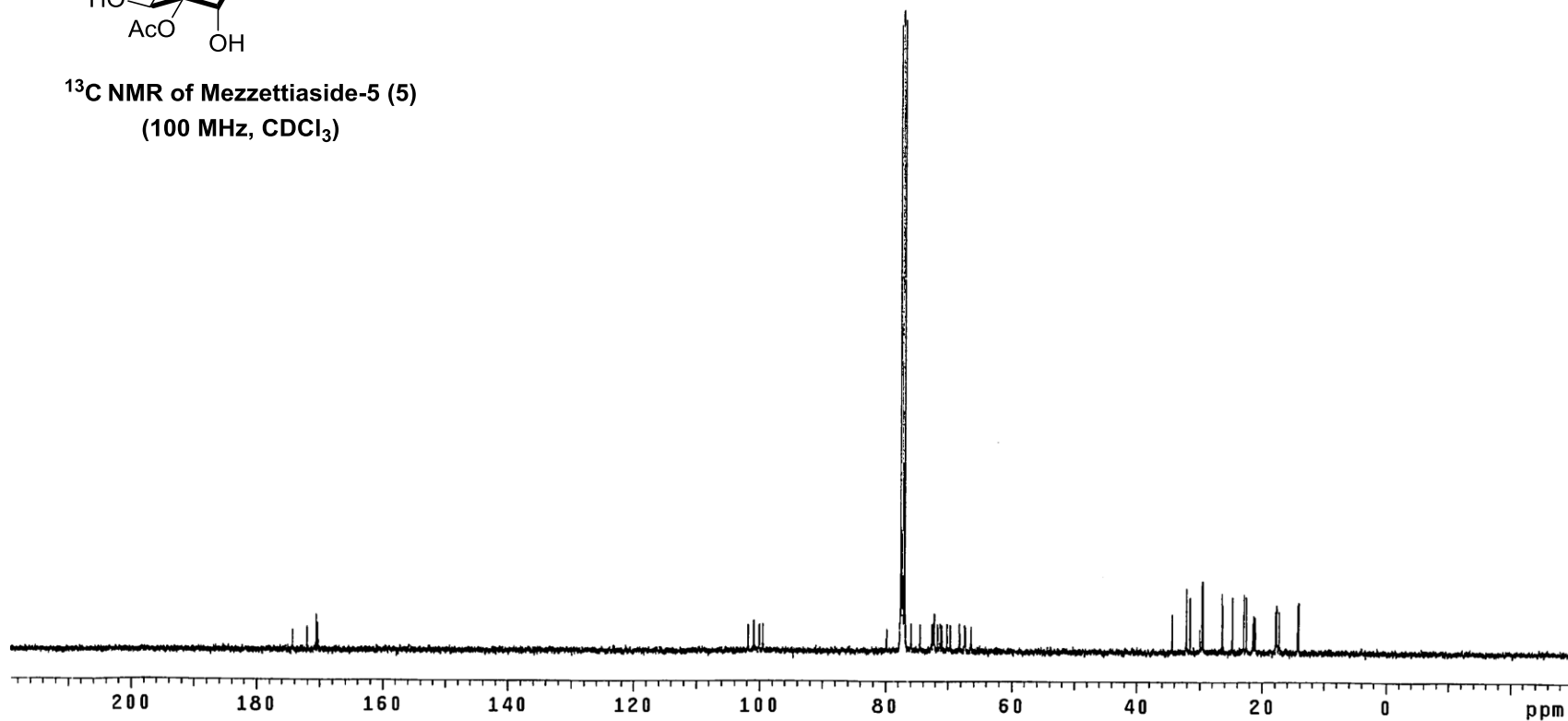


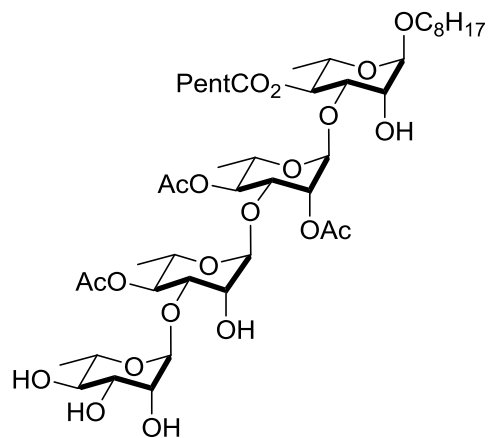
**<sup>1</sup>H NMR of Mezzettiaside-5 (5)**  
**(400 MHz, CDCl<sub>3</sub>)**



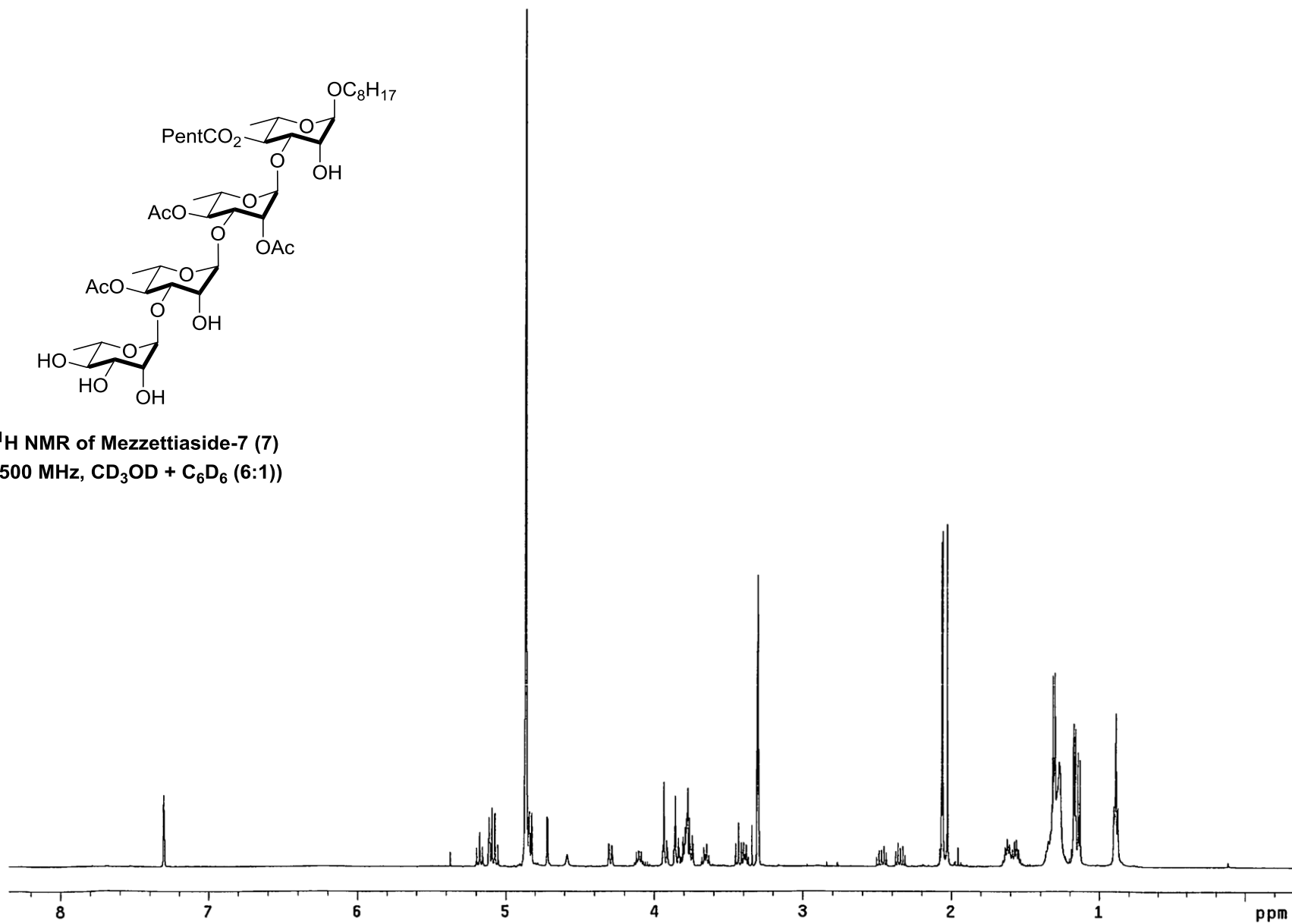


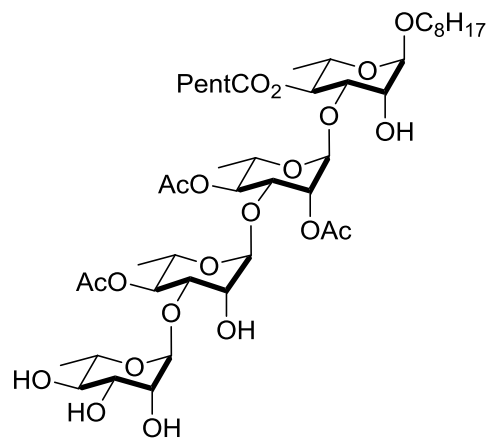
**<sup>13</sup>C NMR of Mezzettiaside-5 (5)**  
**(100 MHz, CDCl<sub>3</sub>)**



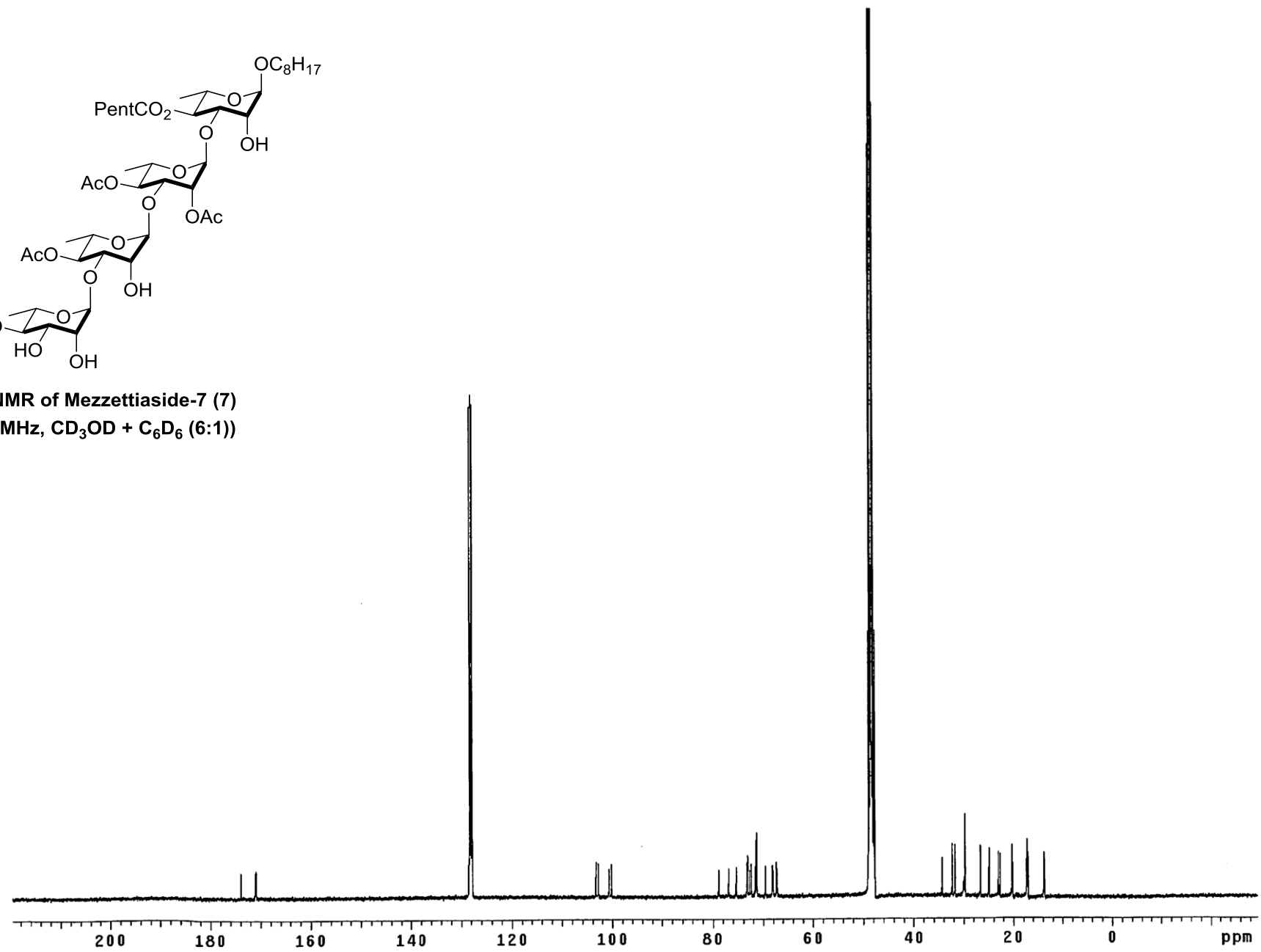


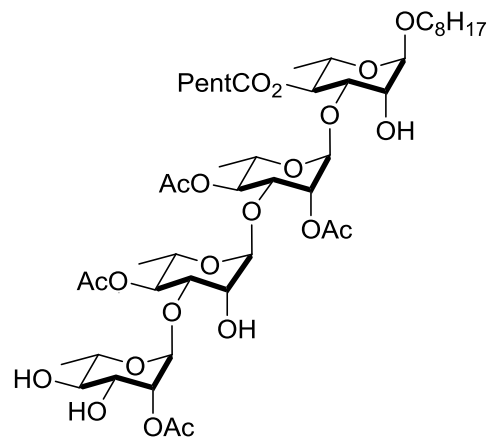
**<sup>1</sup>H NMR of Mezzettiaside-7 (7)**  
**(500 MHz, CD<sub>3</sub>OD + C<sub>6</sub>D<sub>6</sub> (6:1))**



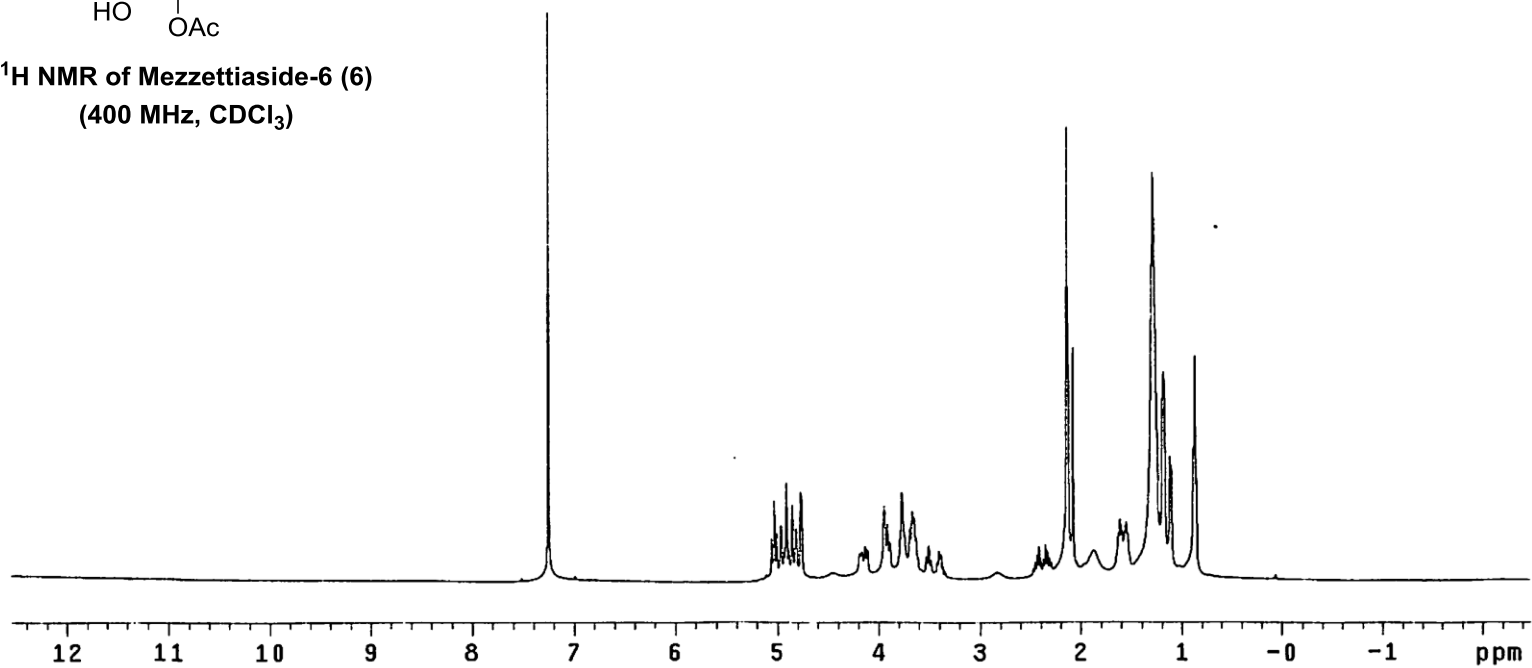


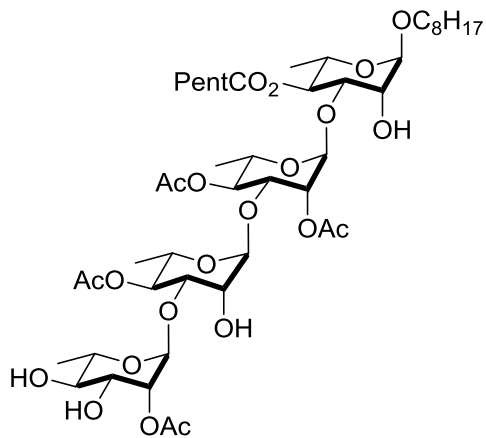
**<sup>13</sup>C NMR of Mezzettiaside-7 (7)**  
**(100 MHz, CD<sub>3</sub>OD + C<sub>6</sub>D<sub>6</sub> (6:1))**



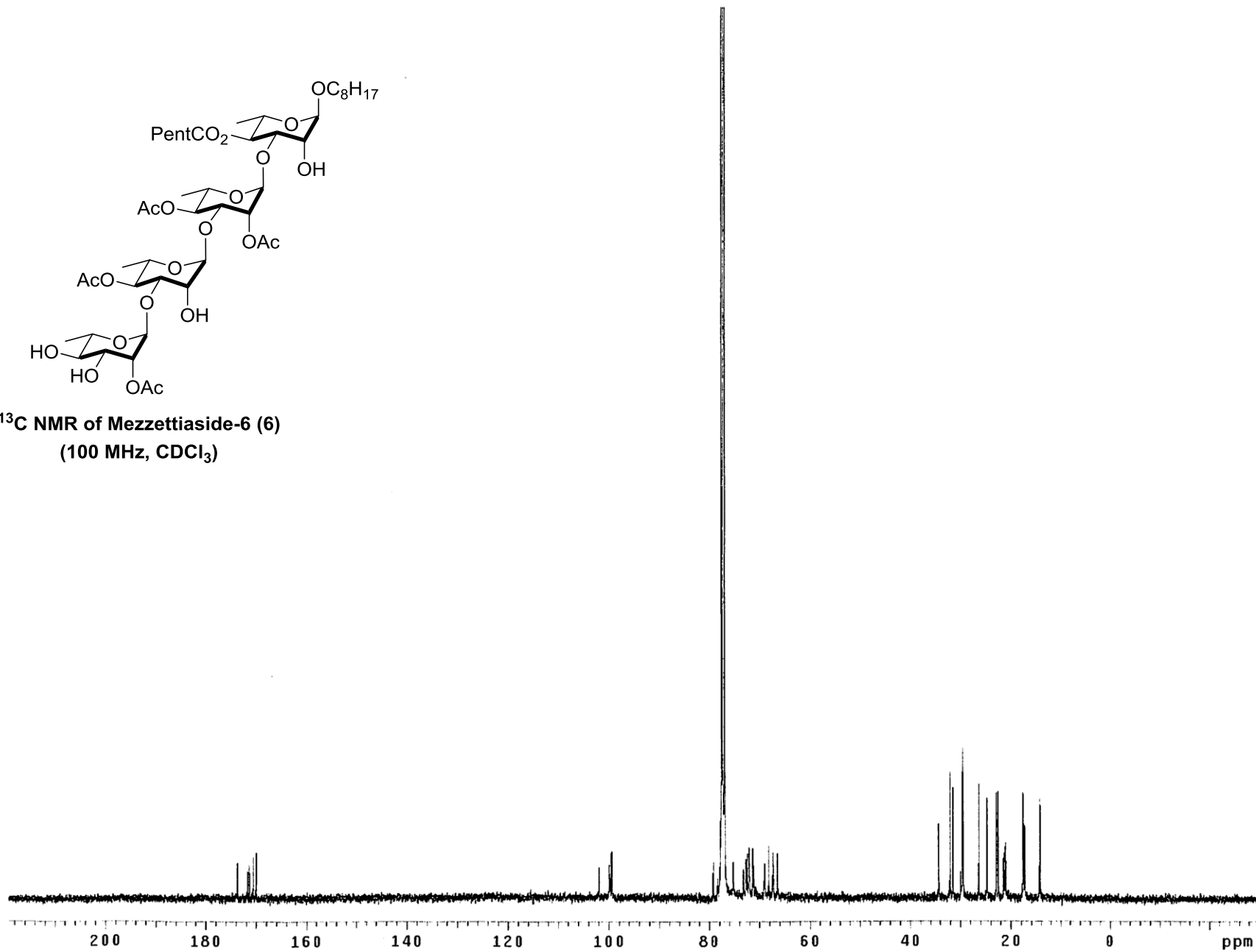


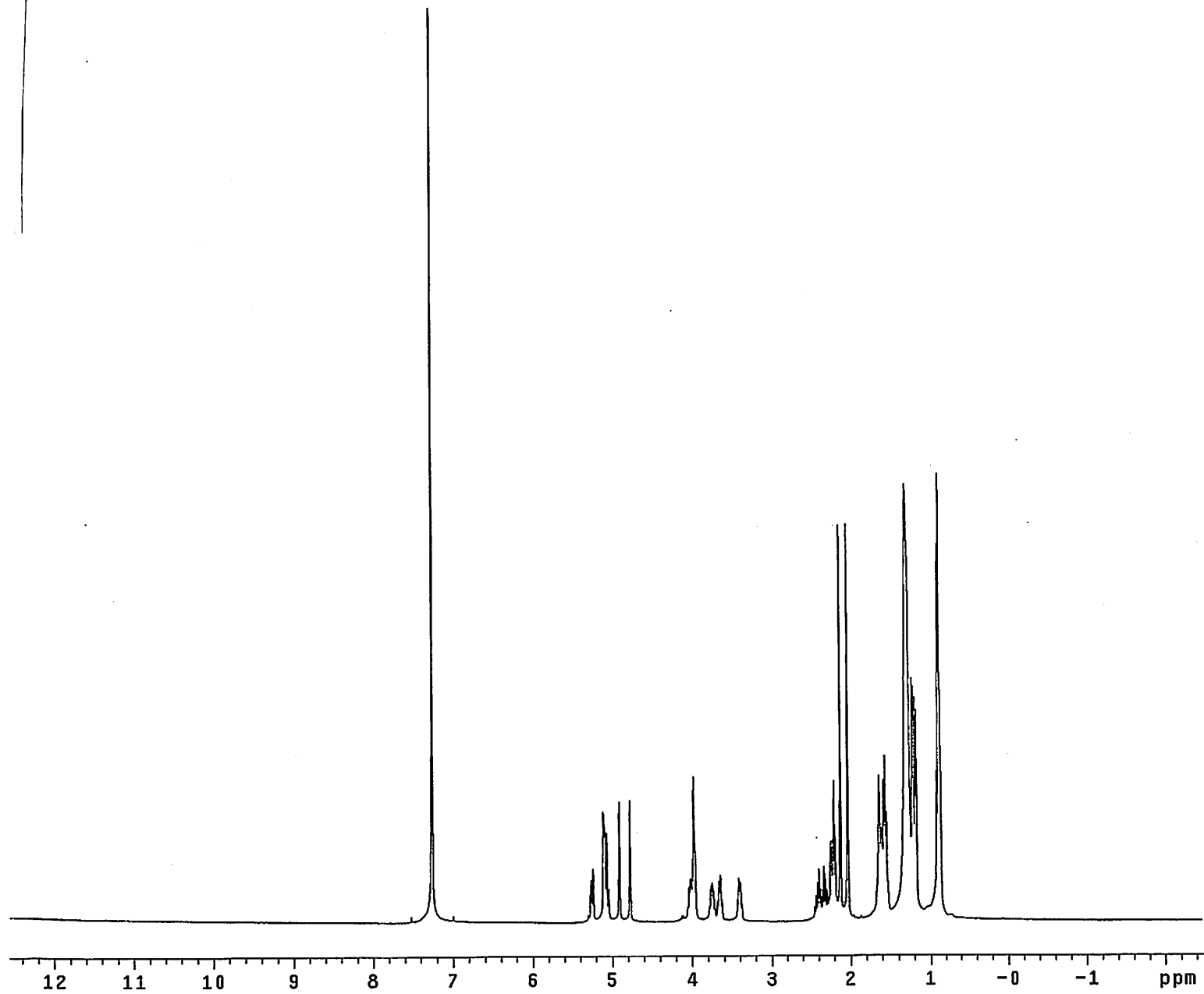
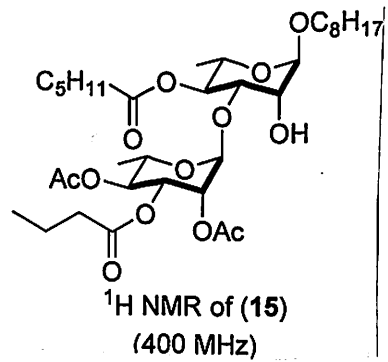
**<sup>1</sup>H NMR of Mezzettiaside-6 (6)**  
(400 MHz, CDCl<sub>3</sub>)



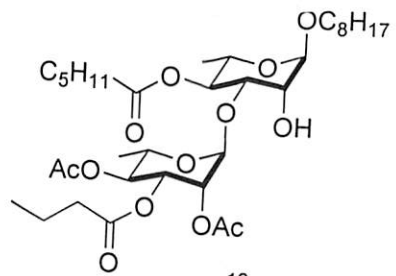


**<sup>13</sup>C NMR of Mezzettiaside-6 (6)**  
**(100 MHz, CDCl<sub>3</sub>)**









<sup>13</sup>C NMR of (15)  
(100 MHz)

