Electronic Supplementary Information

Synthetic Glycosidase for Precise Hydrolysis of Oligosaccharides and Polysaccharides

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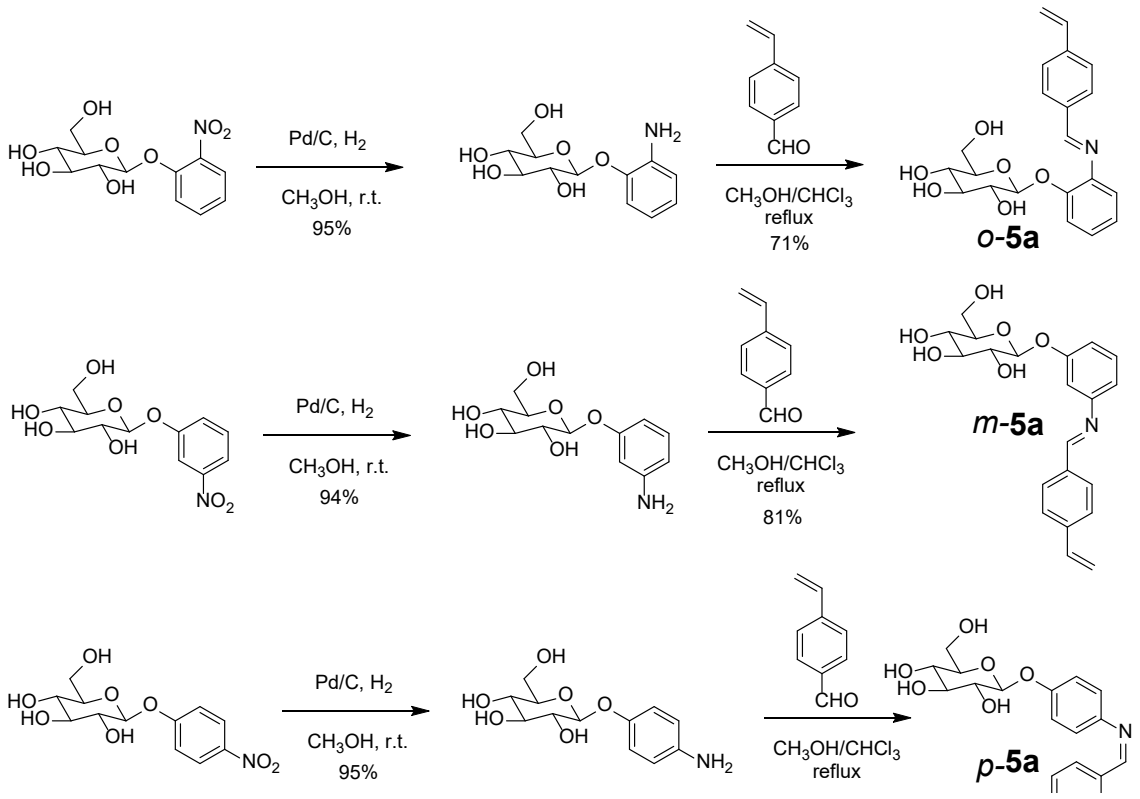
General

All organic solvents and reagents were of ACS-certified grade or higher grade, and were purchased from Fisher Scientific. Ultrapure water (18.2 MU; Millipore Co., USA) was used throughout the experiment to prepare buffers and nanoparticles. Flash column chromatography was performed on SiliFlash P60 silica gel (40-63 μm, 60 Å). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400, Bruker AV III 600 or Varian VXR-400 spectrometer. Chemicals shifts are reported in ppm relative to residual solvent peaks ($CDCl_3$ = 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR, CD_3OD = 3.31 ppm for ^{1}H NMR and 49.00 for ^{13}C NMR). Coupling constants are reported in hertz. High resolution mass spectra (HRMS) were recorded on Agilent QTOF 6540 mass spectrometer with a QTOF detector. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano ZS. Isothermal titration calorimetry (ITC) was performed using a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). LC-MS analysis was performed with a Thermo Scientific HILIC-LC column (4.6 mm, 150 mm) coupled to an Agilent 1200 Series Binary VWD system with an Agilent 6540 UHD Accurate Mass Q-TOF mass spectrometry detector.

Synthesis & Characterization

Syntheses of compounds **1a**,[1] **1b**,[2] **2**,[3] **3**,[1] and **4**[3] were reported previously.

Synthesis of templates o-5a, m-5a and p-5a



Scheme S1. Syntheses of templates *o*-**5a**, *m*-**5a** and *p*-**5a**

Synthesis of *o*-**5a**

2-Aminophenyl-β-D-glucopyranoside. 2-Nitrophenyl-β-D-glucopyranoside (200 mg, 0.66 mmol) was hydrogenated in methanol (10 mL) with Pd/C (40 mg). After 12 h, the catalyst was removed by filtration, and methanol was removed in vacuo. The residue was crystallized from ethanol to a white crystal (171 mg, 95%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 7.36 (d, J = 4.8 Hz, 2H), 7.32 (t, J = 4.8 Hz, 1H), 7.14 (m, 1H), 5.01 (d, J = 7.6 Hz, 1H), 3.90-3.88 (m, 1H), 3.74-3.69 (m, 1H), 3.57-3.46 (m, 2H), 3.44-3.39 (m, 1H), 3.30-3.29 (m, 1H). HRMS (ESI$^+$/QTOF): Calcd for $C_{12}H_{17}NO_6$ m/z: $[M+H]^+$ 272.1129; Found 272.1137.

Compound ***o*-5a.** A solution of 2-Aminophenyl-β-D-glucopyranoside (100 mg, 0.369 mmol) and 4-vinylbenzaldehyde (73 mg, 0.553 mmol) in $CHCl_3$ (0.5 mL) and ethanol (3 mL) was heated to reflux overnight. After the reaction mixture was cooled to room temperature, diethyl ether (30 mL) was added

slowly. The precipitate formed was collected by filtration and washed with cold diethyl ether to yield a white powder (101 mg, 71%). ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) δ 8.65 (s, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.23 (dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.18-7.12 (m, 2H), 7.07-7.03 (m, 1H), 6.81 (dd, J_1 = 18.0 Hz, J_2 = 11.2 Hz, 1H), 5.98 (d, J = 18.0 Hz, 1H), 5.39 (d, J = 11.2 Hz, 1H), 5.30 (d, J = 4.8 Hz, 1H), 5.06 (d, J = 4.8 Hz, 1H), 5.02 (d, J = 4.8 Hz, 1H), 4.94-4.92 (m, 1H), 4.58-4.49 (m, 1H), 4.36 (t, J = 5.2 Hz, 1H), 3.70-3.66 (m, 1H), 3.49-3.38 (m, 2H), 3.26-3.23 (m, 1H), 3.13-3.11 (m, 1H). ^{13}C NMR (100 MHz, 298 K, DMSO-d_6) δ 162.37, 149.79, 142.14, 140.40, 136.57, 136.15, 129.48, 126.91, 123.18, 122.09, 117.83, 109.99, 103.27, 101.78, 77.61, 77.09, 73.86, 70.08, 61.17. HRMS (ESI$^+$/QTOF): Calcd for $C_{21}H_{23}NO_6$ m/z: $[M+H]^+$ 386.1598; Found 386.1600.

Synthesis of *m*-**5a**

3-Aminophenyl-β-D-glucopyranoside. The same procedure for 2-aminophenyl-β-D-glucopyranoside was followed, using 3-nitrophenyl-β-D-glucopyranoside (200 mg, 0.66 mmol) to afford 3-aminophenyl-β-D-glucopyranoside as a white crystal (169 mg, 94%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 7.45 (t, J = 8.4 Hz, 1H), 7.20 (dd, J_1 = 8.4 Hz, J_2 = 2.8 Hz, 1H), 7.15 (t, J = 2.0 Hz, 1H), 7.05 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 4.95 (d, J = 7.6 Hz, 1H), 3.92 (dd, J_1 = 12.0 Hz, J_2 = 2.0 Hz, 1H), 3.72-3.67 (m, 1H), 3.51-3.46 (m, 2H), 3.41-3.39 (m, 1H), 3.31-3.29 (m, 1H). HRMS (ESI$^+$/QTOF) Calcd for $C_{12}H_{17}NO_6$ m/z: $[M+H]^+$ 272.1129; Found 272.1148.

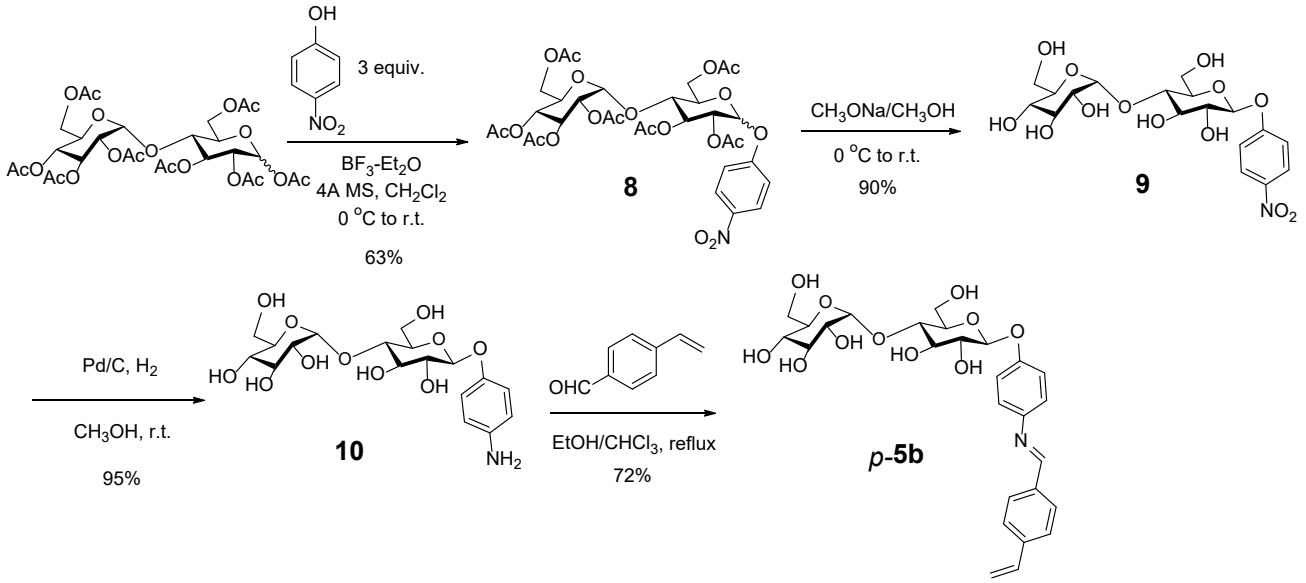
Compound ***m*-5a.** The same procedure for *o*-**5a** was followed, using 3-aminophenyl-β-D-glucopyranoside (100 mg, 0.369 mmol) to afford *m*-**5a** as a white powder (115 mg, 81%). ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) δ 8.61 (s, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.32 (t, J = 8.0 Hz, 1H), 6.95 (t, J = 2.0 Hz, 1H), 6.90 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 2H), 6.81 (dd, J_1 = 18.0 Hz, J_2 = 10.8 Hz, 1H), 5.98 (d, J = 18.0 Hz, 1H), 5.39 (d, J = 10.8 Hz, 1H), 5.33 (d, J = 4.8 Hz, 1H), 5.11 (d, J = 4.4 Hz, 1H), 5.04 (d, J = 5.2 Hz, 1H), 4.92 (d, J = 7.6 Hz, 1H), 4.64 (t, J = 2.4 Hz, 1H), 3.73-3.69 (m, 1H), 3.38-3.58 (m, 1H), 3.29-3.24 (m, 2H), 3.17-3.11 (m, 1H). ^{13}C NMR (100 MHz, 298 K, DMSO-d_6) δ 160.91, 158.63, 153.08, 140.50, 136.54, 135.86, 130.28, 129.51, 126.99, 116.73, 115.54, 114.45, 108.86, 100.83, 77.56, 77.08, 73.71, 70.26, 61.21. HRMS (ESI$^+$/QTOF): Calcd for $C_{21}H_{23}NO_6$ $[M+H]^+$ m/z 386.1598, found: m/z 386.1608. HRMS (ESI$^+$/QTOF) Calcd for $C_{12}H_{17}NO_6$ m/z: $[M+H]^+$ 272.1129; Found 272.1148.

Synthesis of *p*-**5a**

4-Aminophenyl-β-D-glucopyranoside. The same procedure for 2-aminophenyl-β-D-glucopyranoside was followed, using 4-nitrophenyl-β-D-glucopyranoside (200 mg, 0.66 mmol) to afford 3-aminophenyl-β-D-glucopyranoside as a white crystal (171 mg, 95%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 6.95 (d, *J* = 8.8 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 5.27 (d, *J* = 4.0 Hz, 1H), 3.85-3.80 (m, 1H), 3.77-3.71 (m, 3H), 3.54-3.51 (m, 1H), 3.44-3.34 (m, 1H). ^{13}C NMR (100 MHz, 298 K, CD_3OD) δ 150.28, 142.05, 118.37, 116.44, 99.13, 73.59, 72.75, 72.01, 61.00. HRMS (ESI^+/QTOF) Calcd for $C_{12}H_{17}NO_6$ m/z: $[M+H]^+$ 272.1129; Found 272.1162.

Compound ***p*-5a.** The same procedure for *o*-**5a** was followed, using 4-aminophenyl-β-D-glucopyranoside (100 mg, 0.369 mmol) to afford *p*-**5a** as a white powder (111 mg, 78%). ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) δ 8.63 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 6.81 (m, 1H), 5.97 (m, 1H), 5.39-5.36 (m, 3H), 5.07 (d, *J* = 6.4 Hz, 1H), 4.99 (d, *J* = 5.6 Hz, 1H), 4.95 (d, *J* = 5.6 Hz, 1H) 4.50 (m, 1H), 3.65-3.63 (m, 2H), 3.48-3.46 (m, 2H), 3.39-3.37 (m, 1H), 3.22-3.16 (m, 1H). ^{13}C NMR (100 MHz, 298 K, DMSO-d_6) δ 158.85, 156.25, 145.76, 140.14, 136.57, 136.17, 135.46, 129.22, 128.71, 126.97, 122.69, 118.05, 116.49, 98.66, 74.20, 73.51, 72.05, 70.38, 61.14. HRMS (ESI^+/QTOF) Calcd for $C_{21}H_{23}NO_6$ m/z: $[M+H]^+$ 386.1598; Found 386.1620. HRMS (ESI^-/QTOF) Calcd for $C_{21}H_{23}NO_6$ m/z: $[M+HCOO]^-$ 430.1507; Found 430.1493.

Synthesis of template p-5b



Scheme S2. Synthesis of maltose-imine template *p*-**5b**

Synthesis of *p*-**5b**

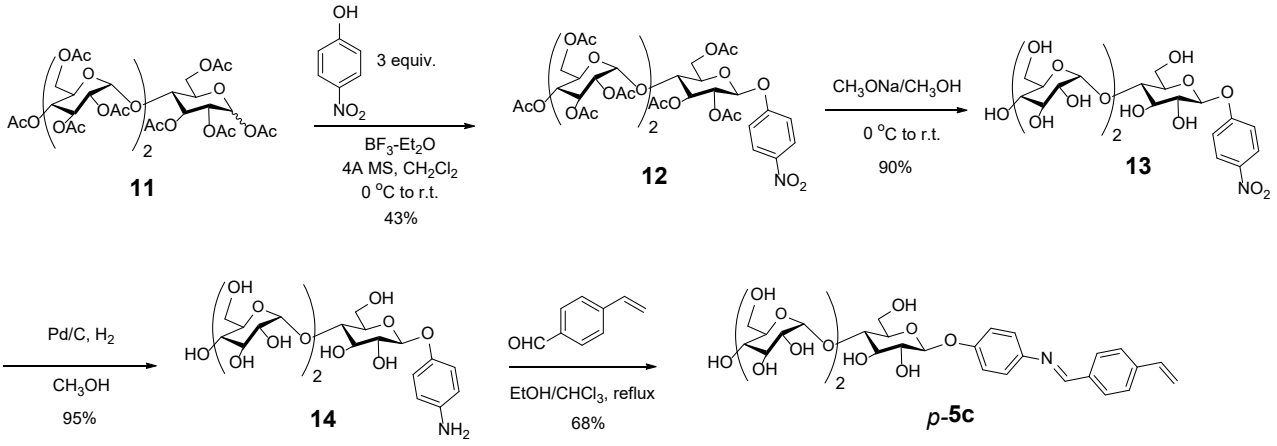
Compound **8.**[4] To a stirred solution of D-maltose octaacetate (500 mg, 0.74 mmol) and 4-nitrophenol (308 mg, 2.21 mmol) in anhydrous CH_2Cl_2 (5 mL) with activated 4 Å molecular sieves at 0 °C was added BF_3•OEt_2 (525 mg, 457 μL, 3.70 mmol). The mixture was warmed to room temperature and stirred for 24 h. A solution of saturated $NaHCO_3$ (20 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic solution was washed with 1 M NaOH (3 × 20 mL) and H_2O (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was crystallized from EtOAc/Hexane and the crude product was purified by column chromatography over silica gel to afford a colorless syrup (353 mg, 63%). ^{1}H NMR (600 MHz, 298 K, $CDCl_3$) δ 8.15 (d, J = 9.0 Hz, 2H), 7.02 (d, J = 9.0 Hz, 2H), 5.39-5.28 (m, 2H), 5.23 (d, J = 7.2 Hz, 1H), 5.11-5.07 (m, 1H), 5.02-4.98 (m, 1H), 4.83-4.80 (m, 1H), 4.46-4.43 (m, 1H), 4.23-4.18 (m, 2H), 4.07-4.00 (m, 3H), 3.93-3.91 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 170.41, 170.16, 170.14, 170.06, 169.88, 169.43, 169.32, 161.06, 143.17, 125.74, 116.57, 97.43, 95.73, 74.95, 74.93, 72.61, 72.58, 71.63, 69.99, 69.19, 68.64, 67.99, 62.63, 61.54, 20.95, 20.83, 20.81, 20.62, 20.53, 20.51, 20.50. HRMS (ESI$^+$/QTOF) Calcd for $C_{32}H_{39}NO_{20}$ m/z: $[M+NH_2]^+$ 775.2404; Found 775.2359. HRMS (ESI$^-$/QTOF) Calcd for $C_{32}H_{39}NO_{20}$ m/z: $[M+HCOO]^-$ 802.2047; Found 802.2105.

Compound **9.** A solution of compound **8** (500 mg, 0.66 mmol) in methanol (10 mL) with a catalytic amount of sodium methoxide was stirred at room temperature for 3 h. The solution was neutralized with Amberlite® IR-120 (H^+) ion-exchange resin, filtered, and concentrated in vacuo. The residue was purified by column chromatography over silica gel to afford compound **9** as a white powder (297 mg, 90%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 8.21 (d, *J* = 9.6 Hz, 2H), 7.23 (d, *J* = 9.6 Hz, 2H), 5.21 (d, *J* = 3.6 Hz, 1H), 5.11 (d, *J* = 7.6 Hz, 1H), 3.93-3.90 (m, 1H), 3.87-3.82 (m, 2H), 3.79-3.63 (m, 13H), 3.58-3.54 (m, 1H), 3.49-3.45 (m, 1H), 3.31-3.29 (m, 2H). ^{13}C NMR (100 MHz, 298 K, CD_3OD) δ 162.36, 142.47, 125.19, 116.29, 101.52, 100.03, 79.26, 76.12, 75.51, 73.62, 73.44, 72.86, 72.70, 70.11, 61.32, 60.42. HRMS (ESI$^-$/QTOF) Calcd for $C_{18}H_{25}NO_{13}$ m/z: $[M-H]^-$ 462.1253; Found 462.1261, $[M+Cl]^-$ 498.1020; Found: m/z 498.1036, $[M+HCOO]^-$ 508.1308; Found 508.1321.

Compound **10.** The same procedure for 2-aminophenyl-β-D-glucopyranoside was followed, using compound **9** (400 mg, 0.86 mmol) to afford compound **10** as a white crystal (354 mg, 95%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 6.93 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 5.20 (d, *J* = 2.4 Hz, 1H), 4.78 (d, *J* = 7.6 Hz, 1H), 4.50 (br, s, 2H), 3.89-3.80 (m, 3H), 3.75-3.65 (m, 6H), 3.54-3.43 (m, 4H), 3.33-3.28 (m, 1H), 3.49-3.45 (m, 1H), 3.31-3.29 (m, 4H). ^{13}C NMR (100 MHz, 298 K, CD_3OD) δ 150.63, 142.11, 117.87, 116.60, 101.95, 101.57, 79.56, 76.12, 75.02, 73.53, 73.39, 73.10, 72.59, 70.23, 61.24, 60.48. HRMS (ESI$^-$/QTOF) Calcd for $C_{18}H_{27}NO_{11}$ m/z: $[M+Cl]^-$ 468.1278; Found: m/z 468.1290, $[M+HCOO]^-$ 478.1566; Found: m/z 478.1569.

Compound ***p*-5b.** The same procedure for *o*-**5a** was followed, using compound **10** (150 mg, 0.35 mmol) and 4-vinylbenzaldehyde (55 mg, 0.42 mmol) to afford *p*-**5b** as a white powder (136 mg, 72%). ^{1}H NMR (600 MHz, 298 K, CD_3OD) δ 8.57 (s, 1H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.18 (d, *J* = 8.4 Hz, 2H), 6.83 (m, 1H), 5.94 (m, 1H), 5.37 (m, 1H), 5.24 (m, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 3.96-3.93 (m, 1H), 3.90-3.86 (m, 4H), 3.80-3.75 (m, 2H), 3.71-3.64 (m, 4H), 3.62-3.54 (m, 2H), 3.51-3.49 (m, 1H), 3.32-3.29 (m, 5H). ^{13}C NMR (150 MHz, 298 K, CD_3OD) δ 159.62, 156.38, 145.94, 140.62, 136.08, 135.78, 135.46, 129.69, 128.71, 126.44, 126.24, 121.80, 117.89, 117.11, 116.50, 116.28, 114.70, 101.54, 100.93, 79.50, 76.25, 75.32, 73.64, 73.42, 73.09, 72.73, 70.14, 61.33, 60.53. HRMS (ESI$^-$/QTOF) Calcd for $C_{27}H_{33}NO_{11}$ m/z: $[M-H]^-$ 546.1981; Found 546.2021, $[M+Cl]^-$ 582.1748; Found 582.1802, $[M+HCOO]^-$ 592.2036; Found: m/z 592.2088.

Synthesis of template p-5c



Scheme S3. Synthesis of template *p*-**5c**

Synthesis of *p*-**5c**

Compound **12.** The same procedure for **8** was followed, using D-maltotriose acetate (600 mg, 0.62 mmol) and 4-nitrophenol (259 mg, 1.86 mmol) to afford **12** as a colorless syrup (201 mg, 43%). ^{1}H NMR (600 MHz, 298 K, $CDCl_3$) δ 8.15 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 5.39-5.28 (m, 2H), 5.23 (d, *J* = 7.2 Hz, 1H), 5.11-5.07 (m, 1H), 5.02-4.98 (m, 1H), 4.83-4.80 (m, 1H), 4.46-4.43 (m, 1H), 4.23-4.18 (m, 2H), 4.07-4.00 (m, 3H), 3.93-3.91 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 170.41, 170.16, 170.14, 170.06, 169.88, 169.43, 169.32, 161.06, 143.17, 125.74, 116.57, 97.43, 95.73, 74.95, 74.93, 72.61, 72.58, 71.63, 69.99, 69.19, 68.64, 67.99, 62.63, 61.54, 20.95, 20.83, 20.81, 20.62, 20.53, 20.51, 20.50. HRMS (ESI$^+$/QTOF) Calcd for $C_{44}H_{55}NO_{28}$ m/z: $[M+NH_2]^+$ 1063.3249; Found 1063.3253.

Compound **13.** The same procedure for **9** was followed, using compound **12** (500 mg, 0.48 mmol) to afford **13** as a white powder (282 mg, 90%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 8.21 (d, *J* = 9.2 Hz, 2H), 7.24 (d, *J* = 9.2 Hz, 2H), 5.21 (d, *J* = 3.6 Hz, 1H), 5.11 (d, *J* = 5.2 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 3.88-3.80 (m, 8H), 3.78-3.69 (m, 8H), 3.54-3.49 (m, 15H), 3.34-3.30 (m, 3H). ^{13}C NMR (100 MHz, 298 K, CD_3OD) δ 162.37, 142.49, 125.21, 116.31, 101.48, 101.25, 96.72, 92.42, 80.40, 79.88,79.82, 79.23, 76.11, 75.50, 73.66, 73.57, 73.52, 73.36, 72.80, 72.32, 72.04, 71.98, 70.07, 61.30,

60.76, 60.49. HRMS (ESI$^-$/QTOF) Calcd for $C_{24}H_{35}NO_{18}$ m/z: $[M+HCOO]^-$ 670.1836; Found: m/z 670.1857.

Compound **14.** The same procedure for **10** was followed, using compound **13** (400 mg, 0.64 mmol) to afford **14** as a white powder (354 mg, 95%). ^{1}H NMR (400 MHz, 298 K, CD_3OD) δ 6.91 (d, *J* = 8.8 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 5.20-5.15 (m, 1H), 4.74 (d, *J* = 7.6 Hz, 1H), 4.48 (d, *J* = 7.6 Hz, 1H), 3.89-3.70 (m, 15H), 3.68-3.58 (m, 5H), 3.52-3.34 (m, 5H), 3.30-3.26 (m, 5H). ^{13}C NMR (100 MHz, 298 K, CD_3OD) δ 150.72, 142.16, 117.89, 116.26, 102.13, 101.41, 96.74, 92.42, 80.42, 79.90, 79.86, 79.57, 76.27, 75.24, 75.19, 73.66, 73.34, 73.23, 73.16, 72.82, 72.35, 72.04, 71.93, 71.87, 70.22, 70.05, 61.03, 60.72. HRMS (ESI$^-$/QTOF) Calcd for $C_{24}H_{37}NO_{16}$ m/z: $[M+Cl]^-$ 630.1806; Found 630.1847, $[M+HCOO]^-$ 640.2094; Found 640.2134.

Compound ***p*-5c.** The same procedure for *o*-**5a** was followed, using compound **14** (100 mg, 0.17 mmol) and 4-vinylbenzaldehyde (27 mg, 0.20 mmol) to afford *p*-**5c** as a white powder (81 mg, 68%). ^{1}H NMR (600 MHz, 298 K, CD_3OD) δ 8.54 (s, 1H), 7.86 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 9.2 Hz, 2H), 7.15 (d, *J* = 9.2 Hz, 2H), 6.79 (m, 1H), 5.90 (m, 1H), 5.34 (m, 1H), 5.24 (m, 1H), 5.22 (d, *J* = 3.6 Hz, 1H), 5.16-5.14 (m, 4H), 5.10 (d, *J* = 3.6 Hz, 1H), 4.95 (d, *J* = 8.0 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 3.90-3.68 (m, 8H), 3.68-3.59 (m, 5H), 55-3.45 (m, 5H), 3.34-3.30 (m, 4H), 3.19-3.15 (m, 1H). ^{13}C NMR (150 MHz, 298 K, CD_3OD) δ 159.66, 156.40, 145.95, 140.64, 136.09, 135.48, 128.70, 126.24, 121.79, 117.12, 114.68, 101.48, 101.45, 101.41, 101.26, 100.91, 96.73, 92.42, 80.41, 80.01, 79.85, 75.23, 74.43, 73.66, 73.57, 73.52, 73.33, 73.32, 72.94, 72.50, 72.35, 72.03, 71.89, 70.23, 70.06, 61.31, 60.76. HRMS (ESI$^-$/QTOF) Calcd for $C_{33}H_{43}NO_{16}$ m/z: $[M-H]^-$ 708.2509; Found 708.2484, $[M+Cl]^-$ 744.2276; Found 744.2247, $[M+HCOO]^-$ 754.2564; Found 754.2578.

[1]H NMR comparison of imine-templates** p-**5a**, p-**5b** and p-**5c

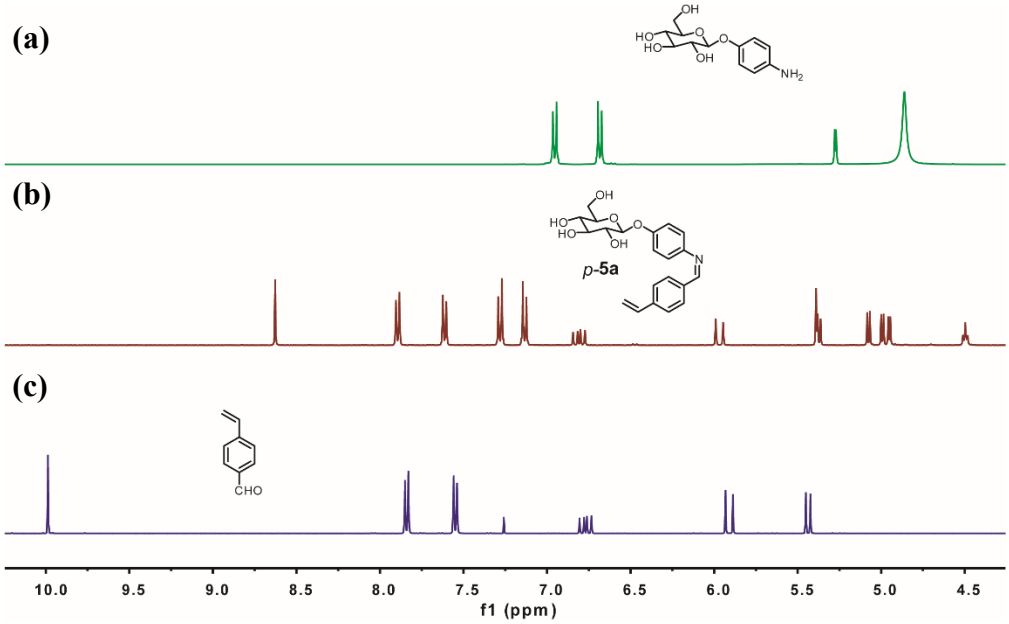


Figure S1. Partial stacked ^{1}H NMR spectra of (a) 4-aminophenyl-β-D-glucopyranoside in CD_3OD, (b) imine template *p*-**5a** in DMSO-d_6 and (c) 4-vinylbenzaldehyde in $CDCl_3$.

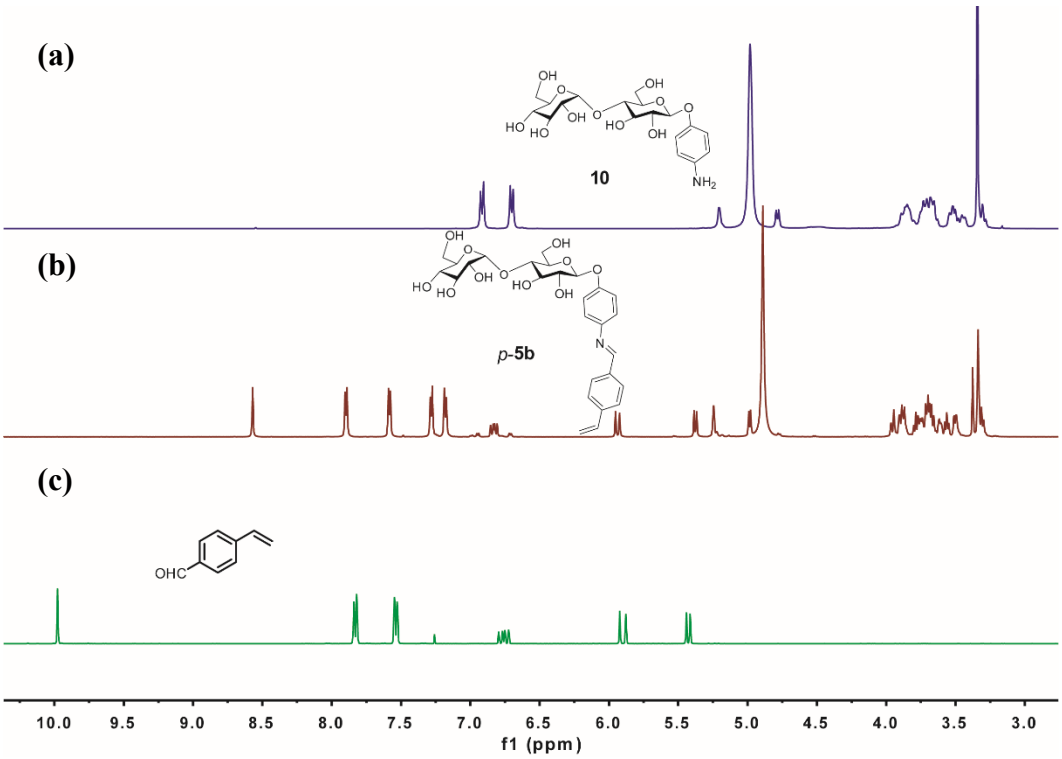


Figure S2. Partial stacked ^{1}H NMR spectra of (a) compound **10** in CD_3OD, (b) imine template *p*-**5b** in CD_3OD and 4-vinylbenzaldehyde in $CDCl_3$.

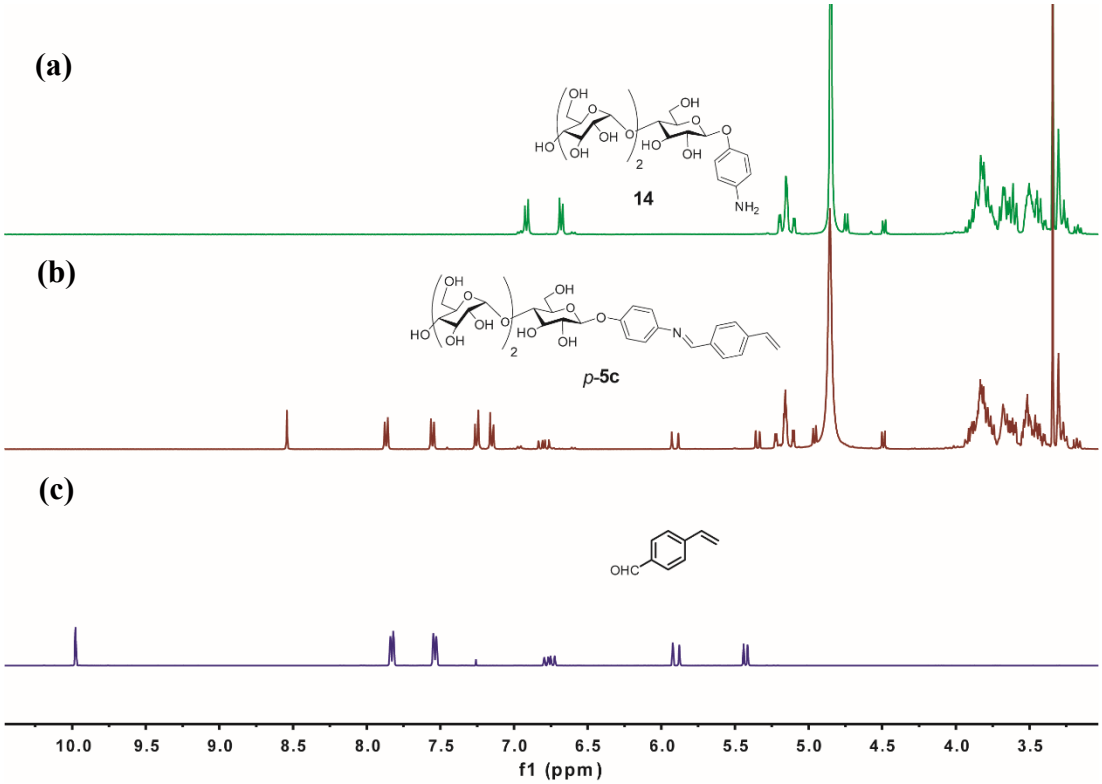


Figure S3. Partial stacked ^{1}H NMR spectra of (a) compound **14** in CD_3OD, (b) imine template *p*-**5c** in CD_3OD and (c) 4-vinylbenzaldehyde in $CDCl_3$.

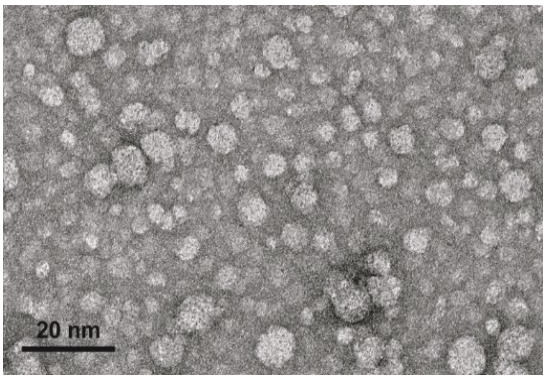


Figure S4. TEM image of typical MINPs (with some particles in the aggregated form caused by the negative staining).

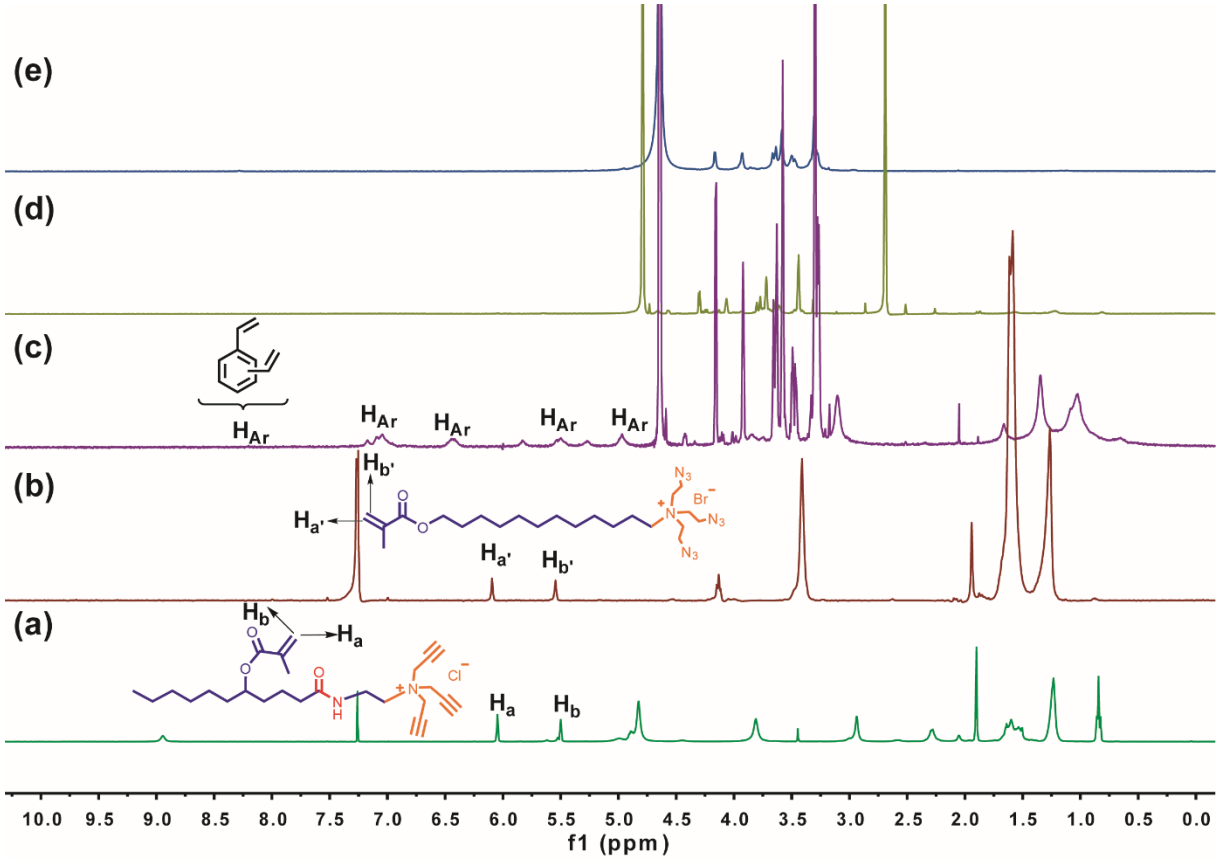


Figure S5. ^{1}H NMR spectra of (a) surfactant **1b** in $CDCl_3$, (b) surfactant **2** in $CDCl_3$ (c) alkynyl-SCM in D_2O, (d) MINP-CHO(*p*-**G1**) in D_2O before solvent washing and (e) final MINP(*p*-**G1**+**7h**) in D_2O after post-modification and purification.

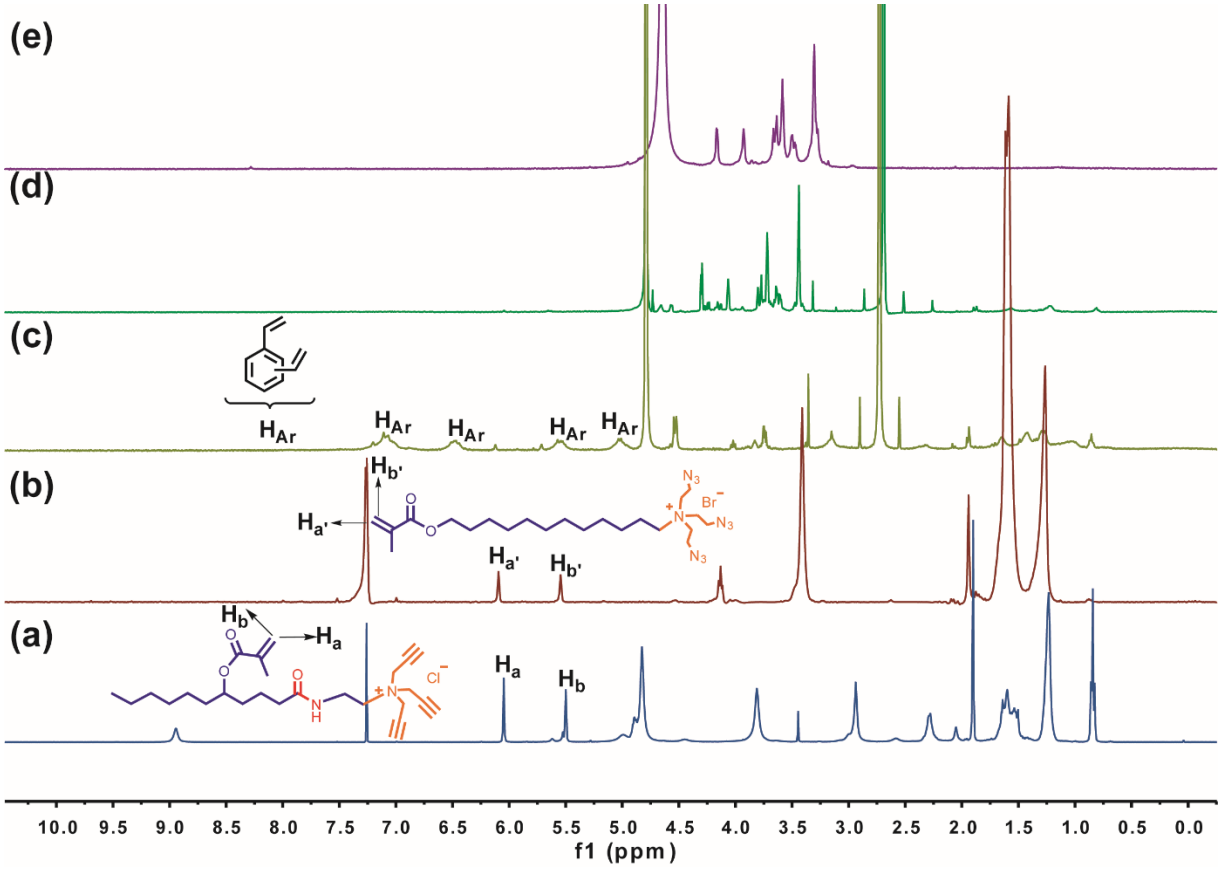


Figure S6. ^{1}H NMR spectra of (a) surfactant **1b** in $CDCl_3$, (b) surfactant **2** in $CDCl_3$ (c) alkynyl-SCM in D_2O, (d) MINP-CHO(*p*-**G2**) in D_2O before solvent washing and (e) final MINP(*p*-**G2**+**7h**) in D_2O after post-modification and purification.

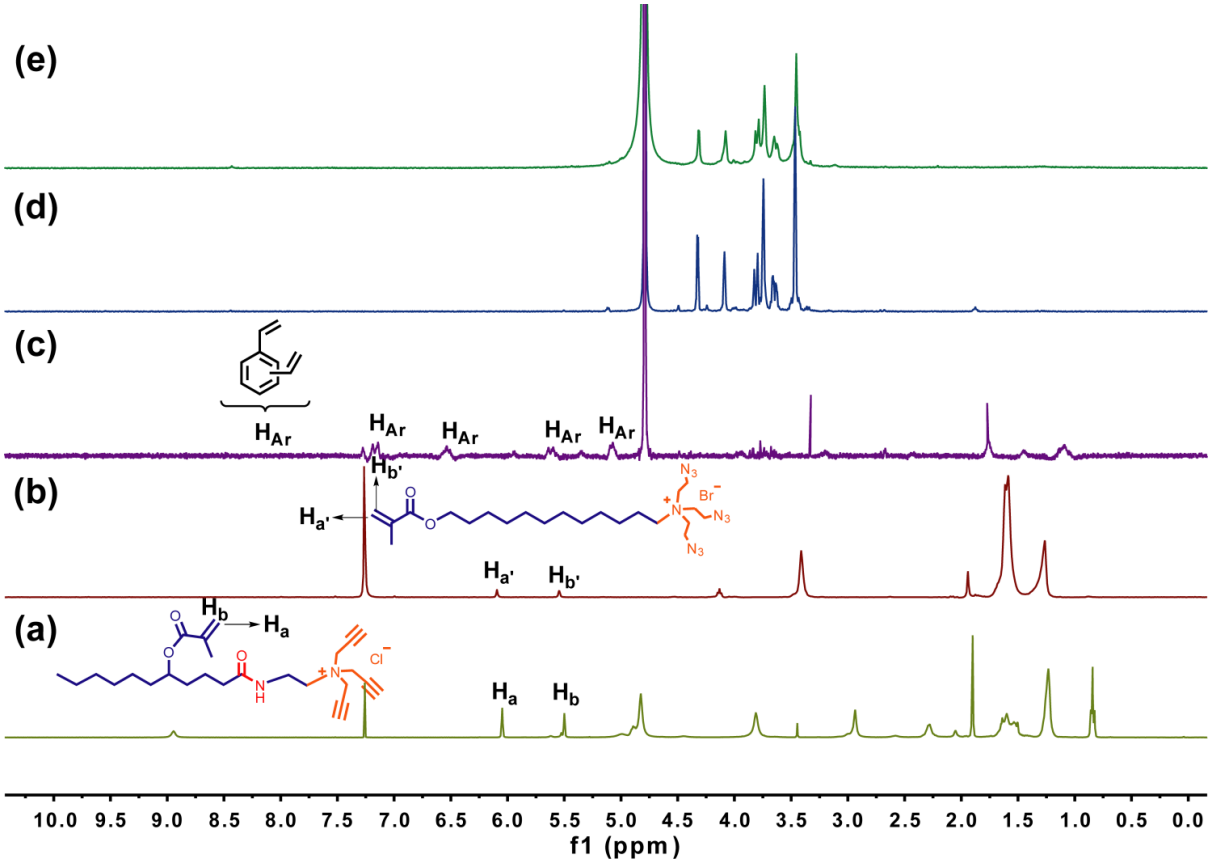


Figure S7. ^{1}H NMR spectra of (a) surfactant **1b** in $CDCl_3$, (b) surfactant **2** in $CDCl_3$ (c) alkynyl-SCM in D_2O, (d) MINP-CHO(*p*-**G3**) in D_2O before solvent washing and (e) final MINP(*p*-**G3**+**7h**) in D_2O after post-modification and purification.

Dynamic Light Scattering

Particle size of MINP was determined on a Malvern Zetasizer Nano ZS using the Zetasizer software according to the Stokes-Einstein equation (1). The volume of a spherical nanoparticle (V_{D_h}) was calculated from equation (2). Assuming a density of 1.37 g/cm^3 (the density of protein), the molecular weight of the particle can be calculated using equation (3).[5] A nanoparticle with hydrodynamic diameter of 4.87 nm has a calculated molecular weight of 50 kDa.

$$D_h = \frac{k_B T}{6\pi\eta D_t} \quad (1)$$

in which D_h is the hydrodynamic diameter, D_t the translational diffusion coefficient measured by dynamic light scattering, T the temperature, k_B the Boltzmann's constant, and η is dynamic viscosity of water (0.890 cP at 298 K).

$$V_{D_h} = \frac{4\pi}{3}\left(\frac{D_h}{2}\right)^3 \quad (2)$$

$$\text{Mw in dalton} = \left(\frac{D_h}{0.132}\right)^3 \quad (3)$$

in which D_h is the hydrodynamic diameter in nm.

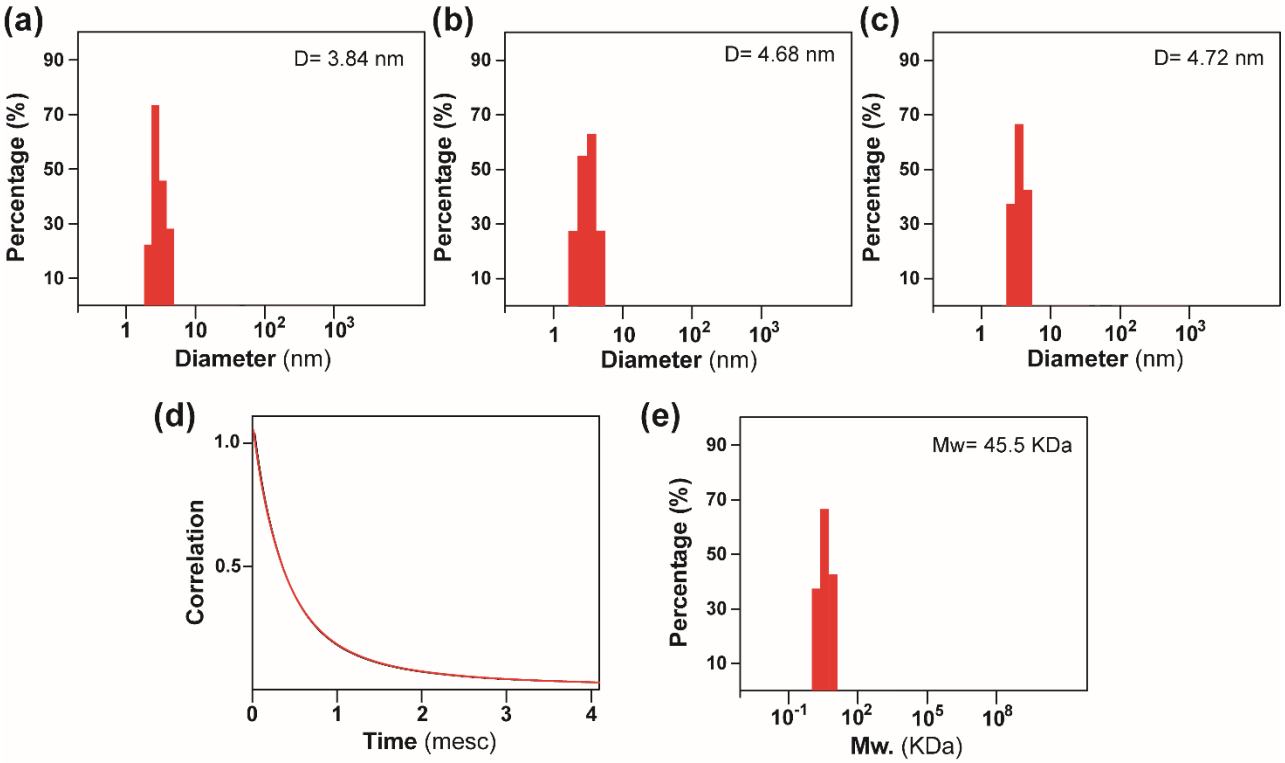


Figure S8. Distribution of the hydrodynamic diameters of the nanoparticles in water at 298 K as determined by DLS for (a) alkynyl-SCM, (b) MINP-CHO(*p*-**G1**) before post-modification and (c) final MINP(*p*-**G1**+**7h**) in H_2O after post-modification and purification (d) The correlation curve and (e) the corresponding molecular weight of the final MINP(*p*-**G1**+**7h**) based on the DLS size. If each unit of building block for the MINP is assumed to contain 0.6 molecules of compound **1b** (M_W = 508 g/mol), 0.4 molecules of compound **2** (M_W = 558 g/mol), one molecule of DVB (M_W = 130 g/mol) and 0.6 molecules of ligand **3** (M_W = 264 g/mol), 0.02 molecules of 6-vinylbenzoxaborole (M_W = 160 g/mol), and 0.02 molecules of compound **7h** (M_W = 139 g/mol) the molecular weight of the MINP(*p*-**G1**+**7h**) translates to 55 [= 45500 / (0.6×508 + 0.4×558 + 0.6×264 + 1.0×130 + 0.02×160 + 0.02×139)] of such units.

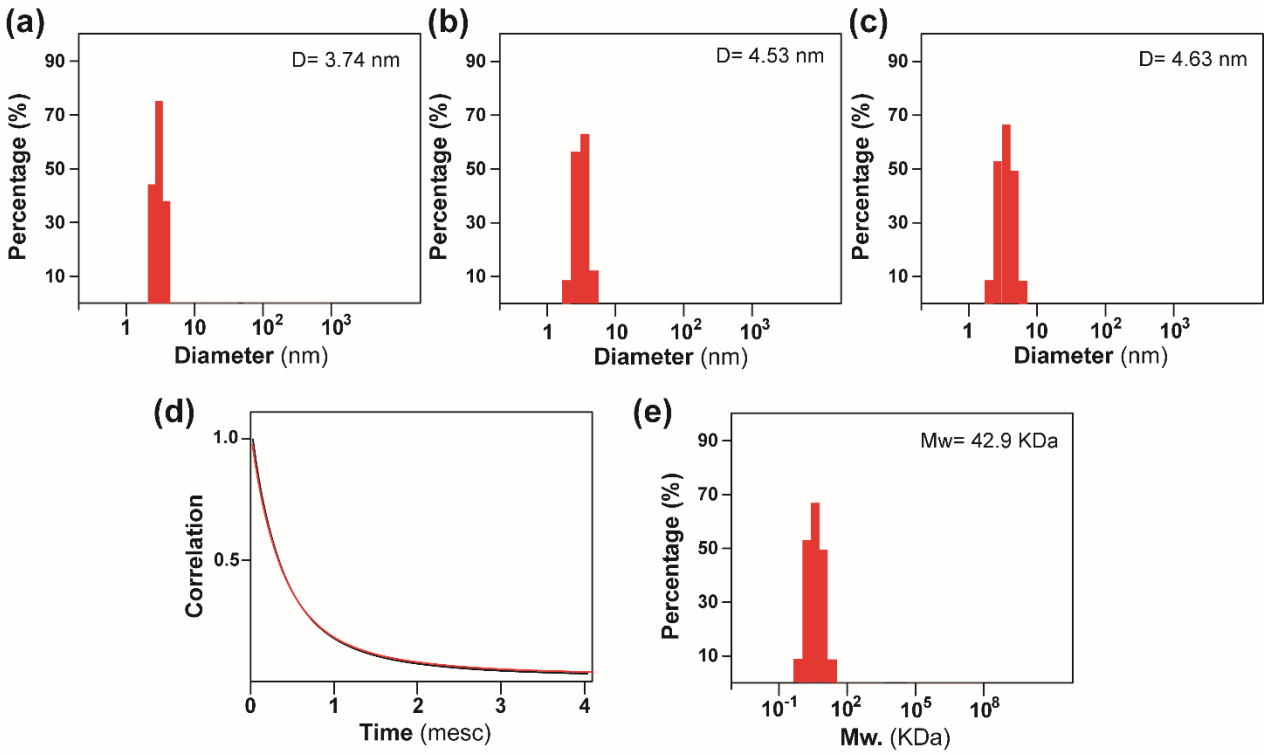


Figure S9. Distribution of the hydrodynamic diameters of the nanoparticles in water at 298 K as determined by DLS for (a) alkynyl-SCM, (b) MINP-CHO(*p*-**G2**) before post-modification and (c) final MINP(*p*-**G2**+**7h**) in H_2O after post-modification and purification (d) The correlation curve and (e) the corresponding molecular weight of the final MINP(*p*-**G2**+**7h**) based on the DLS size. If each unit of building block for the MINP is assumed to contain 0.6 molecules of compound **1b** (M_W = 508 g/mol), 0.4 molecules of compound **2** (M_W = 558 g/mol), one molecule of DVB (M_W = 130 g/mol) and 0.6 molecules of ligand **3** (M_W = 264 g/mol), 0.02 molecules of 6-vinylbenzoxaborole (M_W = 160 g/mol), and 0.02 molecules of compound **7h** (M_W = 139 g/mol) the molecular weight of the MINP(*p*-**G2**+**7h**) translates to 52 [= 42900 / (0.6×508 + 0.4×558 + 0.6×264 + 1.0×130 + 0.02×160 + 0.02×139)] of such units.

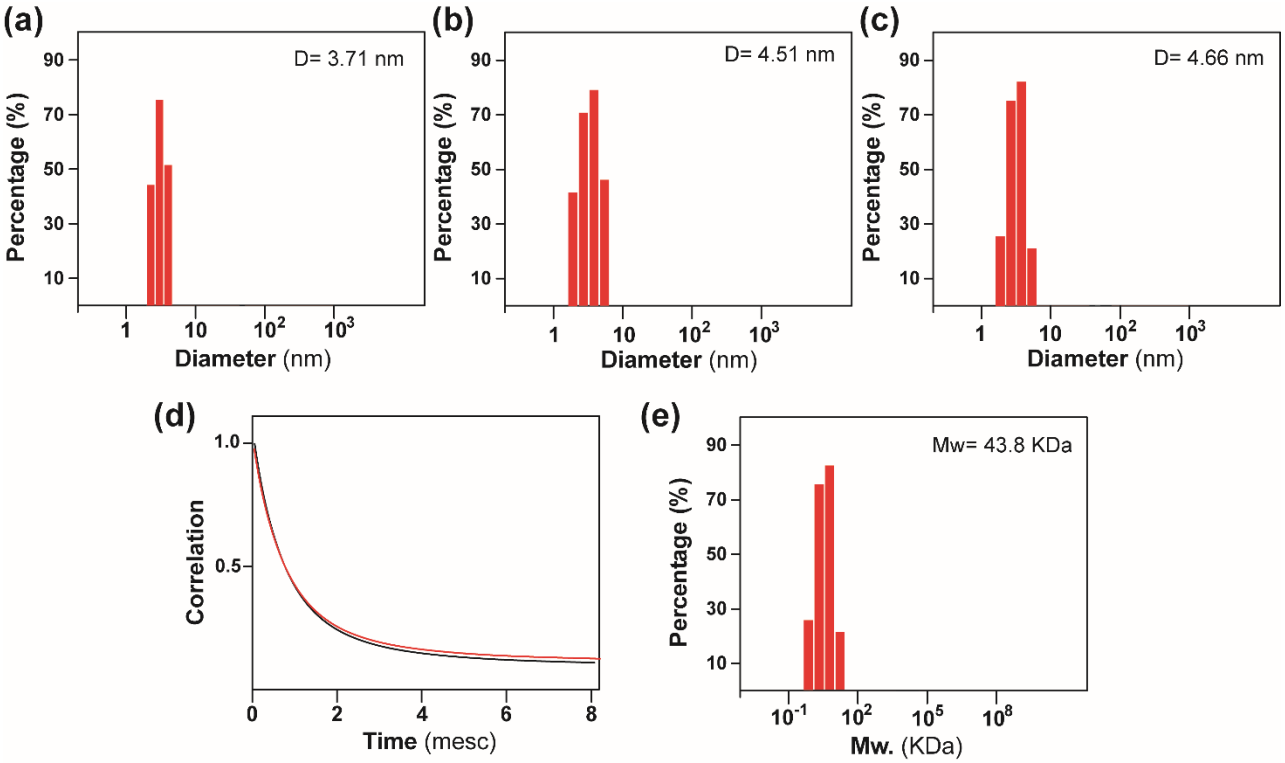
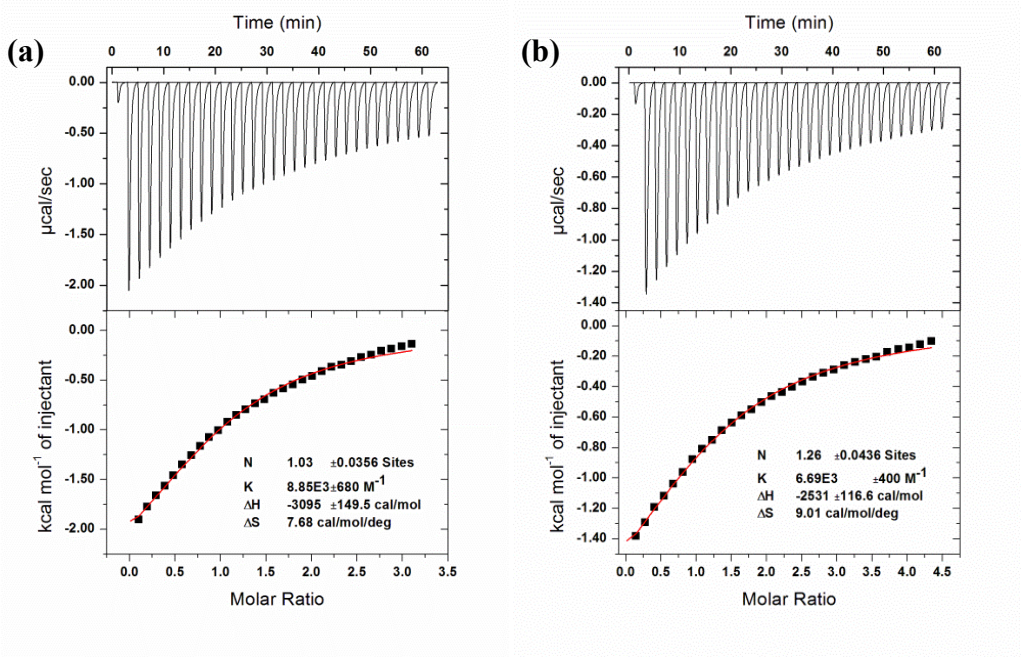


Figure S10. Distribution of the hydrodynamic diameters of the nanoparticles in water at 298 K as determined by DLS for (a) alkynyl-SCM, (b) MINP-CHO(*p*-**G3**) before post-modification and (c) final MINP(*p*-**G3**+**7h**) in H_2O after post-modification and purification (d) The correlation curve and (e) the corresponding molecular weight of the final MINP(*p*-**G3**+**7h**) based on the DLS size. If each unit of building block for the MINP is assumed to contain 0.6 molecules of compound **1b** (M_W = 508 g/mol), 0.4 molecules of compound **2** (M_W = 558 g/mol), one molecule of DVB (M_W = 130 g/mol) and 0.6 molecules of ligand **3** (M_W = 264 g/mol), 0.02 molecules of 6-vinylbenzoxaborole (M_W = 160 g/mol), and 0.02 molecules of compound **7h** (M_W = 139 g/mol) the molecular weight of the MINP(*p*-**G3**+**7h**) translates to 53 [= 43800 / (0.6×508 + 0.4×558 + 0.6×264 + 1.0×130 + 0.02×160 + 0.02×139)] of such units.

ITC Binding Studies

Procedure: The determination of binding constants by ITC followed standard procedures at 298.15 K.[6-8] In general, a solution of an appropriate sugar in 10 mM HEPES buffer (pH 7.4) was injected in equal steps into 1.43 mL of the corresponding MINP in the same solution. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of the MINP to the guest. The smooth solid line is the best fit of the experimental data to the sequential binding of N binding site on the MINP. The heat of dilution for the guest, obtained by titration carried out beyond the saturation point, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

ITC titration curves:



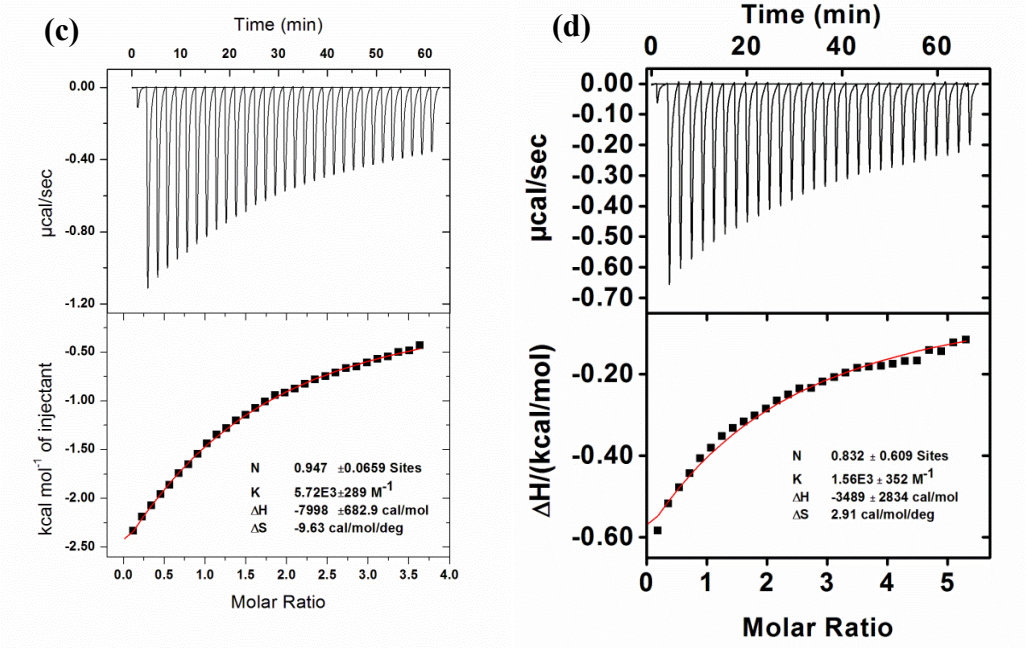


Figure S11. ITC curves obtained at 298 K from titration of MINP-CHO(*p*-**G1**) with (a) glucose, (b) maltose, (c) maltotriose and (d) maltohexaose in HEPEs buffer (10 mM, pH 7.4). The data correspond to entries 1-4 in Table 1, respectively.

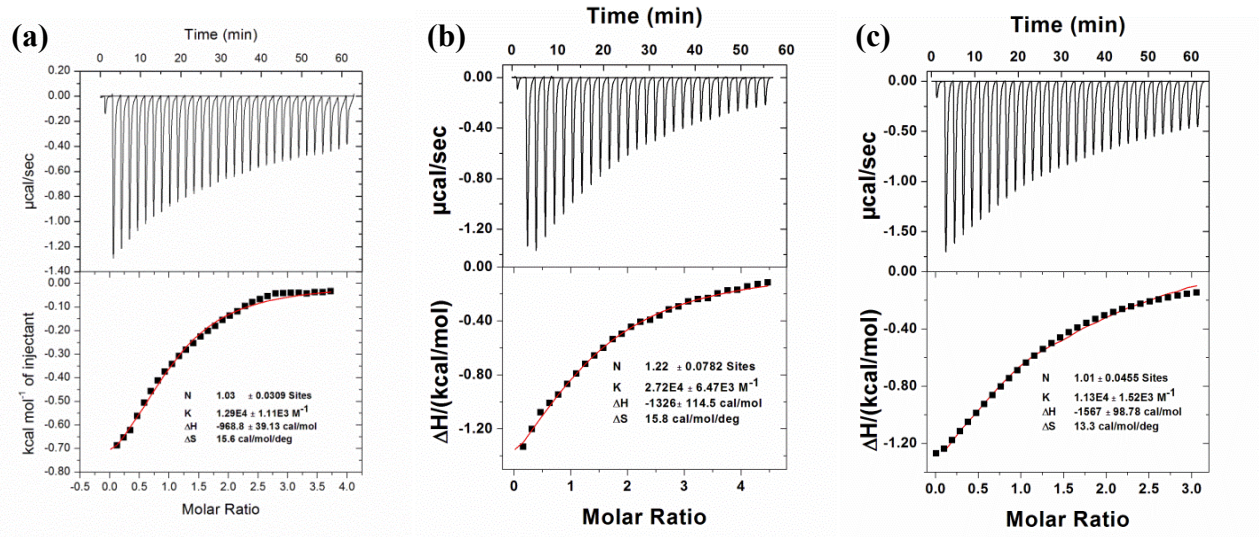


Figure S12. ITC curves obtained at 298 K from titration of MINP-CHO(*p*-**G2**) with (a) glucose, (b) maltose and (c) maltotriose in HEPEs buffer (10 mM, pH 7.4). The data correspond to entries 5-7 in Table 1, respectively.

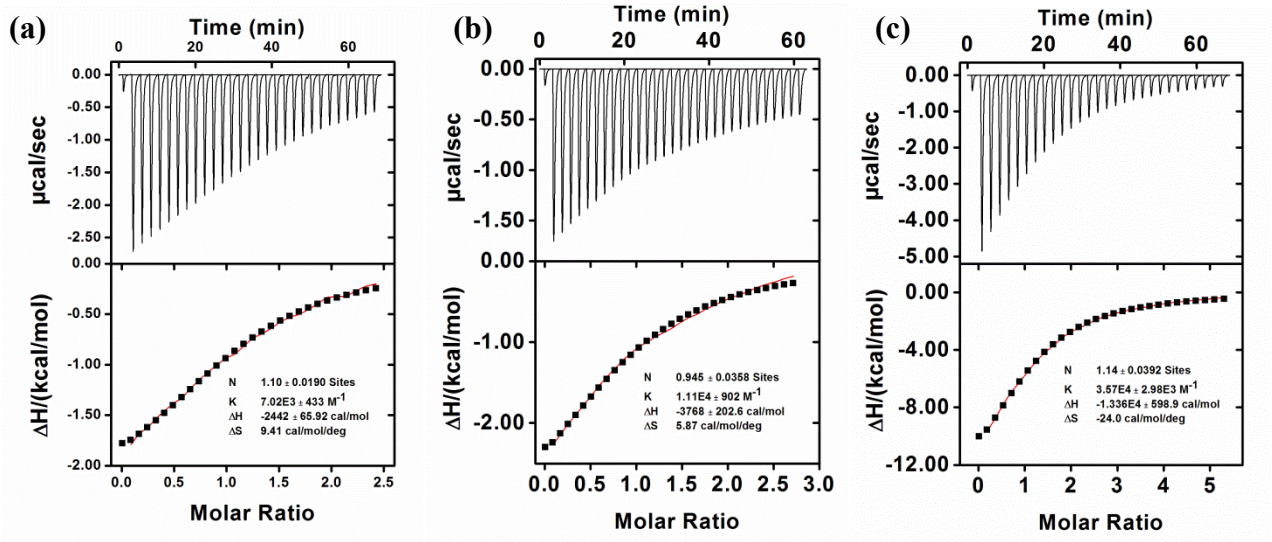


Figure S13. ITC curves obtained at 298 K from titration of MINP-CHO(*p*-**G3**) with (a) glucose, (b) maltose and (c) maltotriose in HEPEs buffer (10 mM, pH 7.4). The data correspond to entries 8-10 in Table 1, respectively.

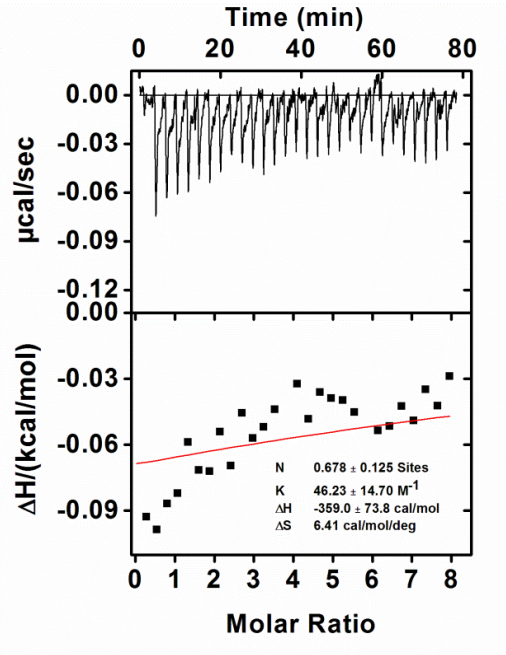


Figure S14. ITC curves obtained at 298 K from titration of NINP prepared with 1.0 equiv. of **4** with glucose in HEPEs buffer (10 mM, pH 7.4). The data correspond to entry 11 in Table 1.

Table S1. Binding of Oligosaccharides by MINPs determined by Isothermal titration calorimetry (ITC).[a]

Entry	MINP	Oligosaccharides	K_a (10^3 M^{-1})	ΔG (kcal/mol)	N[b]
1	MINP(*p*-**G1**+**7h**)	maltose	7.99 ± 0.56	-5.32	1.27 ± 0.04
2	MINP(*p*-**G1**+**7h**)	cellobiose	1.88 ± 0.11	-4.46	0.99 ± 0.16
3	MINP(*p*-**G1**+**7h**)	sucrose	1.83 ± 0.11	-4.45	1.75 ± 0.13
4	MINP(*p*-**G1**+**7h**)	maltulose	2.99 ± 1.29	-4.74	0.97 ± 0.42
5	MINP(*p*-**G1**+**7h**)	lactose	0.20 ± 0.04	-3.12	1.89 ± 0.61
6	MINP(*p*-**G1**+**7h**)	xylobiose	＜0.05	--[c]	--[c]
7	MINP(*p*-**G1**+**7h**)	maltotriose	5.62 ± 0.57	-5.11	1.29 ± 0.08
8	MINP(*p*-**G2**+**7h**)	maltotriose	11.50 ± 0.50	-5.54	1.44 ± 0.01
9	MINP(*p*-**G1**+**7h**)	cellotriose	2.62 ± 0.31	-4.66	1.88 ± 0.14
10	MINP(*p*-**G2**+**7h**)	cellotriose	3.64 ± 0.34	-4.85	0.64 ± 0.11

[a] The FM/template ratio in the MINP synthesis was 1:1. The cross-linkable surfactants were a 3:2 mixture of **1b** and **2**. The titrations were performed in water at 298 K. [b] N is the average number of binding site per nanoparticle measured by ITC curve fitting. [c] Binding was extremely weak. Because the binding constant was estimated from ITC, -ΔG and N are not listed.

ITC titration curves:

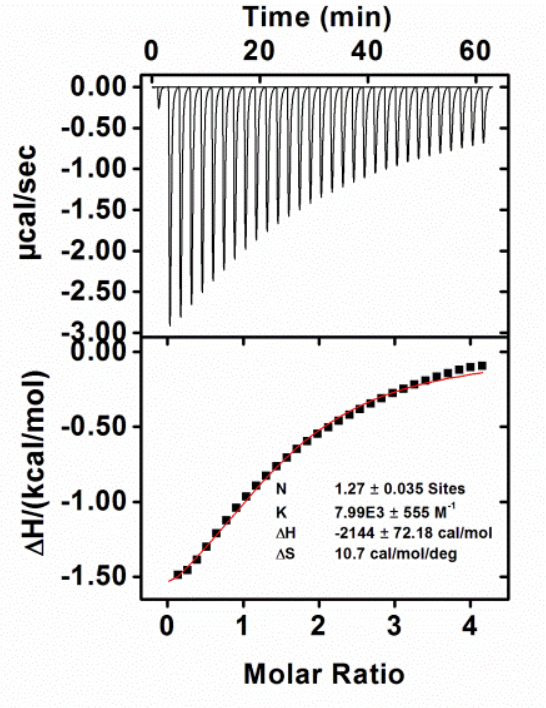


Figure S15. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with maltose in water. The data correspond to entry 1 in Table S1.

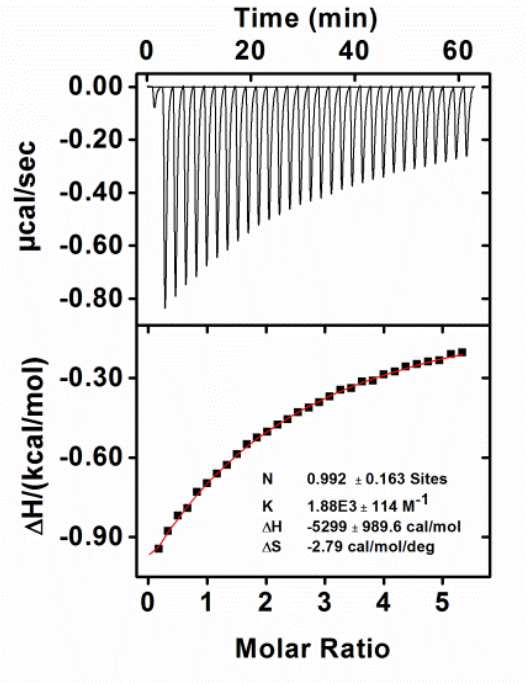


Figure S16. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with cellobiose in water. The data correspond to entry 2 in Table S1.

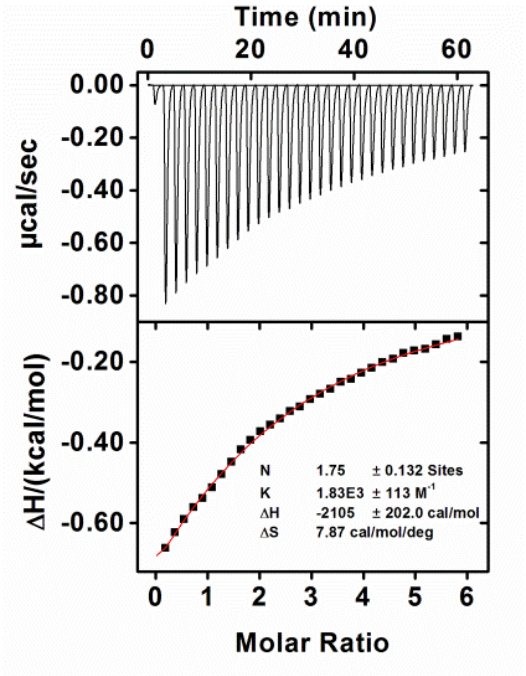


Figure S17. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with sucrose in water. The data correspond to entry 3 in Table S1.

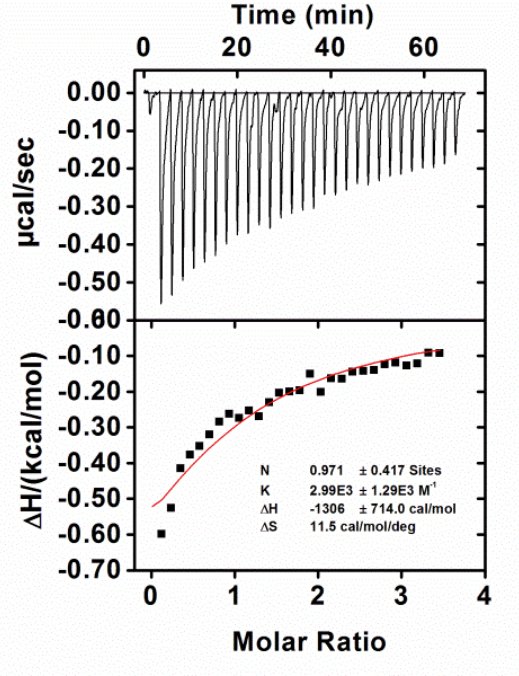


Figure S18. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with maltulose in water. The data correspond to entry 4 in Table S1.

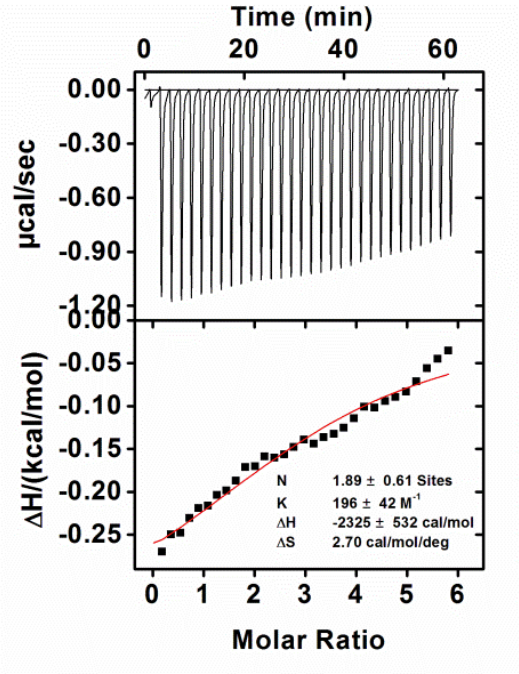


Figure S19. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with lactose in water. The data correspond to entry 5 in Table S1.

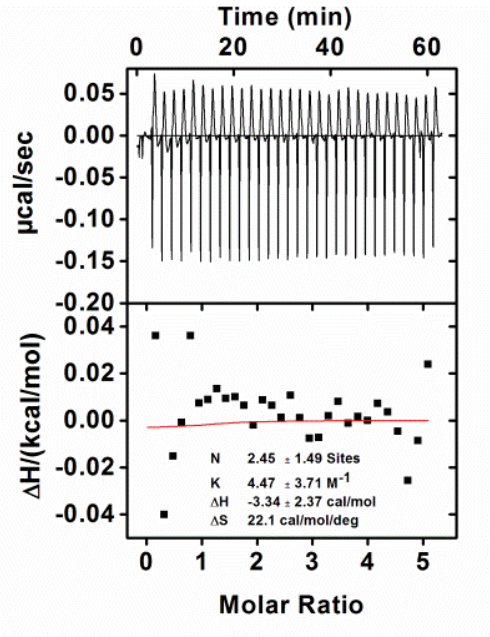


Figure S20. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with xylobiose in water. The data correspond to entry 6 in Table S1.

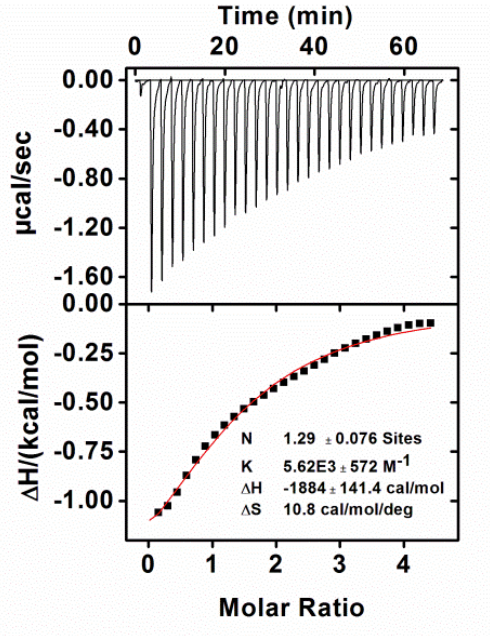


Figure S21. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with maltotriose in water. The data correspond to entry 7 in Table S1.

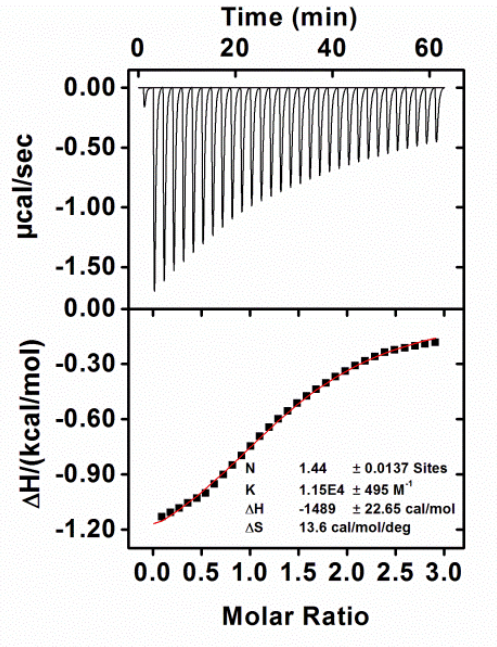


Figure S22. ITC curves obtained at 298 K from titration of MINP(*p*-**G2**+**7h**) with maltotriose in water. The data correspond to entry 8 in Table S1.

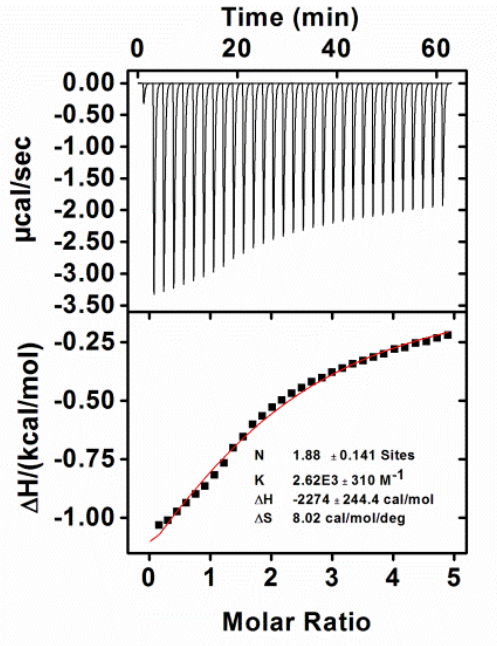


Figure S23. ITC curves obtained at 298 K from titration of MINP(*p*-**G1**+**7h**) with cellotriose in water. The data correspond to entry 9 in Table S1.

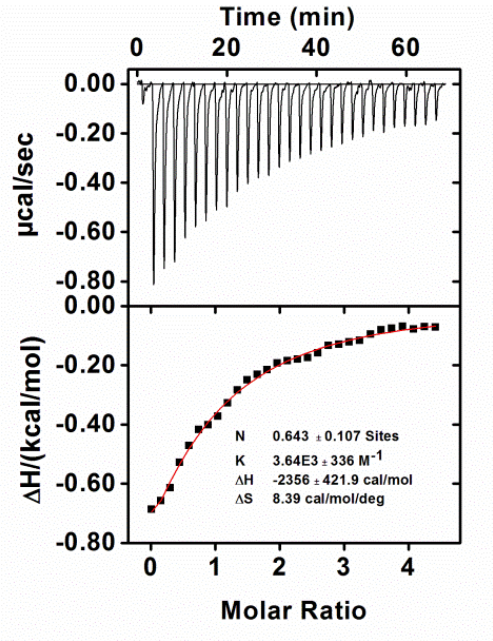


Figure S24. ITC curves obtained at 298 K from titration of MINP(*p*-**G2**+**7h**) with cellotriose in water. The data correspond to entry 10 in Table S1.

Catalytic Hydrolysis of Oligosaccharide by MINPs

Analysis of catalytic hydrolysis reactions using LC/MS

Hydrolysis of maltose and maltohexaose was monitored by LC-MS analysis using an Agilent 1200 Series Binary VWD system with an Agilent 6540 UHD Accurate Mass Q-TOF mass detector. Separation of the products was performed on a Thermo Scientific HILIC-LC column (4.6 mm, 150 mm) at 60 ºC. For quantitative analysis, injection volumes were adjusted for the signal intensity to stay within the linear range of the calibration curve. All samples were centrifuged at 20,000 RPM before analysis to remove the MINP particles (to avoid column blockage over extended usage). Comparison with non-centrifuged samples showed no change in glycan concentration during the centrifugation step. The mobile phase was a mixture of acetonitrile and water, with 0.1% formic acid. Product peaks were identified by a high-resolution mass detector in the negative ion mode.

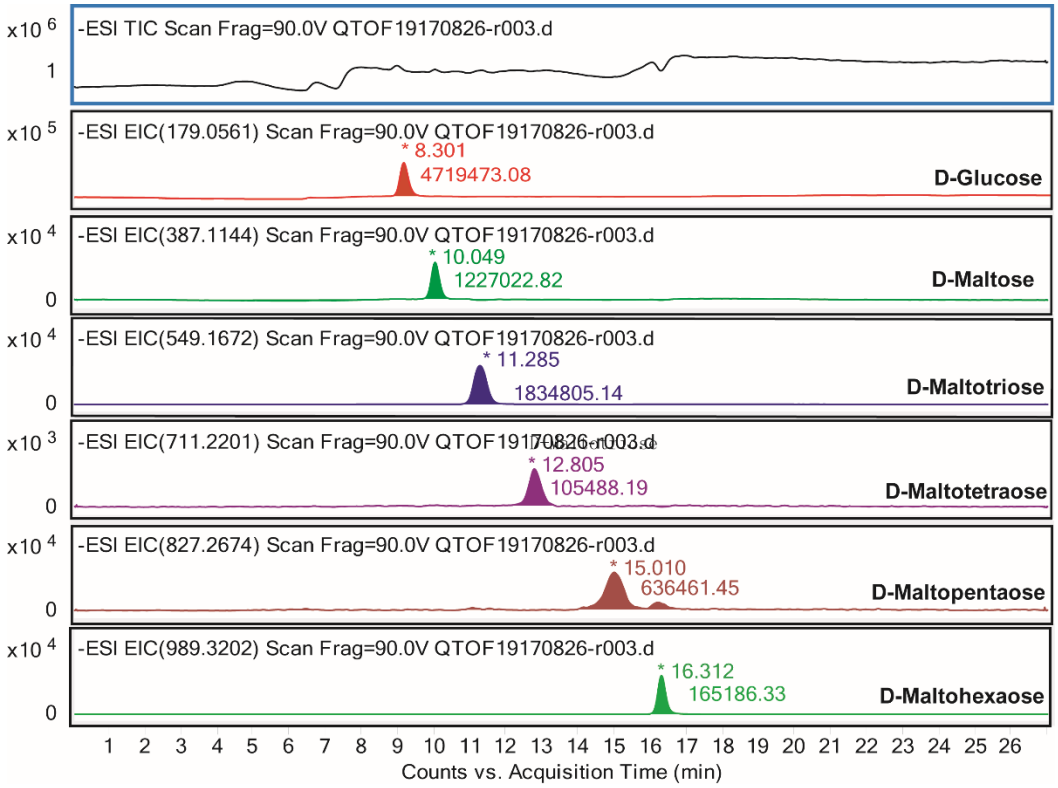


Figure S25. LC-MS analysis of a glycan mixture, with the extracted ion chromatography (EIC) spectra showing the glucose peak (red) at 8.30 min, maltose (green) at 10.05 min, maltotriose (blue) at 11.28 min, maltotetraose (purple) at 12.80 min, maltopentaose (brown) at 15.01 min, and maltohexaose (light green) at 16.31 min.

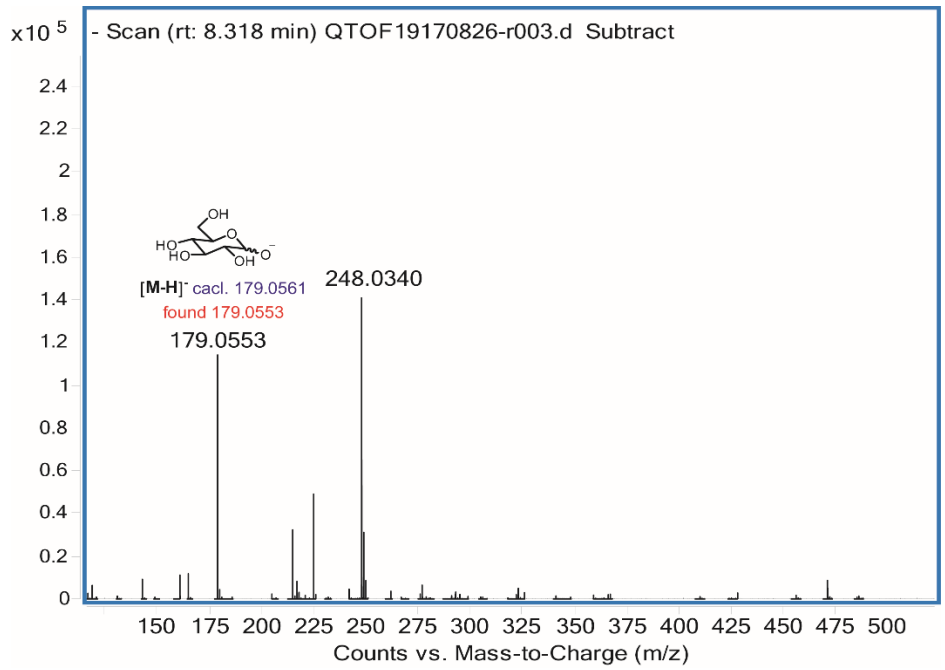


Figure S26. HR-MS spectrum of D-glucose at 8.3 min.

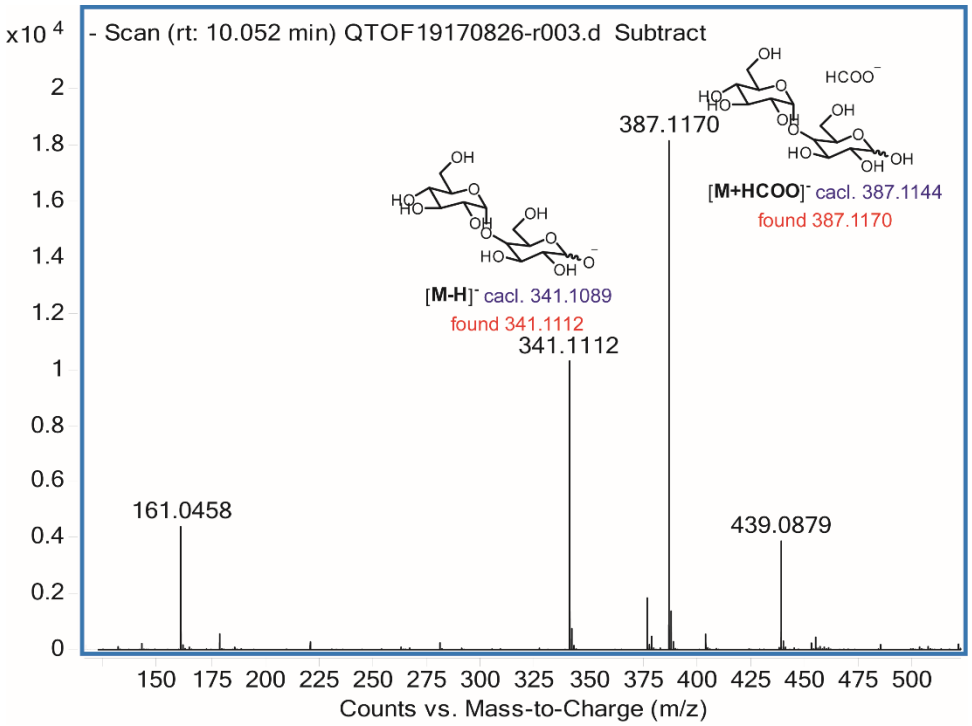


Figure S27. HR-MS spectrum of D-maltose at 10.0 min.

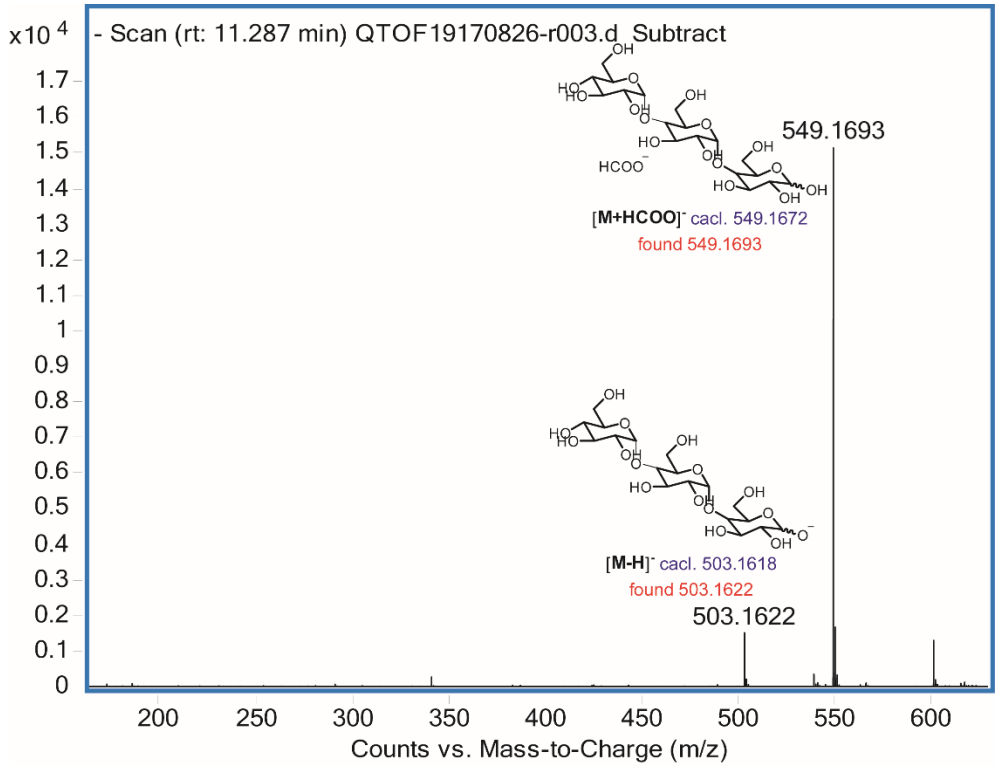


Figure S28. HR-MS spectrum of D-maltotriose at 11.3 min.

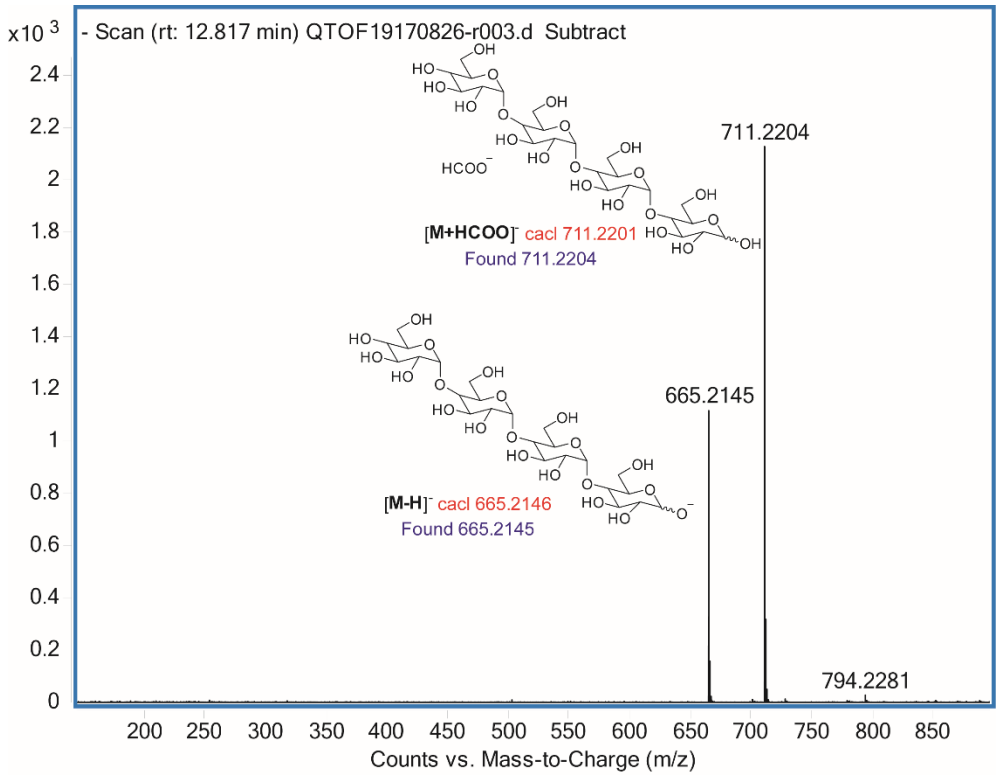


Figure S29. HR-MS spectrum of D-maltotetraose at 12.8 min.

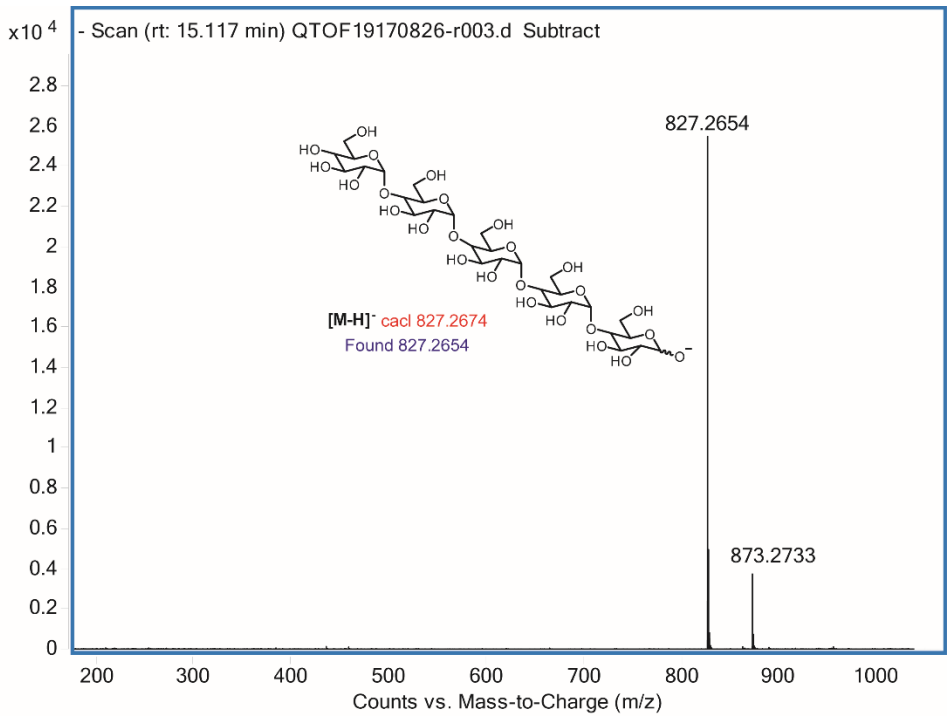


Figure S30. HR-MS spectrum of D-maltopentaose at 15.1 min.

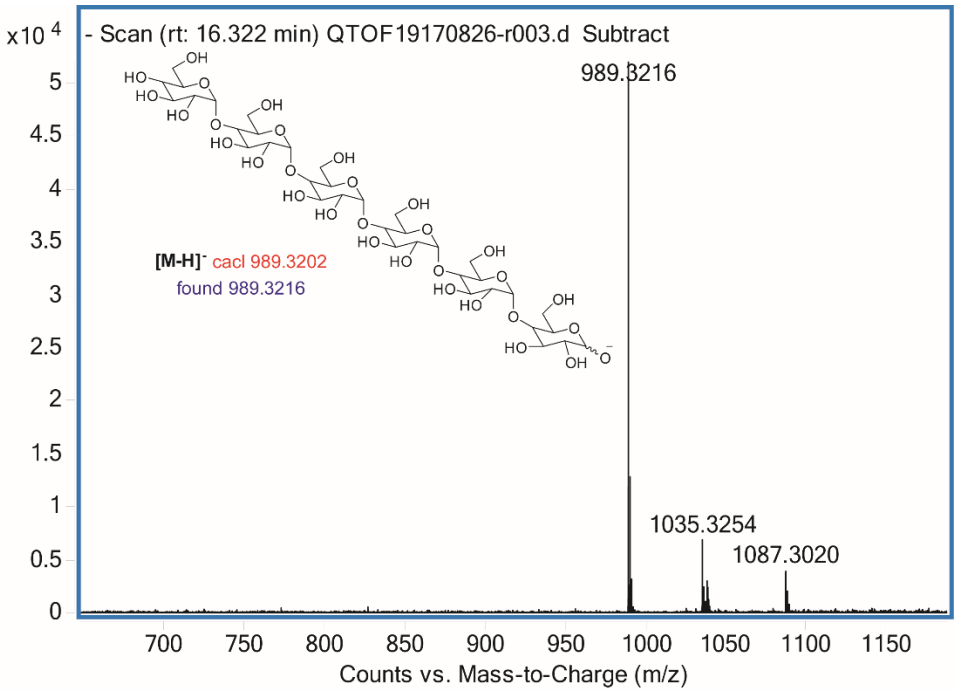


Figure S31. HR-MS spectrum of D-maltohexaose at 16.3 min.

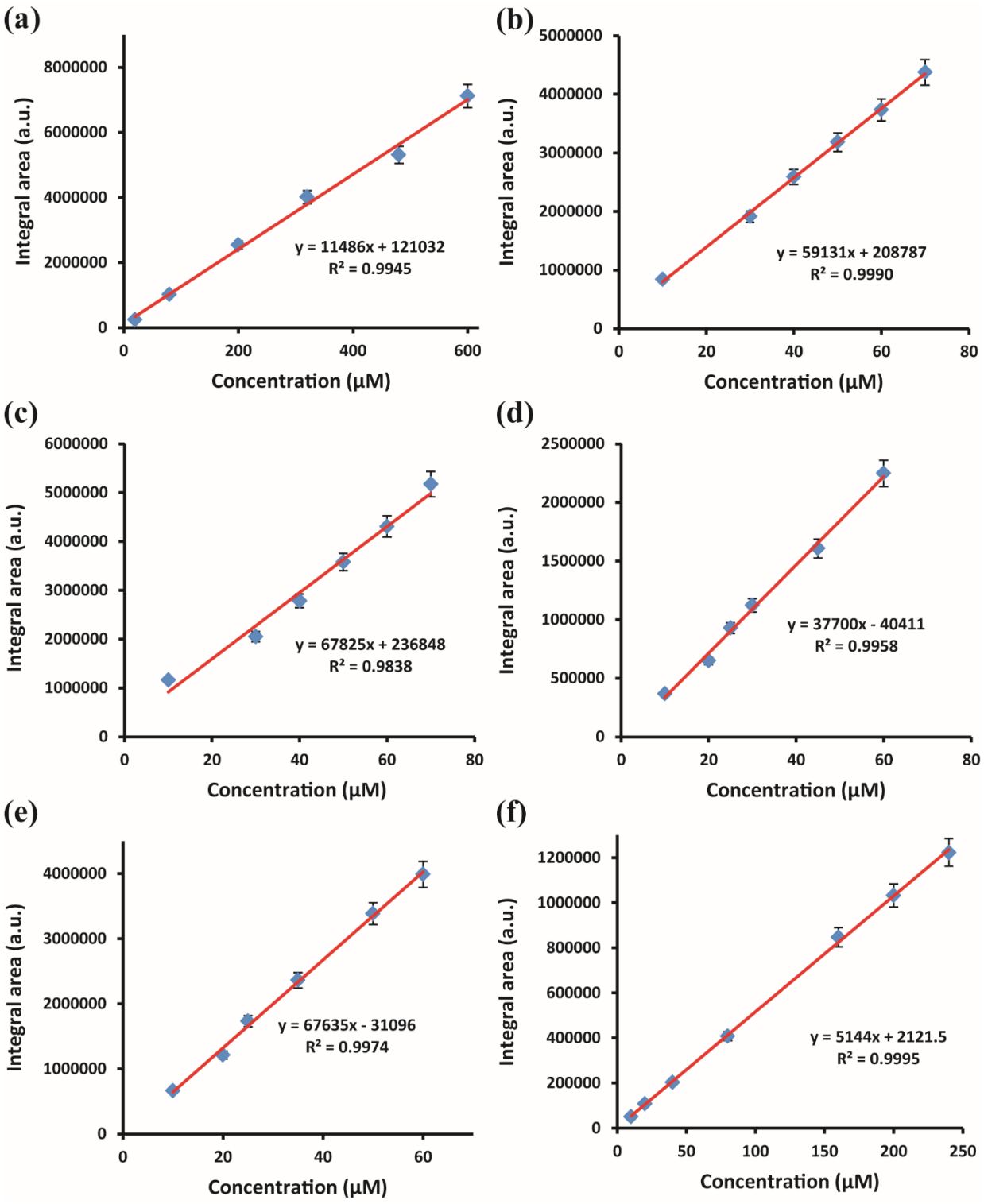


Figure S32. Calibration curves of (a) D-glucose, (b) D-maltose, (c) D-maltotriose (d) D-maltotetraose, (e) D-maltopentaose and (f) D-maltohexaose generated from LCMS analysis by using gallic acid as the internal standard.

pH Effect of Maltohexaose Hydrolysis

Table S2. Hydrolysis of maltohexaose (**G6**) catalyzed by MINPs at different pH. [a]

Entry	MINP [c]	Temp. (°C) & Time (h)	pH	Product Concentration (μM)		
				G1	**G2**	**G3**
1	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	Water	6 ± 2	144 ± 17	7 ± 2
2	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	7.5 [b]	n.d.	n.d.	n.d.
3	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	7.0 [b]	trace [d]	79 ± 8	2 ± 1
4	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	6.5 [c]	5± 2	122 ± 14	2 ± 1
5	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	6.0 [c]	trace [d]	157 ± 23	7 ± 4
6	MINP(*p*-**G2**+*p*-**7h**)	60 / 24	5.5 [c]	13 ± 4	139 ± 16	11 ± 2

[a] MINPs were prepared with surfactants **1b** and **2**. Reactions were performed in duplicates with 0.1 mM maltohexaose (**G6**) and 20 μM MINP in 1.0 mL water or buffer. Yields were determined by LC-MS using calibration curves generated from authentic samples (Figure S32). [b] HEPES buffer was used for the hydrolysis. [c] MES buffer was used for the hydrolysis. [d] The product concentration ＜1 μM. n.d. = not detected by MS.

Full Characterization of the Hydrolyzed Products in the Maltohexaose (G6) Hydrolysis

Table S3. Hydrolysis of maltohexaose (**G6**) in water catalyzed by MINPs. [a]

Entry	MINP [c]	Temp. (°C)	Time (h)	Product Concentration (μM) [b]					
				G1	**G2**	**G3**	**G4**	**G5**	**G6**
1	MINP(*p*-**G1**+*p*-**7h**)	60	24	253 ± 41	12 ± 4	42 ± 8	trace [b]	trace [b]	57 ± 11
2	MINP(*p*-**G1**+*p*-**7h**)	90	48	421 ± 78	17 ± 6	24 ± 5	trace [b]	10 ± 3	32 ± 7
3	MINP(*p*-**G2**+*p*-**7h**)	60	24	11 ± 4	117 ± 21	14 ± 3	11 ± 2	trace [b]	62 ± 12
4	MINP(*p*-**G2**+*p*-**7h**)	90	48	15 ± 4	158 ± 28	13 ± 4	21 ± 4	trace	47 ± 10
5	MINP(*p*-**G3**+*p*-**7h**)	60	24	14 ± 6	17 ± 5	98 ± 17	trace [b]	trace [b]	53 ± 11
6	MINP(*p*-**G3**+*p*-**7h**)	90	48	21 ± 7	14 ± 4	133 ± 24	trace [b]	trace [b]	34 ± 8

[a] MINPs were prepared with surfactants **1b** and **2**. Reactions were performed in duplicates at 0.1 mM maltohexaose (**G6**) and 20 μM MINP in 1.0 mL water. Yields were determined by LC-MS using calibration curves generated from authentic samples (Figure S32). Typically, estimated errors of product concentration were ± 20%. [b] The product concentration ＜1 μM.

Table S4. Hydrolysis of maltohexaose (**G6**) in MES buffer (pH = 6.0) catalyzed by MINPs. [a]

Entry	MINP [c]	Temp. (°C)	Time (h)	Product Concentration (μM) [b]					
				G1	**G2**	**G3**	**G4**	**G5**	**G6**
1	MINP(*p*-**G1**+*p*-**7h**)	60	24	271± 45	25 ± 11	24 ± 8	10 ± 4	14 ± 3	49 ± 11
2	MINP(*p*-**G1**+*p*-**7h**)	90	48	462 ± 82	17 ± 4	29 ± 9	14 ± 4	10 ± 4	25 ± 7
3	MINP(*p*-**G1**+*p*-**7h**)	90	96	518 ± 91	trace [b]	14 ± 4	trace [b]	7± 4	13 ± 4
4	MINP(*p*-**G2**+*p*-**7h**)	60	24	trace [b]	157 ± 23	7 ± 4	31 ± 11	trace [b]	55 ± 14
5	MINP(*p*-**G2**+*p*-**7h**)	90	48	trace [b]	245 ± 34	12 ± 4	18 ± 4	trace [b]	21 ± 9
6	MINP(*p*-**G3**+*p*-**7h**)	60	24	14 ± 5	17 ± 4	98 ± 31	trace [b]	trace [b]	48 ± 16
7	MINP(*p*-**G3**+*p*-**7h**)	90	48	35 ± 8	45 ± 10	175 ± 24	trace [b]	trace [b]	13 ± 7

[a] MINPs were prepared with surfactants **1b** and **2**. Reactions were performed in duplicates at 0.1 mM maltohexaose (**G6**) and 20 μM MINP in 1.0 mL buffer. Yields were determined by LC-MS using calibration curves generated from authentic samples (Figure S32). Typically, estimated errors of product concentration were ± 20%. [b] The product concentration ＜1 μM.

Table S5. Hydrolysis of maltohexaose (**G6**) in water catalyzed by MINPs. [a]

Entry	MINP [c]	Dialysis tubing	Product Concentration (μM) [b]					
			G1	**G2**	**G3**	**G4**	**G5**	**G6**
1	MINP(*p*-**G1**+*p*-**7h**)	No	253±41	12±4	42±8	trace [c]	trace [c]	57±11
2	MINP(*p*-**G1**+*p*-**7h**)	Yes	521±38	14±6	21±5	n.d. [b]	6±2	7±2
3	MINP(*p*-**G2**+*p*-**7h**)	No	11±4	117±21	14±3	11±2	trace [c]	62±12
4	MINP(*p*-**G2**+*p*-**7h**)	Yes	n.d. [b]	268±31	n.d. [b]	8±2	n.d. [b]	5±1
5	MINP(*p*-**G3**+*p*-**7h**)	No	14±6	17±5	98±17	trace [c]	trace [c]	53±11
6	MINP(*p*-**G3**+*p*-**7h**)	Yes	26±12	24±9	144±17	n.d. [b]	n.d. [b]	19±8

[a] MINPs were prepared with surfactants **1b** and **2**. Reactions were performed in duplicates at 0.1 mM maltohexaose (**G6**) and 20 μM MINP in 1.0 mL water at 60 ºC for 24 h. Yields were determined by LC-MS using calibration curves generated from authentic samples (Figure S32). [b] n.d. = not detected. [c] Trace is the oligosaccharide concentration was ＜1 μM.

Michaelis−Menten Kinetics

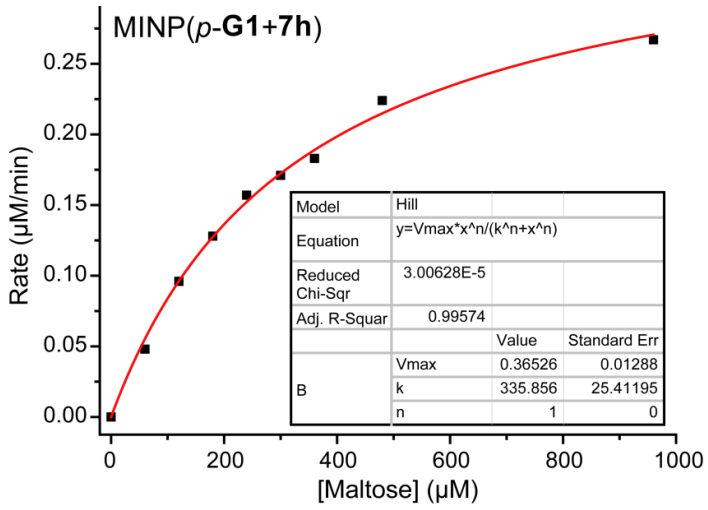


Figure S33. Michaelis–Menten plots of maltose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 ℃ in H_2O. The data corresponds to entry 1 in Table 4.

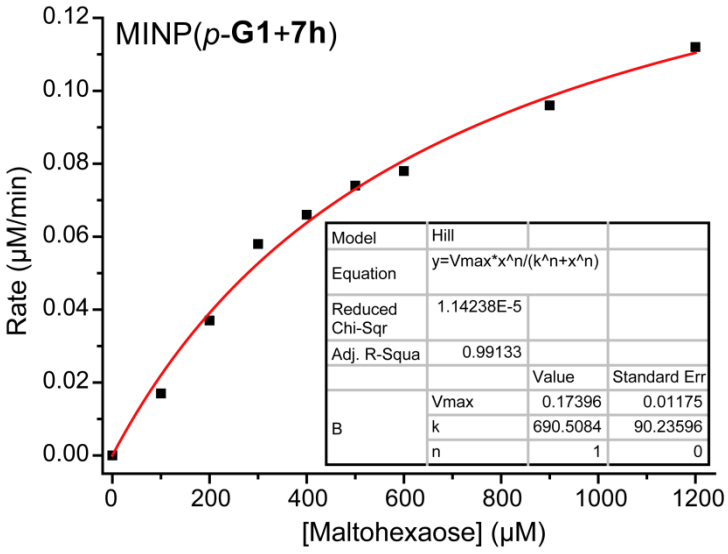


Figure S34. Michaelis–Menten plots of maltohexaose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 ℃ in H_2O. The data corresponds to entry 2 in Table 4.

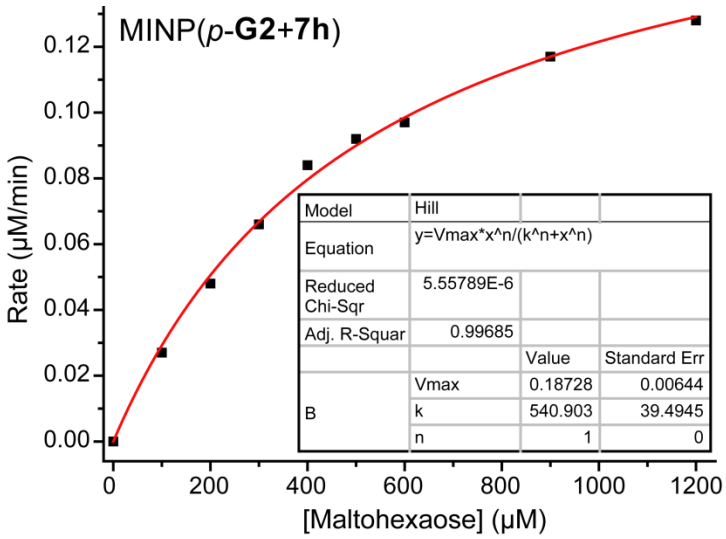


Figure S35. Michaelis–Menten plots of maltohexaose hydrolysis catalyzed by MINP(*p*-**G2**+**7h**) at 60 °C in H_2O. The data corresponds to entry 3 in Table 4.

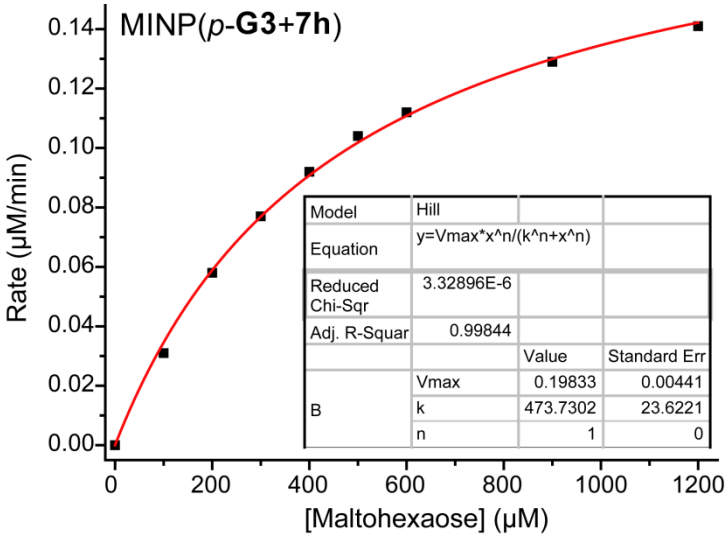


Figure S36. Michaelis–Menten plots of maltohexaose hydrolysis catalyzed by MINP(*p*-**G3**+**7h**) at 60 °C in H_2O. The data corresponds to entry 4 in Table 4.

Product Inhibition Study

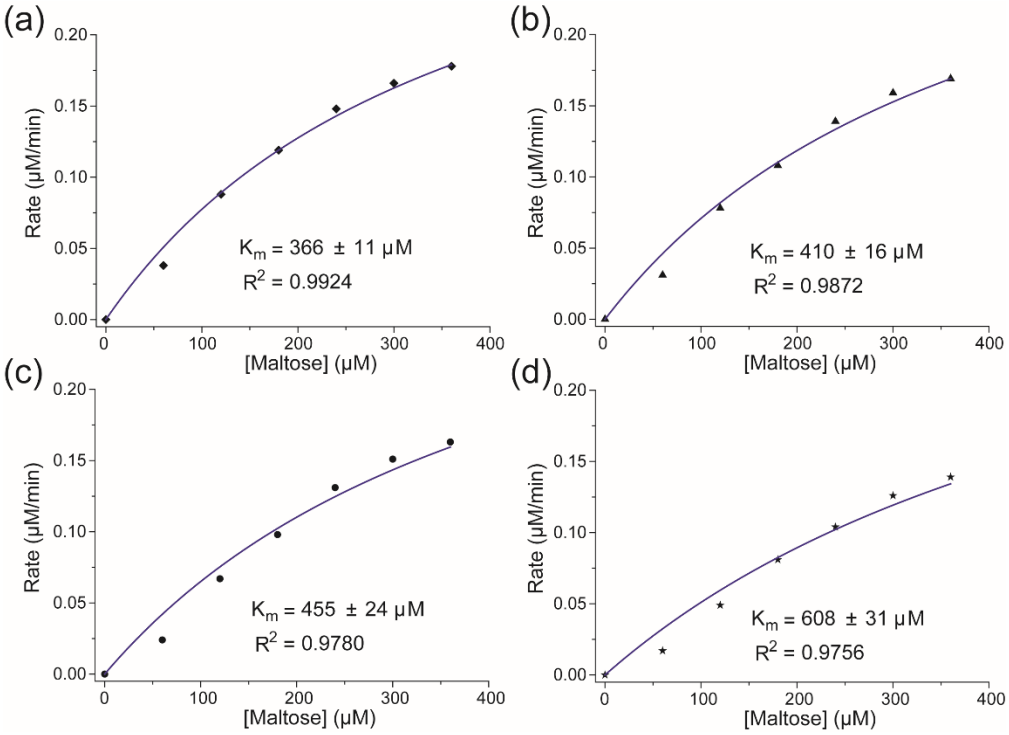


Figure S37. Michaelis–Menten curves for MINP(*p*-**G1**+**7h**) (20 μM) in maltose hydrolysis in the presence of (a) 20 μM, (b) 40 μM, (c) 80 μM, and (d) 160 μM glucose at 60 °C in H_2O.

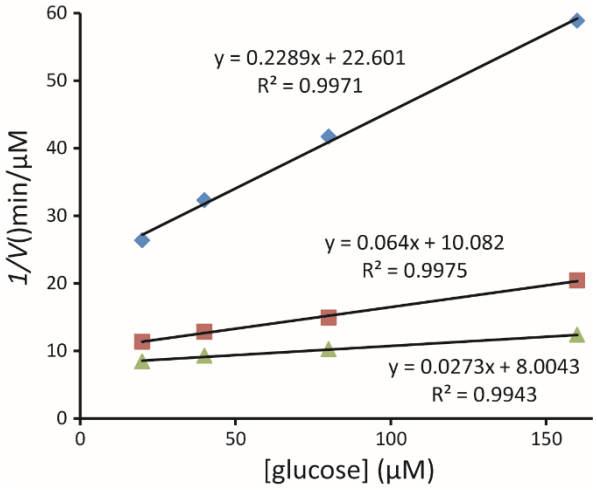


Figure S38. The linear curve fitting of 1/V against [glucose] in MINP(*p*-**G1**+**7h**)-catalyzed maltose hydrolysis in the presence of 20-160 μM glucose at 60 °C in H_2O. The average K_i is calculated to be 68.30 μM.

Substrate Selectivity Study of MINP Catalysts

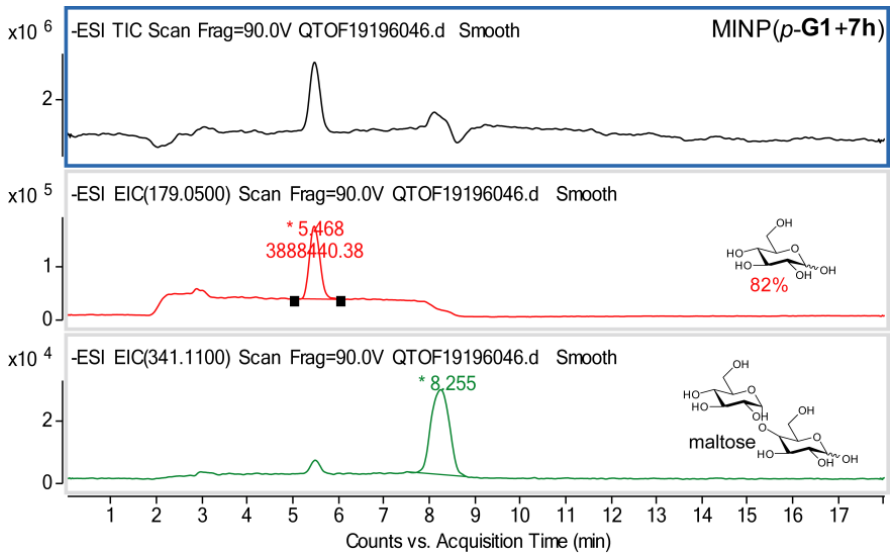


Figure S39. LC-MS analysis of maltose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

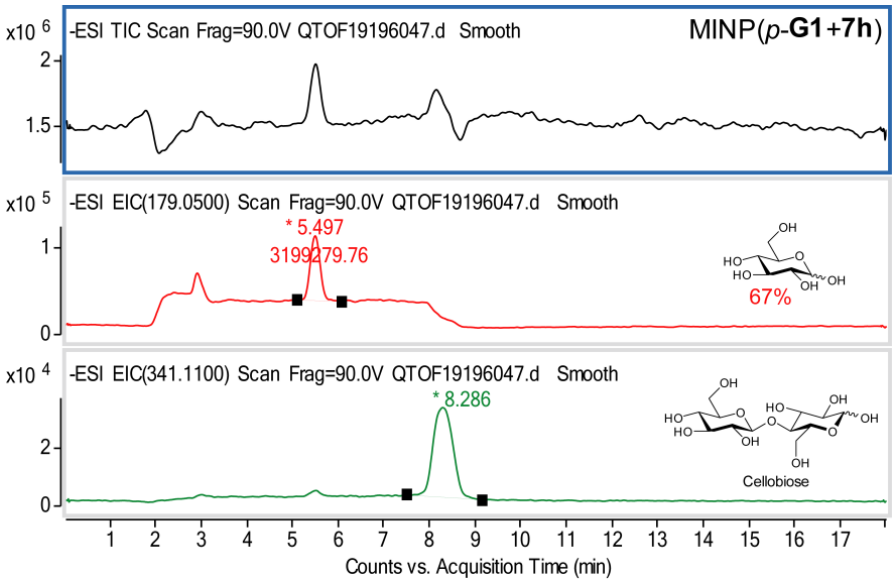


Figure S40. LC-MS analysis of cellobiose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

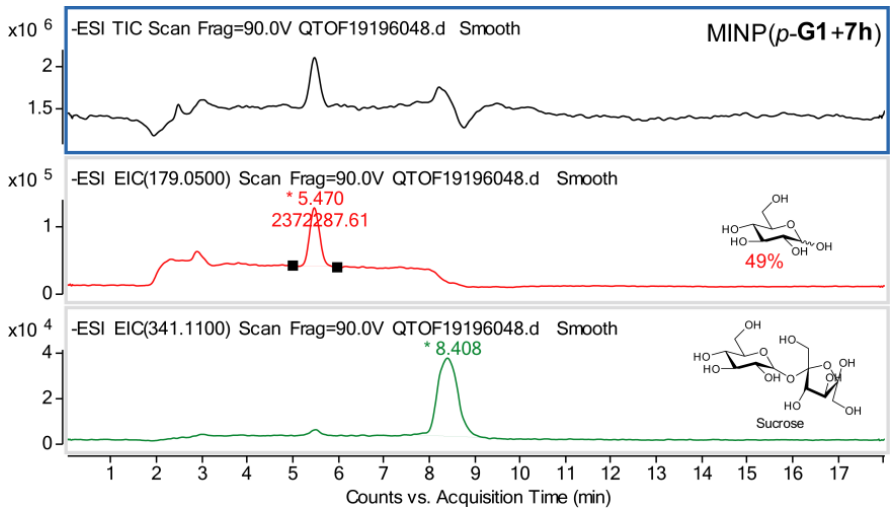


Figure S41. LC-MS analysis of sucrose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

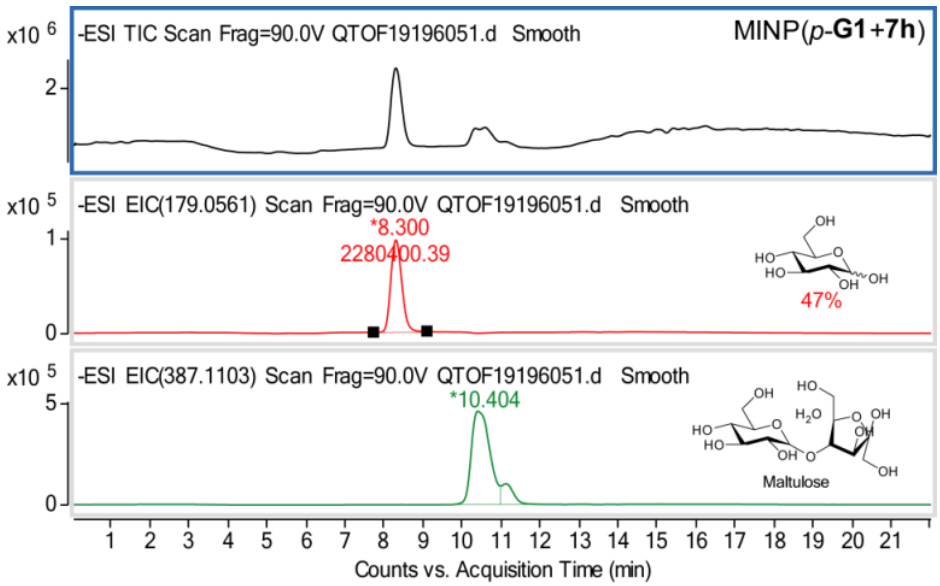


Figure S42. LC-MS analysis of maltulose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

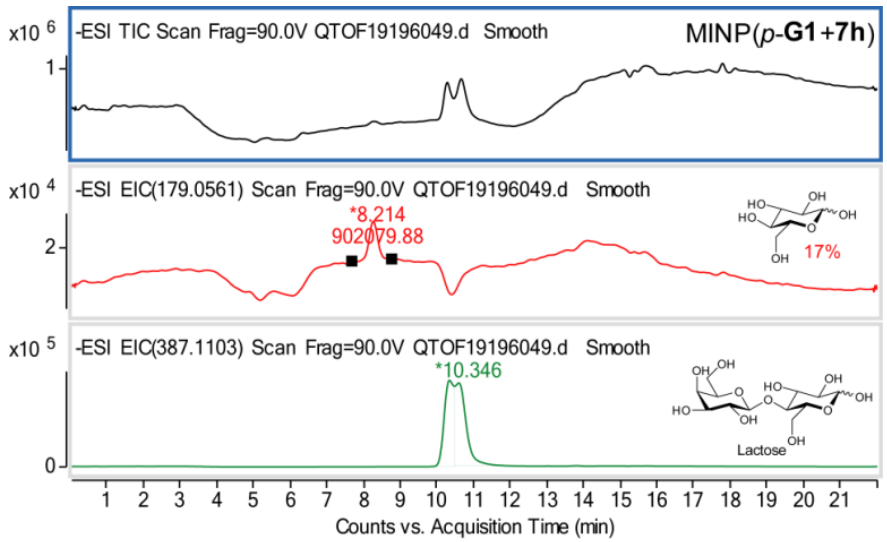


Figure S43. LC-MS analysis of lactose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

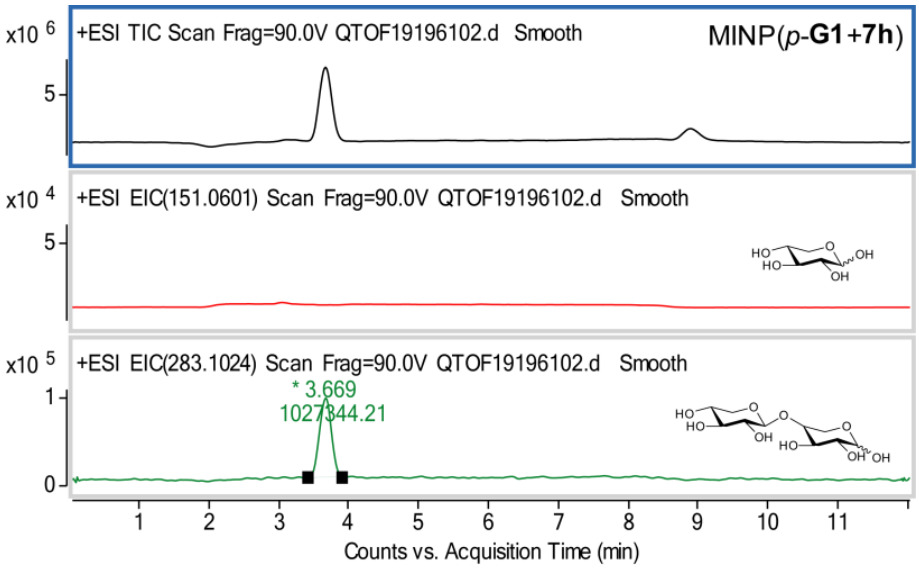


Figure S44. LC-MS analysis of xylobiose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. Product could not be detected.

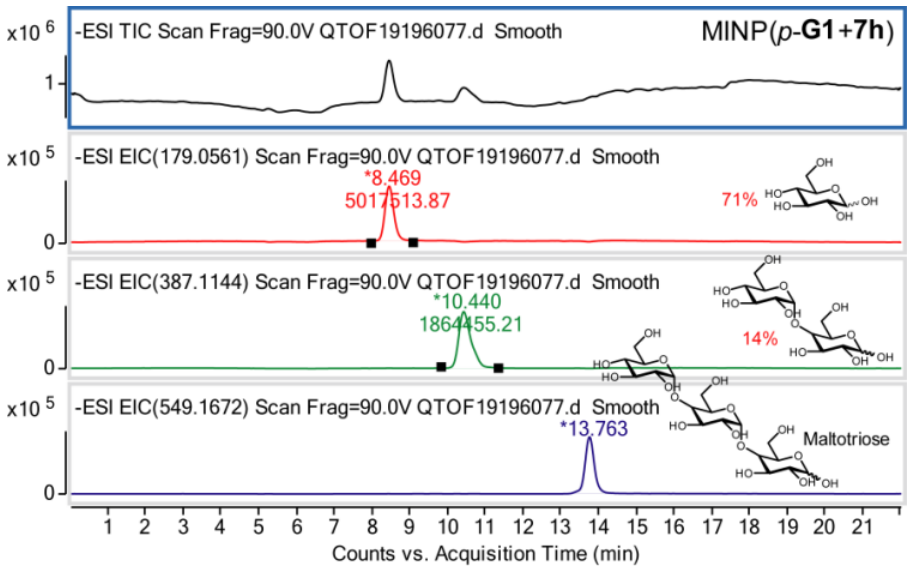


Figure S45. LC-MS analysis of maltotriose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

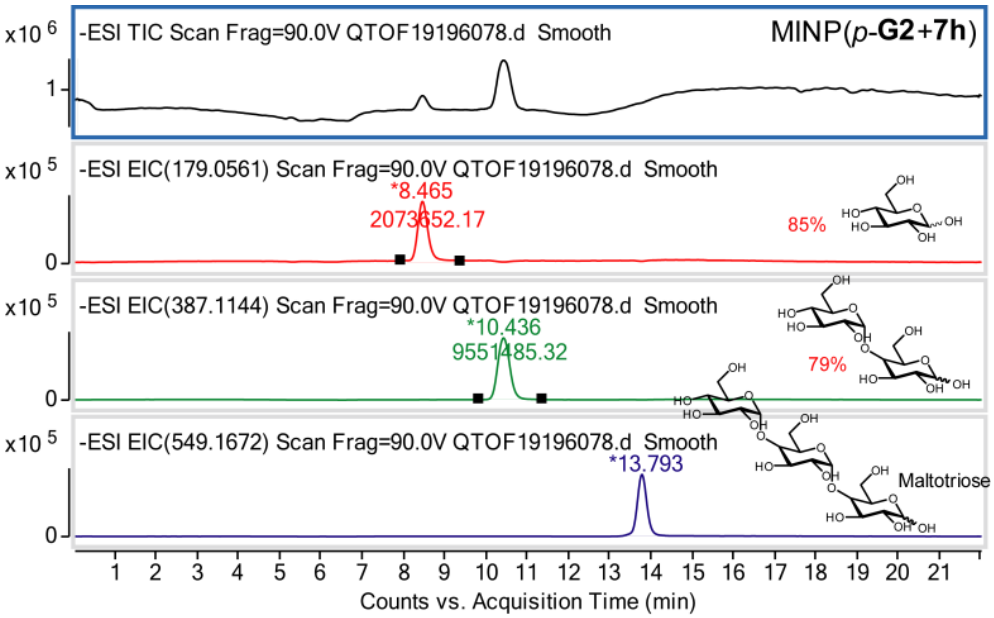


Figure S46. LC-MS analysis of maltotriose hydrolysis catalyzed by MINP(*p*-**G2**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

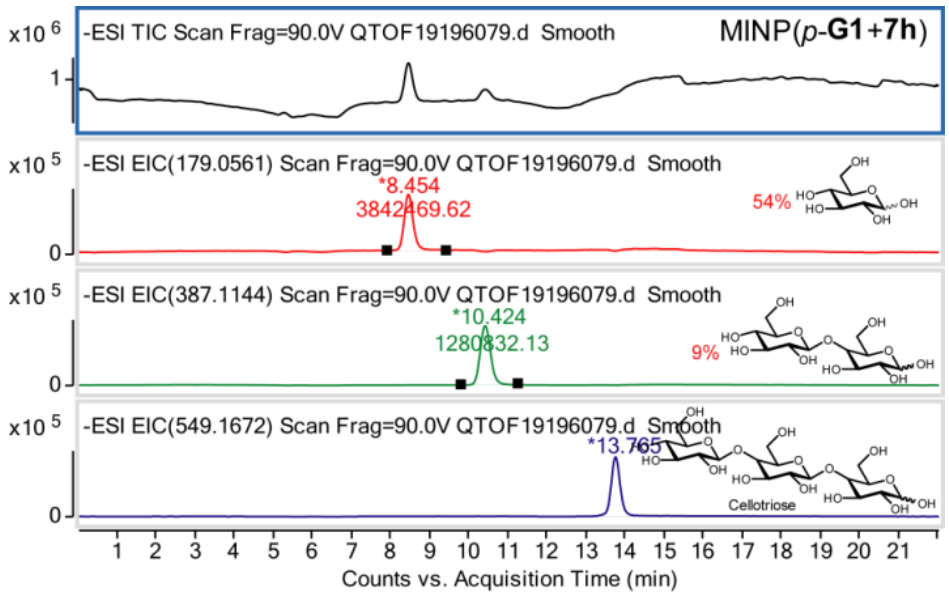


Figure S47. LC-MS analysis of cellotriose hydrolysis catalyzed by MINP(*p*-**G1**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

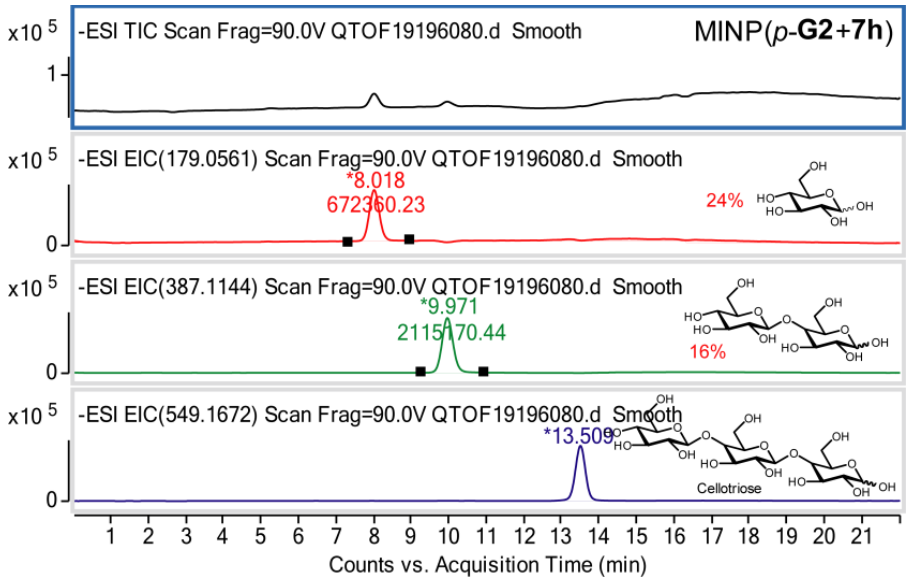
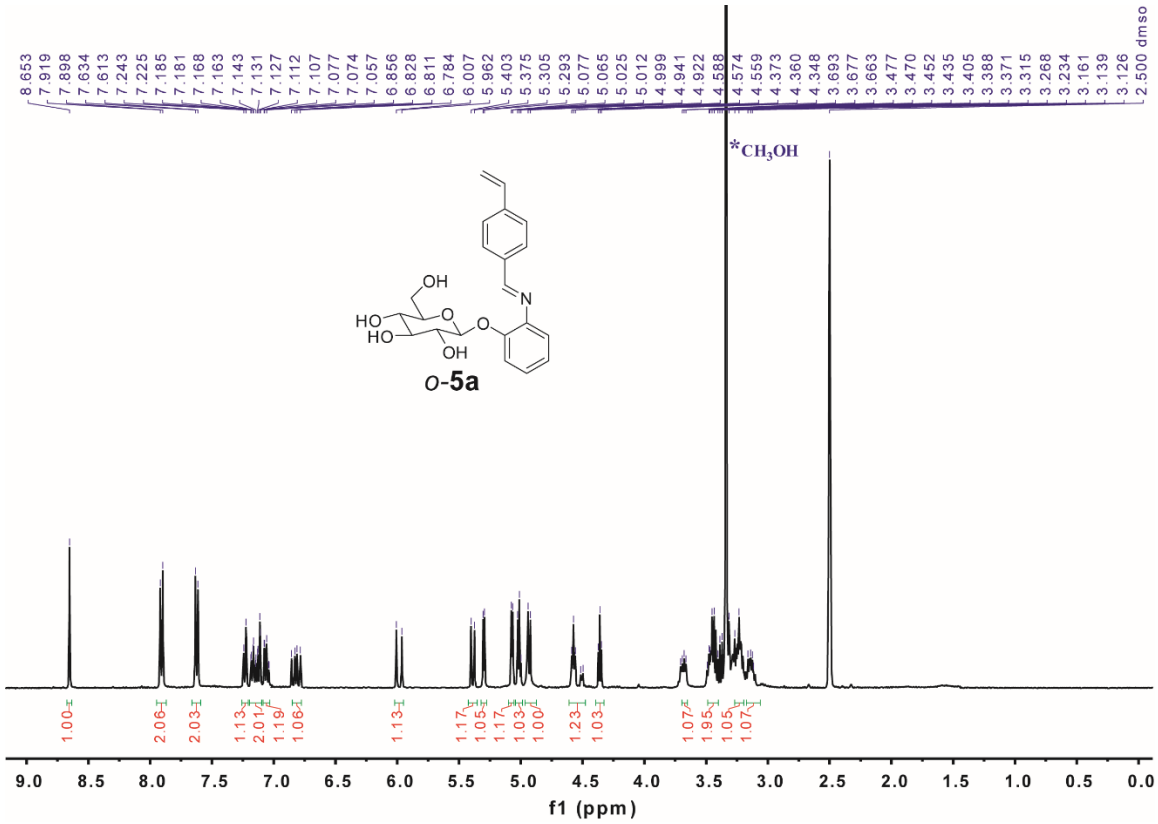
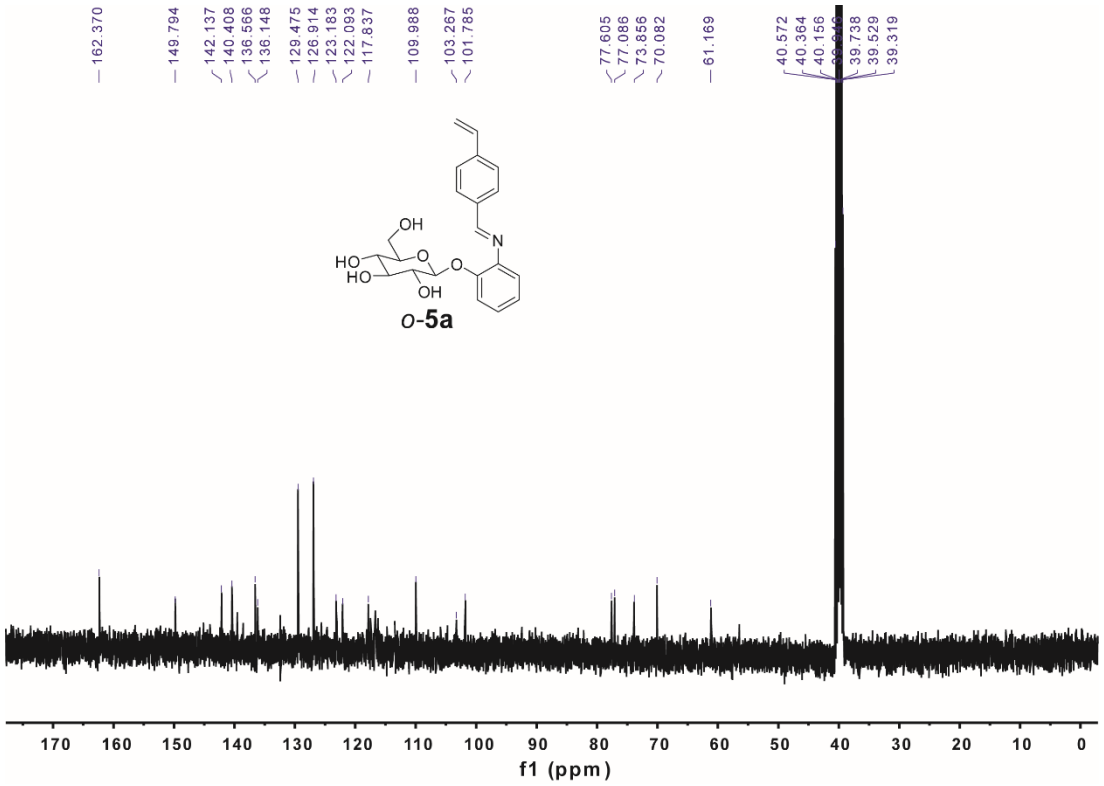
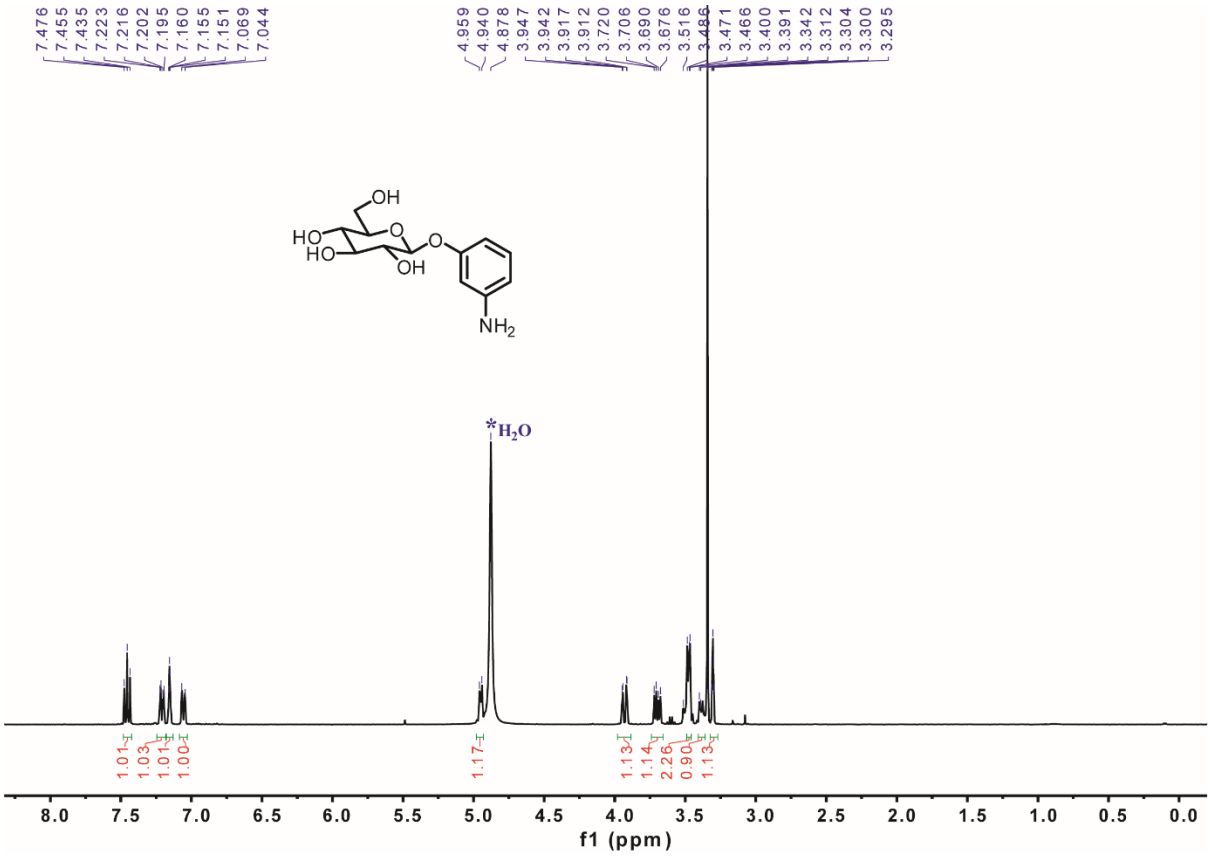


Figure S48. LC-MS analysis of cellotriose hydrolysis catalyzed by MINP(*p*-**G2**+**7h**) at 60 °C in water for 24 h. TIC, total ion chromatography. EIC, extracted ion chromatography. The concentration of product was calculated by using glucose calibration curve (Figure S32) generated from authentic samples.

^{1}H & ^{13}C NMR Spectra



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4.878
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3.942
3.917
3.912
3.720
3.706
3.690
3.676
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3.486
3.471
3.466
3.400
3.391
3.342
3.312
3.304
3.300
3.295
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HO
HO
O
OH
O
NH2
*H2O
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1.03
1.01
1.00
1.17
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1.14
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6.0
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1.0
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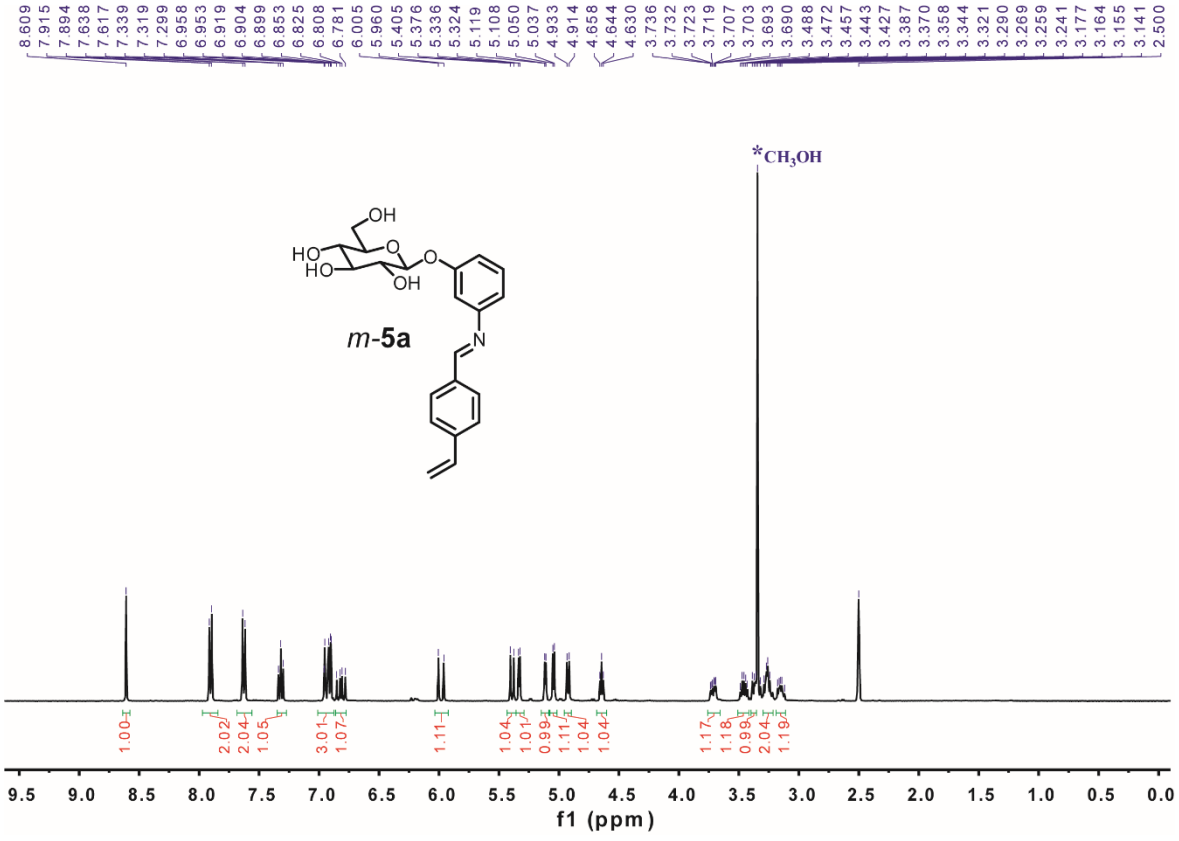
0.0
f1 (ppm)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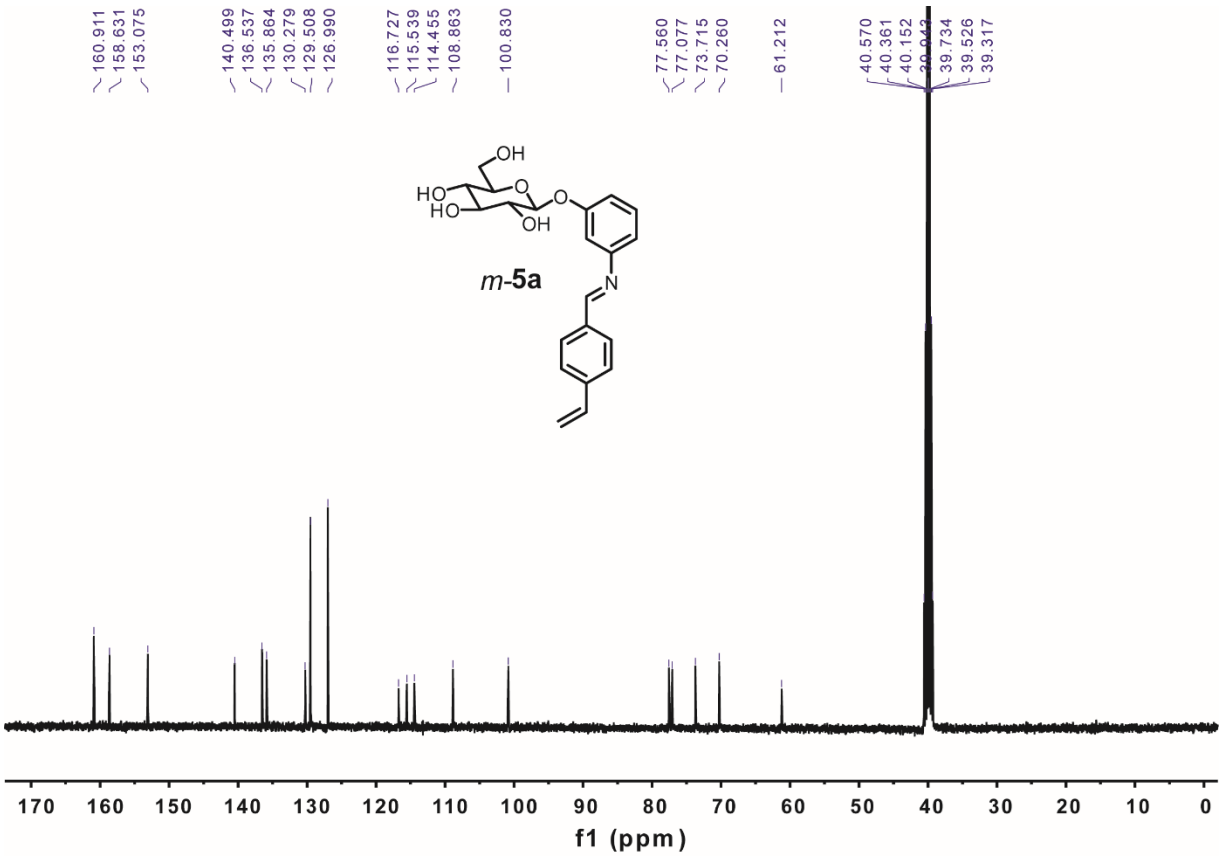
f1 (ppm)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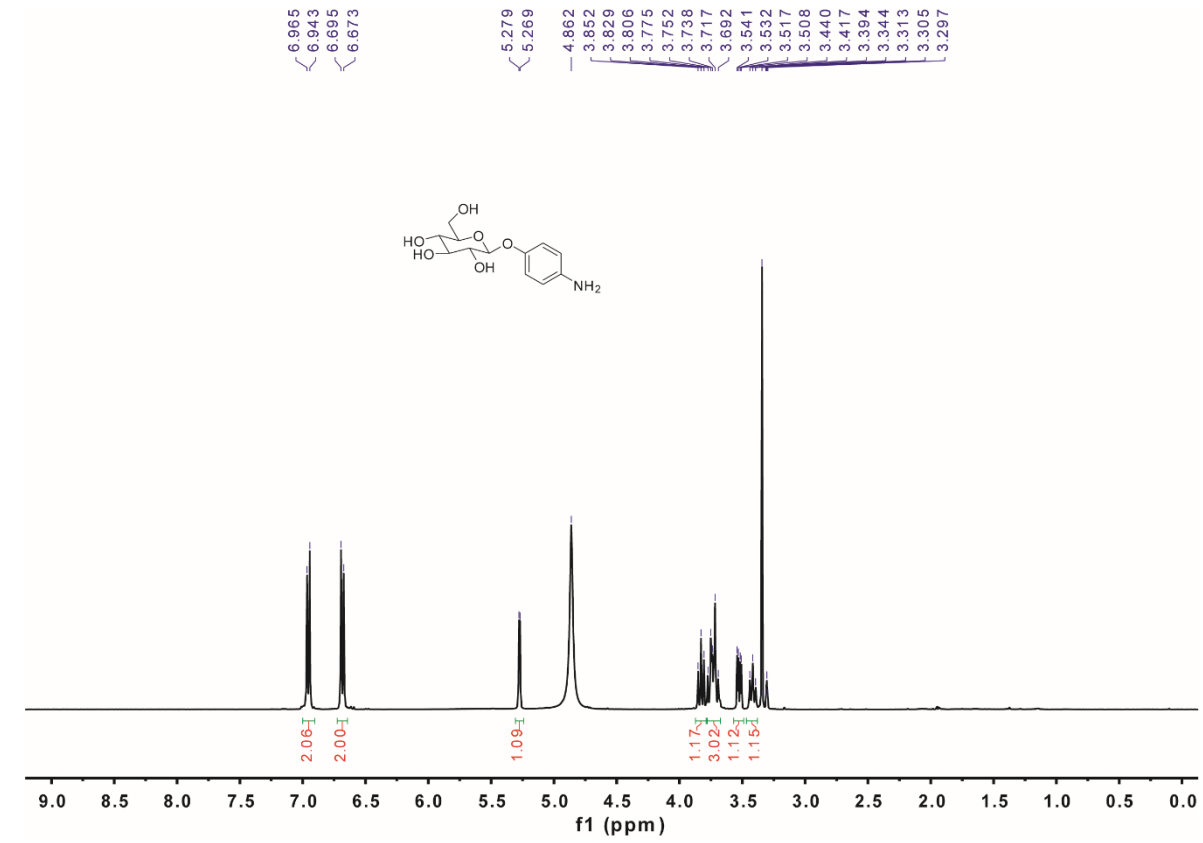
m-5a
f1 (ppm)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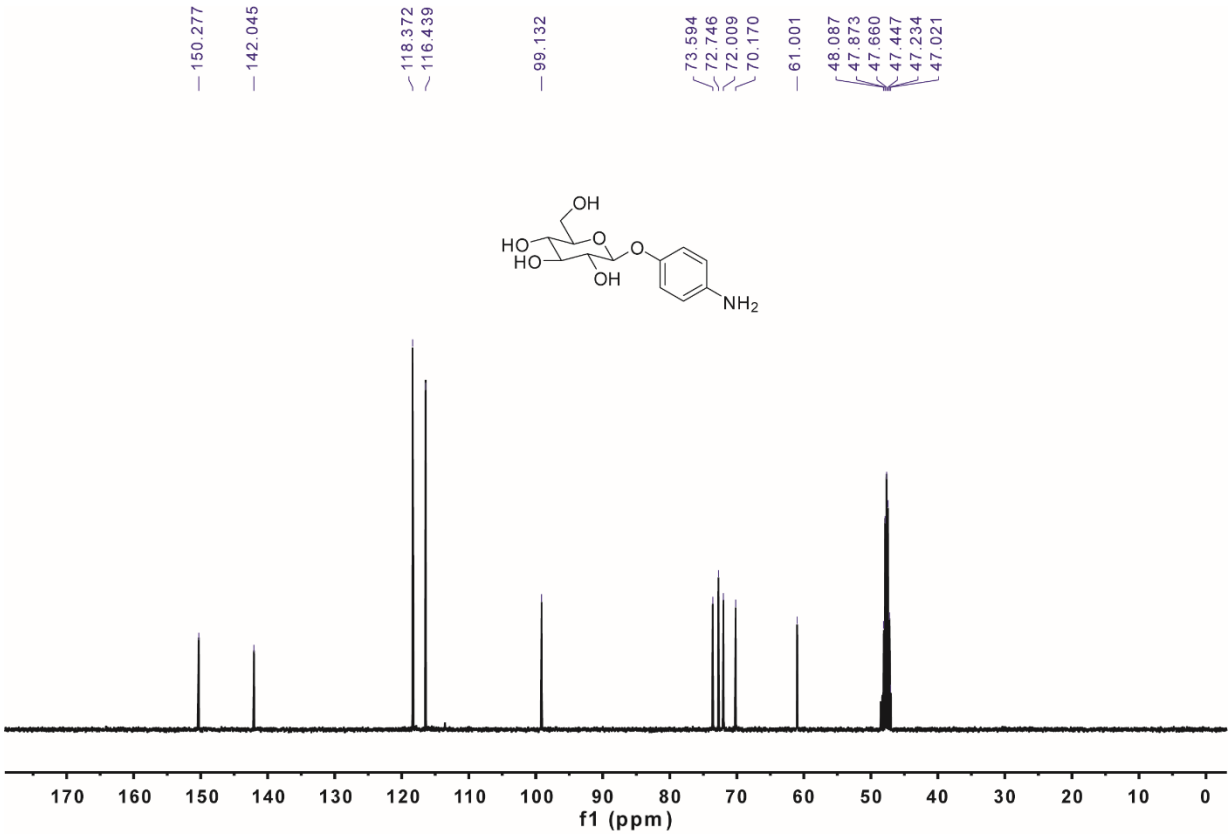
f1 (ppm)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O
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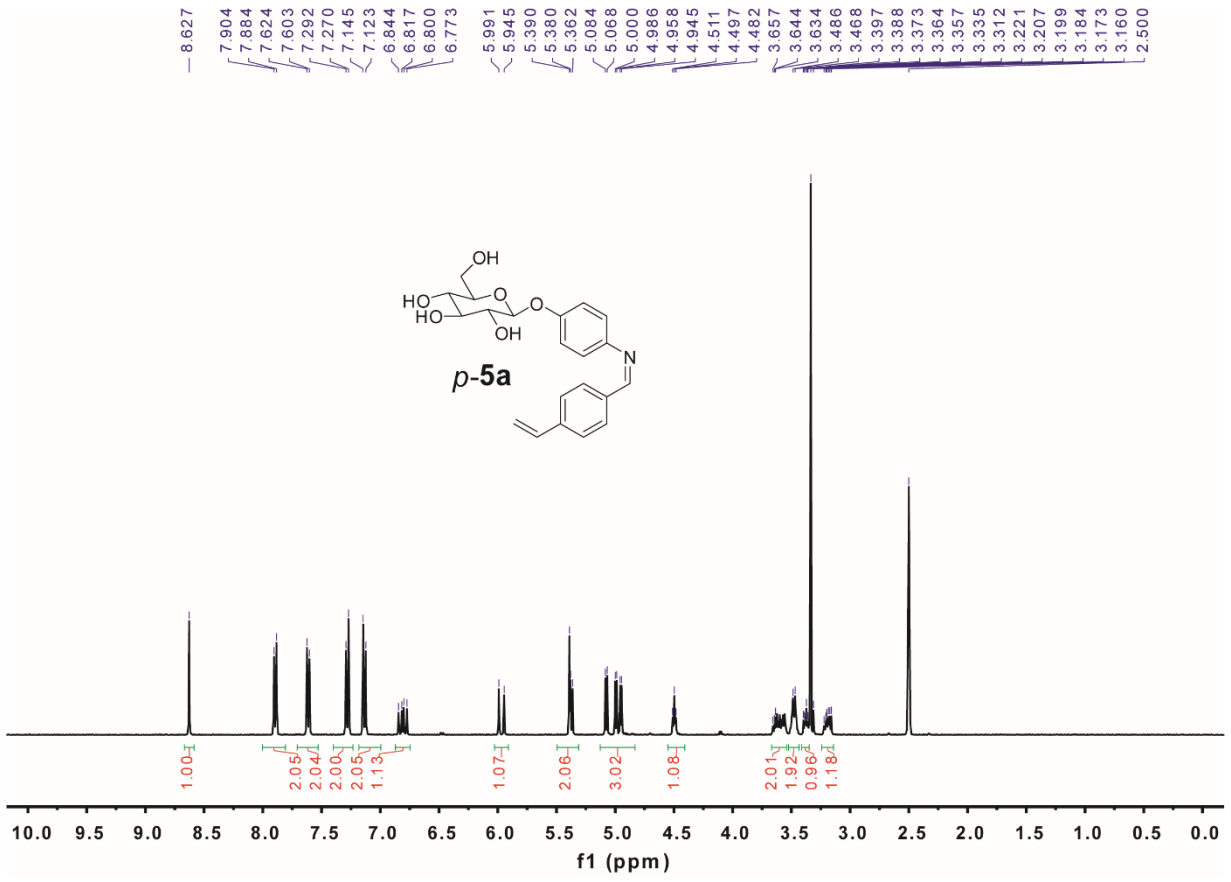
f1 (ppm)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2.06
3.02
1.08
2.01
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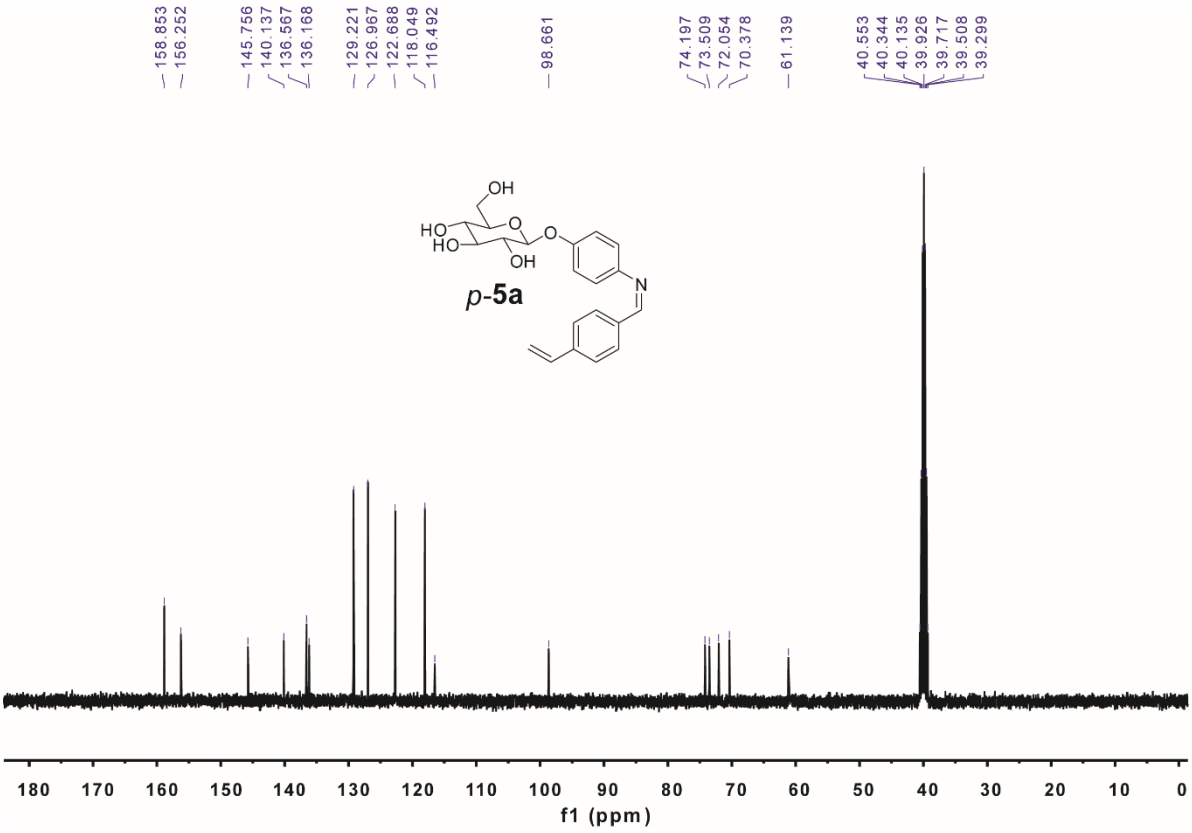
1.18
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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98.661
74.197
73.509
72.054
70.378
61.139
40.553
40.344
40.135
39.926
39.717
39.508
39.299
OH
HO
HO
O
O
OH
N
p-5a
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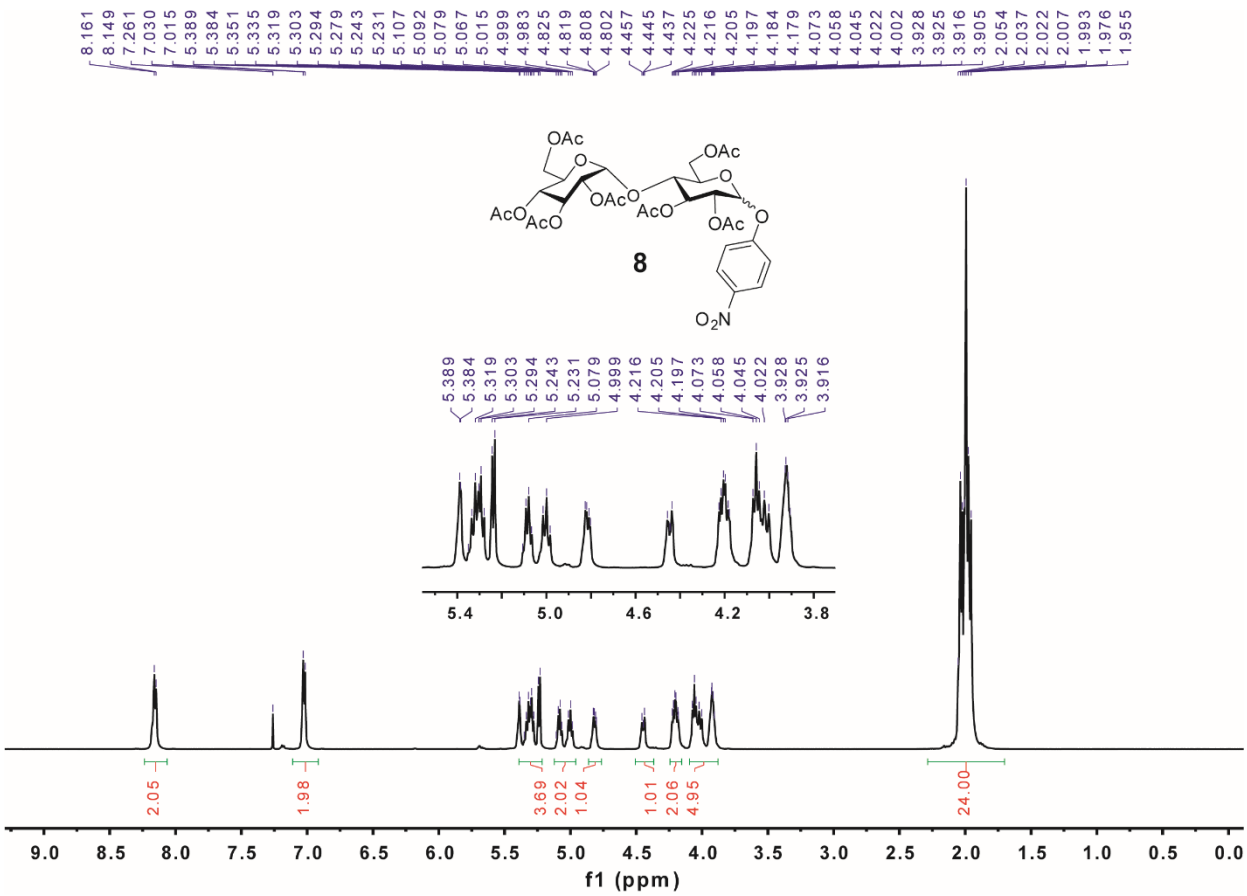
f1 (ppm)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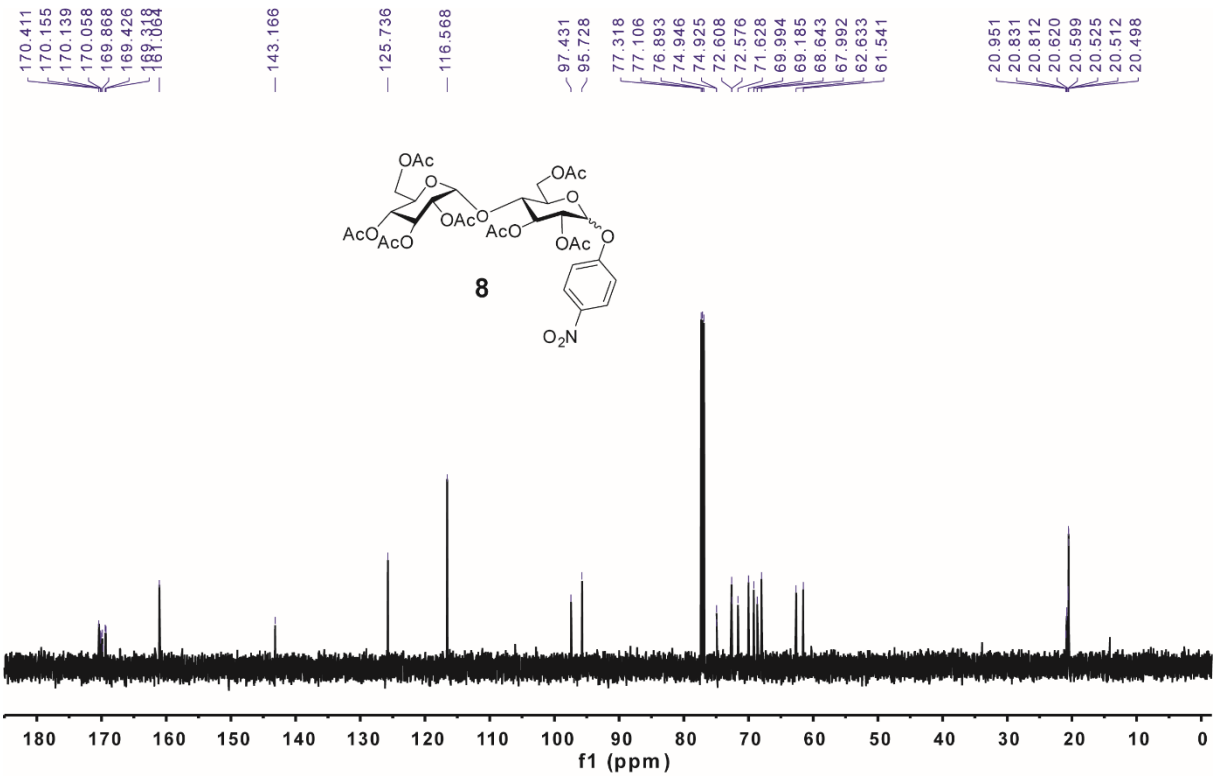
5.389
5.384
5.351
5.335
5.319
5.303
5.294
5.279
5.243
5.231
5.107
5.092
5.079
5.067
5.015
4.999
4.983
4.825
4.819
4.808
4.802
4.457
4.445
4.437
4.225
4.216
4.205
4.197
4.184
4.179
4.073
4.058
4.045
4.022
4.002
3.928
3.925
3.916
3.905
2.054
2.037
2.022
2.007
1.993
1.976
1.955
OAc
AcO
AcO
OAc
AcO
OAc
OAc
O
O2N
8
5.4 5.0 4.6 4.2 3.8
2.05
1.98
3.69
2.02
1.04
1.01
2.06
4.95
24.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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169.426
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125.736
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97.431
95.728
77.318
77.106
76.893
74.946
74.925
72.608
72.576
71.628
69.994
69.185
68.643
67.992
62.633
61.541
20.951
20.831
20.812
20.620
20.599
20.525
20.512
20.498
OAc
AcO
8
O_2N
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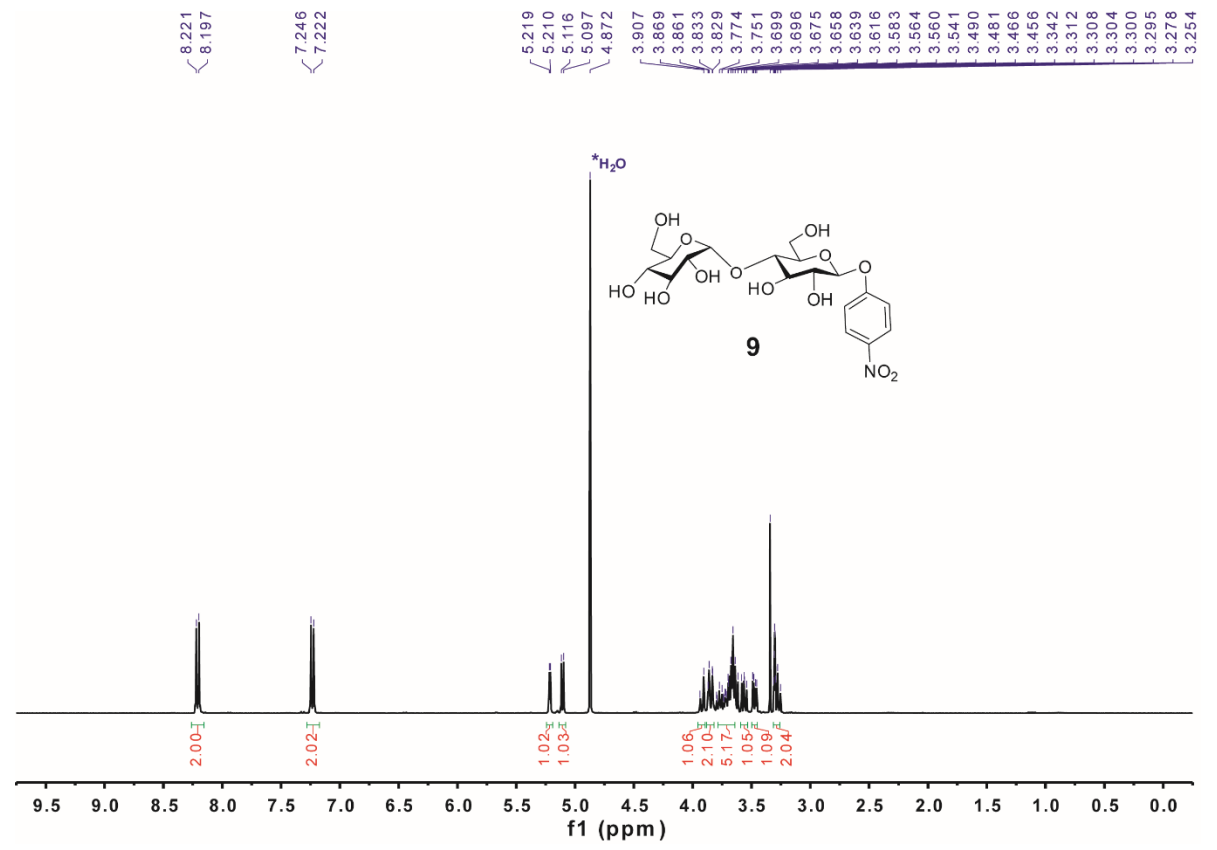
f1 (ppm)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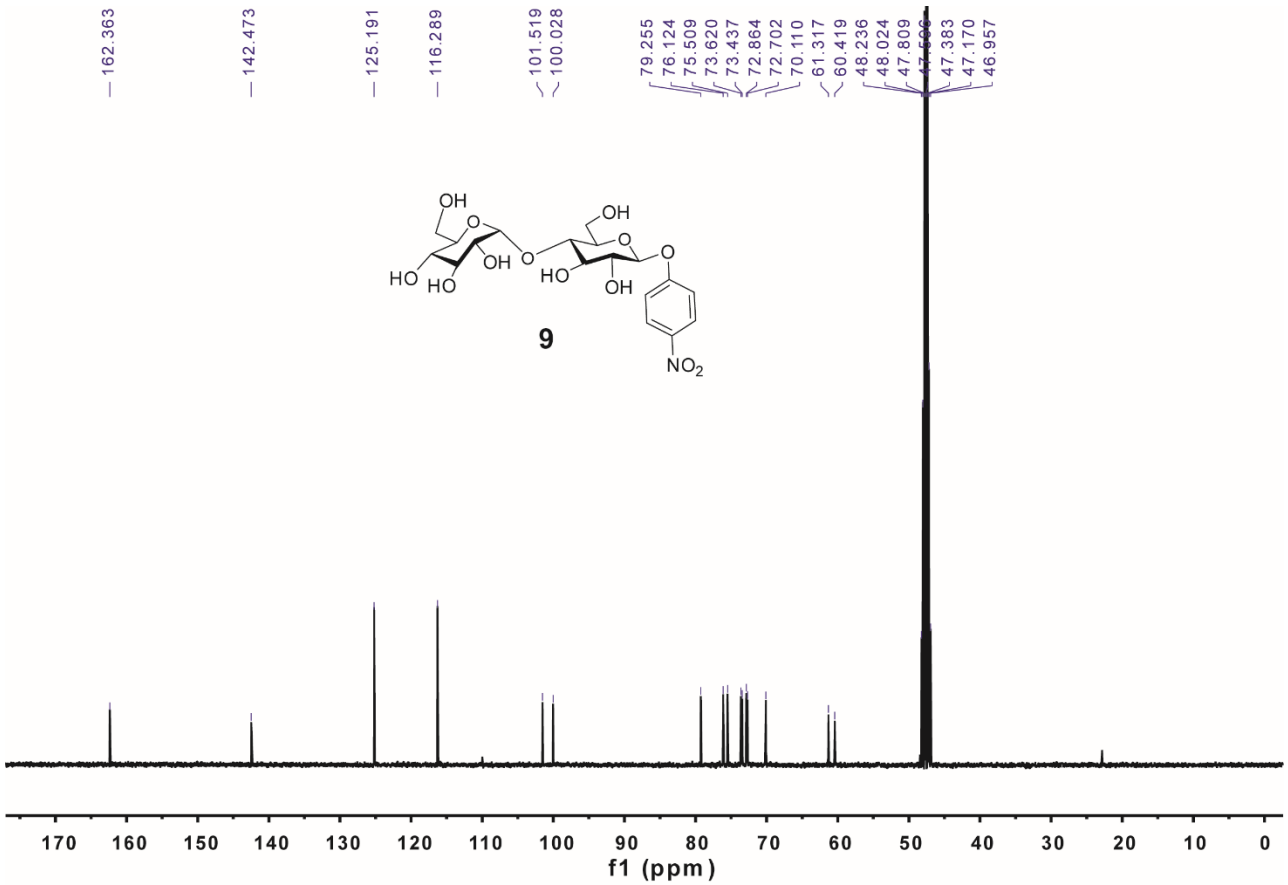
7.222
5.219
5.210
5.116
5.097
4.872
3.907
3.869
3.861
3.833
3.829
3.774
3.751
3.699
3.696
3.675
3.658
3.639
3.616
3.583
3.564
3.560
3.541
3.490
3.481
3.466
3.456
3.342
3.312
3.308
3.304
3.300
3.295
3.278
3.254
*H_2O
OH
HO
9
NO_2
2.00
2.02
1.02
1.03
1.06
2.10
5.17
1.05
1.09
2.04
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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100.028
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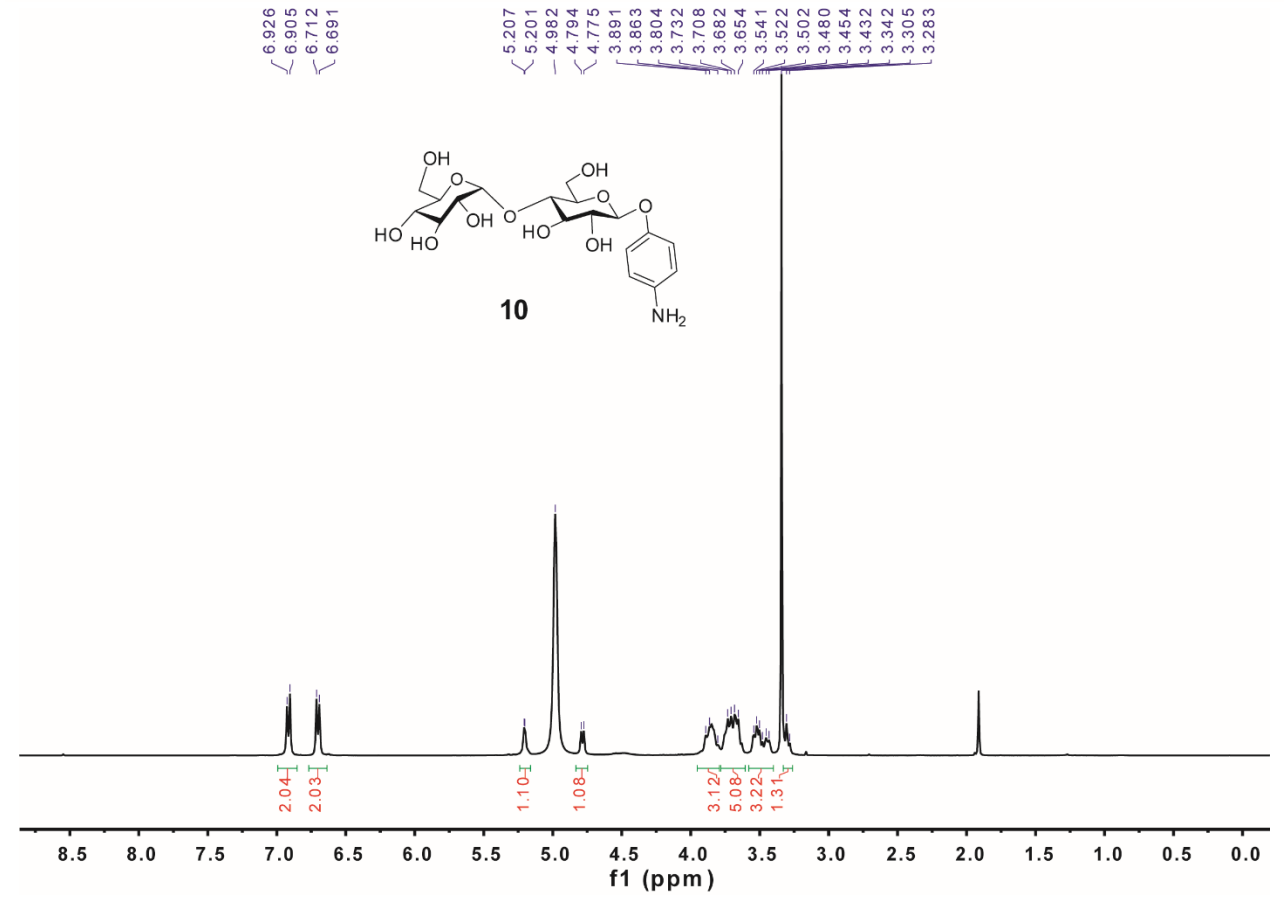
76.124
75.509
73.620
73.437
72.864
72.702
70.110
61.317
60.419
48.236
48.024
47.809
47.596
47.383
47.170
46.957
OH
HO
HO
OH
O
OH
HO
OH
O
O
NO2
9
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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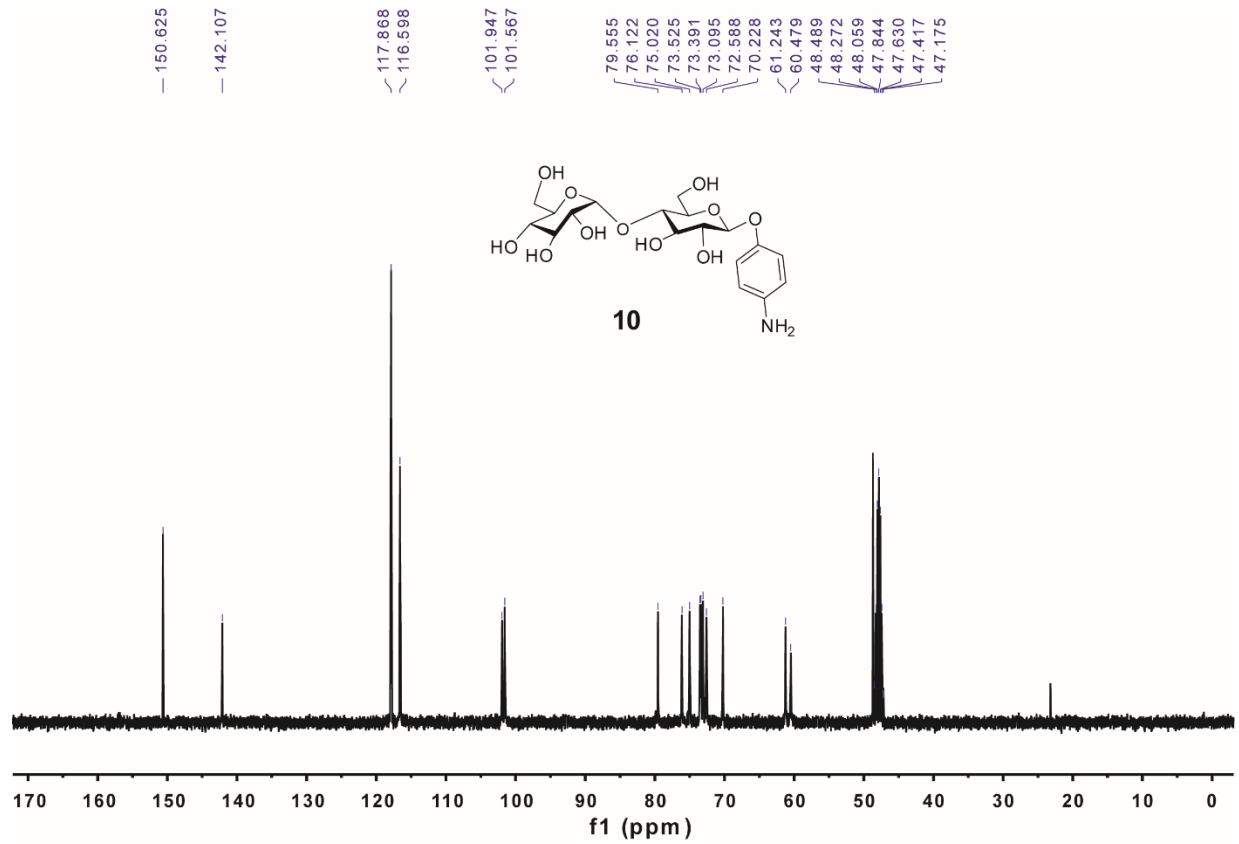
6.905
6.712
6.691
5.207
5.201
4.982
4.794
4.775
3.891
3.863
3.804
3.732
3.708
3.682
3.654
3.541
3.522
3.502
3.480
3.454
3.432
3.342
3.305
3.283
OH
HO
HO
OH
O
OH
HO
OH
O
O
NH2
10
2.04
2.03
1.10
1.08
3.12
5.08
3.22
1.31
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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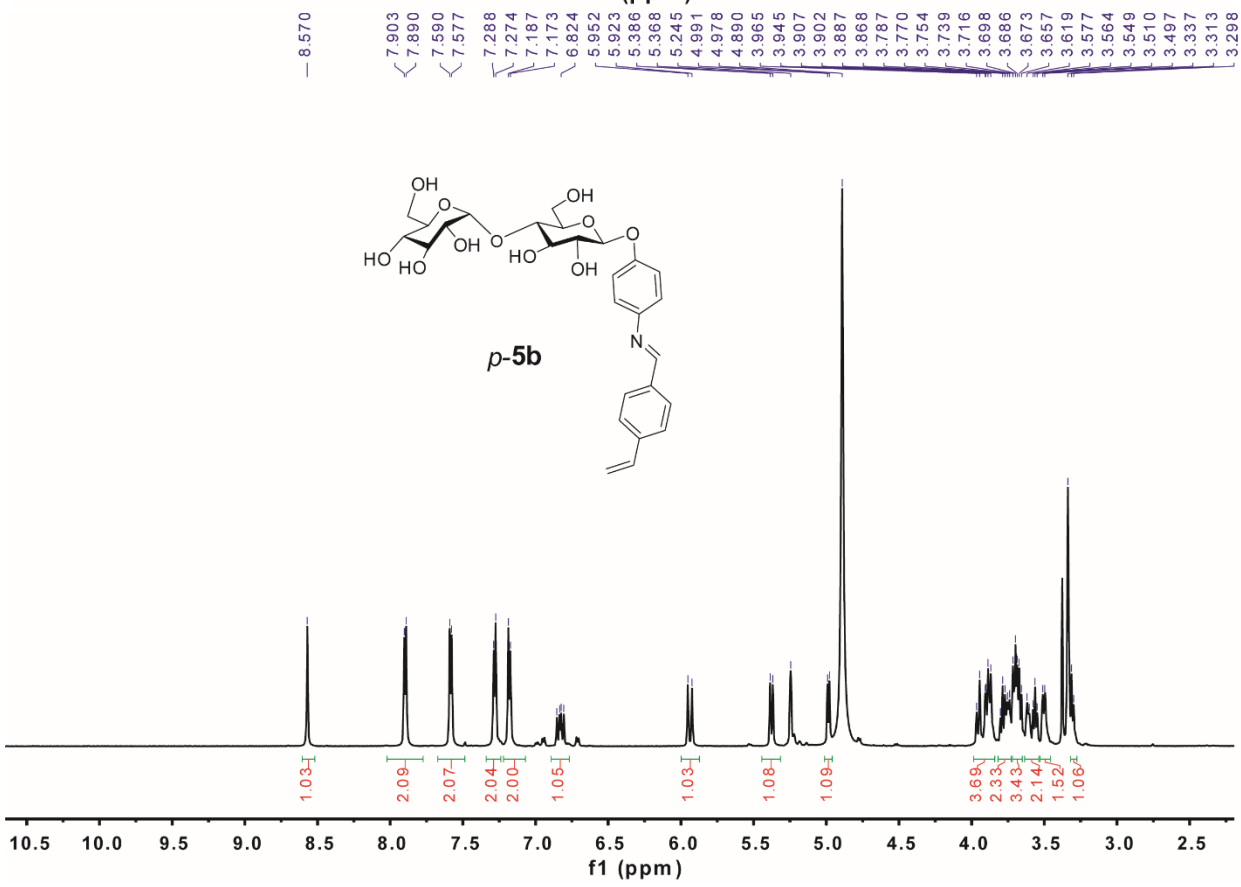
117.868
116.598
101.947
101.567
79.555
76.122
75.020
73.525
73.391
73.095
72.588
70.228
61.243
60.479
48.489
48.272
48.059
47.844
47.630
47.417
47.175
10
f1 (ppm)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7.590
7.577
7.288
7.274
7.187
7.173
6.824
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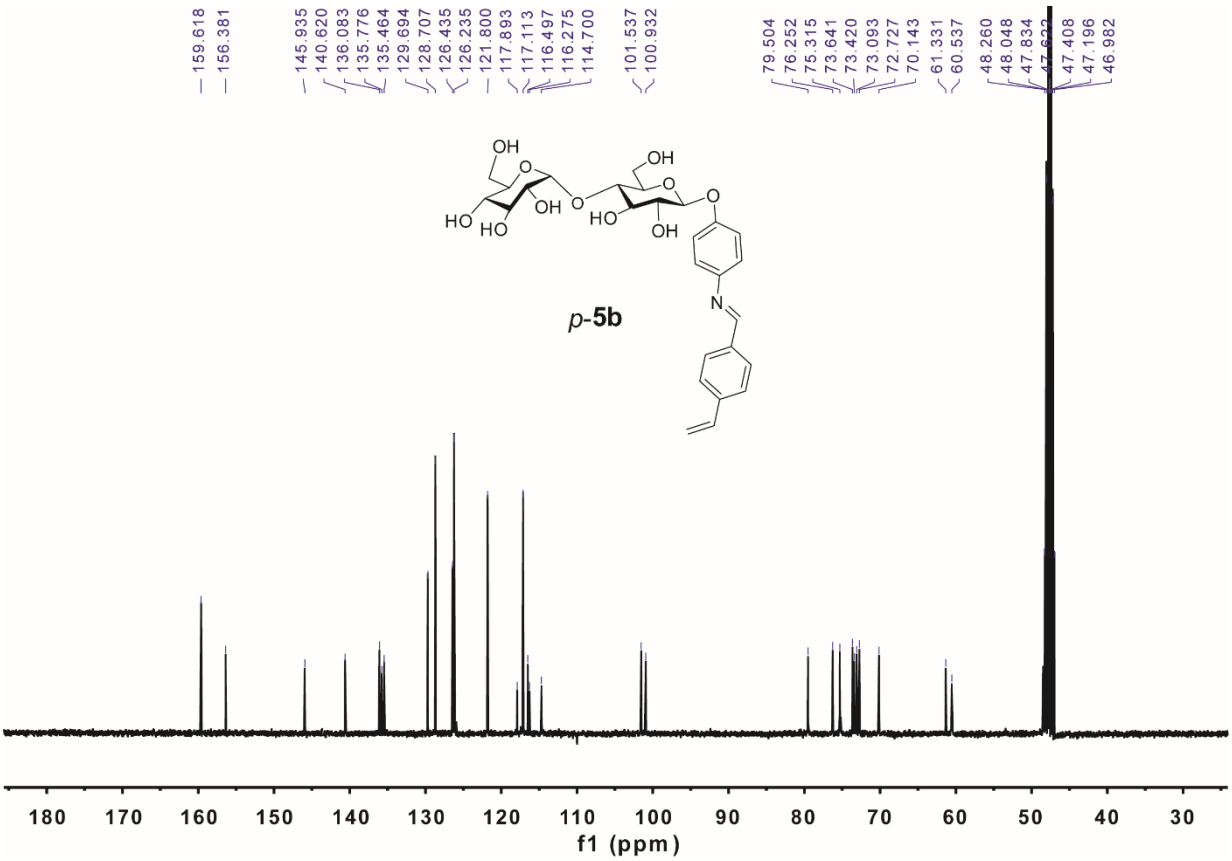
5.923
5.386
5.368
5.245
4.991
4.978
4.890
3.965
3.945
3.907
3.902
3.887
3.868
3.787
3.770
3.754
3.739
3.716
3.698
3.686
3.673
3.657
3.619
3.577
3.564
3.549
3.510
3.497
3.337
3.313
3.298
p-5b
1.03
2.09
2.07
2.04
2.00
1.05
1.03
1.08
1.09
3.69
2.33
3.43
2.14
1.52
1.06
f1 (ppm)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126.235
121.800
117.893
117.113
116.497
116.275
114.700
101.537
100.932
79.504
76.252
75.315
73.641
73.420
73.093
72.727
70.143
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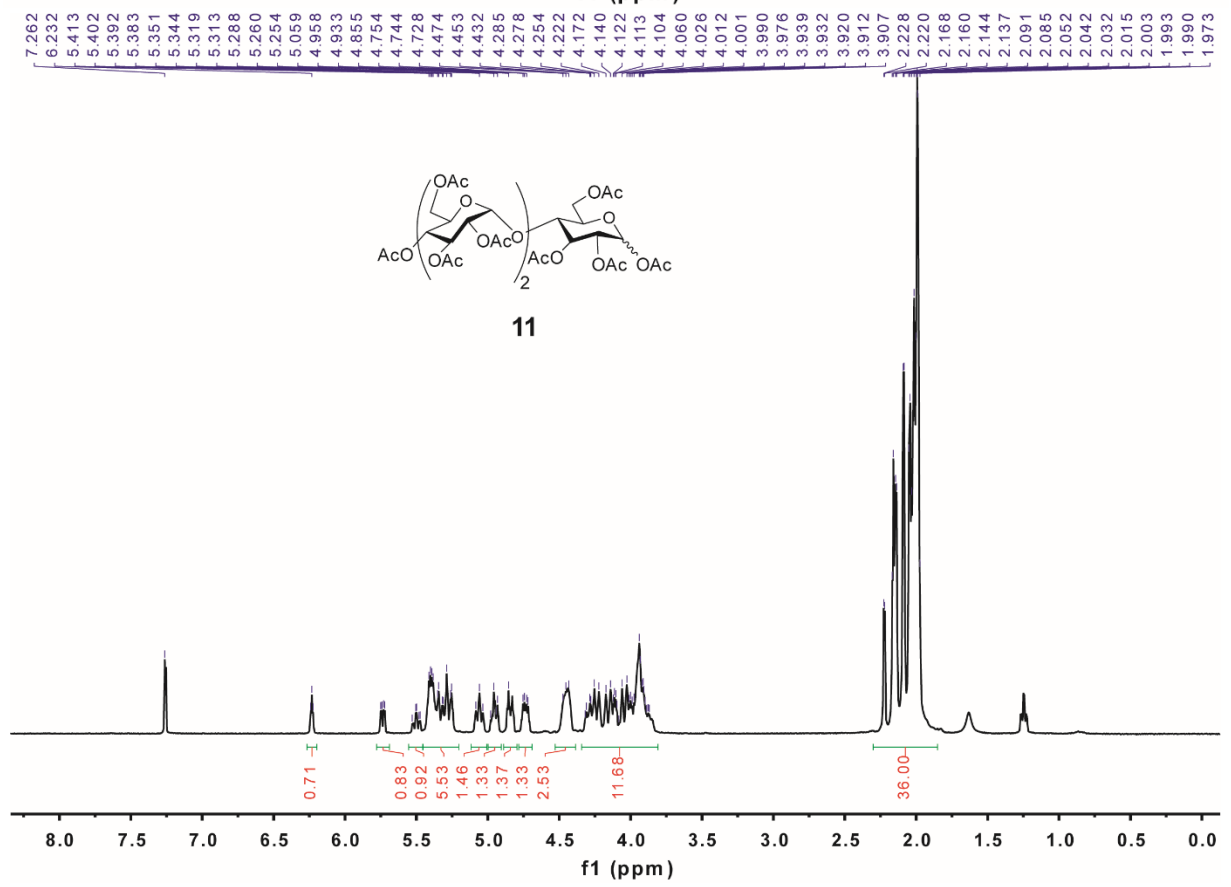
60.537
48.260
48.048
47.834
47.622
47.408
47.196
46.982
p-5b
f1 (ppm)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5.344
5.319
5.313
5.288
5.260
5.254
5.059
4.958
4.933
4.855
4.754
4.744
4.728
4.474
4.453
4.432
4.285
4.278
4.254
4.222
4.172
4.140
4.122
4.113
4.104
4.060
4.026
4.012
4.001
3.990
3.976
3.939
3.932
3.920
3.912
3.907
2.228
2.220
2.168
2.160
2.144
2.137
2.091
2.085
2.052
2.042
2.032
2.015
2.003
1.993
1.990
1.973
11
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0.83
0.92
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1.33
1.37
1.33
2.53
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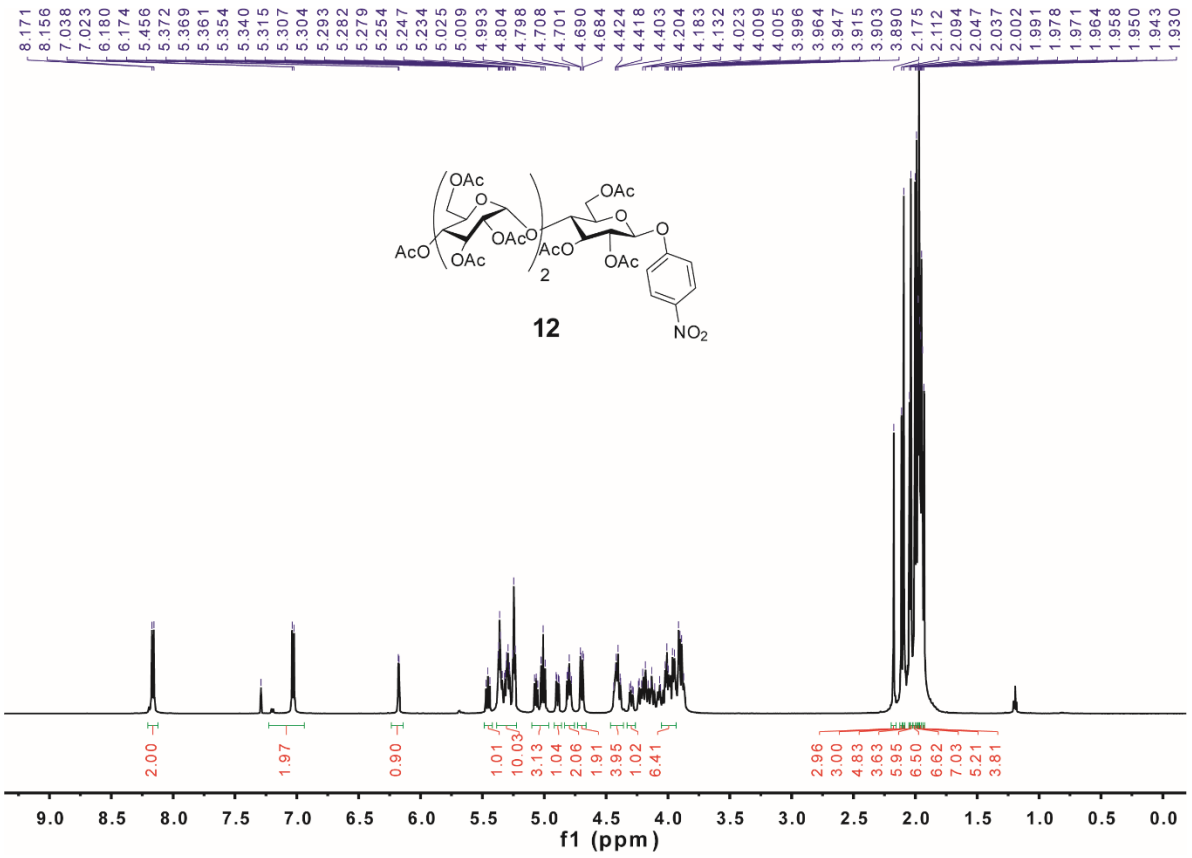
36.00
f1 (ppm)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5.372
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5.354
5.340
5.315
5.307
5.304
5.293
5.282
5.279
5.254
5.247
5.234
5.025
5.009
4.993
4.804
4.798
4.708
4.701
4.690
4.684
4.424
4.418
4.403
4.204
4.183
4.132
4.023
4.009
4.005
3.996
3.964
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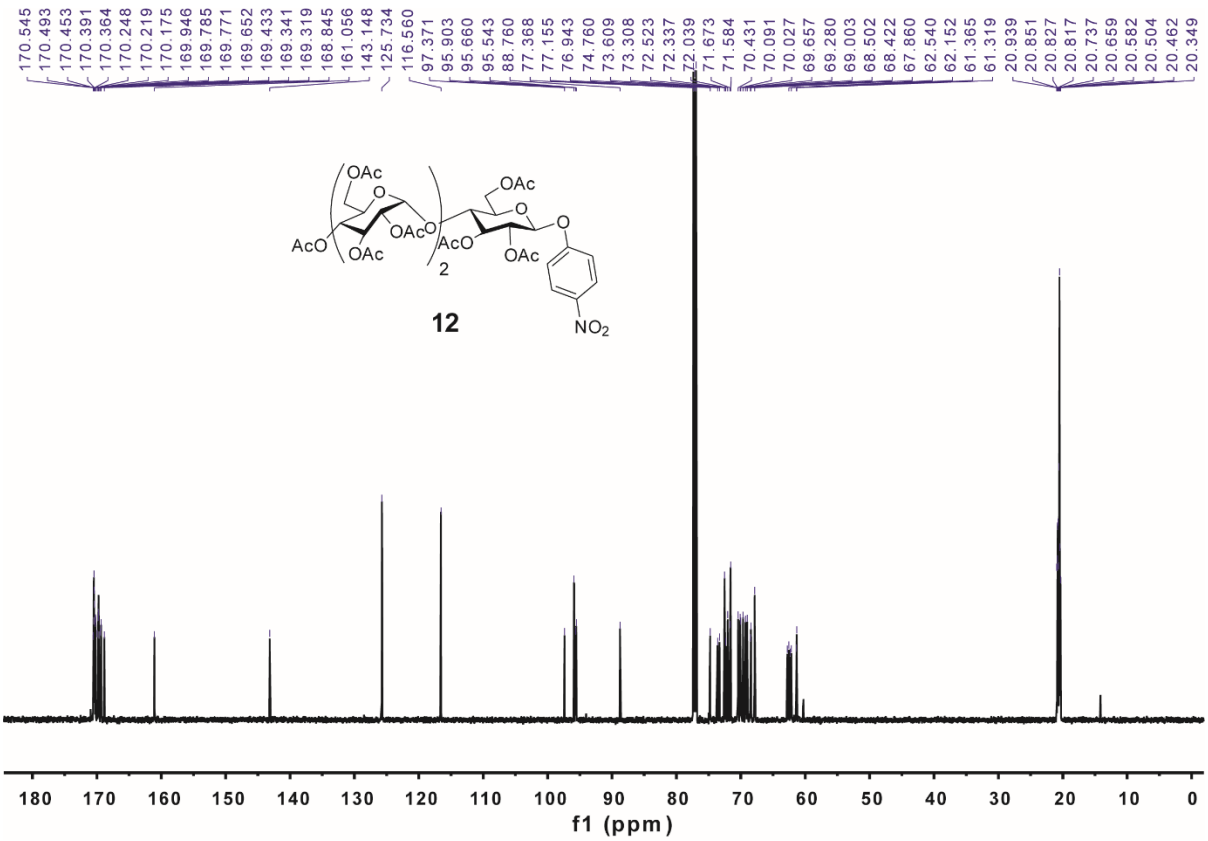
3.903
3.890
2.175
2.112
2.094
2.047
2.037
2.002
1.991
1.978
1.971
1.964
1.958
1.950
1.943
1.930
OAc
AcO
OAc
OAc
AcO
OAc
OAc
OAc
2
12
NO2
2.00
1.97
0.90
1.01
10.03
3.13
1.04
2.06
1.91
3.95
1.02
6.41
2.96
3.00
4.83
3.63
5.95
6.50
6.62
7.03
5.21
3.81
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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169.433
169.341
169.319
168.845
161.056
143.148
125.734
116.560
97.371
95.903
95.660
95.543
88.760
77.368
77.155
76.943
74.760
73.609
73.308
72.523
72.337
72.039
71.673
71.584
70.431
70.091
70.027
69.657
69.280
69.003
68.502
68.422
67.860
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62.152
61.365
61.319
20.939
20.851
20.827
20.817
20.737
20.659
20.582
20.504
20.462
20.349
OAc
AcO
OAc
OAc
AcO
OAc
OAc
OAc
2
12
NO2
180
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150
140
130
120
110
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90
80
70
60
50
40
30
20
10
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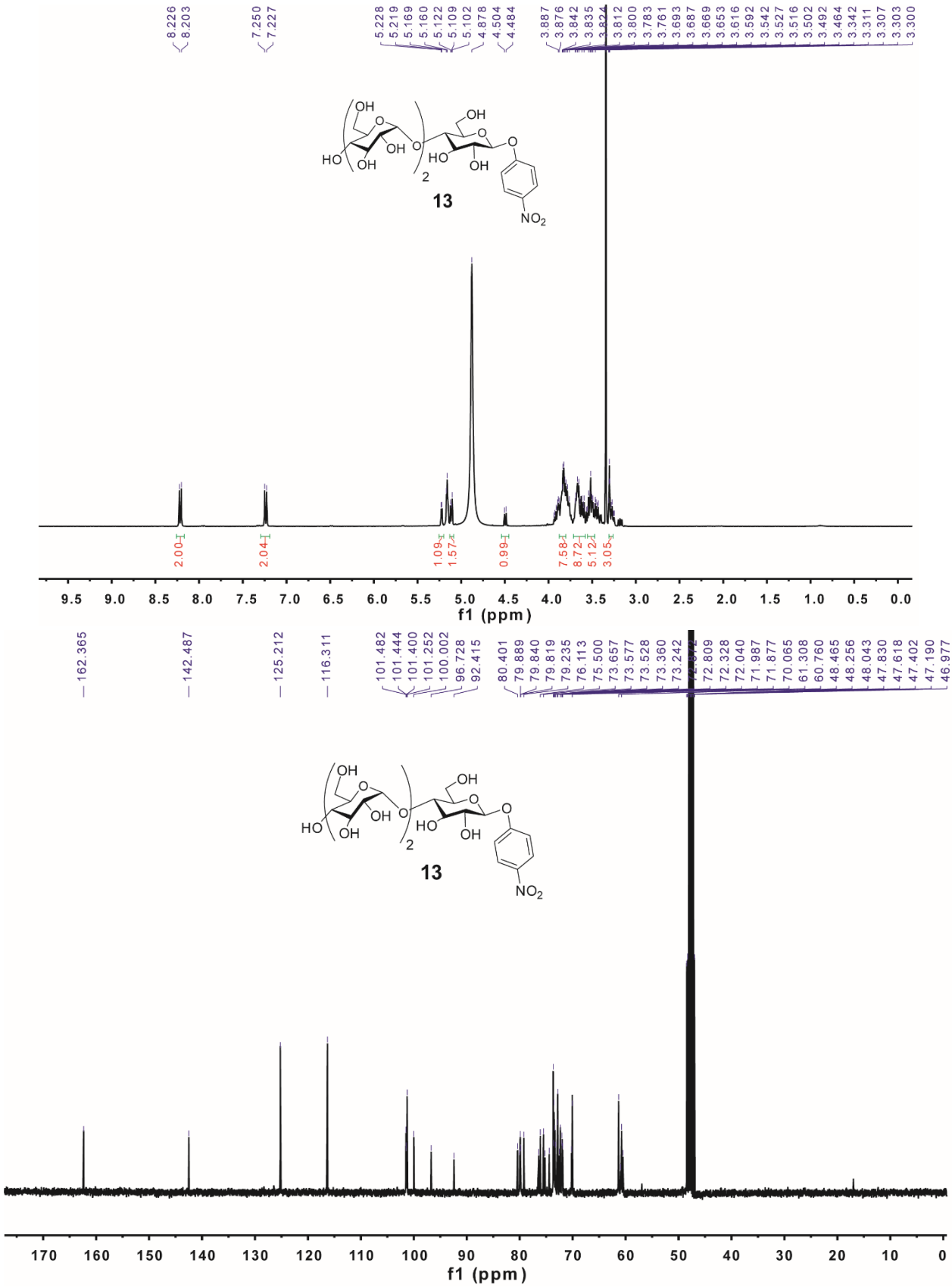
f1 (ppm)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5.219
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5.160
5.122
5.109
5.102
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4.504
4.484
3.887
3.876
3.842
3.835
3.824
3.812
3.800
3.783
3.761
3.693
3.687
3.669
3.653
3.616
3.592
3.542
3.527
3.516
3.502
3.492
3.464
3.342
3.311
3.307
3.303
3.300
13
2.00
2.04
1.09
1.57
0.99
7.58
8.72
5.12
3.05
f1 (ppm)
162.365
142.487
125.212
116.311
101.482
101.444
101.400
101.252
100.002
96.728
92.415
80.401
79.889
79.840
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79.235
76.113
75.500
73.657
73.577
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73.242
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72.040
71.987
71.877
70.065
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60.760
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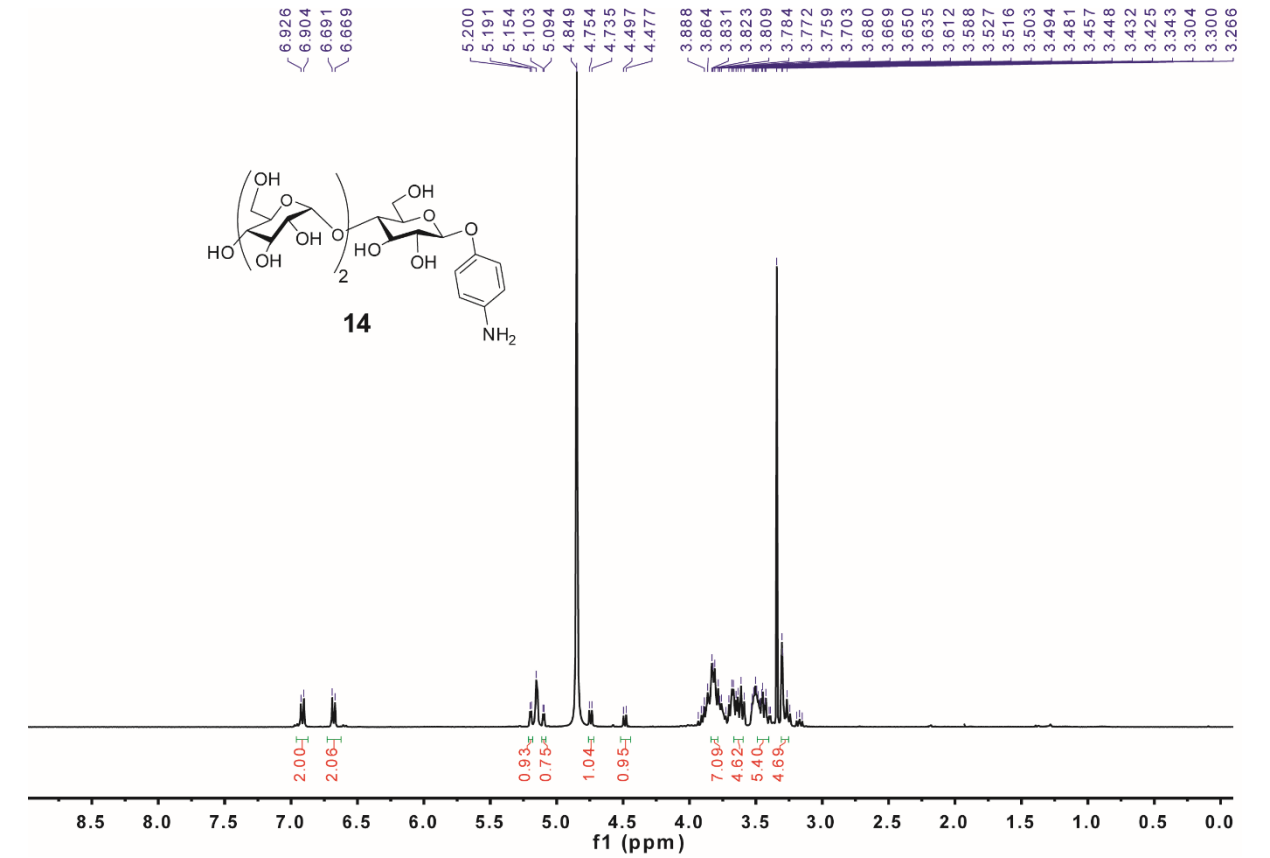
47.830
47.618
47.402
47.190
46.977
13
f1 (ppm)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4.735
4.497
4.477
3.888
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3.823
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3.784
3.772
3.759
3.703
3.680
3.669
3.650
3.635
3.612
3.588
3.527
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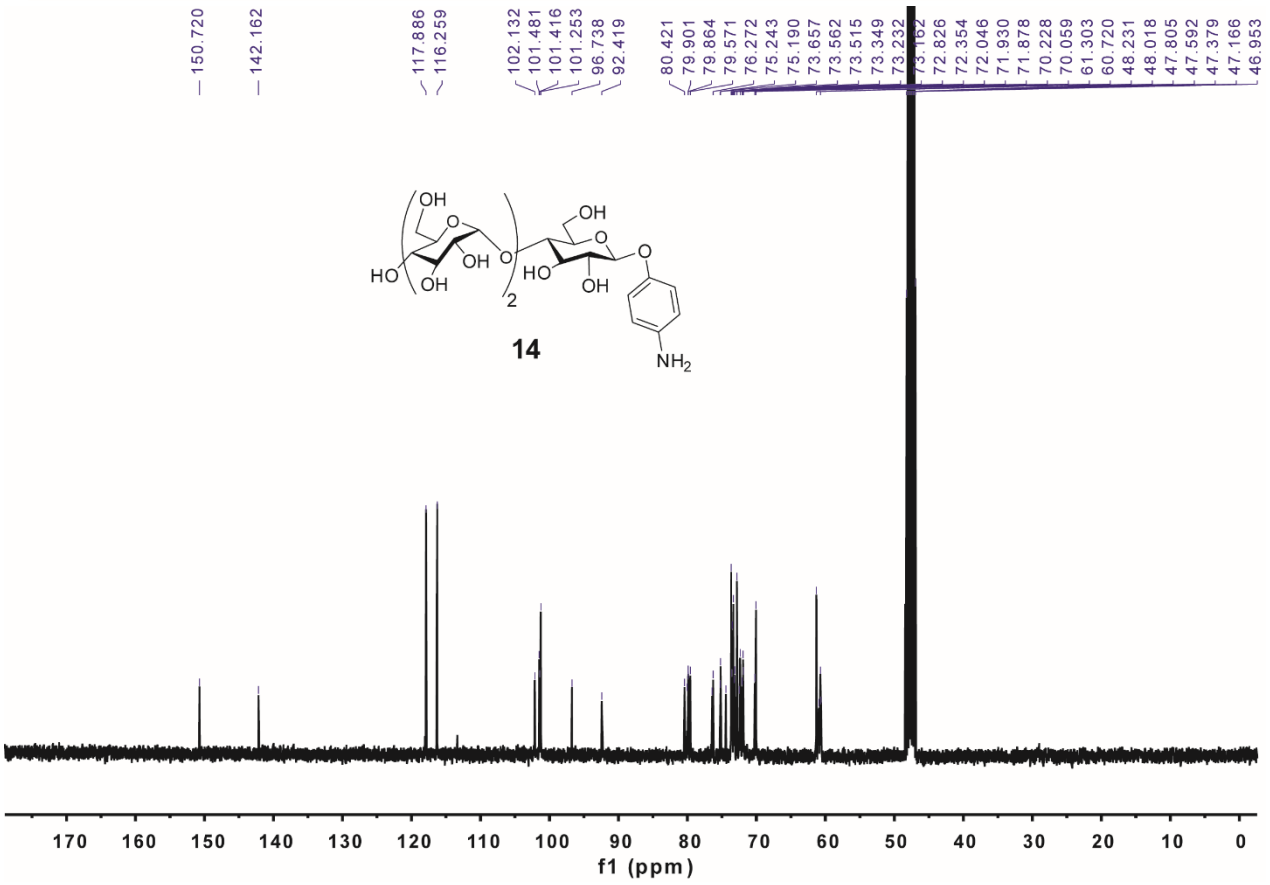
3.494
3.481
3.457
3.448
3.432
3.425
3.343
3.304
3.300
3.266
OH
O
OH
HO
OH
O
HO
OH
O
2
NH2
14
2.00
2.06
0.93
0.75
1.04
0.95
7.09
4.62
5.40
4.69
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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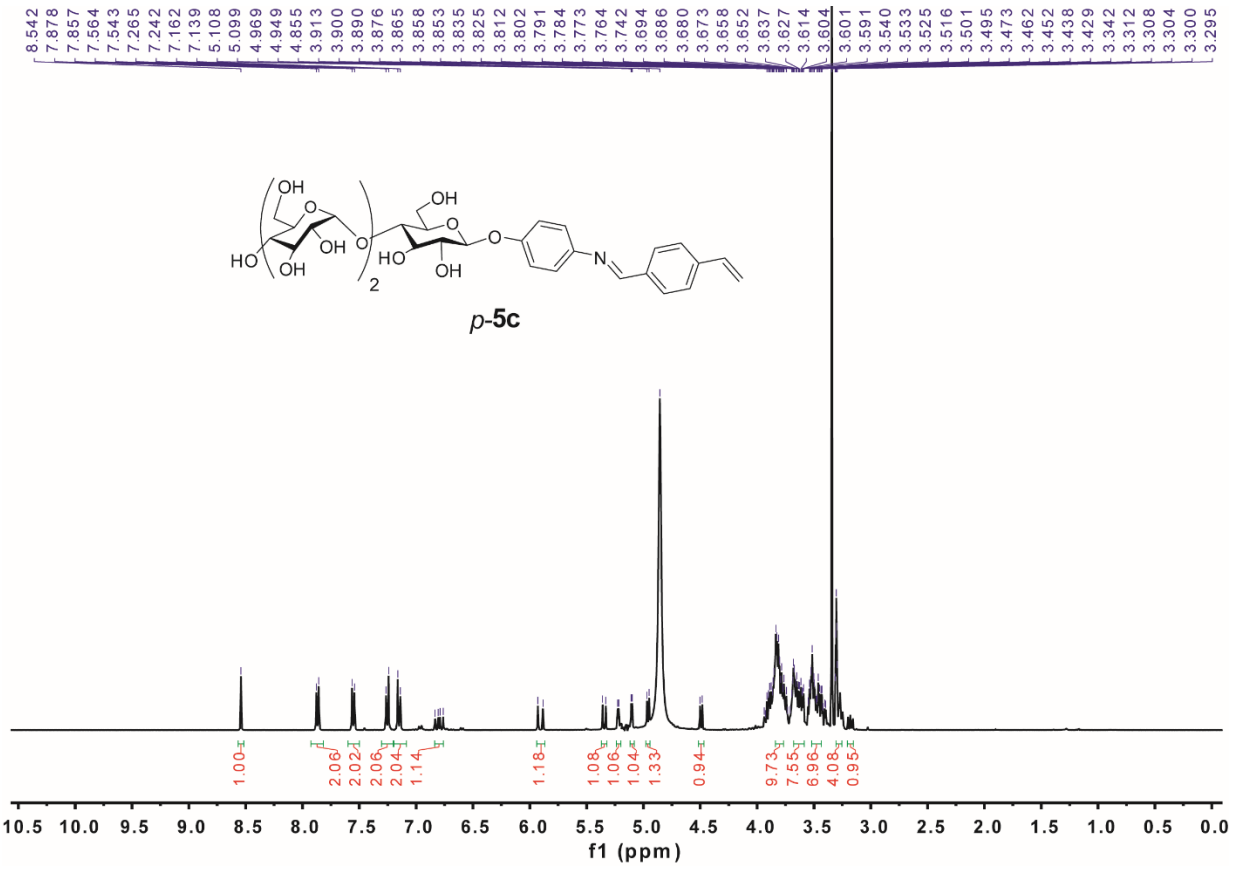
79.864
79.571
76.272
75.243
75.190
73.657
73.562
73.515
73.349
73.232
73.162
72.826
72.354
72.046
71.930
71.878
70.228
70.059
61.303
60.720
48.231
48.018
47.805
47.592
47.379
47.166
46.953
OH
O
OH
HO
OH
O
HO
OH
O
2
NH2
14
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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5.108
5.099
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4.949
4.855
3.913
3.900
3.890
3.876
3.865
3.858
3.853
3.835
3.825
3.812
3.802
3.791
3.784
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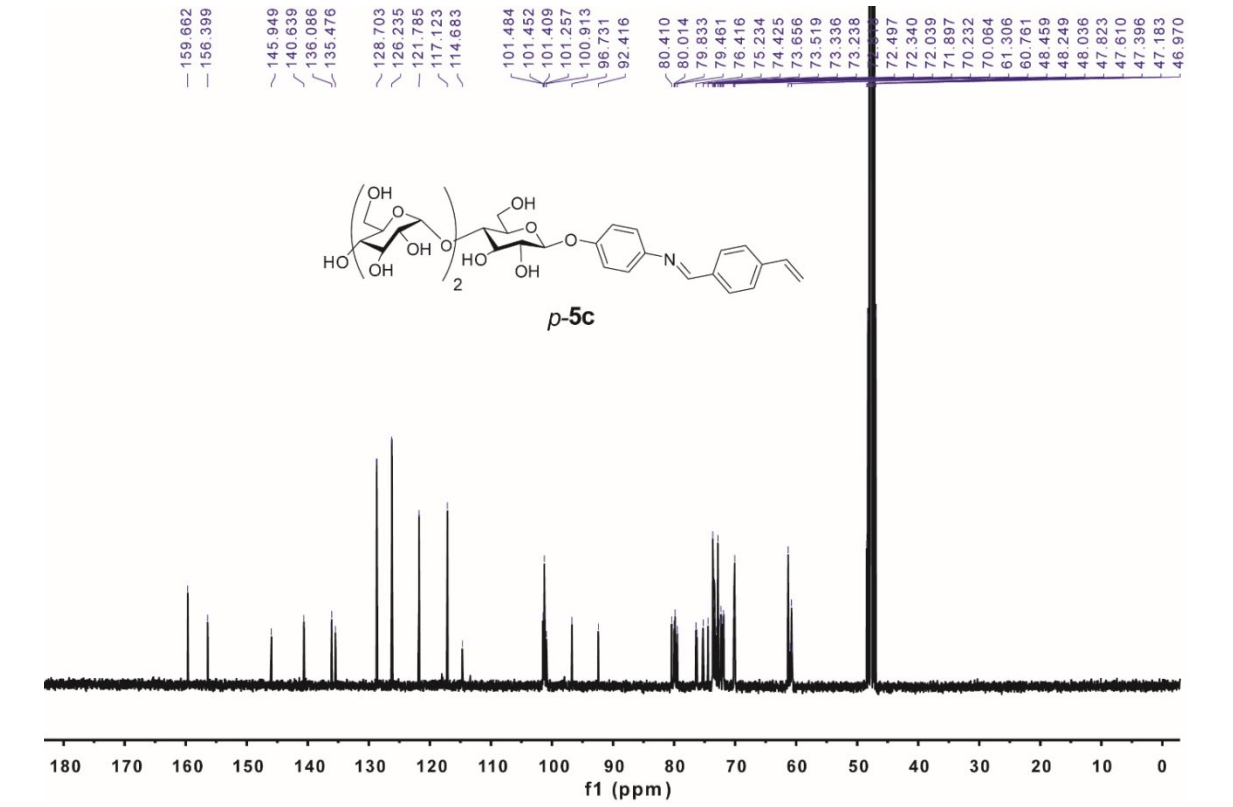
3.680
3.673
3.658
3.652
3.637
3.627
3.614
3.604
3.601
3.591
3.540
3.533
3.525
3.516
3.501
3.495
3.473
3.462
3.452
3.438
3.429
3.342
3.312
3.308
3.304
3.300
3.295
OH
O
HO
OH
OH
O
OH
O
HO
OH
2
O
N
p-5c
1.00
2.06
2.02
2.06
2.04
1.14
1.18
1.08
1.06
1.04
1.33
0.94
9.73
7.55
6.96
4.08
0.95
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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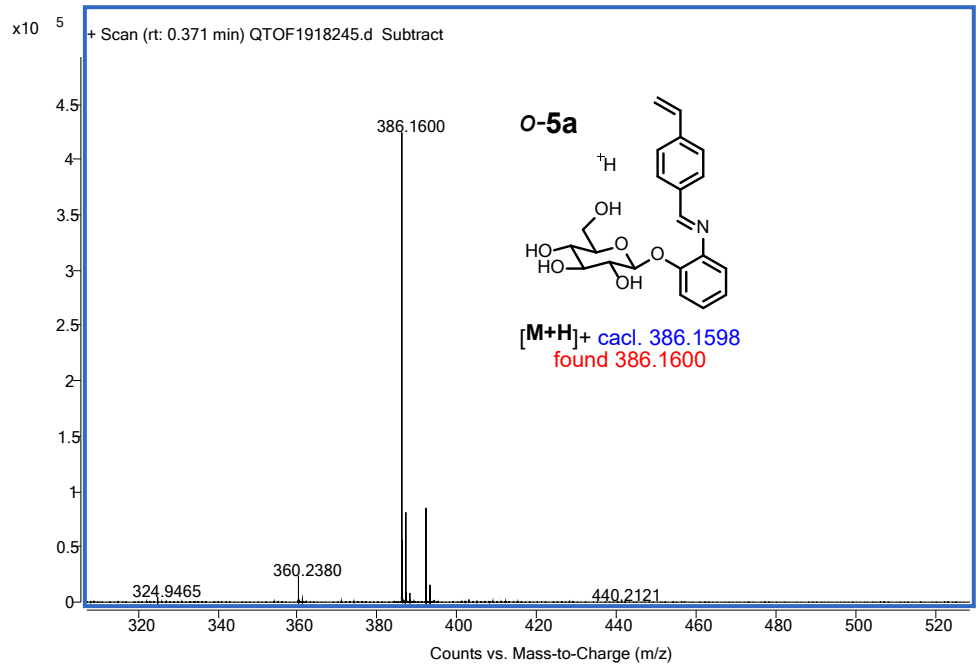
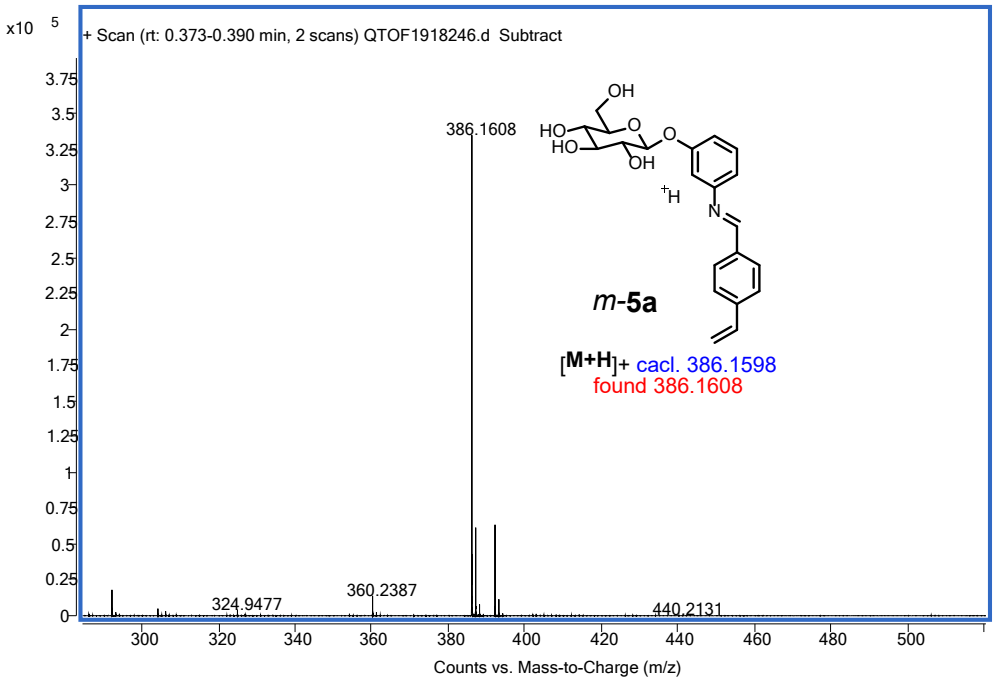
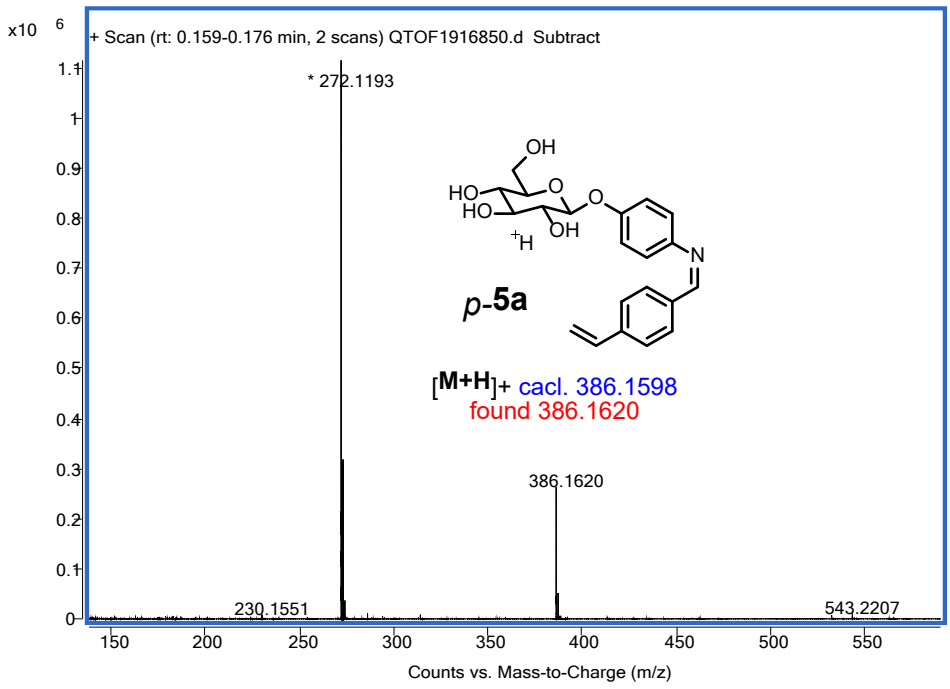
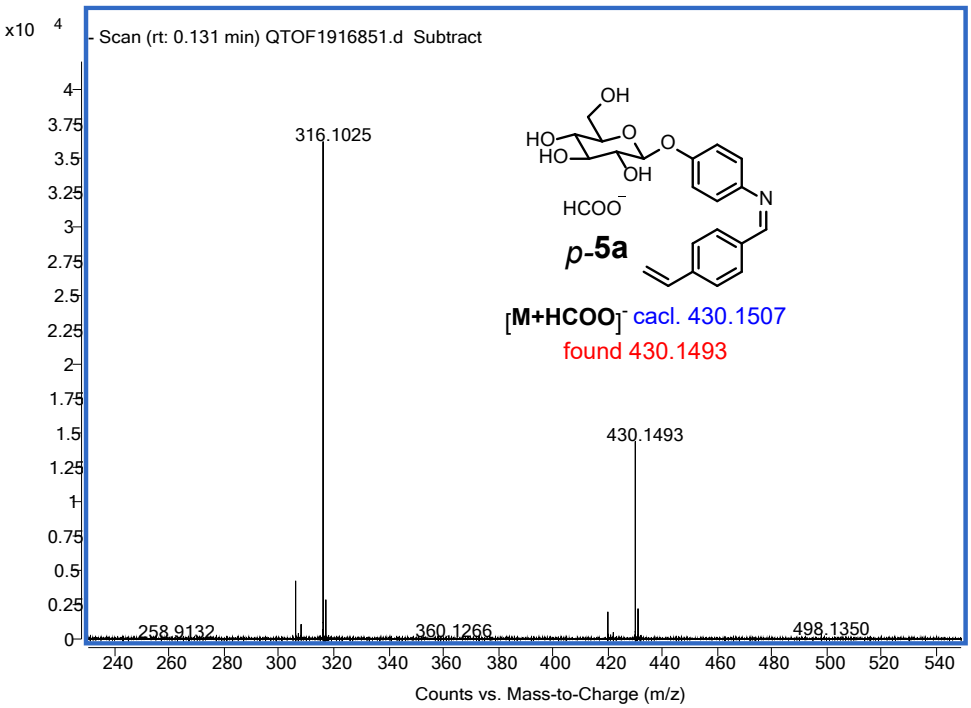
117.123
114.683
101.484
101.452
101.409
101.257
100.913
96.731
92.416
80.410
80.014
79.833
79.461
76.416
75.234
74.425
73.656
73.519
73.336
73.238
72.497
72.340
72.039
71.897
70.232
70.064
61.306
60.761
48.459
48.249
48.036
47.823
47.610
47.396
47.183
46.970
OH
O
HO
OH
OH
O
OH
O
HO
OH
2
O
N
p-5c
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

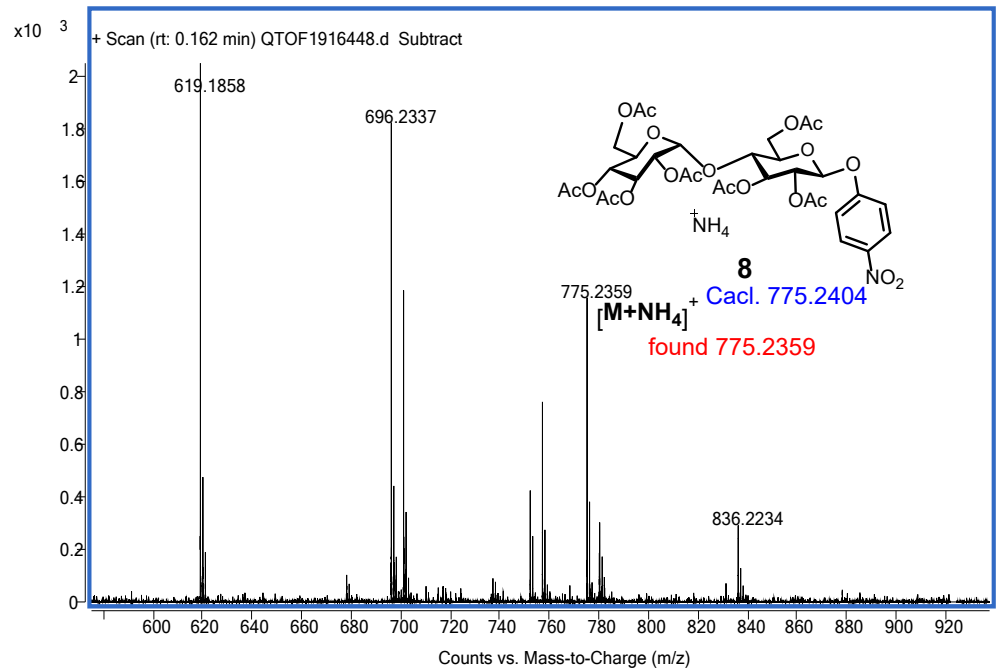
ESI-MS Spectra

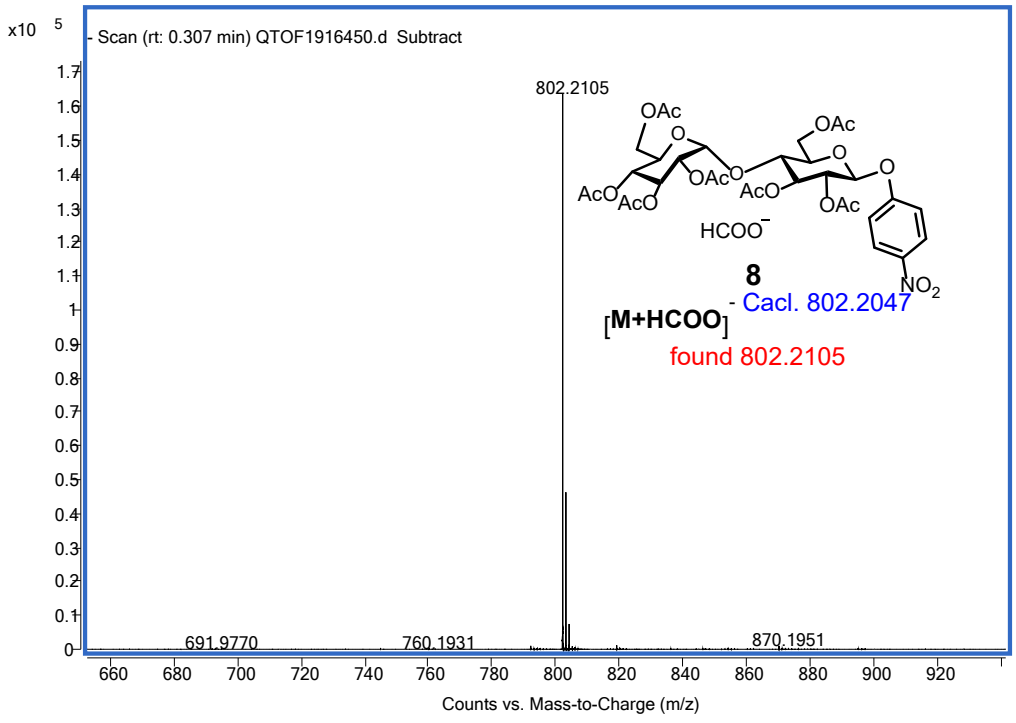


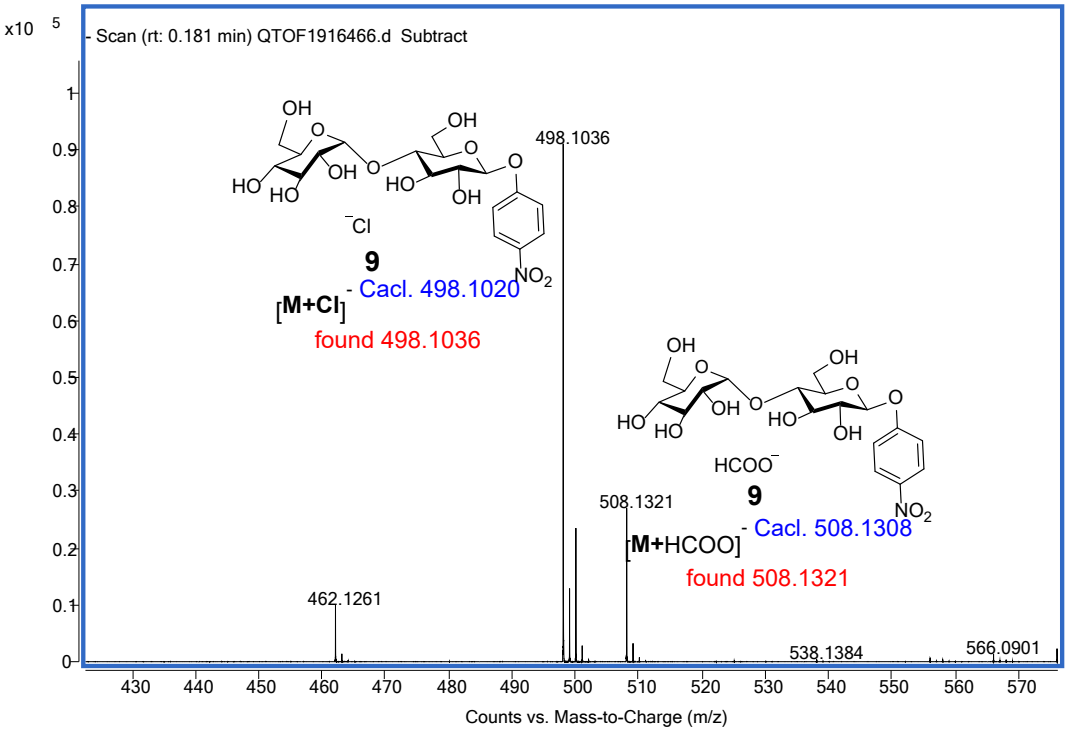


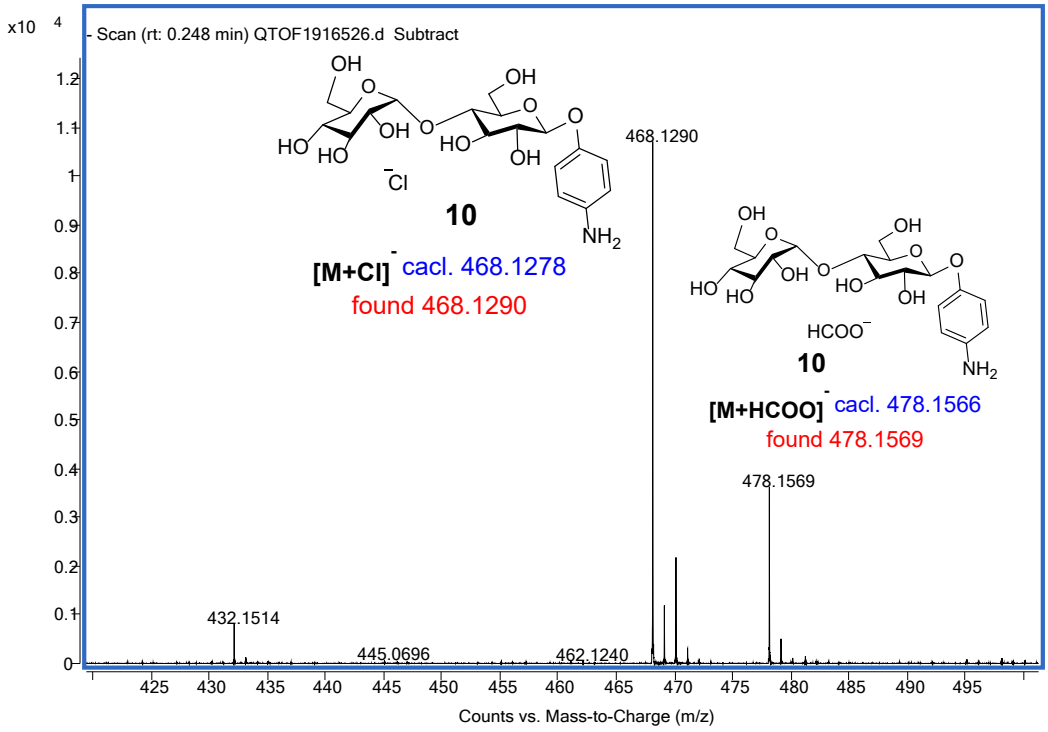

x10 6
+ Scan (rt: 0.159-0.176 min, 2 scans) QTOF1916850.d Subtract
* 272.1193
p-5a
[M+H]+ cacl. 386.1598
found 386.1620
386.1620
230.1551
543.2207
Counts vs. Mass-to-Charge (m/z)

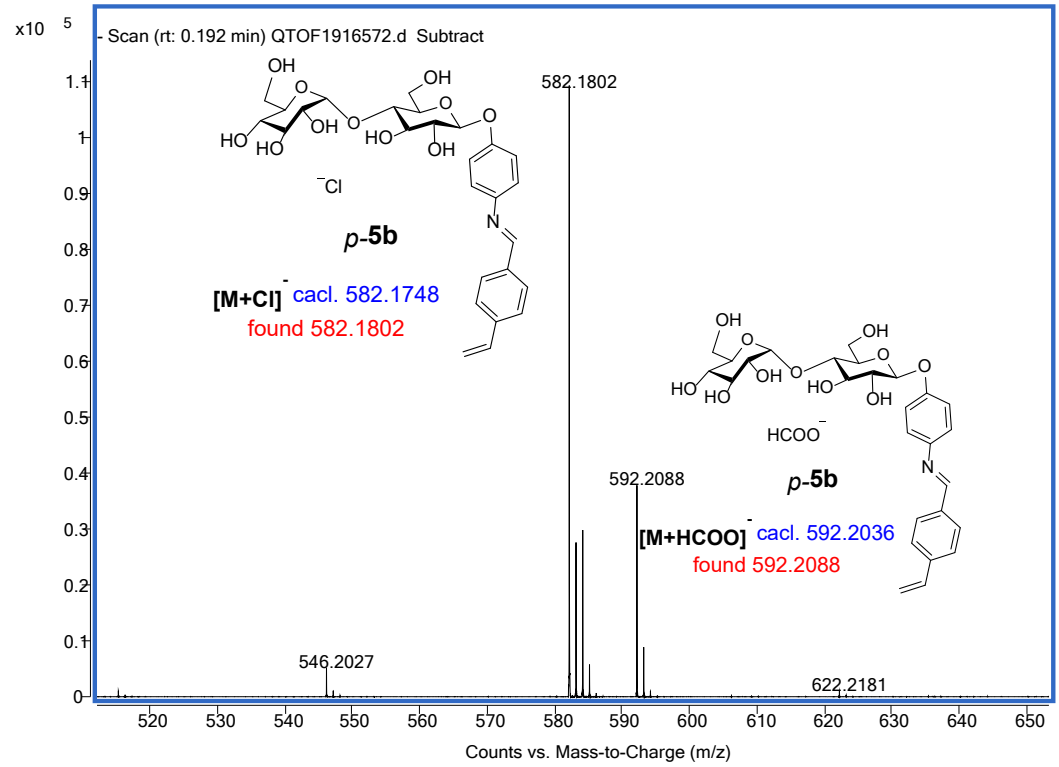

x10 4
- Scan (rt: 0.131 min) QTOF1916851.d Subtract
316.1025
HCOO−
p-5a
[M+HCOO]- cacl. 430.1507
found 430.1493
430.1493
258.9132
360.1266
498.1350
Counts vs. Mass-to-Charge (m/z)

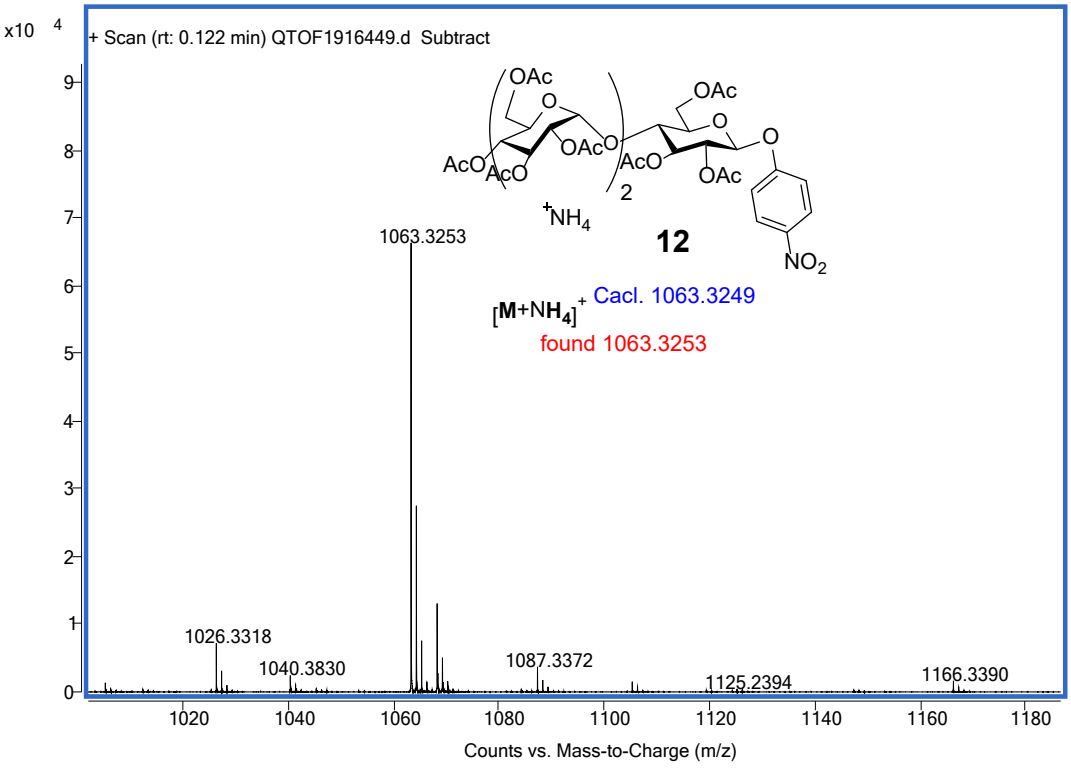

x10 3
+ Scan (rt: 0.162 min) QTOF1916448.d Subtract
619.1858
696.2337
775.2359
836.2234
8
[M+NH4]+ Cacl. 775.2404
found 775.2359
Counts vs. Mass-to-Charge (m/z)

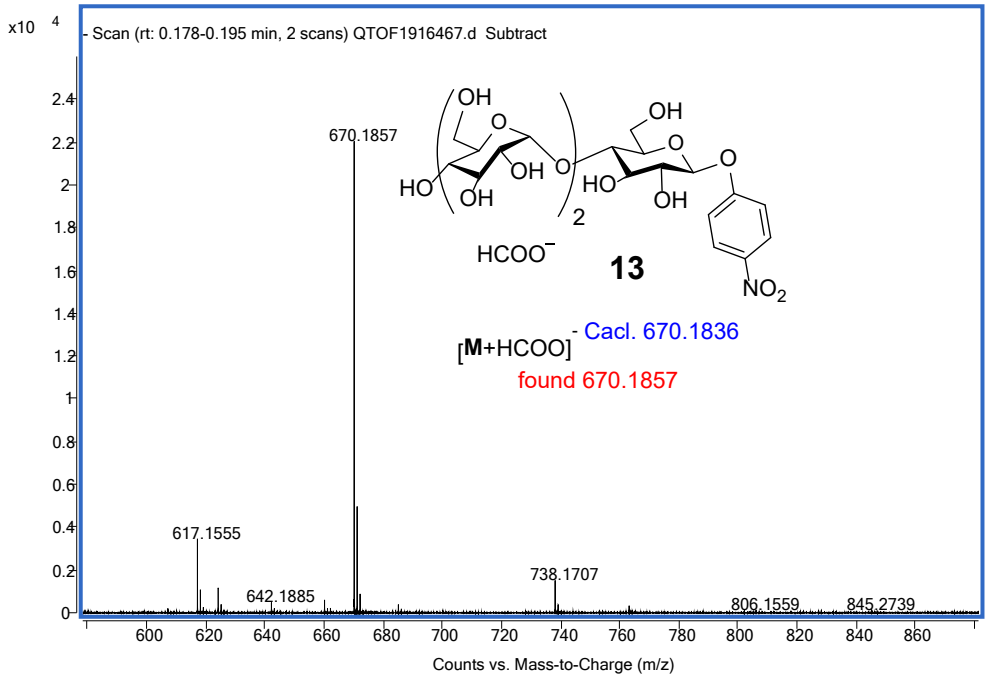

x10 5
- Scan (rt: 0.307 min) QTOF1916450.d Subtract
802.2105
691.9770
760.1931
870.1951
8
[M+HCOO]- Cacl. 802.2047
found 802.2105
Counts vs. Mass-to-Charge (m/z)

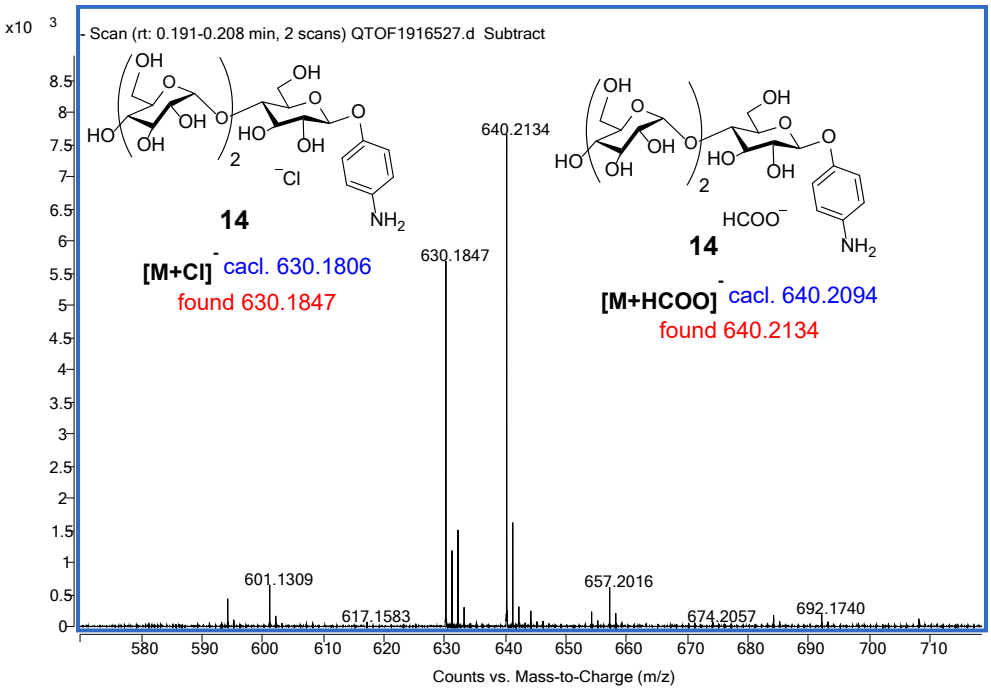

x10 5
- Scan (rt: 0.181 min) QTOF1916466.d Subtract
498.1036
9
[M+Cl]- Cacl. 498.1020
found 498.1036
508.1321
HCOO−
9
[M+HCOO]- Cacl. 508.1308
found 508.1321
462.1261
538.1384
566.0901
Counts vs. Mass-to-Charge (m/z)

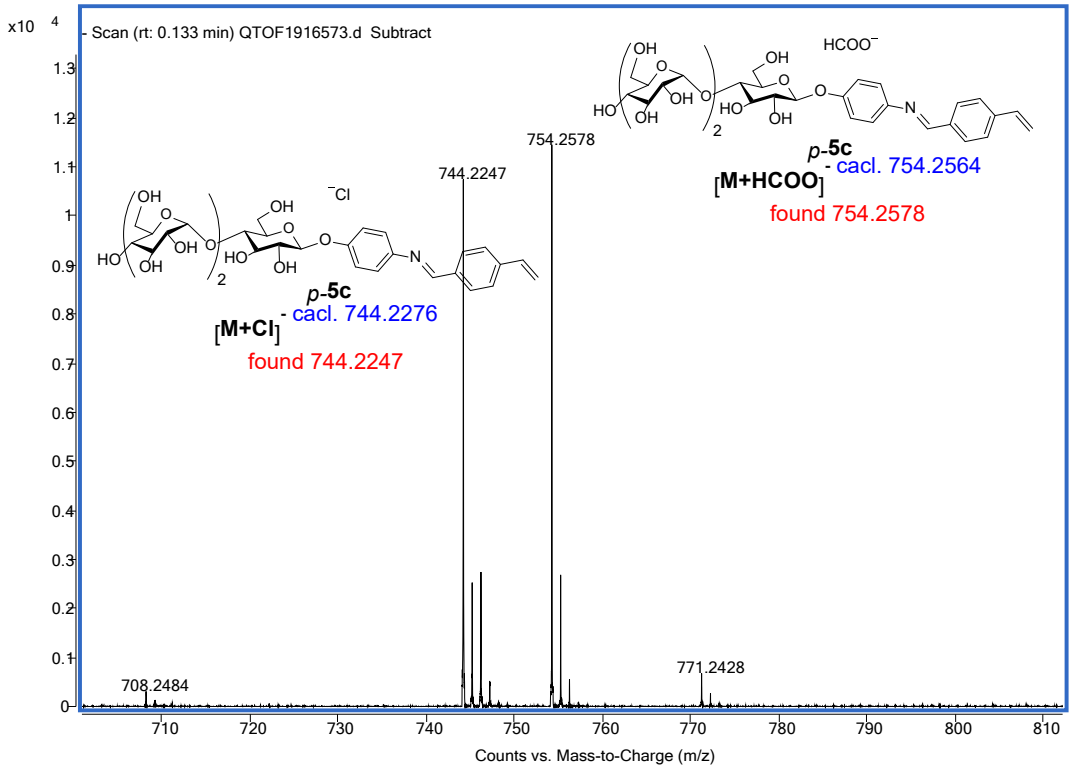

x10 4
- Scan (rt: 0.248 min) QTOF1916526.d Subtract
468.1290
10
[M+Cl]- cacl. 468.1278
found 468.1290
HCOO−
10
[M+HCOO]- cacl. 478.1566
found 478.1569
478.1569
432.1514
445.0696
462.1240
Counts vs. Mass-to-Charge (m/z)


x10 5
- Scan (rt: 0.192 min) QTOF1916572.d Subtract
582.1802
592.2088
546.2027
622.2181
p-5b
[M+Cl]- cacl. 582.1748
found 582.1802
p-5b
[M+HCOO]- cacl. 592.2036
found 592.2088
Counts vs. Mass-to-Charge (m/z)


x10 4
+ Scan (rt: 0.122 min) QTOF1916449.d Subtract
1063.3253
1026.3318
1040.3830
1087.3372
1125.2394
1166.3390
12
[M+NH4]+ Cacl. 1063.3249
found 1063.3253
Counts vs. Mass-to-Charge (m/z)


x10 4
- Scan (rt: 0.178-0.195 min, 2 scans) QTOF1916467.d Subtract
670.1857
617.1555
642.1885
738.1707
806.1559
845.2739
HCOO⁻
13
[M+HCOO]⁻ Cacl. 670.1836
found 670.1857
Counts vs. Mass-to-Charge (m/z)


x10 3
- Scan (rt: 0.191-0.208 min, 2 scans) QTOF1916527.d Subtract
14
⁻Cl
[M+Cl]⁻ cacl. 630.1806
found 630.1847
630.1847
640.2134
14
HCOO⁻
[M+HCOO]⁻ cacl. 640.2094
found 640.2134
601.1309
617.1583
657.2016
674.2057
692.1740
Counts vs. Mass-to-Charge (m/z)


x10 4
- Scan (rt: 0.133 min) QTOF1916573.d Subtract
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
744.2247
754.2578
708.2484
771.2428
710
720
730
740
750
760
770
780
790
800
810
Counts vs. Mass-to-Charge (m/z)
p-5c
[M+Cl]- cacl. 744.2276
found 744.2247
p-5c
[M+HCOO]- cacl. 754.2564
found 754.2578

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