Supporting Information

**Table S1** FPLC purification scheme of rHPLOE

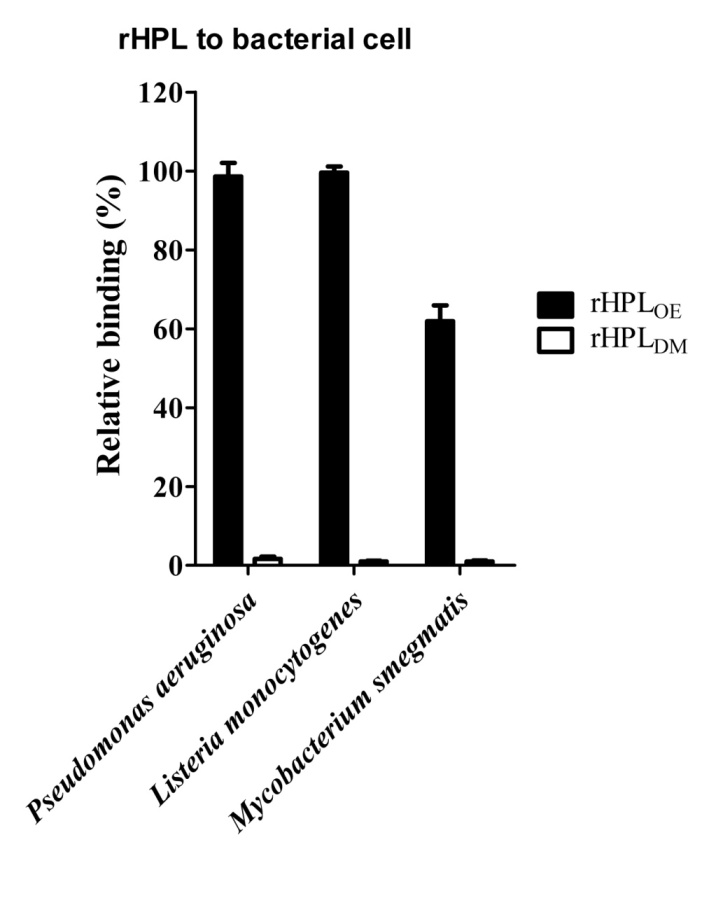
|  |  |  |  |
| --- | --- | --- | --- |
|  | Starting volume | Primed solution | Flow rate |
| Binding | 0 mL | Sample in resuspension buffer | 2 mL/min |
| Wash I | 75 mL | 95% Binding Buffer  5% Elution Buffer | 3 mL/min |
| Wash II | 135 mL | 90% Binding Buffer  10% Elution Buffer | 3 mL/min |
| Wash III | 210 mL | 85% Binding Buffer  15% Elution Buffer | 3 mL/min |
| Wash IV | 285 mL | 70% Binding Buffer  30% Elution Buffer | 3 mL/min |
| Elution | 360 mL | 60% Binding Buffer  40% Elution Buffer | 3 mL/min |
| Equilibrium | 510 mL | Binding Buffer | 3 mL/min |
| Method end | 560 L |  |  |

\*Column: HisTrap™ HP immobilized metal ion affinity chromatography

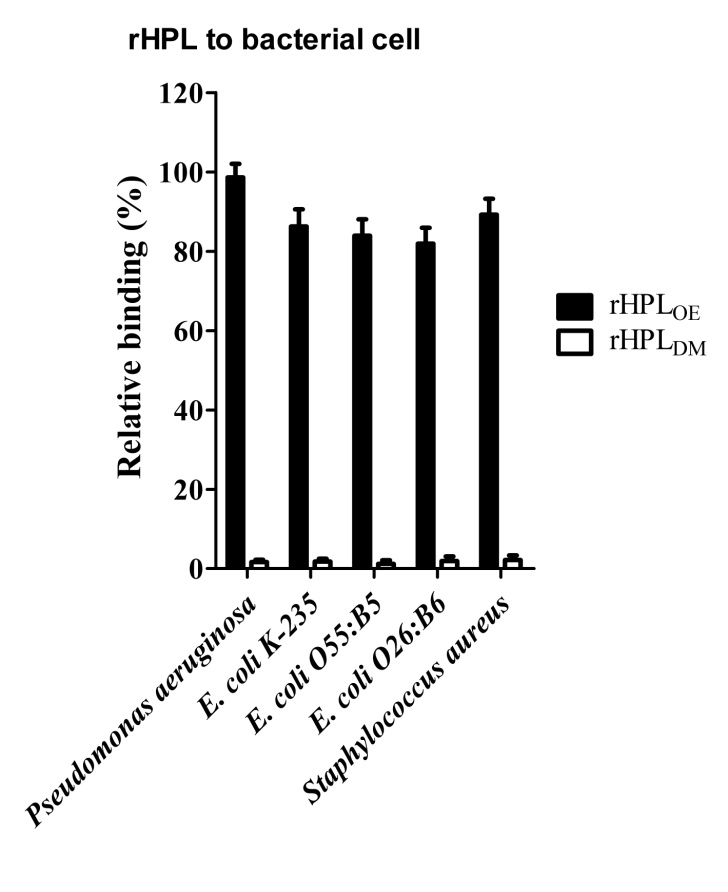
\*Resuspension buffer: 20 mM Tris, 200 mM NaCl, pH 7.2

\*Binding buffer: 20 mM Tris, 200 mM NaCl, pH 7.2

\*Elution buffer: 20 mM Tris, 200 mM NaCl, 2 M imidazole, pH7.2

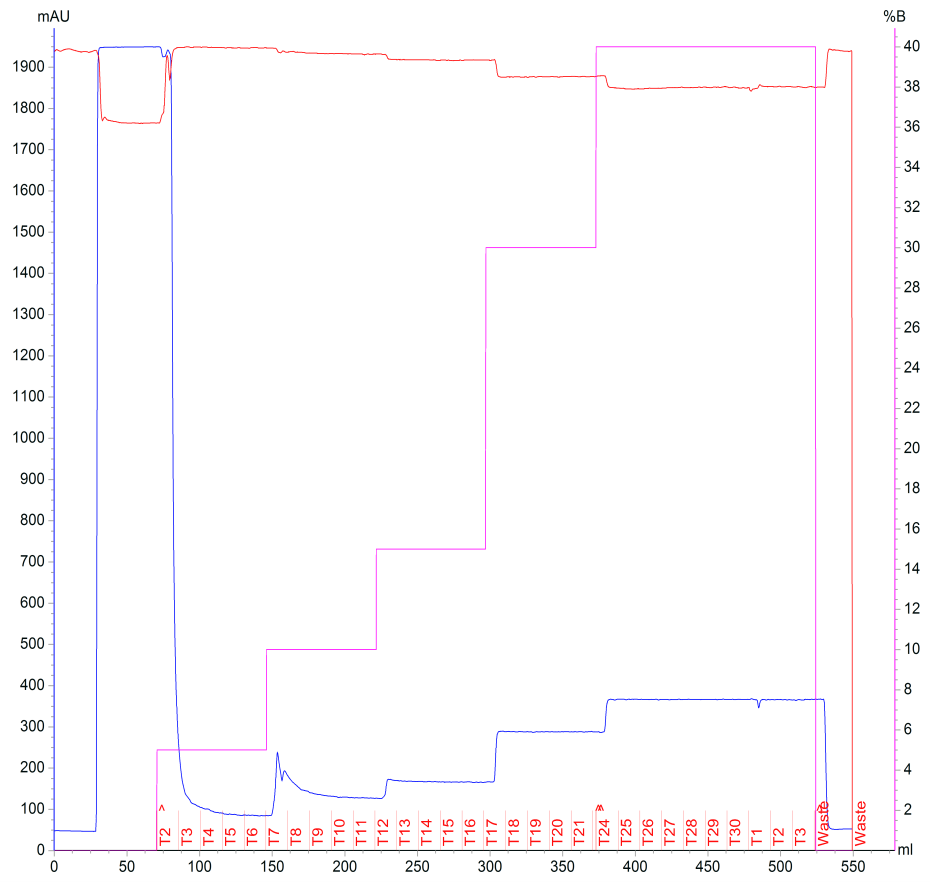


(A)

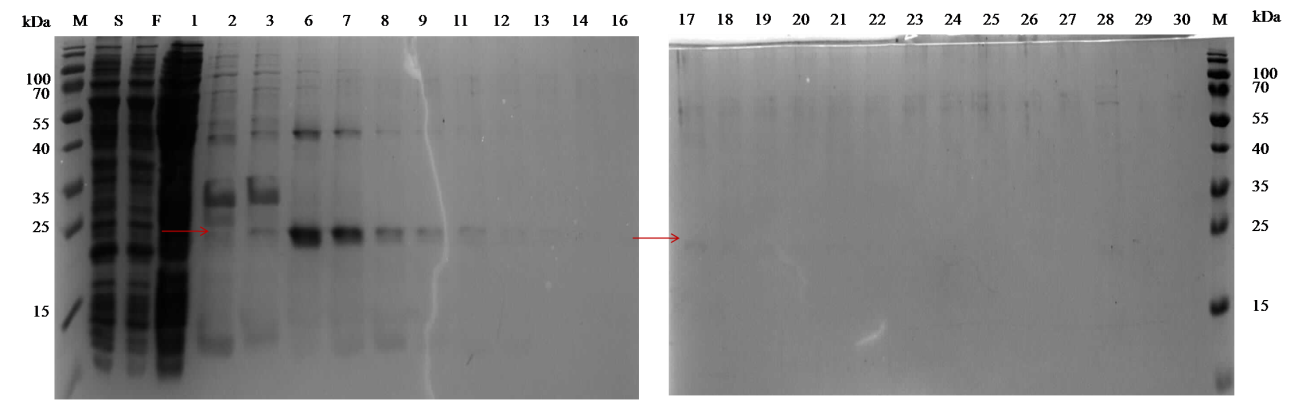
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(B)

**Figure S1.** Bacterial cell or PAMP binding activity of rHPLOE and rHPLDM. Bacterial cell (A) or PAMP (B) binding activity of rHPLOE or rHPLDM was determined by direct ELISA. 1 μM rHPLOE or rHPLDM was applied to 96-well plate coated with bacterial cell (5 × 107 cells each well) or PAMP (0.5 μg each well). PBS buffer was applied as blank. Anti-His monoclonal antibody and anti-mouse IgG polyclonal antibody conjugated HRP was used to detect the binding. Each value was the average of three measurements where presented data was mean ± SD. All means were compared by One-way ANOVA. \*\*\*P<0.001 versus the rHPLOE group.

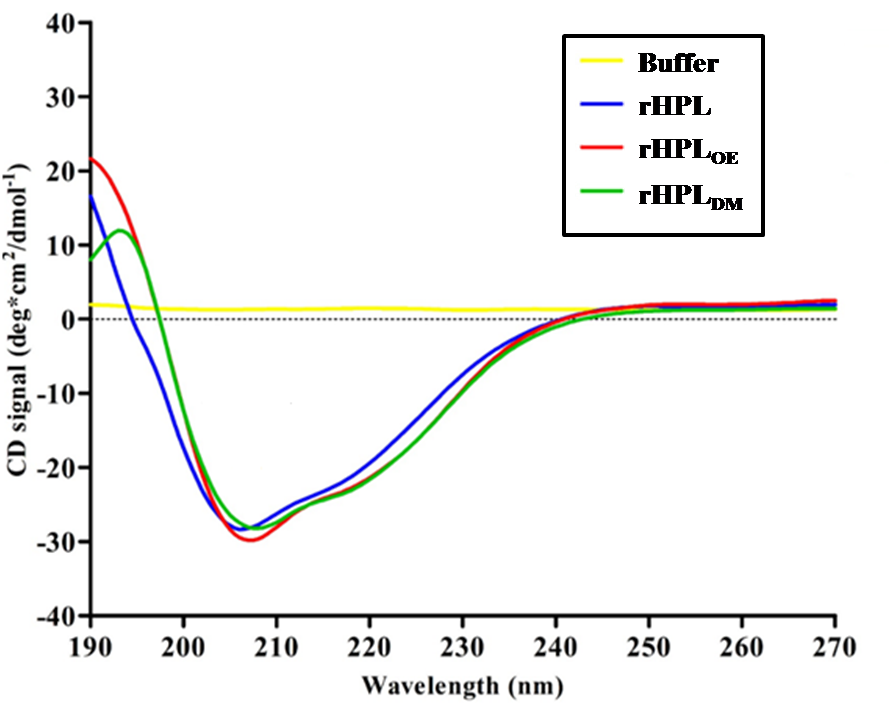


(A)



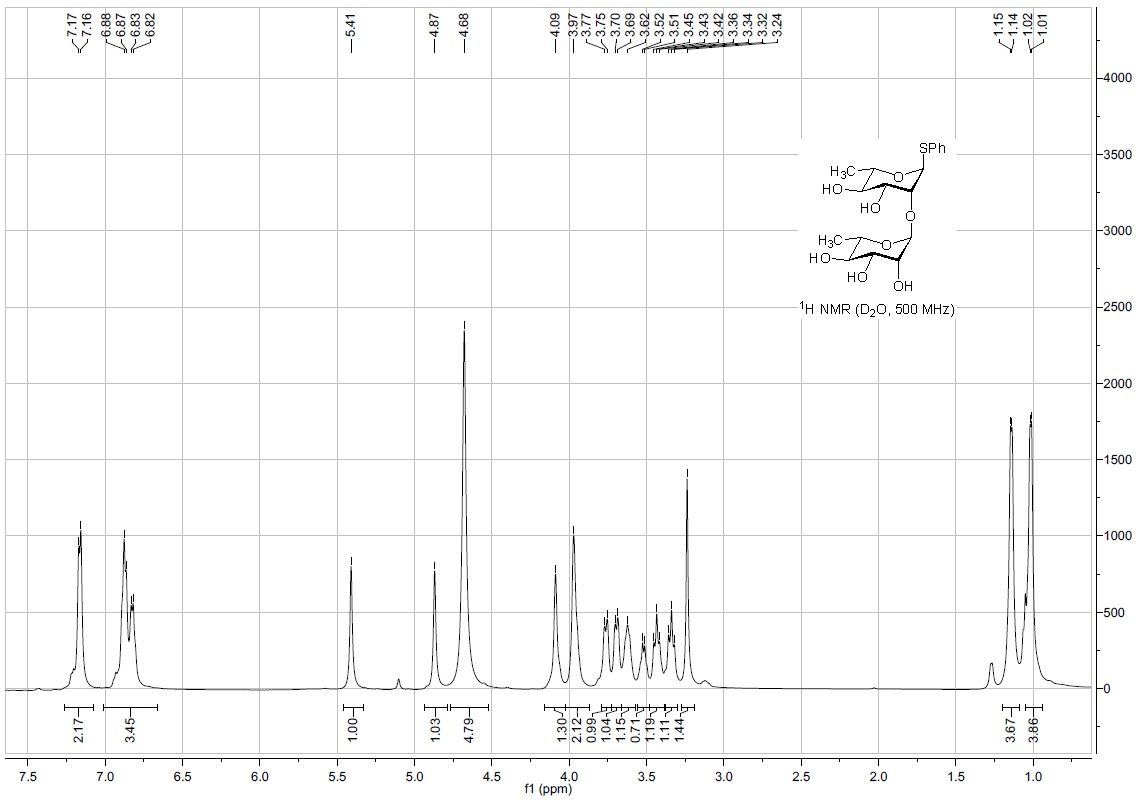
(B)

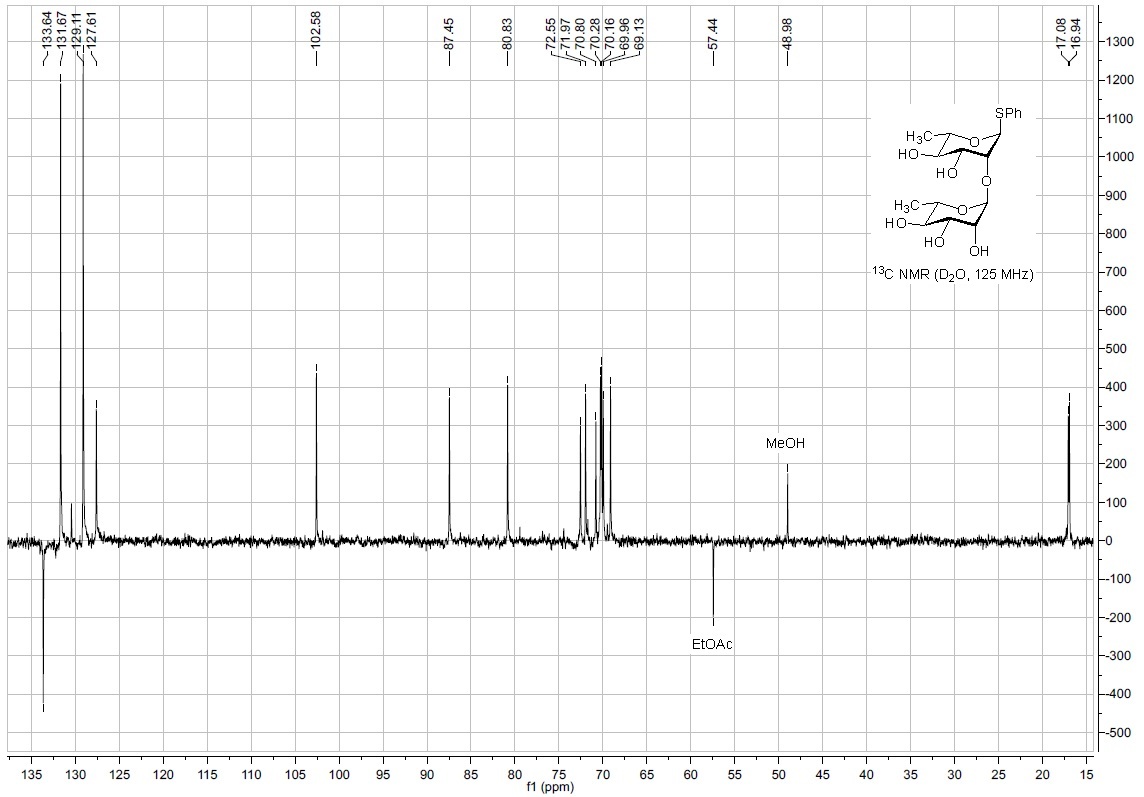
**Figure S2.** Purification and characterization of rHPLOE with HisTrap™ HP immobilized metal ion affinity chromatography. (A) Chromatographic profile of rHPLOE purification. 90 milliliters of *E. coli*cell lysate containing induced rHPLOE was loaded onto nickel column and separated with a gradient of imidazole (purple trace) at a flow rate of 2 mL per minute at 26 °C and UV absorbance at 280 nm (blue trace) was monitored by ÄKTA™ start chromatography systems. Fractions were automatically collected every 15 mL (T2 to T33 fraction No. 1 to fraction number No. 32 (B) Purification efficiency analysis of rHPLOE. Collected fractions were analyzed by 15% (w/v) SDS-PAGE. The molecular weight of rHPL was approximately 19 kDa. Lane M: molecular weight marker; Lane S: soluble protein extracts in supernatant from total *E. coli* lysate; Lane F: flow-through of unbound supernatant; Lanes 1 to 30: fraction number (fraction number 2 to 5 were collected as “wash”, fraction number 6 to 30 were collected as “eluent”).

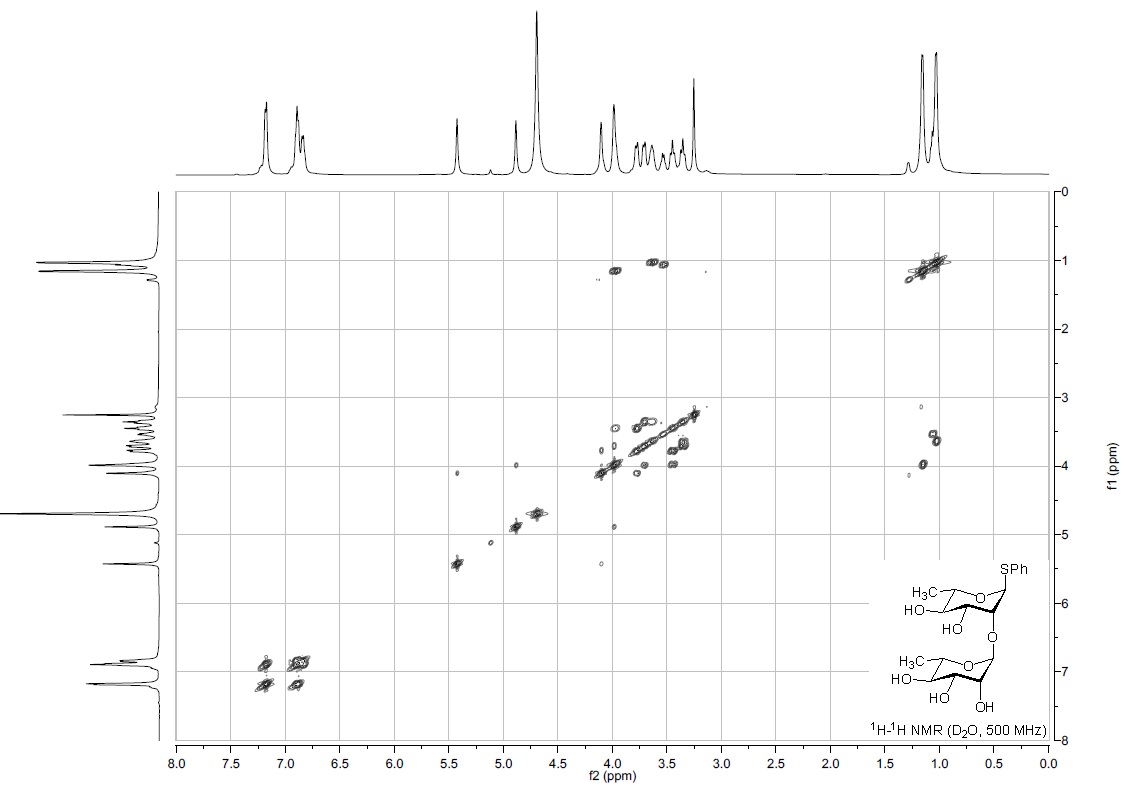


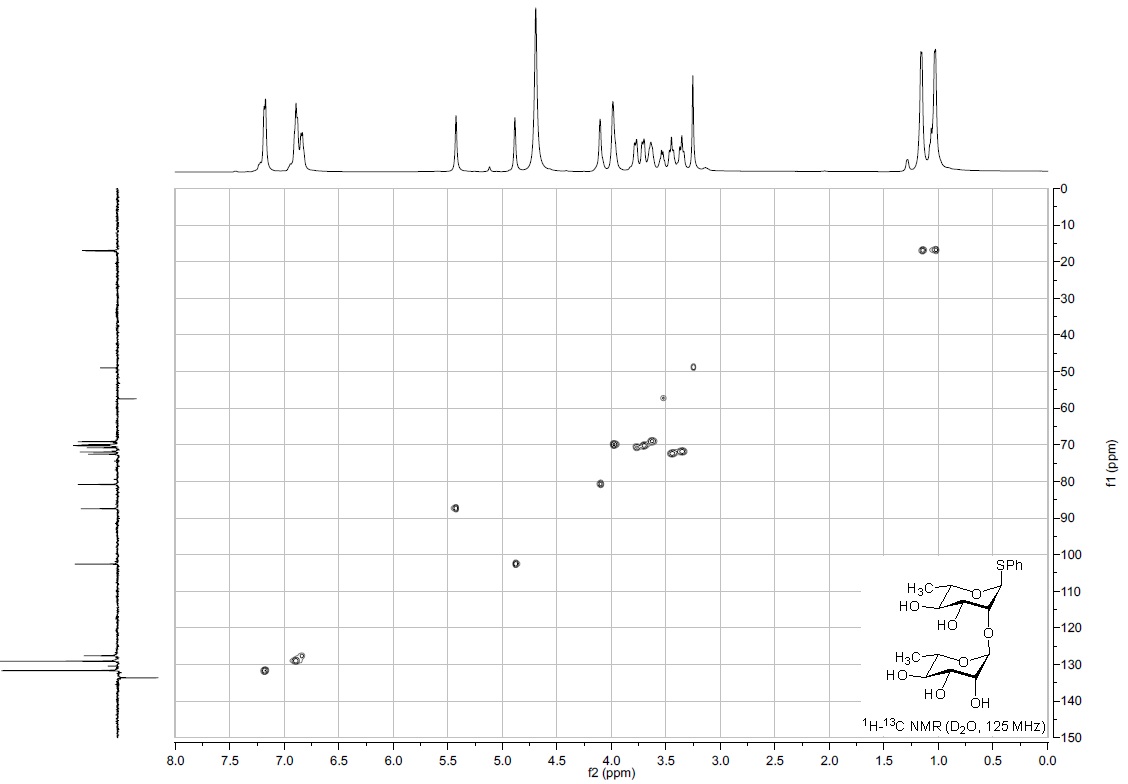
**Figure S3.** Secondary structure analysis of rHPLOE, rHPLDM and rHPL using circular dichroism (CD). The CD spectrum of 50 μM rHPLOE, rHPLDM or rHPL in 20 mM sodium phosphate (pH 7.2) was determined at 25 ºC. CD spectra were recorded on an Aviv CD spectrometer (model202) equipped with a 450-W xenon arc lamp. Far-UV spectral analysis at 190 to 270 nm was performed in a rectangular quartz cuvette with a 0.1 cm path length at 25 °C using a scan rate of4 nm/sand a bandwidth of 0.5 nm. Each spectrum was the average of three consecutive scans and was baseline-corrected by subtracting the spectrum of buffer alone at the same temperature. The values were indicated as the mean and smoothed with 10 of neighbors on each size to average.





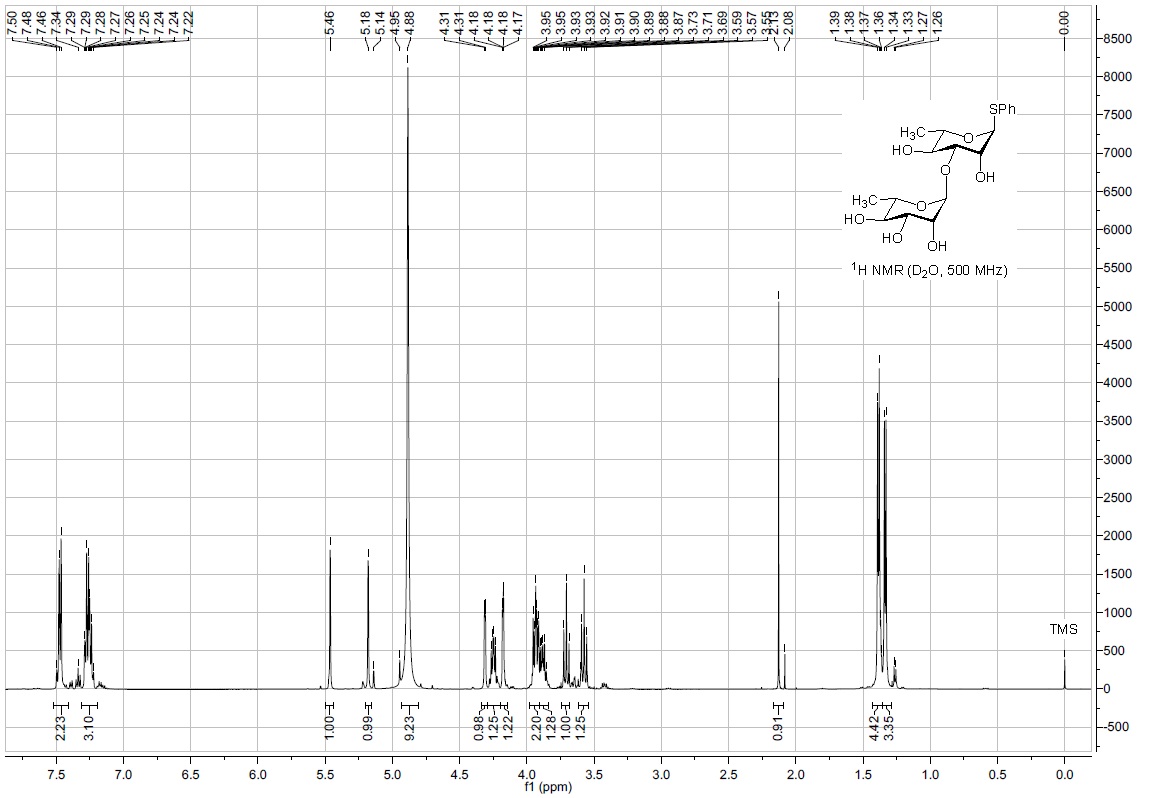


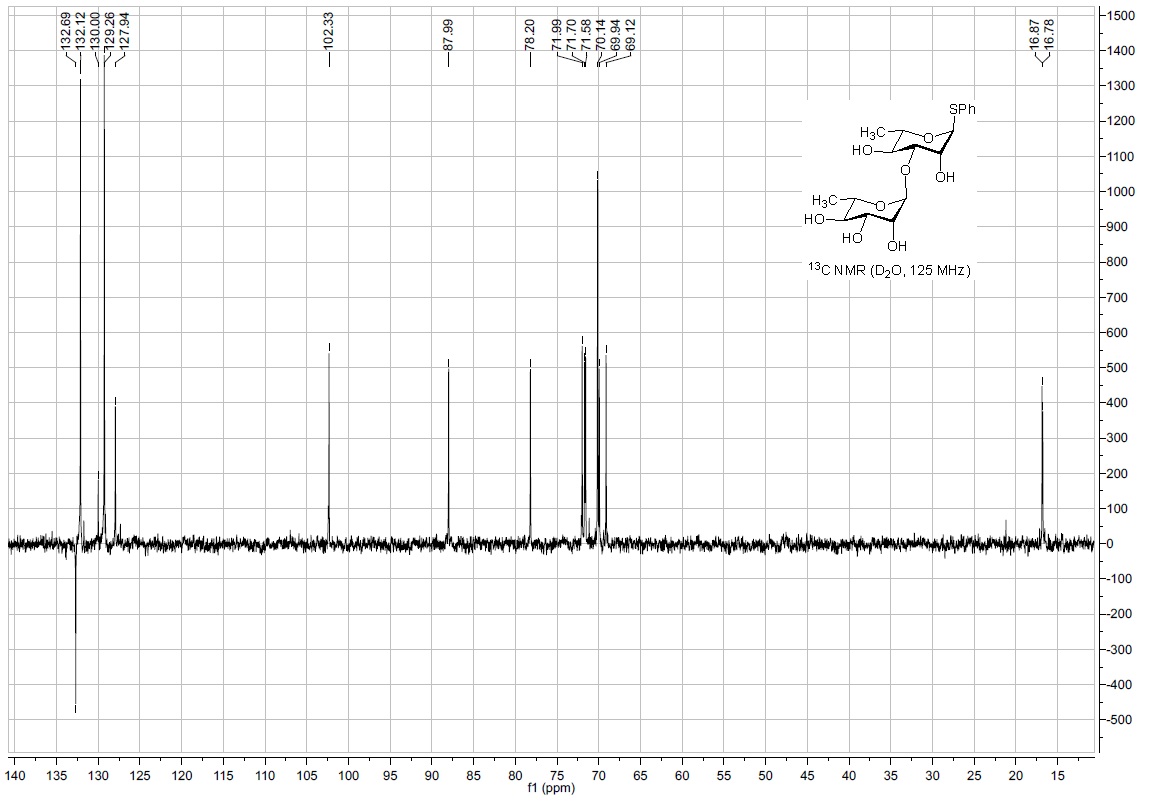


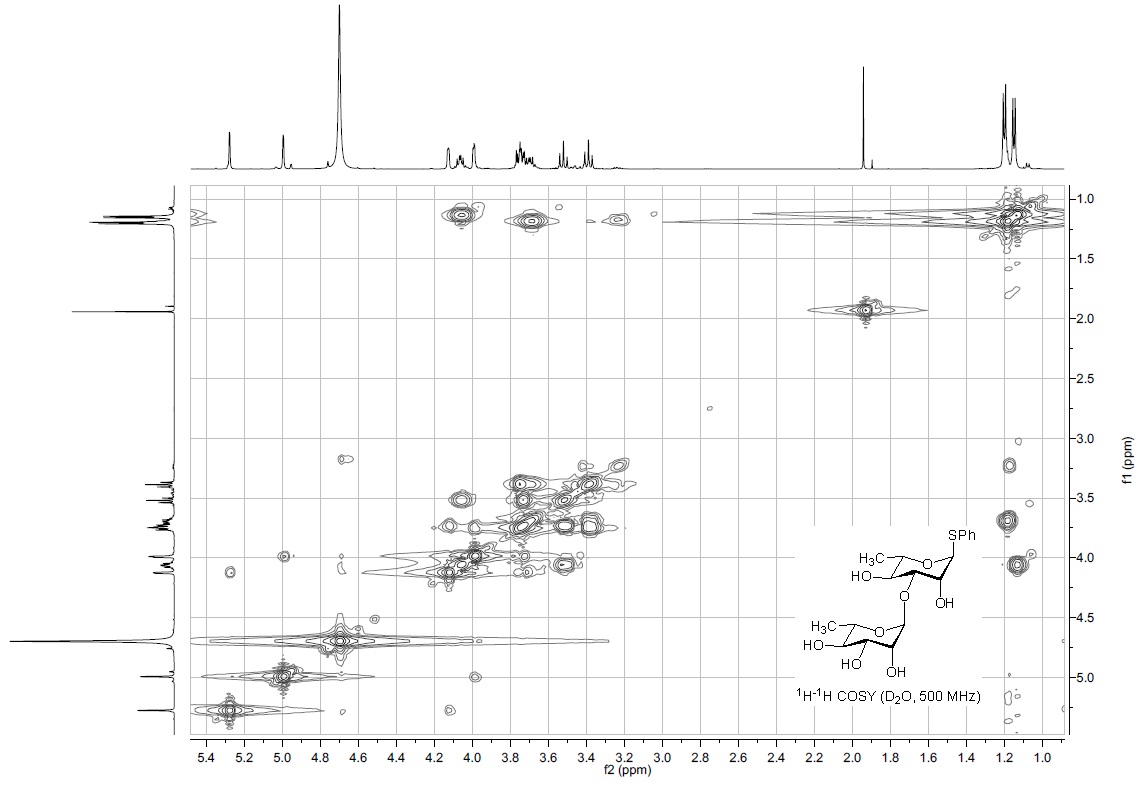


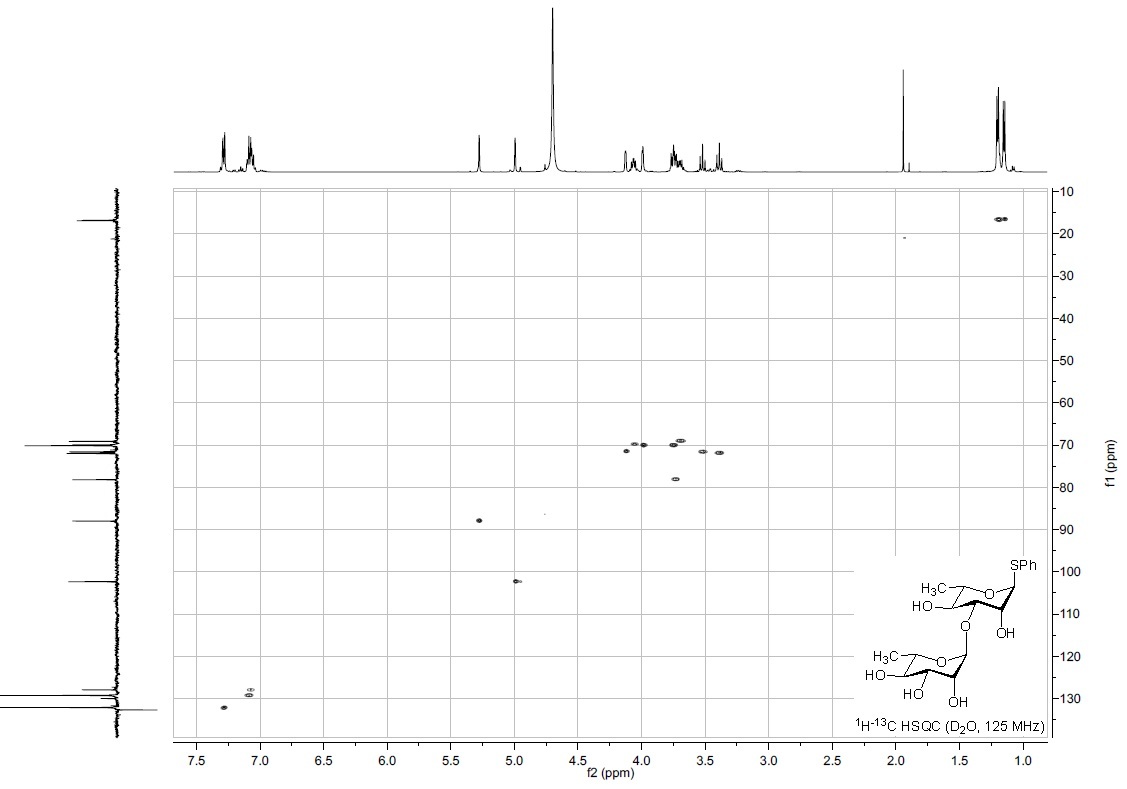
**Figure S4.** Structure and NMR spectra (1H and 13C) of pehnylthio-1-2-rhamnobioside. Phenyl α-l-rhamnopyranosyl-(1→2)-1-thio-α-l-rhamnopyranoside. The title compound was isolated as a colourless syrup. [α]D25 −169.3 (*c* 0.13, MeOH); *R*f = 0.45 (75:25 CH2Cl2/MeOH); 1H NMR (500 MHz, D2O) *δ* 7.17-6.82 (m, 5H, arom), 5.41 (s, 1H, H-1), 4.87 (s, 1H, H-1’), 4.09 (s, 1H, H-2), 3.97 (s, 2H, H-2’, H-5), 3.76 (d, *J* = 8.4 Hz, 1H, H-3), 3.69 (d, *J* = 8.1 Hz, 1H, H-3’), 3.63-3.60 (m, 1H, H-5’), 3.43 (t, *J* = 8.9 Hz, 1H, H-4), 3.34 (t, *J* = 9.1 Hz, 1H, H-4’), 1.14 (d, *J* = 4.1 Hz, 3H, C*H*3), 1.02 (d, *J* = 4.0 Hz, 3H, C*H*3’) ppm; 13C NMR (125 MHz, D2O) *δ* 133.6 (1C, Cq arom), 131.7, 129.1, 127.6 (5C, arom), 102.6 (1C, C-1’), 87.5 (1C, C-1), 80.8 (1C, C-2), 72.6 (1C, C-4), 72.0 (1C, C-4’), 70.8 (1C, C-3), 70.3 (1C, C-2’), 70.2 (1C, C-3’), 70.0 (1C, C-5), 69.1 (1C, C-5’), 17.1, 16.9 (2C, 2 x *C*H3) ppm. MS (UHR ESI-QTOF): *m/z* calcd for C18H26NaO8S: 425.1241 [M+Na]+; found: 425.1246.





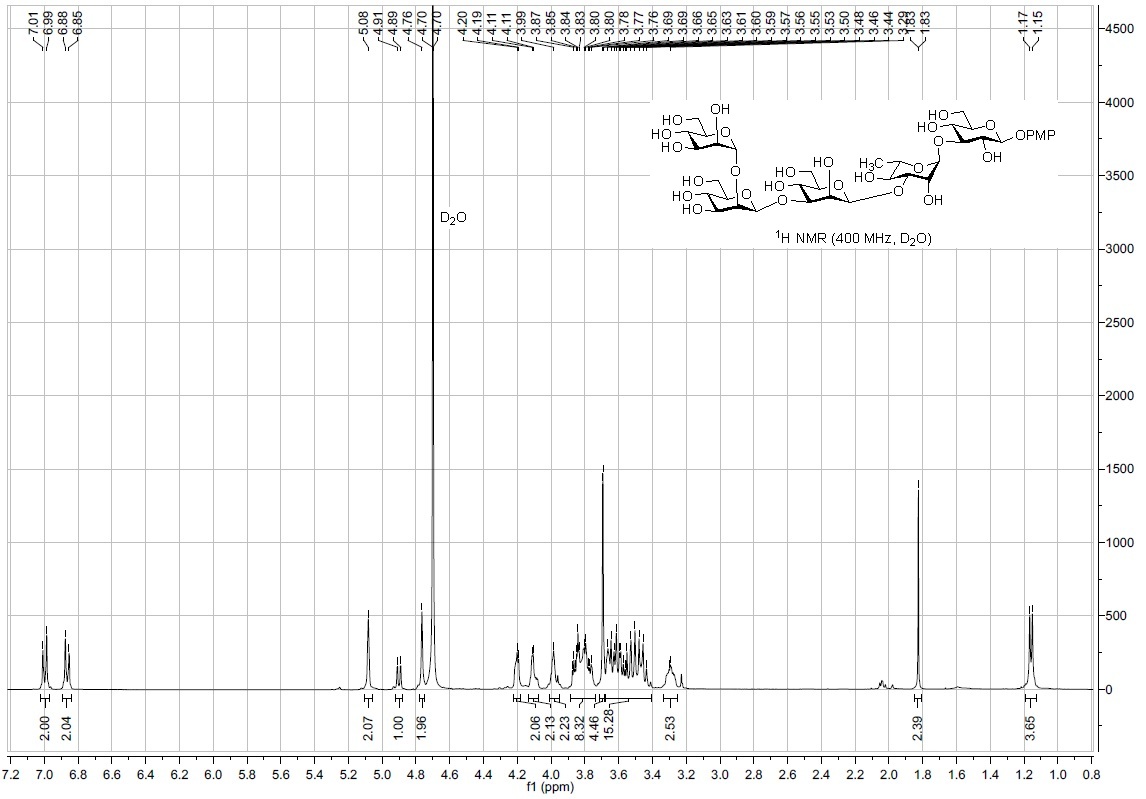


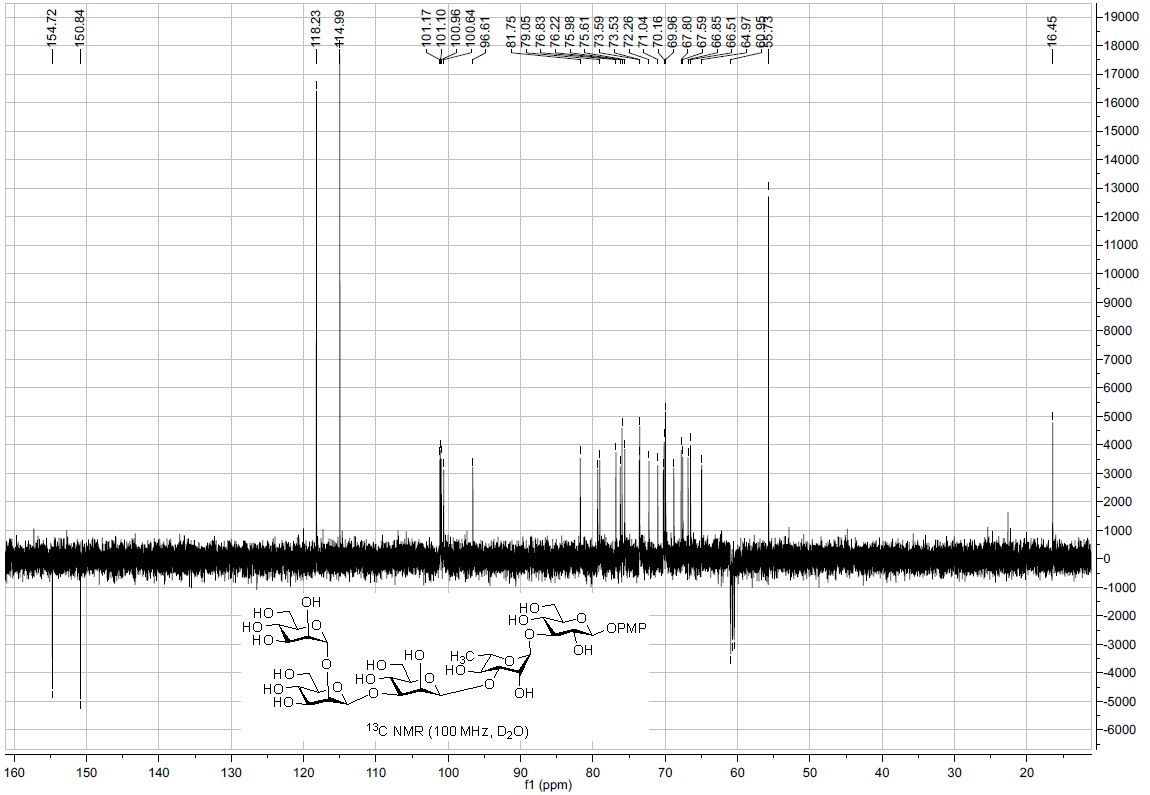


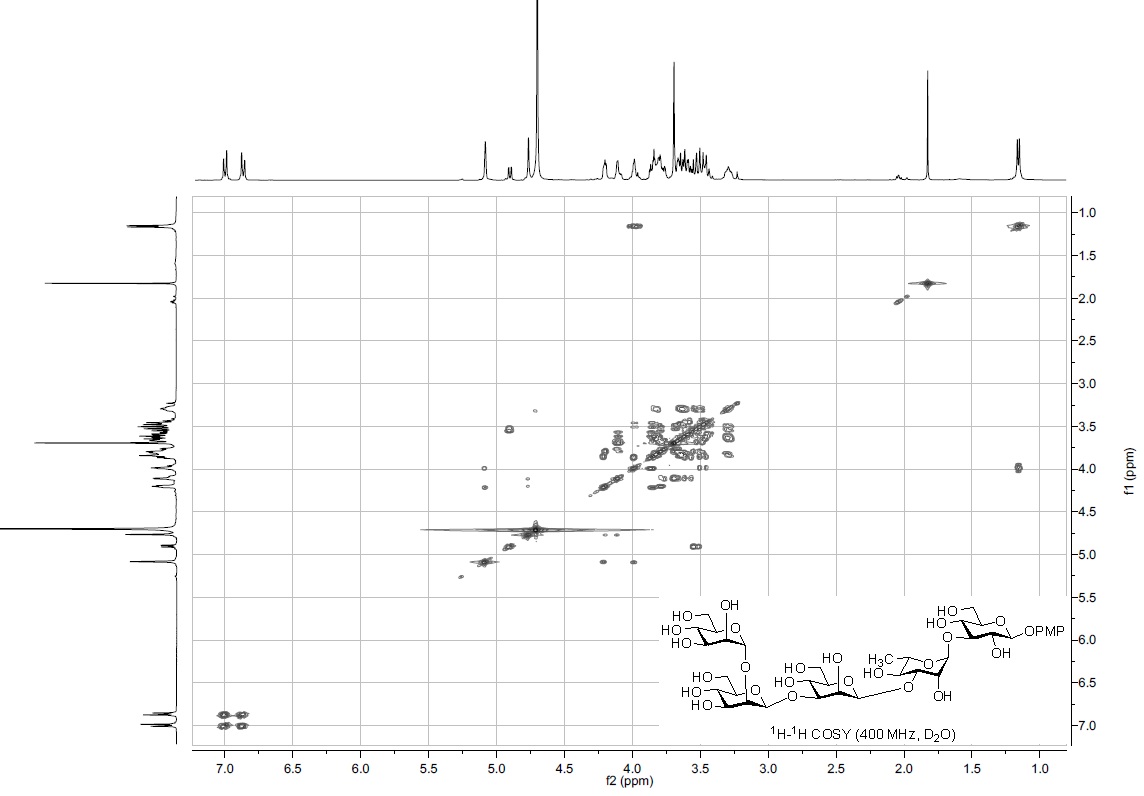


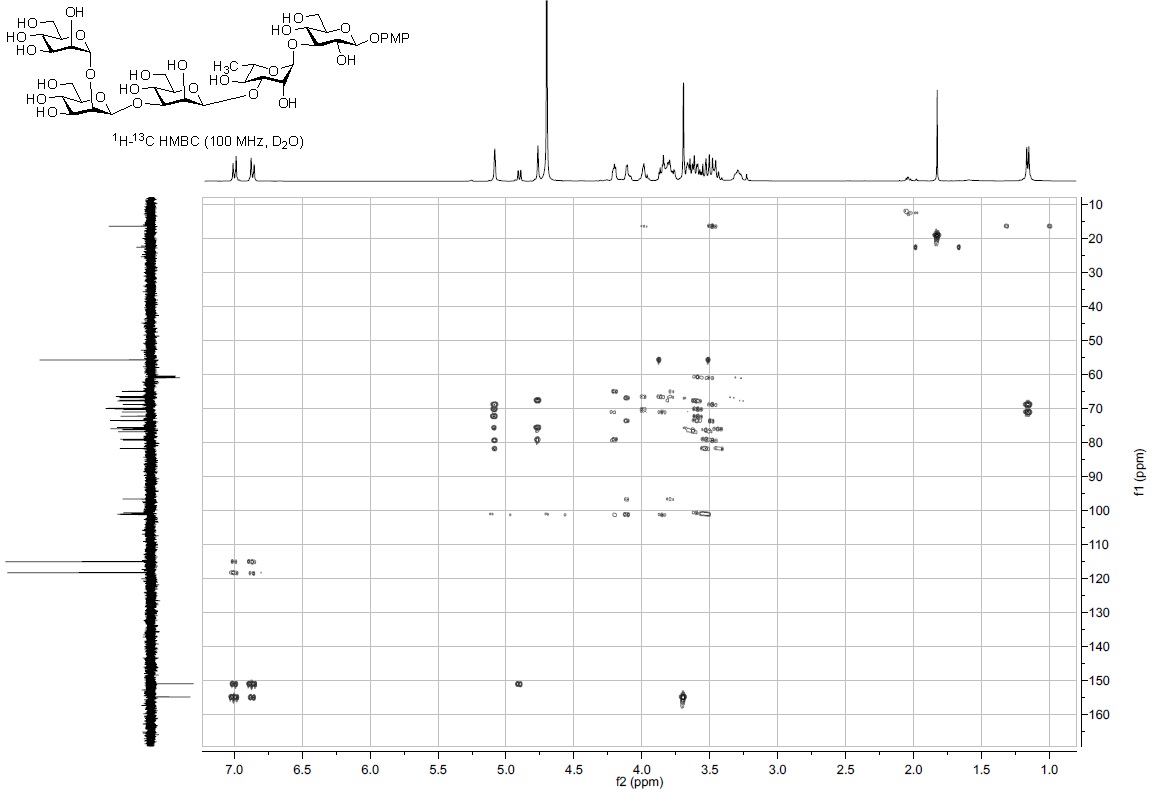
**Figure S5.** Structure and NMR spectra (1H and 13C) of phenylthio-1-3-rhamnobioside. Phenyl α-l-rhamnopyranosyl-(1→3)-1-thio-α-l-rhamnopyranoside. The title compound was isolated as a colourless syrup. [α]D25 −165.0 (c 0.14, MeOH); Rf = 0.54 (75:25 CH2Cl2/MeOH); 1H NMR (500 MHz, D2O) δ 7.50-7.22 (m, 5H, arom), 5.46 (s, 1H, H-1), 5.18 (s, 1H, H-1’), 4.31 (dd, *J* = 1.4 Hz, *J* = 2.7 Hz, 1H, H-2), 4.25 (dq *J* = 6.2 Hz, *J* = 12.4 Hz, 1H, H-5’), 4.18 (dd, *J* = 1.5 Hz, *J* = 3.1 Hz, 1H, H-2’), 3.95-3.91 (m, 2H, H-3, H-3’), 3.90-3.86 (m, 1H, H-5), 3.71 (t, *J* = 9.6 Hz, 1H, H-4’), 3.57 (t, *J* = 9.6 Hz, 1H, H-4), 1.39 (d, *J* = 6.2 Hz, 3H, CH3), 1.34 (d, *J* = 6.2 Hz, 3H, CH3’) ppm; 13C NMR (125 MHz, D2O) δ 132.7 (1C, Cq arom), 132.1, 129.3, 127.9 (5C, arom), 102.3 (1C, C-1’), 88.0 (1C, C-1), 78.2 (1C, C-3), 72.0 (1C, C-4), 71.7 (1C, C-4’), 71.6 (1C, C-2), 70.1 (2C, C-2’, C-3’), 69.9 (1C, C-5’), 69.1 (1C, C-5), 16.9, 16.8 (2C, 2 x CH3) ppm; MS (UHR ESI-QTOF): m/z calcd for C18H26NaO8S: 425.1241 [M+Na]+; found: 425.1244.

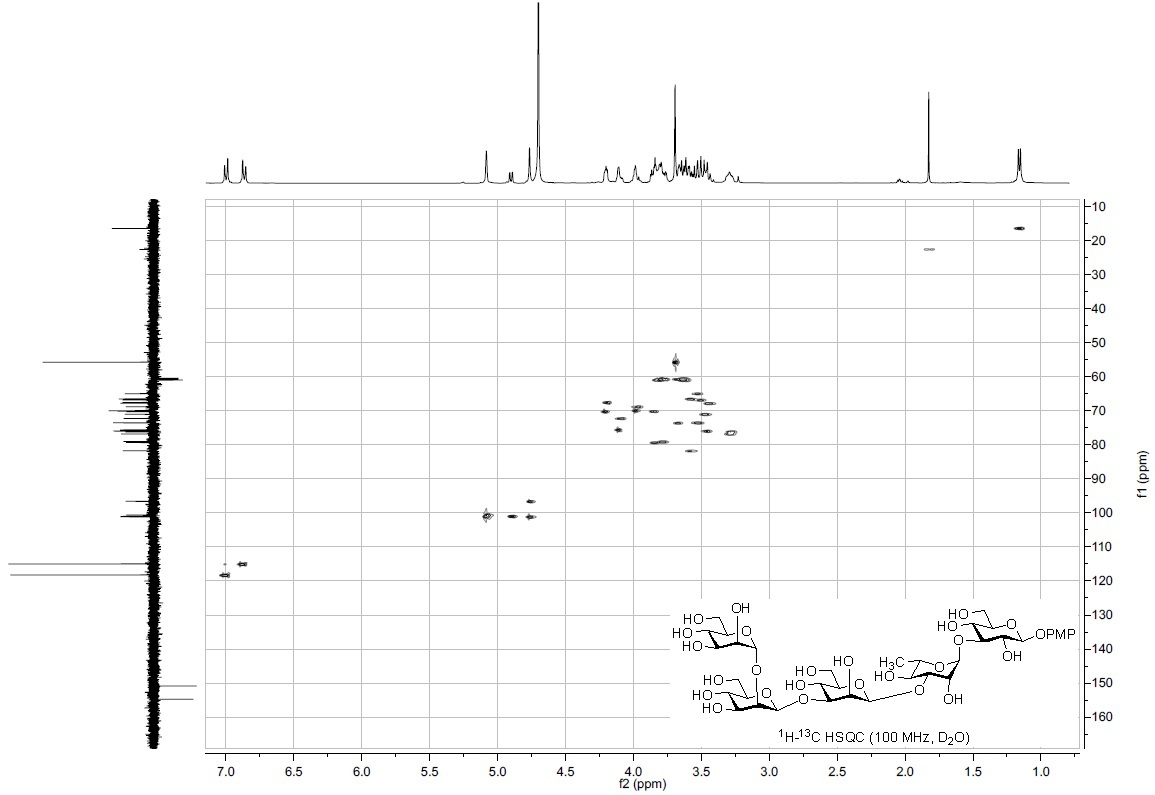




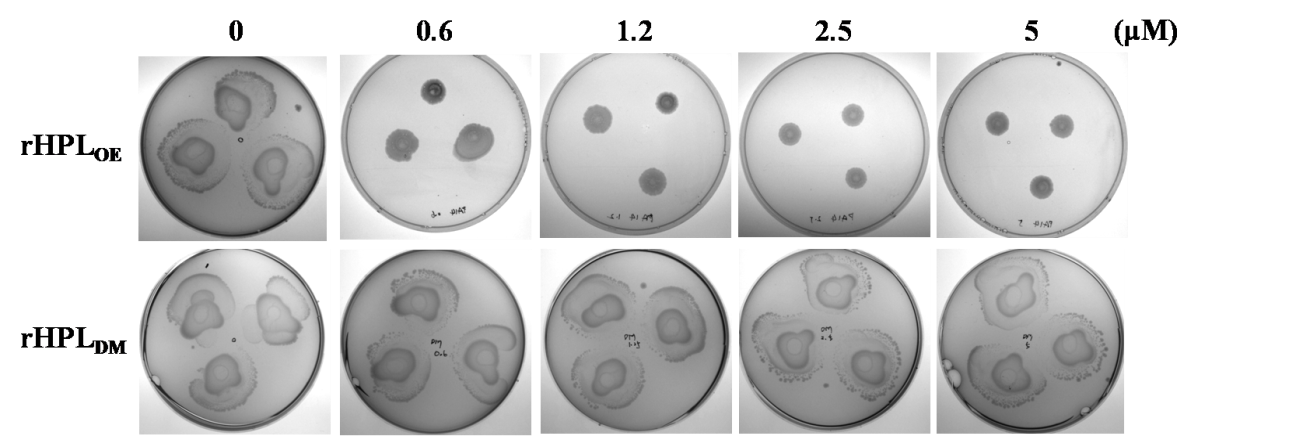




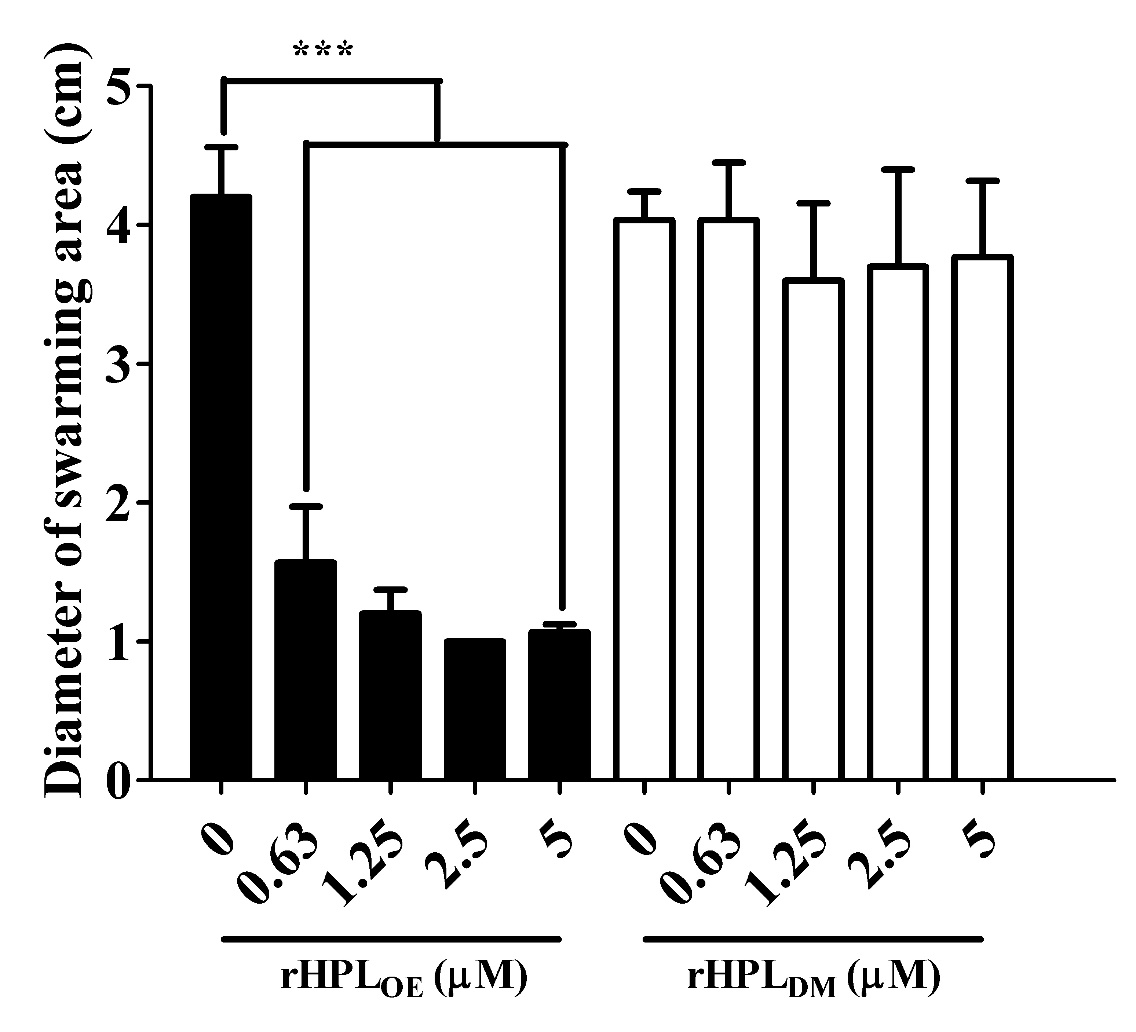




**Figure S6.** Structure and NMR spectra (1H and 13C) of Psl-pentasaccharide. 4-Methoxyphenyl α-d-mannopyranosyl-(1→2)-β-d-mannopyranosyl-(1→3)-β-d-mannopyranosyl-(1→3)-α-l-rhamnopyranosyl-(1→3)-β-d-glucopyranoside. The title compound was isolated as a white solid. [α]D25 −13.5 (c 0.14, MeOH); Rf = 0.29 (7:6:1 CH2Cl2/MeOH/H2O); 1H NMR (400 MHz, D2O) δ 7.01-6.85 (m, 4H, arom), 5.08 (s, 2H, H-1-II., H-1-V.), 4.90 (d, J1,2 = 7.7 Hz, 1H, H-1-I.), 4.76 (s, 2H, H-1-III., H-1-IV.), 4.21-4.19 (m, 2H, H-2-III., H-2-V.), 4.11-4.08 (m, 2H, H-2-IV., H-5-V.), 3.99-3.96 (m, 2H, H-2-II., H-5-II.), 3.87-3.83 (m, 2H, H-3-V., H-3-II.), 3.80-3.76 (m, 5H, H-3-III., 4 x H-6a), 3.69 (s, 3H, OCH3), 3.66-3.44 (m, 13H, H-2-I., H-3-I., H-4-I., H-5-I., H-4-II., H-4-III., H-3-IV., H-4-IV., H-4-V., 4 x H-6b), 3.32-3.27 (m, 2H, H-5-III., H-5-IV.), 1.16 (d, *J* = 6.2 Hz, 3H, CH3) ppm; 13C NMR (100 MHz, D2O) δ 154.7, 150.8 (2C, 2 x Cq arom), 118.2, 115.0 (4C, arom), 101.2 (1C, C-1-III.), 101.1 (1C, C-1-V.), 101.0 (1C, C-1-I.), 100.6 (1C, C-1-II.), 96.6 (1C, C-1-IV.), 81.8 (1C, C-3-I.), 79.4 (1C, C-3-V.), 79.1 (1C, C-3-III.), 76.8 (1C, C-5-III.), 76.2 (1C, C-5-IV.), 76.0 (1C, C-5-I.), 75.6 (1C, C-2-IV.), 73.6 (1C, C-3-IV.), 73.5 (1C, C-2-I.), 72.3 (1C, C-5-V.), 71.0 (1C, C-4-V.), 70.3 (1C, C-2-V.), 70.2 (1C, C-3-III.), 70.0 (1C, C-2-II.), 68.8 (1C, C-5-II.), 67.8 (1C, C-4-I.), 67.6 (1C, C-2-III.), 66.8 (1C, C-4-IV.), 66.5 (1C, C-4-II.), 65.0 (1C, C-4-III.), 61.0, 60.9, 60.7, 60.5 (4C, 4 x C-6), 55.7 (1C, OCH3), 16.5 (1C, 1 x CH3) ppm; MS (UHR ESI-QTOF): m/z calcd for C37H58NaO26: 941.3109 [M+Na]+; Found: 941.3112.

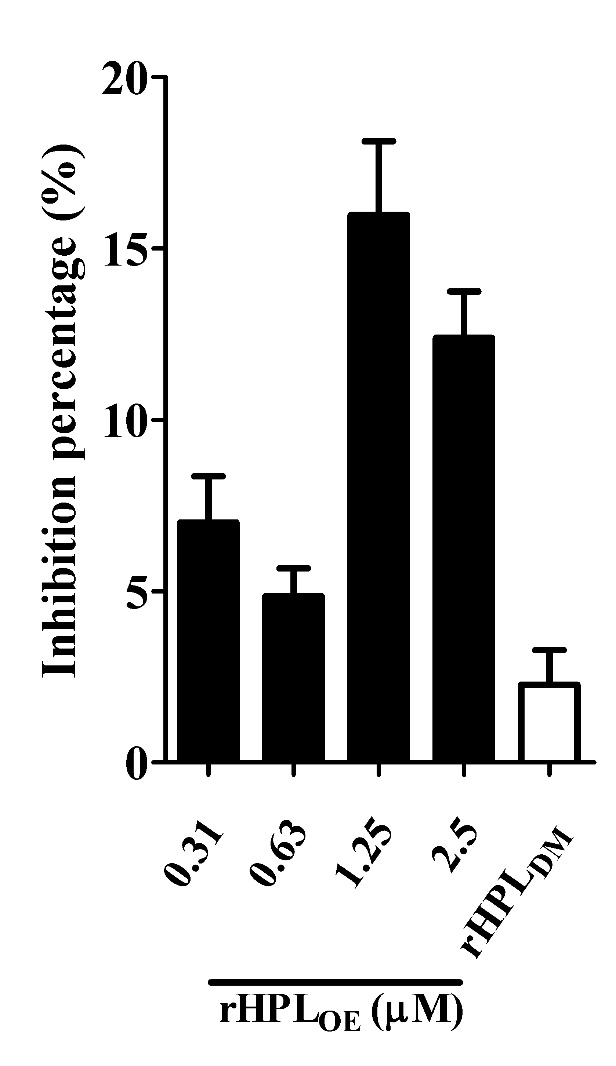


(A)

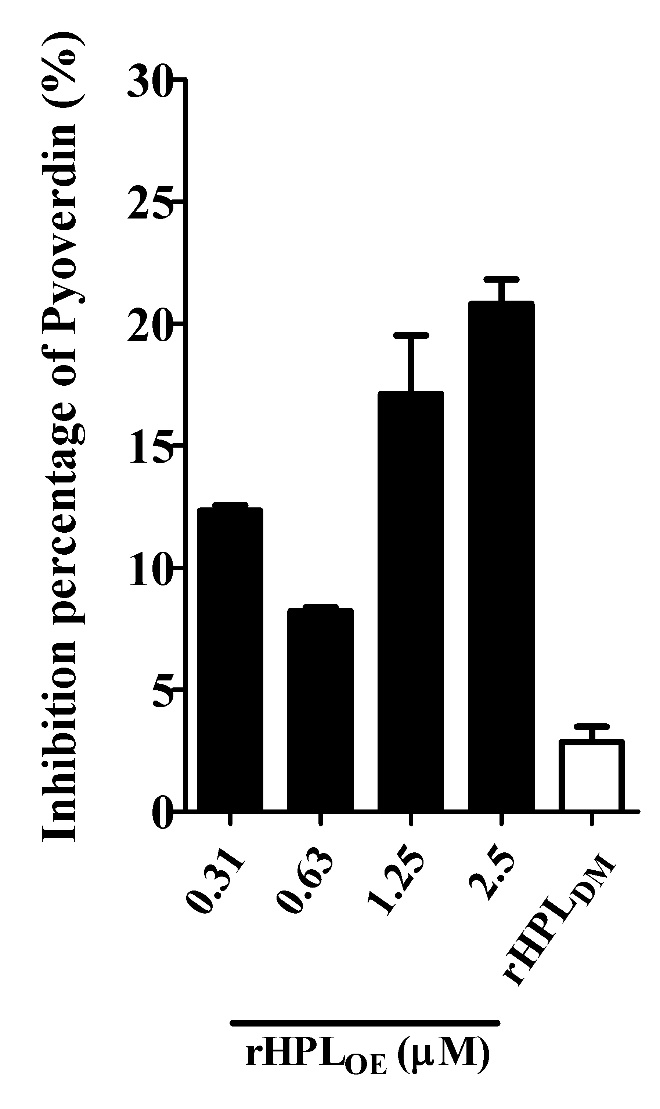


(B)

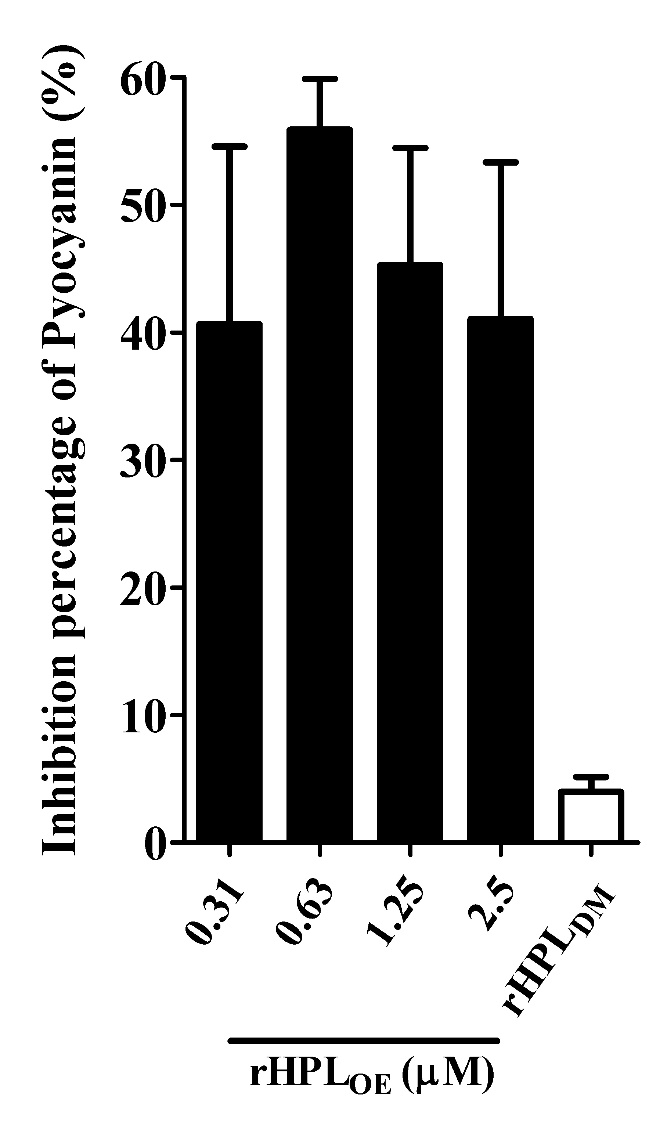
**Figure S7.** Inhibitory effect on the swarming motility of *P. aeruginosa* PA14 by rHPLOE or rHPLDM. The swarming area of *P. aeruginosa* PA14 treated with different concentrations of (A) rHPLOE or rHPLDM. The diameter of the swarming area of PA14 treated with different concentrations of rHPLOE or rHPLDM was measured after 72 h (B). Each value was the average of three measurements, where the presented data was the mean ± SD. All means were compared by one-way ANOVA. \*\*\**P*<0.001 versus the buffer-treated group.



(A)



(B)



(C)

**Figure S8.** Down-regulation effect on the extracellular protease activities or QS-factors of *P. aeruginosa* PA14 by rHPLOE. (A) Extracellular protease activities of *P. aeruginosa* PA14 were measured by the azocasein degradation assay. (B) Secreted pyoverdine was detected by a fluorescence spectrophotometer using 405 nm as the excitation wavelength and 465 nm as the emission wavelength. (C) Secreted pyocyanin was extracted by chloroform and detected by a fluorescence spectrophotometer at 520 nm. The value of the buffer-treated group was set as 0%. All values were expressed as the percentage inhibition with respect to the buffer-treated control. Each value was the average of a triplicate assay, where the presented data was the mean ± SD. \**P*<0.05, \*\**P*< 0.01, \*\*\**P*<0.001 versus the buffer-treated group.