Supporting Information

Experimental

Reagents

Horse myoglobin, horseradish peroxidase, RNase B, asialofetuin, 2, 5-dihydroxyhenzoic acid and trifluoroacetic acid were purchased from Sigma (St. Louis, MO). Bovine serum albumin was purchased from Bovogen (BovoStar grade; Australia). SDS, acrylamide, N, N'-methylene-bisacrylamide, iodoacetamide were obtained from Fluka, acetonitrile from Merck (Darmstadt, Germany), and trypsin from Promega (sequence level). Solutions, unless otherwise stated, were prepared using deionized (Milli-Q, Millipore, Bedford, MA) water. Other materials (analytical grade) were commercially available

Synthesis of aminophenylboronic acid-functionalized magnetic nanoparticles

Synthesis route of aminophenylboronic acid-functionalized magnetic nanoparticles was shown in **Scheme S2**. The synthesis of boronic acid magnetic nanoparticles involved two steps including preparation of amine-functionalized magnetic particles and surface modification. First, amine-functionalized magnetic particles were synthesized according to previous method with some modification. Typically, 1, 6-hexadiamine (3.6 g), anhydrous sodium acetate (4.0 g), $FeCl_3 \cdot 6H_2O$ (1.0 g) were dissolved in ethylene glycol (30 mL) under magnetic stirring and acquired a homogeneous yellow solution. The solution was then sealed in a Teflon-lined stainless-steel autoclave heated at 198 ^oC for 6 h. The black product settled at the bottom of the autoclave was throughly washed with hot water and ethanol to remove the solvent and unbound 1, 6-hexanediamine, and then vacuum dried at 50 ^oC to gain the black powder for further use. In the next step, in a two-necked round bottom flask, 0.1 g of the amine-functionalized magnetic particles black powder was suspended in 30 mL dry chloroform under ultrasonication vibration. Then, under continuous mechanically stirring, 0.2 mL of hexanedioyl chloride was added into the mixture by injection. After reaction for 4h, with the help of a magnet, the product was quickly collected and washed with dry chloroform (10 mL) for three times

to remove the excess hexanedioyl chloride, and then redispersed in 30 mL of dry chloroform. Into the resultant mixture, 0.2 g of 3-aminobenzeneboronic acid was added. After reaction for 6h under stirring, the product was collected and washed according to the above-mentioned procedure to remove excess 3-aminobenzeneboronic acid, and then vacuum dried to obtain the aminophenylboronic acid-functionalized magnetic nanoparticles.

Preparation of N-linked glycopeptides from asialofetuin and horseradish peroxidase

Because asialofetuin and horseradish peroxidase have been studied extensively and the sites and types of its N-linked glycans have been elucidated, we used them for treatment in this study. 100μg asialofetuin and horseradish peroxidase were dissolved in 0.1mL 50 mM NH_4HCO_3 (pH 8) respectively. Each solution was diluted to 50ng μl^{-1}. The proteins were reduced with DTT (final concentration 10 mM, 60 °C, 30 min), and the reduced cysteine residues were then alkylated with iodoacetamide (final concentration 100 mM, 37 °C, 45 min). Then the solutions were supplemented with trypsin and incubated overnight at 37°C.

Enrichment of Glycopeptides and Glycoproteins Using aminophenylboronic acid-functionalized Magnetic Nanoparticles

As shown in **Scheme S1**, Because boronates reacted reversibly with hydroxyl groups oriented in proper arrangements, and particularly with cis-1,2-diols of diol-containing substances such as nucleotides or glycoproteins, they had been used for separation of glycoproteins or glycopeptides. First, aminophenylboronic acid-functionalized magnetic nanoparticles were washed twice under binding conditions. Then 100μl of tryptic peptides of glycoprotein (5 ng μL^{-1}) reacted with aminophenylboronic acid-immobilized magnetic nanoparticles (about 100 ng) under gentle agitation and slightly alkaline (pH 8.5) binding conditions, and incubated for 90 min at room temperature. Then, the reacted nanoparticles were washed with binding buffer several times using an

external magnet for efficient collection of nanoparticles. After incubation for several minutes at RT under gentle shaking, the nanoparticles were washed and the bound peptide species were eluted under acidic conditions (final volume 10 μL). In the last, 1μL of the elution, 1μL of untreated tryptic peptides (5 ng μL^{-1}) and 1μL of supernatant were directly for MALDI-MS detection. For glycoproteins, 2 μL of glycoproteins (1μg μL^{-1}) and non-glycoproteins (1μg μL^{-1}) mixture reacted with aminophenylboronic acid-functionalized magnetic nanoparticles (about 100 μg) in slightly alkaline (pH 8.5) binding buffer, and the subsequent procedure is the same as stated except for the elution was dried in a SpeedVac for SDS-PAGE.

Mass spectrometry

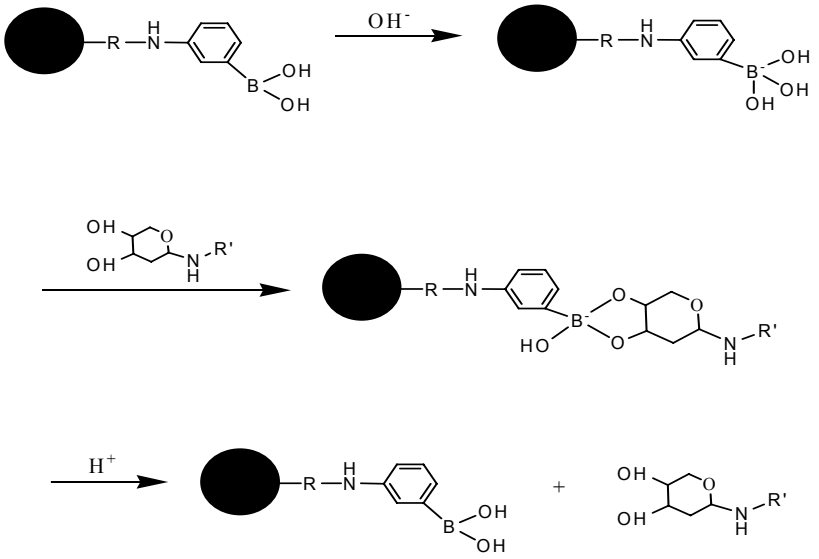
PMF analysis was performed by MALDI-QIT-TOF MS (AXIMA QIT; Shimadzu/Kratos, Manchester, UK). This instrument employs a three-dimensional ion trap with a time of flight mass measurement stage. Matrix-assisted laser desorption of peptides is produced by pulses of light (337 nm, 3-ns pulse width) generated by a nitrogen laser with a maximum pulse rate of 10 Hz. Each profile is composed of the accumulation of two laser shots. Ions are directed into the ion trap by means of an electrostatic lens assembly, designed to ensure high transmission for the MALDI beam into the ion trap and to minimize field strength directly above the sample surface.

For QIT measurements, 1μL of sample was mixed with 1μL of 12.5 mg/mL DHB dissolved in a 0.1% (v/v) TFA and 20% (v/v) acetonitrile solution on a MALDI sample target and dried. The measurements were carried out in positive ion mode. The PeptideMass program (http://www.expasy.org/) was employed to calculate the theoretical ions resulting from tryptic digestion of the protein. The Y-axis of MS spectra presented in figures was expressed as the relative intensity of ion peaks, and in each spectrum the most intense ion peak was set to 100. The X-axis denotes the mass-to-charge ratio or m/z.

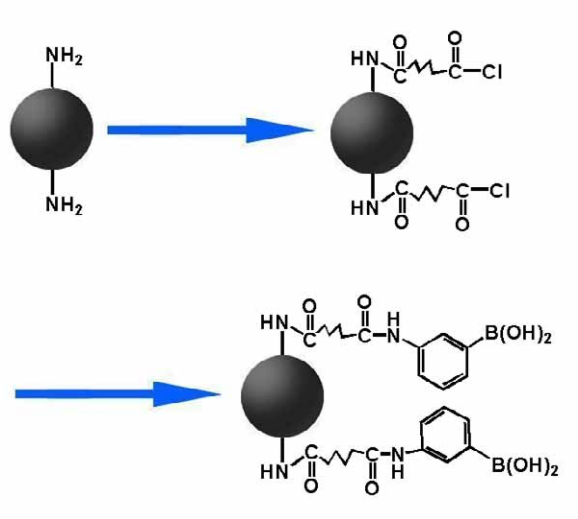
SDS-PAGE

After treatment by the aminophenylboronic acid-functionalized magnetic nanoparticles,

the dried elution of proteins were dissolved in a 0.1% (v/v) TFA and 50% (v/v) acetonitrile solution, then loaded on a 12% SDS-polyacrylamide gel. After visualization with CBB, the gel was scanned for analysis. We have calculated the gray scale of the band on the SDS-PAGE lane to estimate the recovery of the glycoproteins. Multi Gauge software from Fujifilm was used for gray scale calculation. As shown in Figure 5A, the absolute gray scale of HRP band before and after enrichment were 196146.00 and 173748.00, with the deduction of background (91280 and 92180 correspondingly), the gray scale were 104866.00 and 81568.00. Therefore, the recovery was 77.78%. (81568.00/104866.00). Similarly, the absolute gray scale of RNB band before and after enrichment were 186298.00 and 122476.00 with the background of 32120.00 and 32454.00 correspondingly，so the recovery was 58.39%.



Scheme S1. The reaction and elution process with aminophenylboronic acid-functionalized magnetic nanoparticles



Scheme S2. Synthesis route of aminophenylboronic acid-functionalized magnetic nanoparticle.

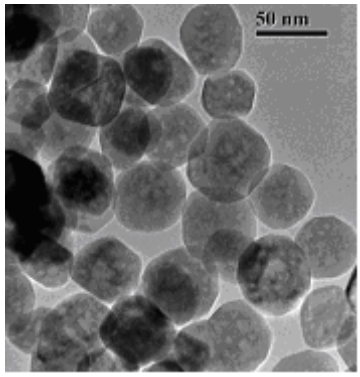


Figure S1. TEM image of the synthesized amine-functionalized magnetic particles

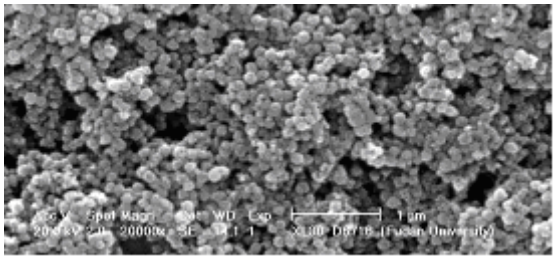


Figure S2. SEM image of the synthesized amine-functionalized magnetic particles

The enrichment of HRP

Experiments similar to those discussed for asialofetuin were performed on another glycoprotein horseradish peroxidase (HRP), which has more oligosaccharide side chains. The comparing of mass spectra before and after treatment of HRP was given in Figure S3 A and B. According to the report {ref 1}, there are nine glycosylation sites in HRP which dominate the mass spectrum, and the masses of many observed glycopeptides species indicated a composition of a xylosylated, core-(α1-3)-fucosylated trimannosyl N-glycan structure for the attached N-glycan moiety. Due to the specialty of the matrix-assisted laser desorption of QIT, there might be some fragment ions of glycopeptides in the MS map, so in Figure S3, the number of glycopeptides are more than 9 and was marked with *. In Figure S3 A, most glycopeptides are hard to detect, yet in Figure S3 B, both the intensities and the S/N ratios of the most glycopeptides have enhanced, i.e., the intensities of *m/z* 4983.9, 4222.6, 3895.5, 3671.5, 3527.5, 3353.3, 3322.3, 2850.3, 2591.1, 2531.9 were increased from 231.4, 898.0, 505.9, 1964.7, 635.3, 886.3, 580.4, 886.3, 721.6 to 1090.2, 2451.0, 3149.0, 10196.1, 3364.7, 6094.1, 3874.5, 14227.5, 4317.6, respectively. an intensity enhancement factor is estimated as 7 for average for these glycopeptides. In Figure S3 B, the %Int. was increased from 7.7mV to 63mV, and in this case, the %Int. enhancement factor could be estimated as 8.

ref 1

[16] M. Wuhrer, C. H. Hokke, A. M. Deelder, *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1741-1748.

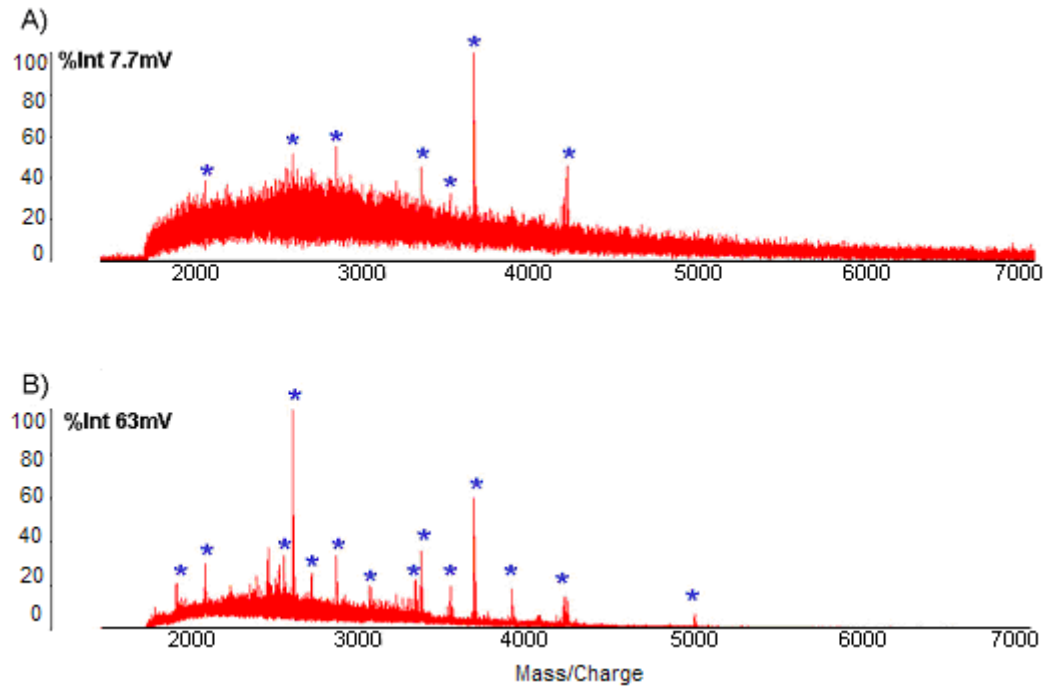


Figure S3. Comparison of MALDI-TOF-MS spectra of HRP. A) The spectrum of supernatant after the aminophenylboronic acid-functionalized magnetic nanoparticles treatment of the tryptic digests of HRP. B) The eluate after aminophenylboronic acid-functionalized magnetic nanoparticles treatment of the tryptic digests of HRP.

The pH conditions for detachment

For elution, different pH conditions were tried. In Figure S4, corresponding mass spectra of water-solutions of 20%ACN-0.5%TFA (pH 2.35, Figure S4A), 20%ACN-1%TFA (pH 2.08, Figure S4B), 20%ACN-2.5%TFA (pH 1.50, Figure S4C), 20%ACN-5%TFA (pH 1.35, Figure S4D) were shown. We had found that the signal intensity and the signal to noise ratio of A was the highest of all, but there were no obvious difference among the four pH conditions.

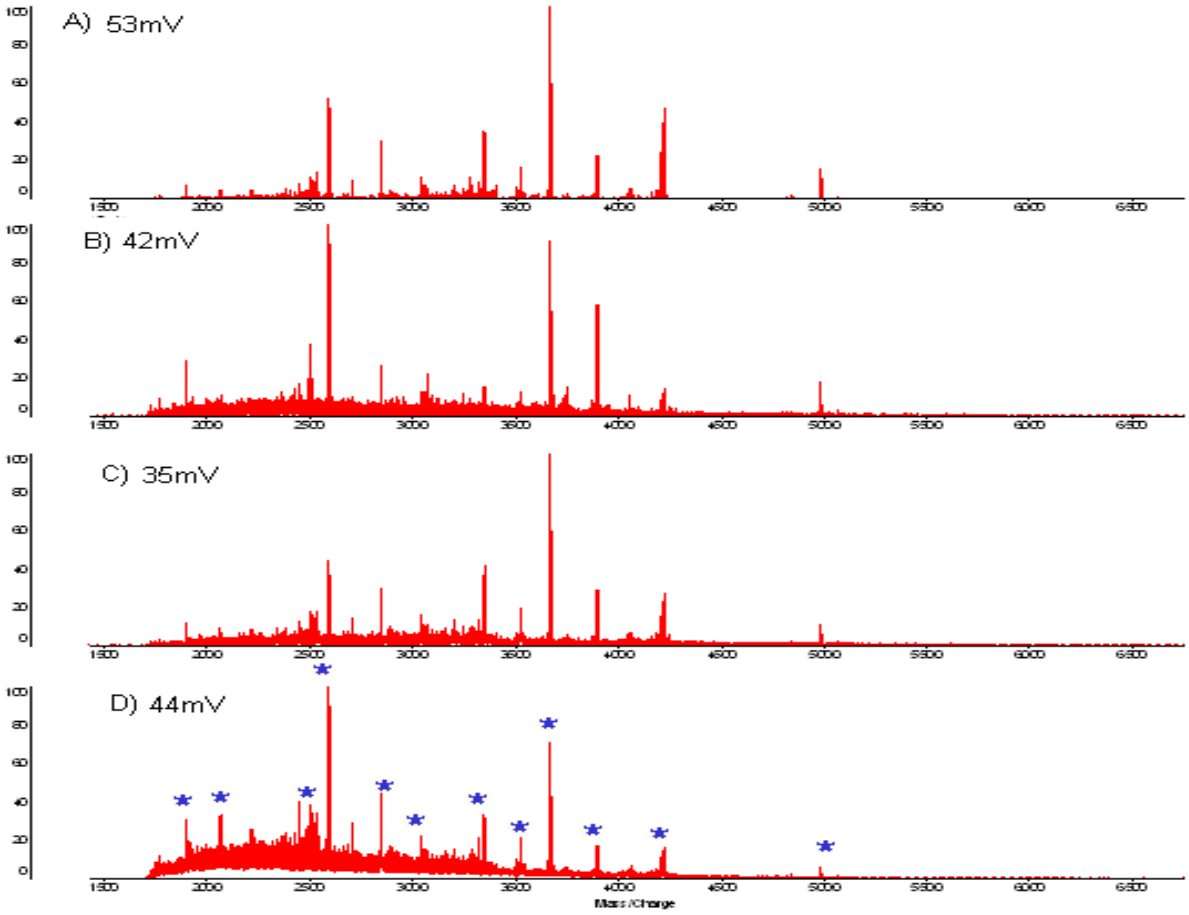


Figure S4. Comparison of MALDI-TOF-MS spectra of eluates of HRP under different pH conditions. A) 20%ACN-0.5%TFA water-solution B) 20%ACN-1%TFA water-solution C)20%ACN-2.5%TFA water-solution D) 20%ACN-5%TFA water-solution. The glycopeptides of Fig 3A were marked with arterisk

The concentration control experiment

In order to estimate which factor is the major one for the improvement of signal-to-noise ratios, no interference of nonglycosylated peptides or concentration of glycopeptides and glycoproteins, control experiments were taken as follow. 100uL of 5ng/uL ASF tryptic solution was concentrated directly to 10uL of 50ng/uL, and 1μL of which were directly for MALDI-MS detection (Figure S5A). It was observed that although signal intensity and signal to noise ratio had enhanced than the case without concentration, the glycopeptides and nonglycosylated peptides were concentrated

simultaneously. Figure S5B showed MALDI-MS spectra of the eluate of ASF digests after aminophenylboronic acid-functionalized magnetic nanoparticles treatment. Comparing Figure S5A with Figure S5B, many nonglycosylated peptides were observed on the A mass spectrum, which would interfere the analysis of glycopeptides. The nonglycosylated peptides would restrain the ionization of glycopeptides, that would also enhance the degree of difficulty for analysis. Few nonglycosylated peptides were observed in Figure S5B , and the intensity and signal to noise ratio were higher than that in Figure S5A. Therefore, no interference of nonglycosylated peptides is the major one for the improvement of signal-to-noise ratios.

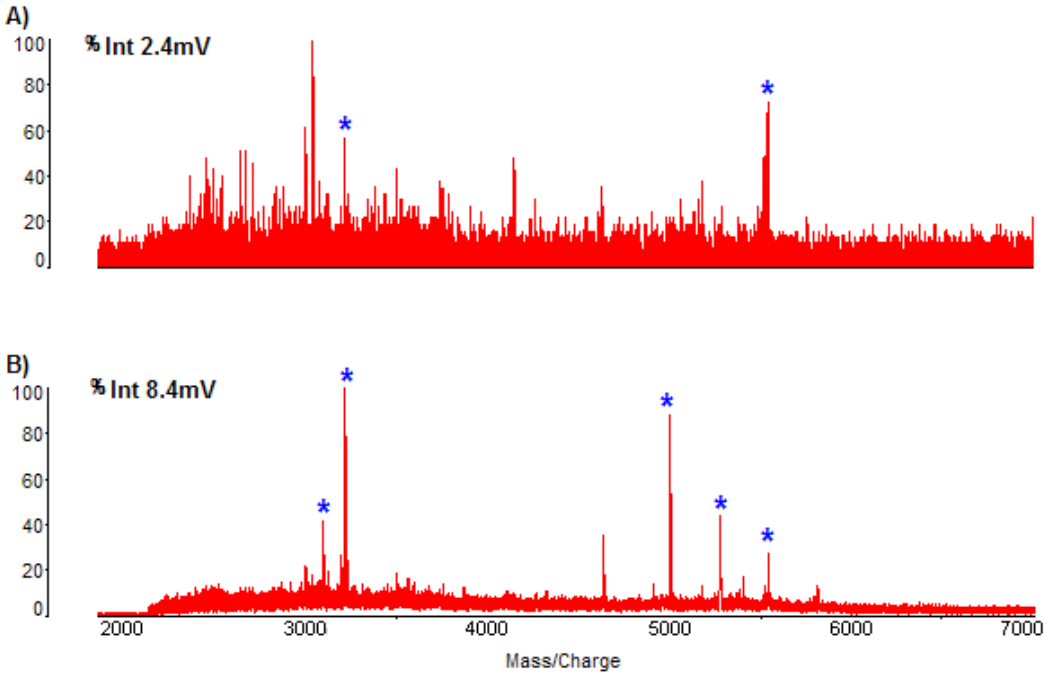


Figure S5. Comparison of MALDI-TOF-MS spectra of ASF. A) The spectrum of concentration tryptic peptides from ASF. (The loading amount was 50 ng) B) The spectrum of eluate after the aminophenylboronic acid-functionalized magnetic nanoparticles treatment of the tryptic digests of ASF. (The loading amount was 50 ng)

The amine-magnetic nanoparticals control experiment

In peptides solution, there are also unselective adsorptions on the surface of

amine-functionalized magnetic particles. But that would not take interferences in our binding and eluting condition. As shown in Figure S6, few peptides were found in the eluate after treated with amine-magnetic nanoparticals by the same way.

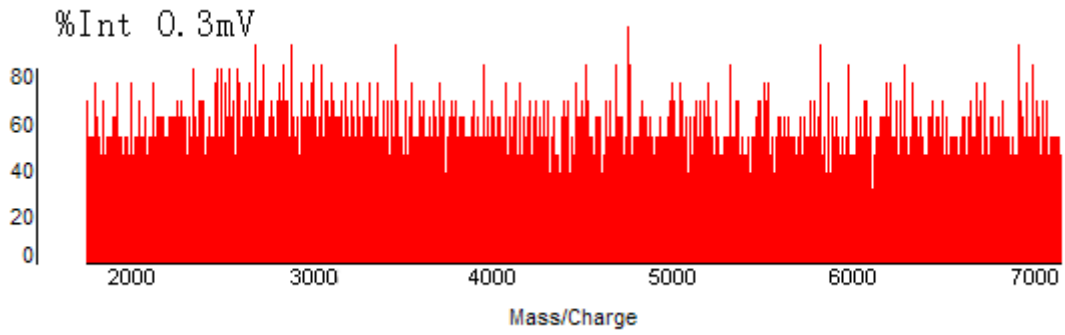


Figure S6. MALDI-TOF-MS spectra of the eluate after amine-magnetic nanoparticals treatment of the tryptic digests of ASF.

Table S1. Molecular masses and proposed oligosaccharide composition of the glycopeptides from asialofetuin after enrichment.

Peptide	Peptide sequence Calculated m/z,[Peptide+H]+	[Glycopeptide+H]+ (Da)	Glycan composition
P1	VVHAVEVALATFNAESNGSYLQLVEISR 3016.6	5004.2(GP1) 4638.9 3220.5	(GlcNAc)5(Man)3(Gal)3 (GlcNAc)4(Man)3(Gal)2 (GlcNAc)1
P2	RPTGEVYDIEIDTLETTCHVLDPEPLANCSVR 3671.8 (Cysteines modified with carboxyamidomethylation)	5278.0(GP2) 4913.2	(GlcNAc)4(Man)3(Gal)2 (GlcNAc)3(Man)3(Gal)1
P3	RPTGEVYDIEIDTLETTCHVLDPEPLANCSVR 3557.7	5544.8(GP3) 5179.8	(GlcNAc)5(Man)3(Gal)3 (GlcNAc)4(Man)3(Gal)2