Electronic Supplementary Information

Simultaneous quadruple-channel optical transduction of a nanosensor for multiplexed qualitative and quantitative analysis of lectins

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1. Experimental

Materials and reagents

The concanavalin A (Con A), wheat germ agglutinin (WGA), peanut agglutinin (PNA), ricinus communis agglutinin (RCA_{120}), pisum sativum agglutinin (PSA), bovine serum albumin (BSA), human blood hemoglobin (HGB) and human Serum were purchased from Sigma-Aldrich (St. Louis, USA). Three octylamine-capped QDs were purchased from Xingzi New Material Technology Development Co., Ltd. The D-mannopyranose, D-galactopyranose and D-*N*-acetyl glucosamine, $MnCl_4 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$ and $CaCl_2$ were obtained from J&K (Beijing, China). Phosphate buffered saline (PBS) was prepared by using 20 mM Na_2HPO_4 and 20 mM KH_2PO_4 solution. Ultrapure water (18.25 MΩ cm^{-1}, 25 °C) was used for all of the experiments. All other chemicals were of analytical reagent grade and were used without further purification.

Instrumentation

Fluorescent and Rayleigh resonance scattering measurements were recorded by a RF-5301PC spectrofluorophotometer (SHIMADZU, Kyoto, Japan). The TEM images were taken from a Tecnai G2 F30 (FEI, US). Nuclear magnetic resonance (NMR) spectra were performed on VARIAN 400 MHz NMR system (Varian, Inc. USA). High performance liquid chromatography-tandem mass spectrometry (HPLC-MS) was performed by an Acquity SQ Detector (Waters, USA). Fourier Transform infrared (FI-IR) spectra were obtained using a Nicolet iS50 FT-IR spectrometer (Thermo Scientific, USA). The hydrodynamic sizes of particles were measured by a Zetasizer Nano ZS90 (Malvern, UK).

2. Supplementary Figures and Tables

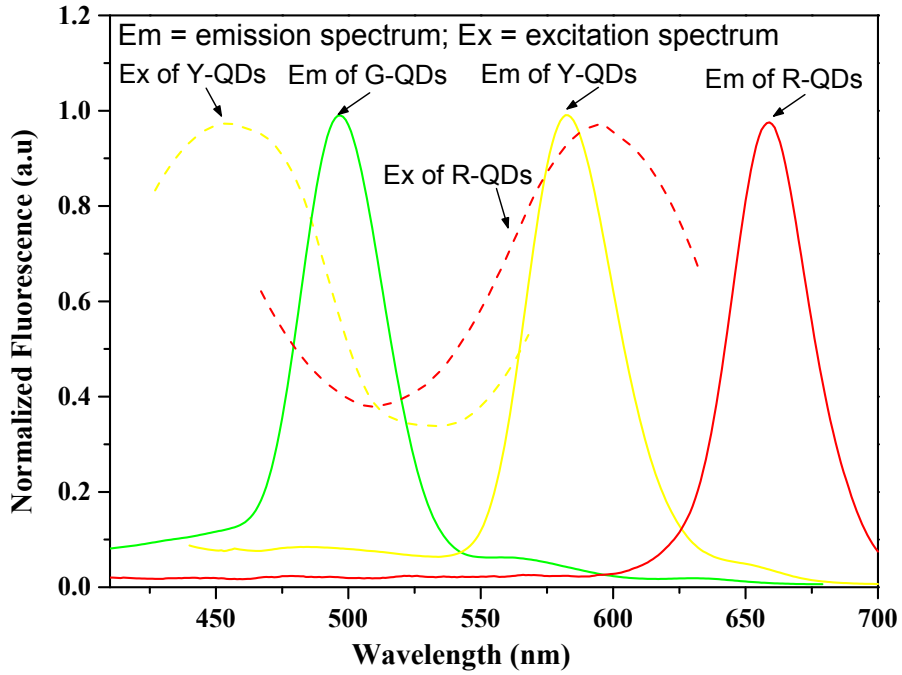


Figure S1. The excitation (dash line) and emission (solid line) spectra of G-, Y- and R-QDs.

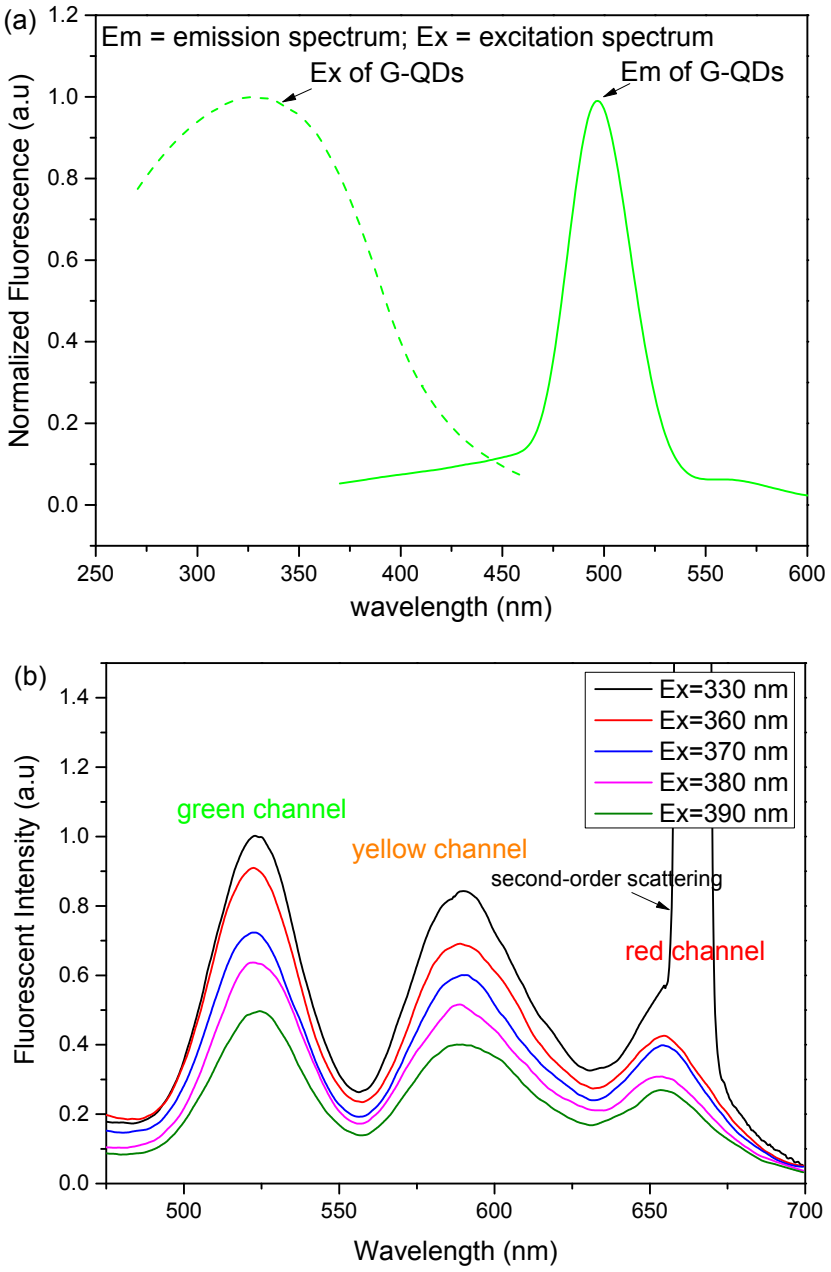


Figure S2. The selection of excitation wavelength in view of (a) emission and excitation spectra of G-QDs and (b) the decreasing emitting intensities of three channels in increasing excitation wavelength.

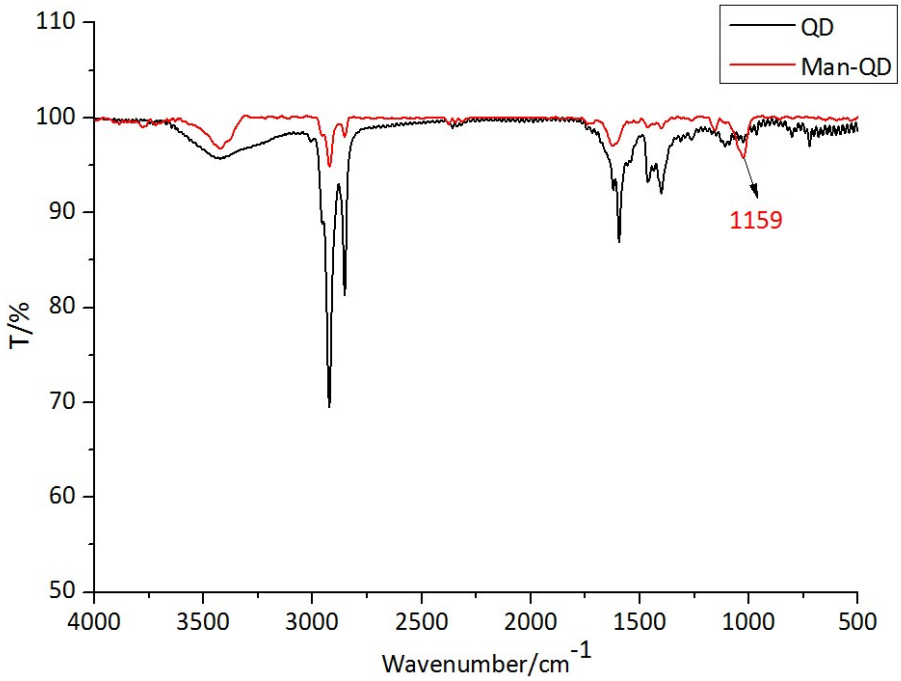


Figure S3. FT-IR spectra of QDs before and after modified with Man.

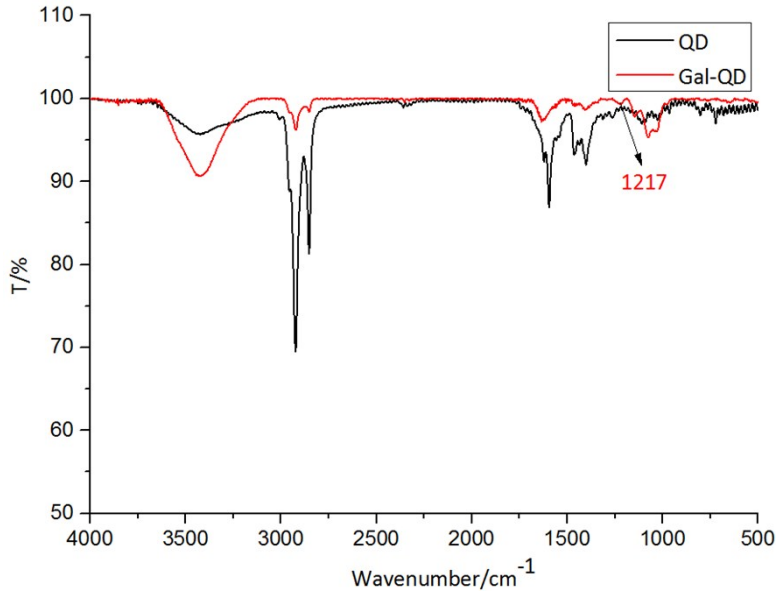


Figure S4.FT- IR spectra of QDs before and after modified with Gal.

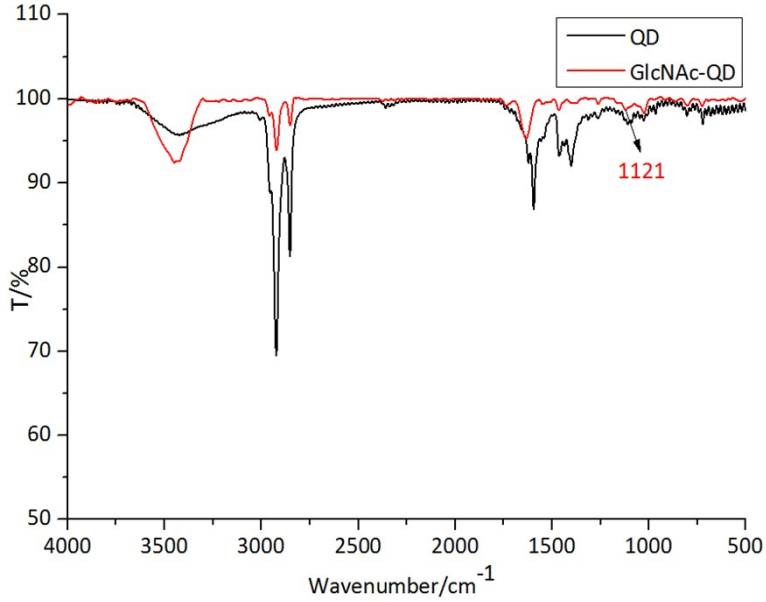


Figure S5. FT-IR spectra of QDs before and after modified with GlcNac.

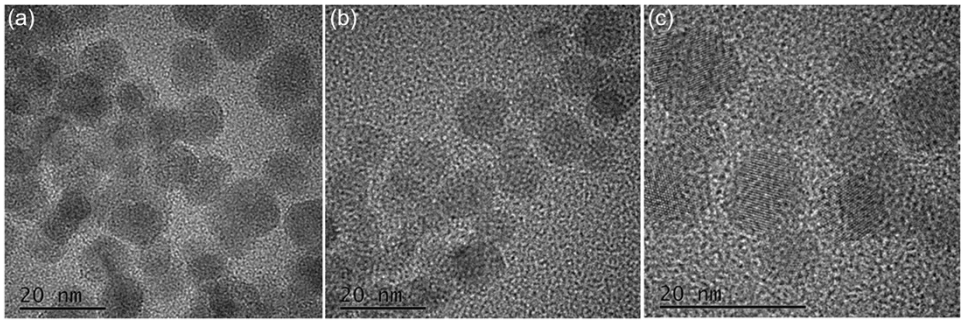


Figure S6. TEM images of (a) Man-G-QDs (b) GlcNac-Y-QDs and (c) Gal-R-QDs.

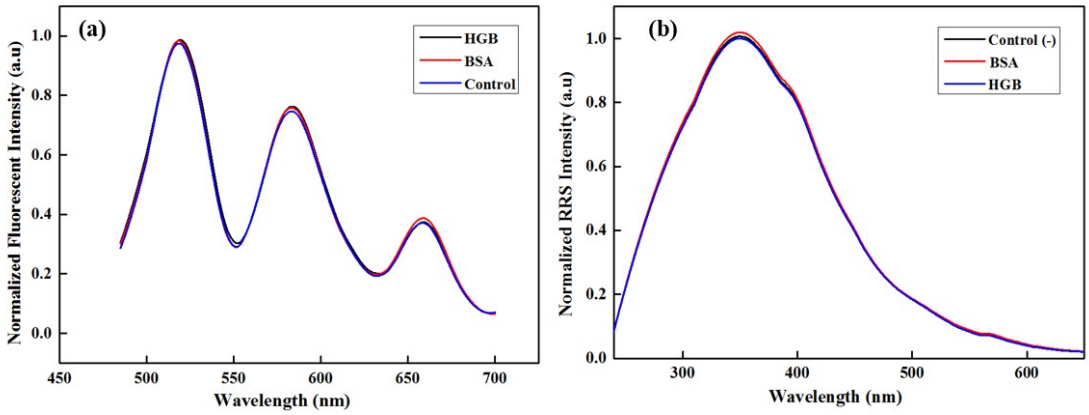


Figure S7. Effect of HGB and BSA on the (a) fluorescence, and (b) RRS spectra of the nanosensor. Control experiment was carried out without addition of any other proteins.

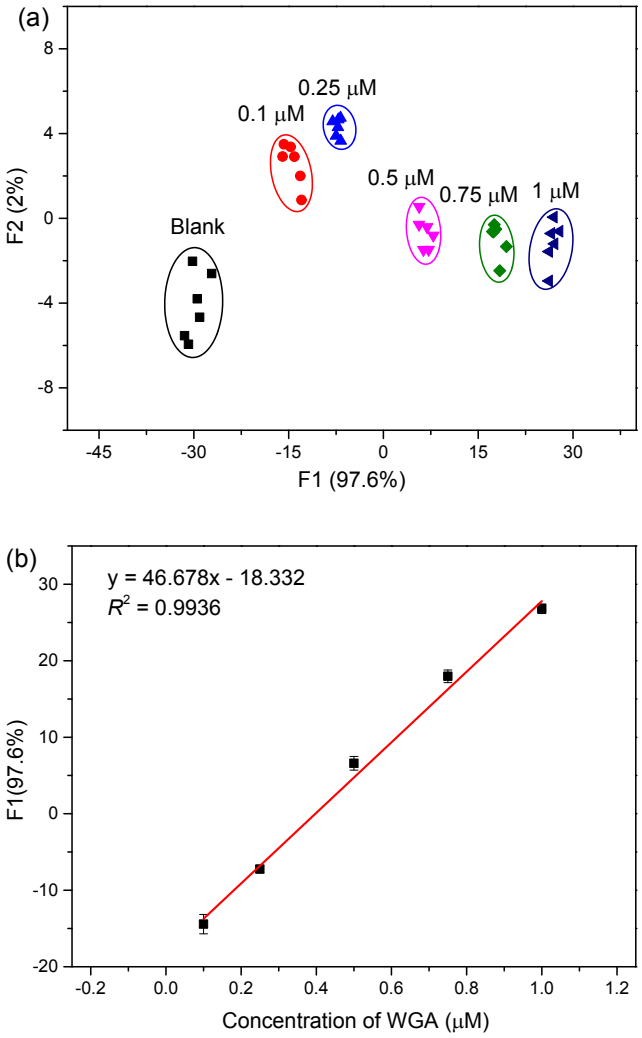


Figure S8. Quantitative analysis of WGA. (a) LDA score plot of WGA with concentrations of 0, 0.1, 0.25, 0.5, 0.75, and 1.0 μM. (b) Liner fitting between factor (1) and WGA.

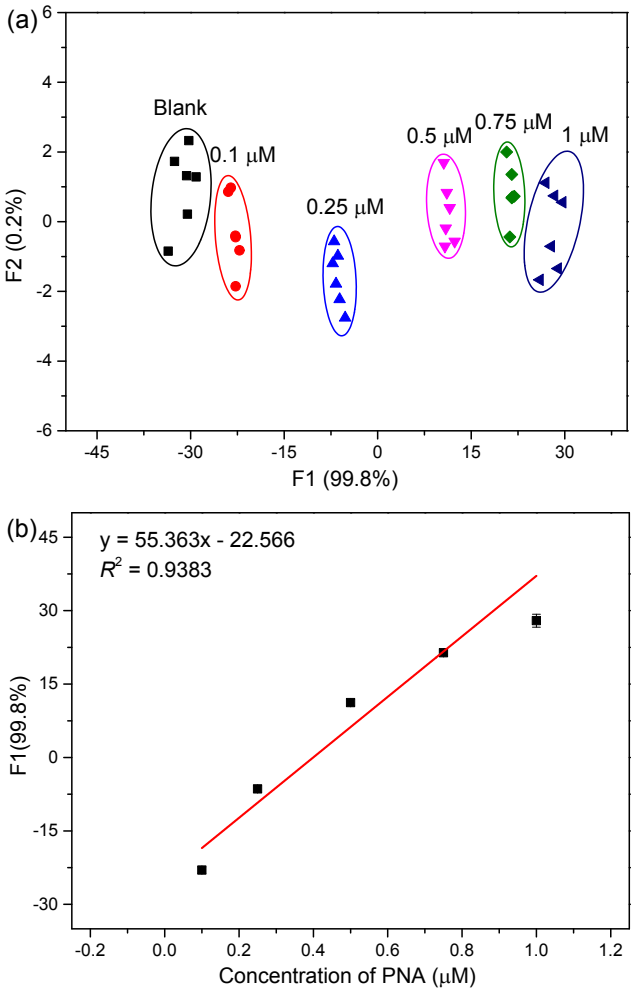


Figure S9. Quantitative analysis of PNA. (a) LDA score plot of PNA with concentrations of 0, 0.1, 0.25, 0.5, 0.75, and 1.0 μM. (b) Liner fitting between factor (1) and PNA.

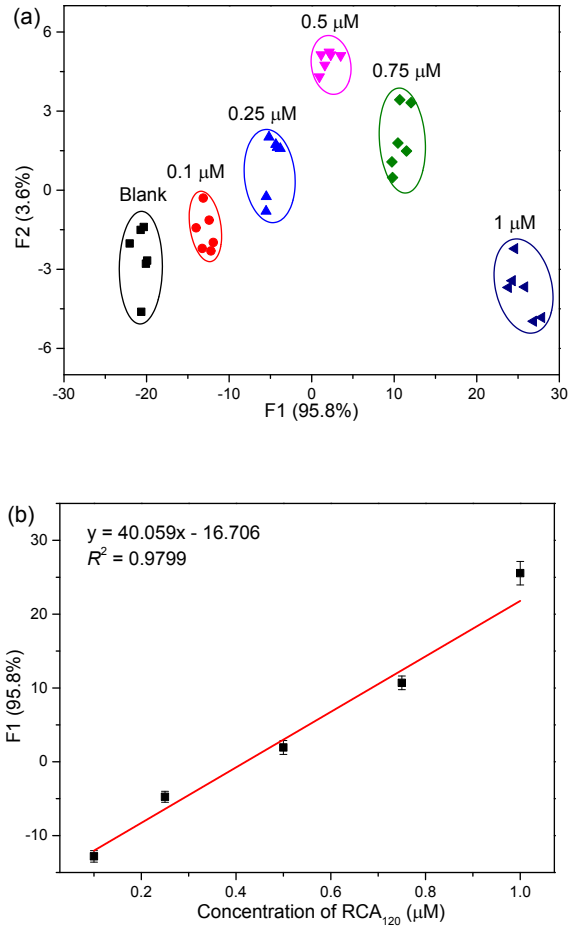


Figure S10. Quantitative analysis of RCA_{120}. (a) LDA score plot of RCA_{120} with concentrations of 0, 0.1, 0.25, 0.5, 0.75, and 1.0 μM. (b) Liner fitting between factor (1) and RCA_{120}.

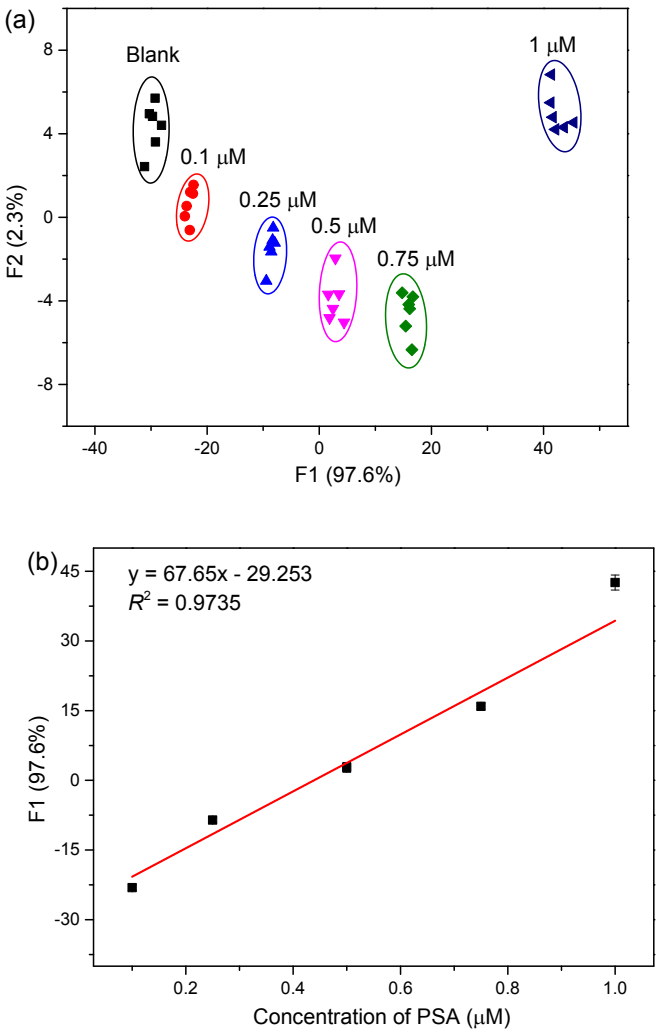


Figure S11. Quantitative analysis of PSA. (a) LDA score plot of PSA with concentrations of 0, 0.1, 0.25, 0.5, 0.75, and 1.0 μM. (b) Liner fitting between factor (1) and PSA.

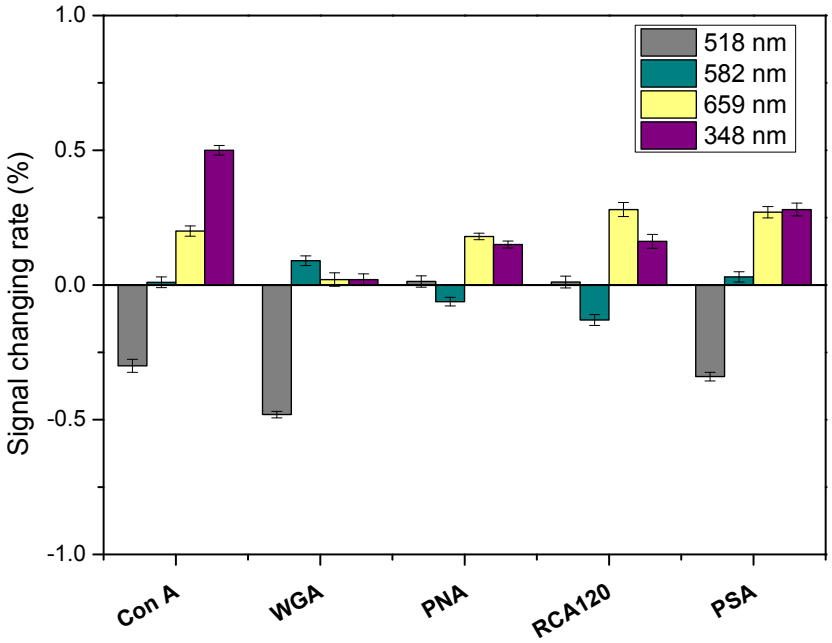


Figure S12. Signal patterns obtained by measuring fluorescent and RRS intensity changes at four channels induced by various lectins (10 nM).

Table S1. The binding constants of the lectins to different saccharides ($\times 10^3$ M^{-1}).

	Man	Gal	GlcNac
Con A	2.1	-	-
PNA	-	0.53	-
RCA_{120}	-	2.2	-
WGA	-	-	0.41
PSA	0.9	-	-

Abbreviations: Man: D-mannopyranose; Gal: D-galactopyranose; GlcNac: D-*N*-acetyl glucosamine. "-" means not reported.

All binding constants are cited from the three references.

1. M. H. Sleiman, R. Csonka, C. Arbez-Gindre, G. A. Heropoulos, T. Calogeropoulou, M. Signorelli, A. Schiraldi, B. R. Steele, D. Fessas and M. Micha-Screttas, *Int J Biol Macromol*, 2015, **80**, 692-701.
2. M. Ambrosi, N. R. Cameron and B. G. Davis, *Org Biomol Chem*, 2005, **3**, 1593-1608.
3. T. K. Dam and C. F. Brewer, *Chem Rev*, 2002, **102**, 387-429.

Table S2. The FRET efficiencies of aggregation induced by lectins (1 μM).

Lectins	FRET efficiency (*E*)
Con A	21.42%
PNA	11.85%
RCA_{120}	16.25%
WGA	33.61%
PSA	40.28%

The FRET efficiency (*E*) is typically measured using the relative fluorescence intensity of the donor, in the absence (F_0) and presence (F) of lectins: $E=(1-F/F_0)\times100\%$ (reference: *J. Phys. Chem. B*, 2011, 115, 13643).

Table S3. The accuracy of the nanosensor in detection of Con A in human serum (n=6).

Added (μM)	0.10	0.50	1.00
Found (μM)	0.097±0.022	0.498±0.013	0.952±0.051
Accuracy	97.60%	99.60%	95.20%

Table S4. The accuracy of the nanosensor in detection of WGA in human serum (n=6).

Added (μM)	0.10	0.50	1.00
Found (μM)	0.096±0.013	0.476±0.013	0.957±0.027
Accuracy	96.60%	95.20%	95.70%

Table S5. The accuracy of the nanosensor in detection of PNA in human serum (n=6).

Added (μM)	0.10	0.50	1.00
Found (μM)	0.098±0.061	0.498±0.031	0.984±0.046
Accuracy	98.20%	99.60%	98.40%

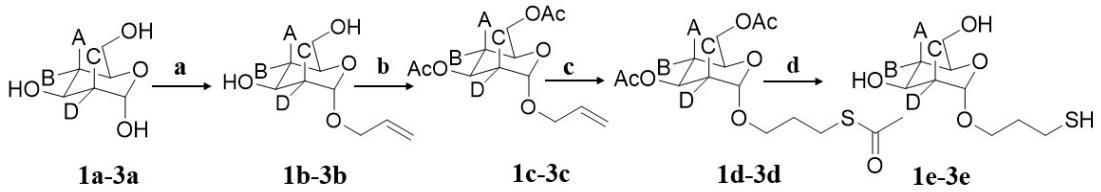
Table S6. The accuracy of the nanosensor in detection of RCA_{120} in human serum (n=6).

Added (μM)	0.10	0.50	1.00
Found (μM)	0.097±0.014	0.487±0.024	0.978±0.043
Accuracy	96.90%	97.40%	97.80%

Table S7. The accuracy of the nanosensor in detection of PSA in human serum (n=6).

Added (μM)	0.10	0.50	1.00
Found (μM)	0.097±0.036	0.484±0.025	0.966±0.042
Accuracy rate	97.30%	96.80%	96.60%

3. Synthesize of 3-mercaptopropyl glycosides



Scheme S1 Synthesis of 3-mercaptopropyl glycosides (Man-SH, Gal-SH and GlcNac-SH). **1a-3a** are D-mannopyranose (Man), D-galactopyranose (Gal) and D-*N*-acetyl glucosamine (GlcNac), respectively. **1e**: Man-SH (A=H, B=OH, C=OH, D=H); **2e**: Gal-SH (A=OH, B=H, C=OH, D=H); **3e**: GlcNac-SH (A=H, B=OH, C=H, D=NHAc)

The following synthesis were carried according to the two references (*Langmuir* 2003, 19, 1522-1531; *Chem. Eur. J.* 2012, 18, 6485-6492).

3.1 Synthesis of *O*-allyl glycosides (1b-3b)

Allyl alcohol (125 mL, 1.84 mol) was cooled to 0 °C, acetyl chloride (10 mL, 14.0 mmol) was added dropwise, and the mixture was stirred at 0 °C for 1 h. After heating to 70 °C, 1a-3a (55.6 mmol) was added and the reaction mixture was stirred under reflux for 5 h. It was neutralized with sodium hydrogen carbonate and filtered over Celite. After three co-distillations with toluene, the crude product was purified by flash column chromatography on silica (ethyl acetate/methanol, 8:2) to yield a colourless solid.

Allyl α-D-mannopyranoside (1b). ^{13}C NMR (101 MHz, D_2O): δ_C = 133.0 ($OCH_2CH=CH_2$), 118.1 ($OCH_2CH=CH_2$), 99.5 (C-1), 72.8 (C-5), 70.6 (C-3), 70.1 (C-2), 68.5 (C-4), 67.3 ($OCH_2CH=CH_2$), 61.8 (C-6) ppm. **^{1}H NMR** (400 MHz, D_2O): δ_H = 6.05 (m, 1H,), 5.37-5.14 (m, 2H), 4.89 (d, 1H), 4.40-4.13 (m, 1H), 4.12 (dd, 1H), 3.97 (dd, 1H), 3.95-3.16 (m, 5H) ppm.

Allyl α-D-galactopyranoside (2b). ^{13}C NMR (101 MHz, D_2O): δ_C = 135.4 ($OCH_2CH=CH_2$), 116.7 ($OCH_2CH=CH_2$), 98.7 (C-1), 71.7 (C-5), 70.0 (C-3), 69.1 (C-2), 68.8 (C-4), 67.6 ($OCH_2CH=CH_2$), 60.9 (C-6) ppm. **^{1}H NMR** (400 MHz, DMSO) δ_H = 5.95-5.77 (m, 1H), 5.32-5.17 (m, 1H), 5.12-5.01 (m, 1H), 5.00-4.83 (m, 1H), 4.61

(d, 1H), 4.13-3.40 (m, 1H), 3.87 (dd, 1H), 3.76-3.55 (m, 2H), 3.54-3.13 (m, 3H) ppm.

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (3b). **^{13}C NMR** (101 MHz, D_2O): δ_C = 174.3 ($NHCOCH_3$), 133.6 ($OCH_2CH=CH_2$), 117.9 ($OCH_2CH=CH_2$), 96.0 (C-1), 71.9 (C-3), 71.0 (C-4), 70.0 (C-5), 68.4 ($OCH_2CH=CH_2$), 60.5 (C-6), 53.6 (C-2), 21.9 ($NHCOCH_3$) ppm. **^{1}H NMR** (400 MHz, DMSO): δ_H =5.79 (m, 1H), 5.17 (d, 1H), 5.04 (d, 1H), 4.34 (d, 1H), 4.24 (dd, 1H), 3.98 (dd, 1H), 3.79 (dd, 1H), 3.61-3.55 (m, 2H), 3.36 (t, 1H), 1.90 (s, 3H) ppm.

3.2 Synthesis of O-allyl glycoside peracetates (1c-3c)

Acetic anhydride (0.75 mL, 8.0 mmol) was added to a solution of 1b-3b (0.80 mmol) in pyridine (10 mL) at room temperature. The reaction mixture was stirred overnight and co-concentrated with toluene (3 x 25 mL). The residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether, 2:3) to afford 1c-3c as a colourless syrup.

Allyl 2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranoside (1c). **^{13}C NMR** (101 MHz, $CDCl_3$): δ_C = 170.6, 170.1, 170.0, 169.6 (4 x $C(O)CH_3$), 133.0 ($OCH_2CH=CH_2$), 118.3 ($OCH_2CH=CH_2$), 95.0 (C-1), 68.7 (C-2), 68.5 (C-3), 68.0, 67.3 ($OCH_2CH=CH_2$, C-4), 67.2 (C-5), 61.8 (C-6), 20.9, 20.7, 20.6, 20.6 (4 x $C(O)CH_3$) ppm. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H = 5.88 (m, 1H), 5.30 (m, 5H), 4.81 (d, 1H), 4.27 (dd, 1H,), 4.15 (m,1H), 4.08 (dd, 1H), 4.03 (m, 2H), 2.14, 2.09, 2.01, 1.95 (each s, each 3H) ppm.

Allyl 2, 3, 4, 6-tetra-*O*-acetyl-α-D-galactopyranoside (2c). **^{13}C NMR** (101 MHz, $CDCl_3$): δ_C = 170.3, 170.3, 170.2, 167.0 (4 x CH_3COO), 133.1 ($OCH_2CH=CH_2$), 118.0 ($OCH_2CH=CH_2$), 95.2 (C-1), 68.7 (C-5), 68.0 (C-3), 67.5 (C-2), 66.3 ($OCH_2CH=CH_2$), 61.7 (C-4), 60.3 (C-6), 21.0, 20.8, 20.7, 20.6 (4 x $C(O)CH_3$) ppm. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H = 5.94-5.70 (m, 1H), 5.43-5.36 (m, 1H), 5.21 (m, 3H), 4.98 (dd, J = 10.5, 3.1 Hz, 1H), 4.45 (d, J = 7.9 Hz, 1H), 4.27 (m, 1H), 4.01 (m, 3H), 4.02-3.88 (m, 1H), 2.04, 2.01, 1.96, 1.90 (each s, each 3H) ppm.

Allyl 2-*N*-acetyl-3, 4, 6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (3c). **^{13}C NMR** (101 MHz, $CDCl_3$): δ_C = 171.4, 170.7, 170.1, 169.3 (4 x CH_3COO), 133.0 ($OCH_2CH=CH_2$), 118.5 ($OCH_2CH=CH_2$), 96.4 (C-1), 71.3 (C-5), 68.7 (C-3), 68.1 (C-4), 67.8 ($OCH_2CH=CH_2$), 61.9 (C-6), 51.76 (C-2), 23.1, 21.4, 20.7, 20.6 (4 x $C(O)CH_3$)

ppm. **^{1}H NMR** (400 MHz, $CDCl_3$,): δ_H =5.82 (dddd, 1 H), 5.73 (d, 1H), 5.24 (dq, 1H), 5.18 (dq, 1H), 5.14 (dd, 1H), 4.95 (dd, 1H), 4.65 (dd, 1H), 4.20 (dd, 1H), 4.14 (ddt, 1H), 3.98 (ddt, 1H), 3.81-3.73 (m, 1H), 3.65-3.57 (m, 2H), 1.98, 1.93, 1.92, 1.85 (each s, each 1H) ppm.

3.3 Synthesis of 3-Thioacetylpropyl glycosides (1d-3d)

To a solution of olefin 1c-3c (5 mmol) in dry THF (25 mL) was added thiolacetic acid (12.5 mmol) and 2, 2'-azobisisobutyronitrile (AIBN, 1 mmol). The reaction mixture was irradiated in a photochemical reactor for 5 h under an atmosphere of nitrogen. Concentration of the reaction mixture, followed by flash chromatography (2:1 Hex/EtOAc) provided compounds 1d-3d as clear oils.

3-Thioacetyl-2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranoside (1d). ^{13}C NMR (101 MHz, D_2O): δ_C = 195.2 ($OCH_2CH_2CH_2S\mathit{C}OCH_3$), 170.4, 170.0, 168.9, 169.4 (4 x $\mathit{C}(O)CH_3$), 95.6 (C-1), 70.5 (C-5), 70.0 (C-3), 68.4 (C-2), 67.2 (C-4), 66.6 ($O\mathit{C}H_2CH_2CH_2SCOCH_3$), 61.7 (C-6), 30.4 ($OCH_2CH_2CH_2SCO\mathit{C}H_3$), 29.0 ($OCH_2CH_2\mathit{C}H_2SCOCH_3$), 25.5 ($OCH_2\mathit{C}H_2CH_2SCOCH_3$), 20.5, 20.5, 20.5, 20.4 (4 x $C(O)\mathit{C}H_3$) ppm. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H= 5.30-5.23 (m, 4H), 4.77 (s, 1H), 4.25-4.21 (m, 1H), 4.08-4.07 (m, 1H), 3.97-3.90 (m, 1H), 3.74-3.68 (m, 1H), 3.47-3.44 (m, 1H), 2.93 (t, 2H), 2.30 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.94-1.86 (m, 2H) ppm.

3-Thioacetyl-2, 3, 4, 6-tetra-*O*-acetyl-α-D-galactopyranoside (2d). ^{13}C NMR (101 MHz, D_2O): δ_C = 195.5 ($OCH_2CH_2CH_2S\mathit{C}OCH_3$), 170.4, 170.2, 170.1 170.0 (4 x $\mathit{C}(O)CH_3$), 96.23 (C-1), 68.5 (C-5), 68.0 (C-3), 67.5 (C-2), 66.7 (C-4), 66.3 ($O\mathit{C}H_2CH_2CH_2SCOCH_3$), 61.7 (C-6), 30.6 ($OCH_2CH_2CH_2SCO\mathit{C}H_3$), 29.1 ($OCH_2CH_2\mathit{C}H_2SCOCH_3$), 25.1 ($OCH_2\mathit{C}H_2CH_2SCOCH_3$), 20.9, 20.8, 20.6, 20.6 (4 x $C(O)\mathit{C}H_3$) ppm. **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H= 5.38 (m, 1H), 5.27-5.24 (m, 1H), 5.06-5.03 (m, 2H), 4.11-4.08 (m, 1H), 4.07-3.96 (m, 2H), 3.66-3.63 (m, 1H), 3.39-3.36 (m, 1H), 2.85 (t, 2H), 2.41 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.84 (s, 3H), 1.81-1.76 (m, 2H).

3-Thioacetyl-2-deoxy-2-acetamido-3, 4, 6-tri-*O*-acetyl-α-D-glucopyranoside (3d). ^{13}C NMR (101MHz, D_2O): δ_C= 195.7 ($OCH_2CH_2CH_2S\mathit{C}OCH_3$), 171.3, 170.7, 170.3,

169.3 (4 x *C*(O)CH_3)，97.3 (C-1), 71.4 (C-5), 68.2 (C-3), 67.8 (C-2), 66.0 (C-4), 62.0 ($OCH_2CH_2CH_2SCOCH_3$), 51.7 (C-6), 30.6 ($OCH_2CH_2CH_2SCOCH_3$), 29.3 ($OCH_2CH_2CH_2SCOCH_3$), 25.5 ($OCH_2CH_2CH_2SCOCH_3$), 23.0, 20.7, 20.7, 20.6 (4 x C(O)CH_3) ppm. **^{1}H NMR** (400MHz,$CDCl_3$):δ_H= 6.07 (d, 1H), 5.17 (t, 1H), 5.02 (t, 1H,), 4.74 (d, 1H), 4.31-4.27 (m, 1H), 4.19-4.13 (m, 1H), 4.06-3.97 (m, 1H), 3.87-3.84 (m, 1H), 3.69-3.62 (m, 1H), 3.40-3.3 (m, 1H), 3.04-2.98 (m, 1H), 2.92-2.86 (m, 1H), 2.35 (s, 3H), 2.05 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.83-1.79 (m, 2H) ppm.

3.5 Mass spectrometry characterization of 1e-3e

The final products (**1e-3e**) were characterized by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS). Considering the property of thiol group, all the MS spectra were collected under negative ion mode.

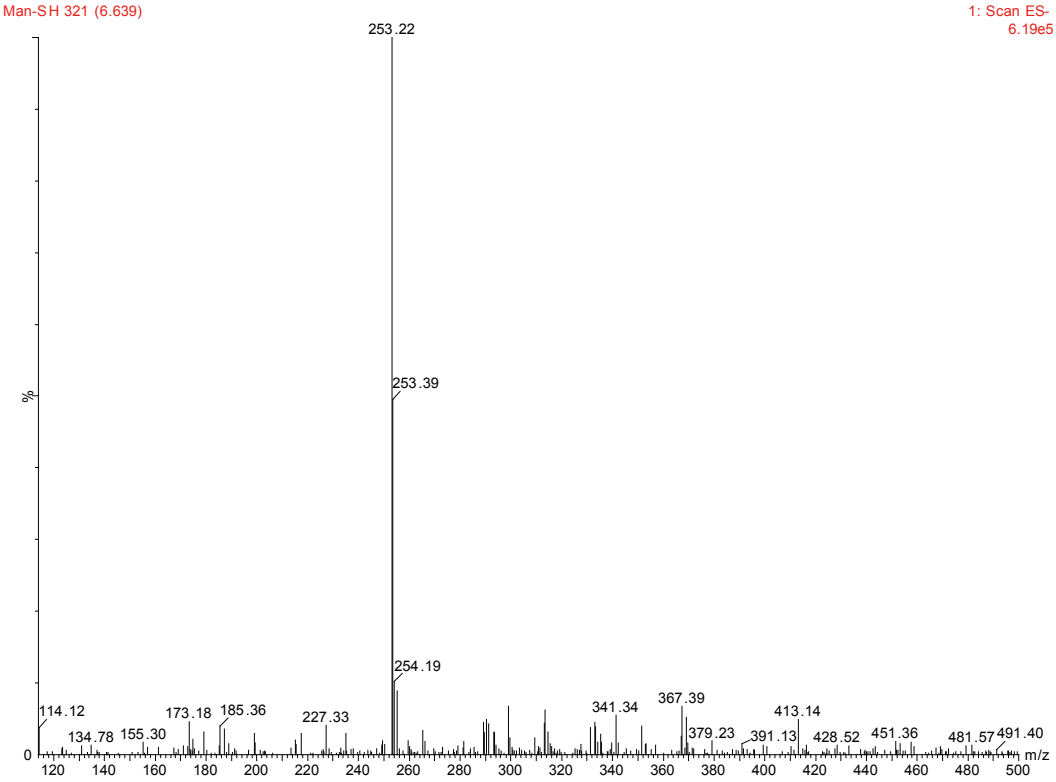


Figure S13 Negative ion mode mass spectrum of **1e** (Man-SH). MS (HPLC-MS) for [M^-]: Calcd. for $C_9H_{17}O_6S^-$ 253.08; found: 253.22.

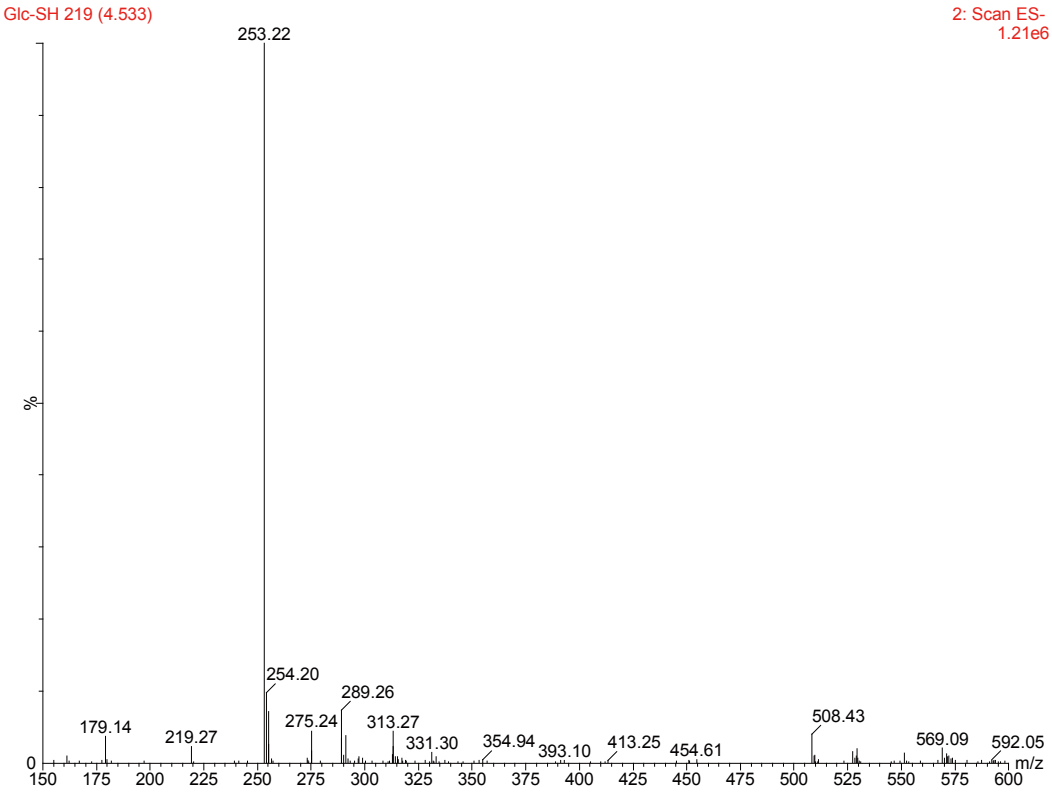


Figure S14 Negative ion mode mass spectrum of **2e** (Gal-SH). MS (HPLC-MS) for [M$^-$]: Calcd. for $C_9H_{17}O_6S^-$ 253.08; found: 253.22.

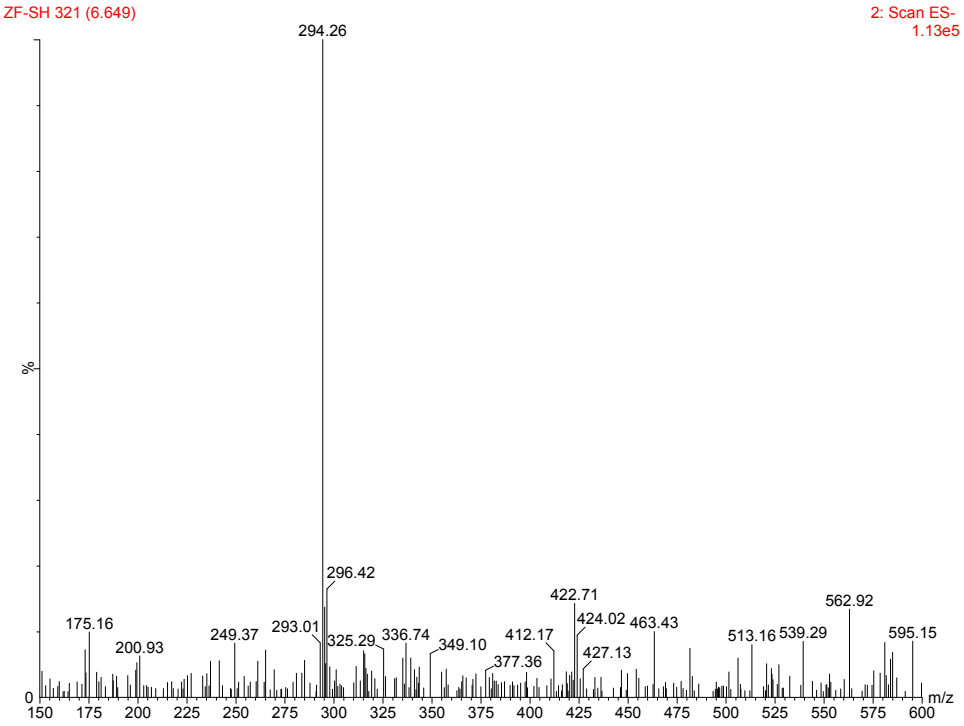


Figure S15 Negative ion mode mass spectrum of **3e** (GlcNac-SH). MS (HPLC-MS) for [M^-]: Calcd. for $C_{11}H_{20}NO_6S^-$ 294.10; found: 294.26.