Supporting information

Figure S1. LOD of DP7 using DHB matrix and HYNIC matrix.

Figure S2. Comparison of ionization ability of HYNIC matrix, DHB matrix and nicotinic acid matrix using Dextran 1000.

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Figure S8. MALDI-TOF-MS spectrum of N-glycans from human serum using HYNIC as matrix.

Figure S9. Tandem mass spectrum of the glycan (m/z 1663.6) from serum.

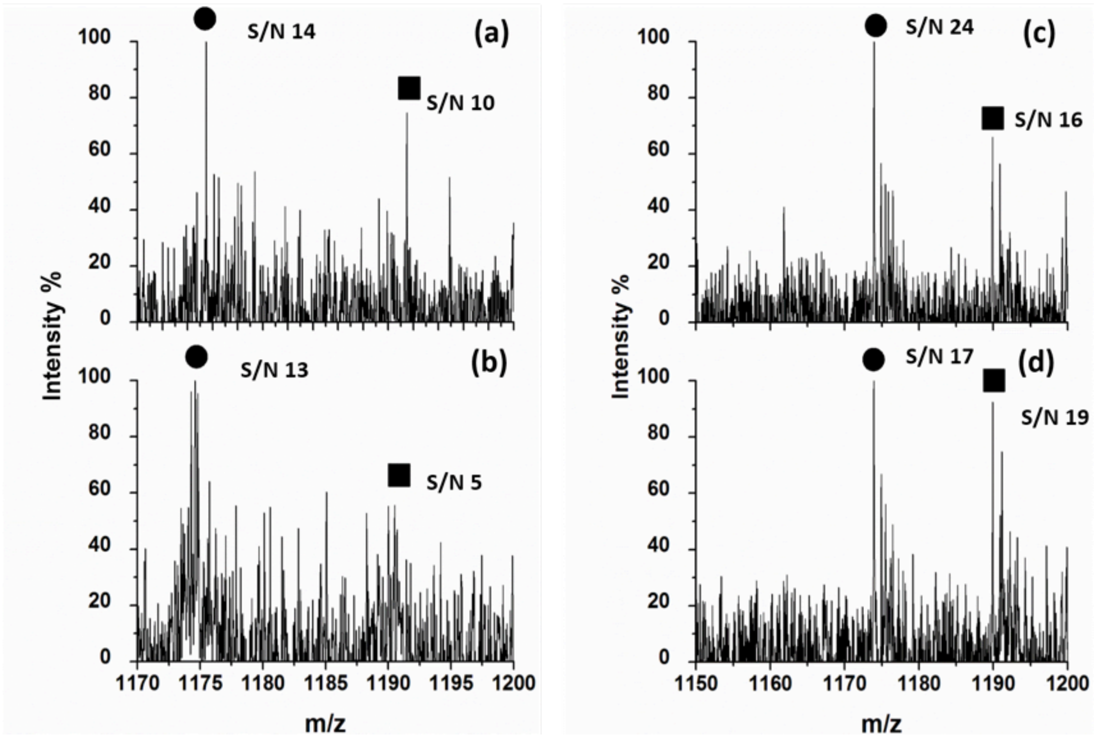


Figure S1. LOD of DP7 using DHB matrix and HYNIC matrix. MALDI-TOF mass spectra of (a) 100 fmol of DP7 with DHB matrix (b) 1 amol of DP7 with HYNIC matrix (c) 500 fmol of DP7 with DHB matrix and (d) 5 amol of DP7 with HYNIC matrix ● denotes $[DP7+Na]^+$ signals. ■ denotes $[DP7+K]^+$ signals.

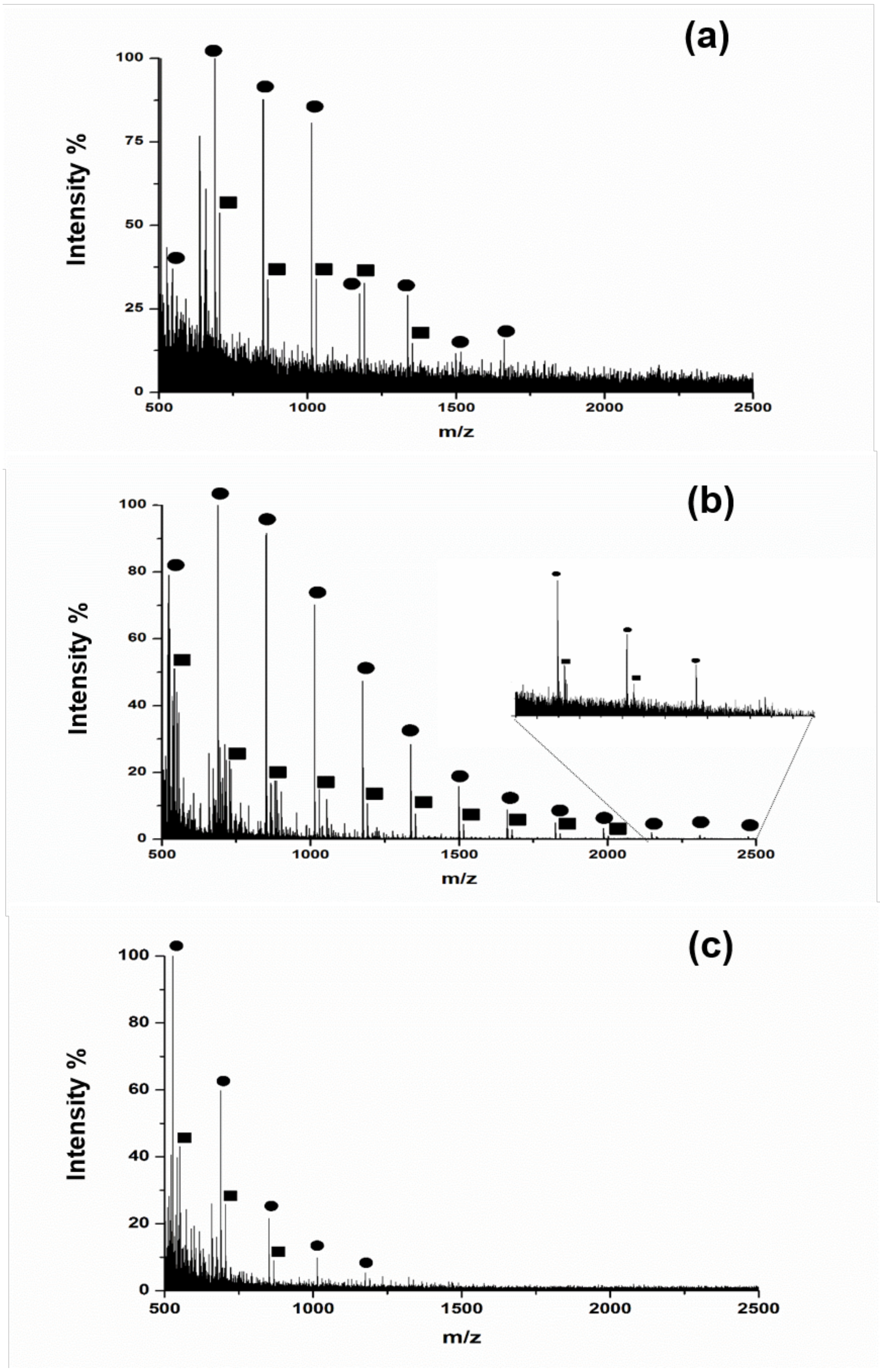


Figure S2. Comparison of ionization ability of HYNIC matrix,DHB matrix and nicotinic acid using Dextran 1000. MALDI-TOF mass spectra of 100 pmol Dextran1000 with (a) DHB matrix, (b) HYNIC matrix (c) and nicotinic acid. ● denotes $[M+Na]^+$ signals. ■ denotes $[M+K]^+$ signals.

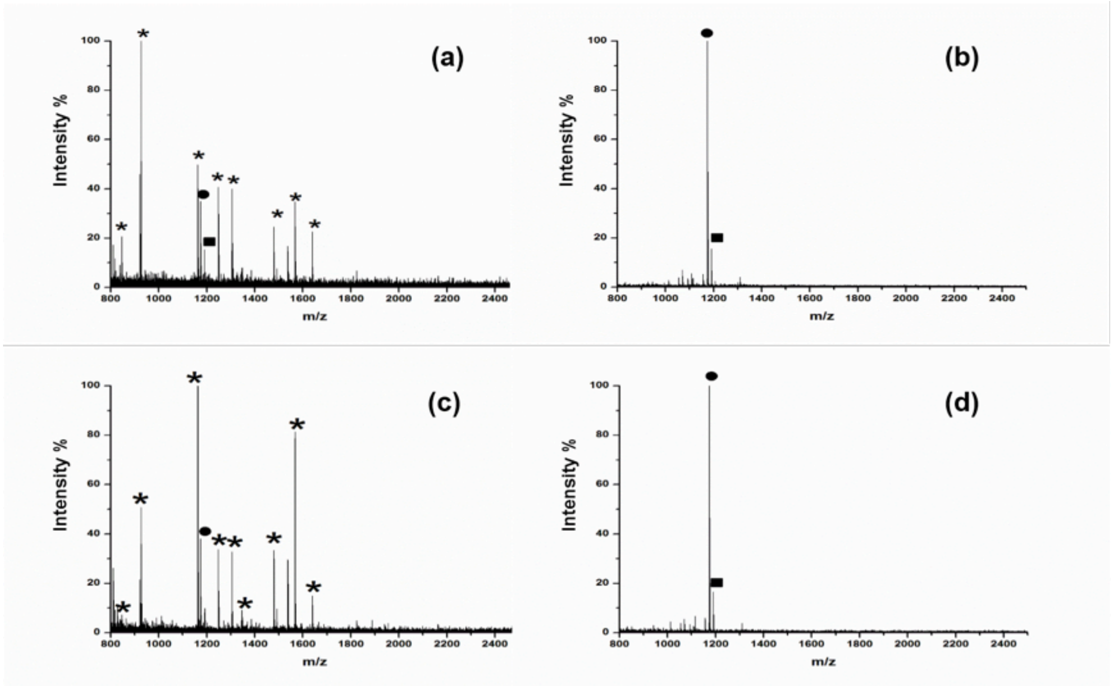


Figure S3. The selective ionization of oligosaccharide from mixture of DP7 and BSA tryptic peptides with different mass ratio. MALDI mass spectra of the mixture of DP7: BSA peptides at (a, b) 1:1 and (c, d) 1:10 using (a, c) DHB and (b,d) HYNIC matrix. * denotes BSA peptides, ● denotes $[DP7+Na]^+$ signals. ■ denotes $[DP7+K]^+$ signals.

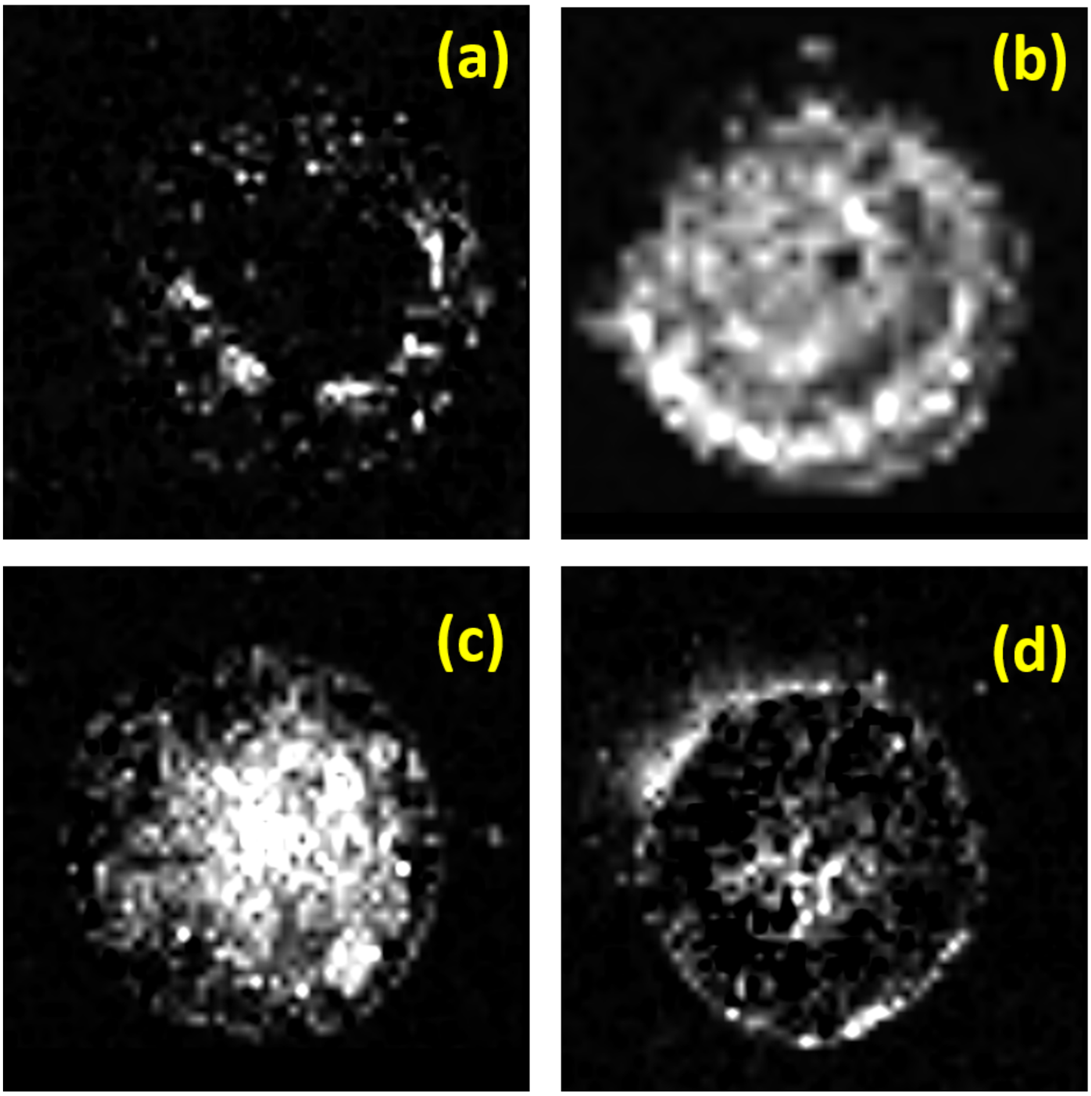


Figure S4. MALDI imagings of DP7 with DHB matrix prepared by different methods. (a) thin layer method (b) thin layer method with acetonitrile recrystallization (c) dried droplet method and (d) double-layer method.

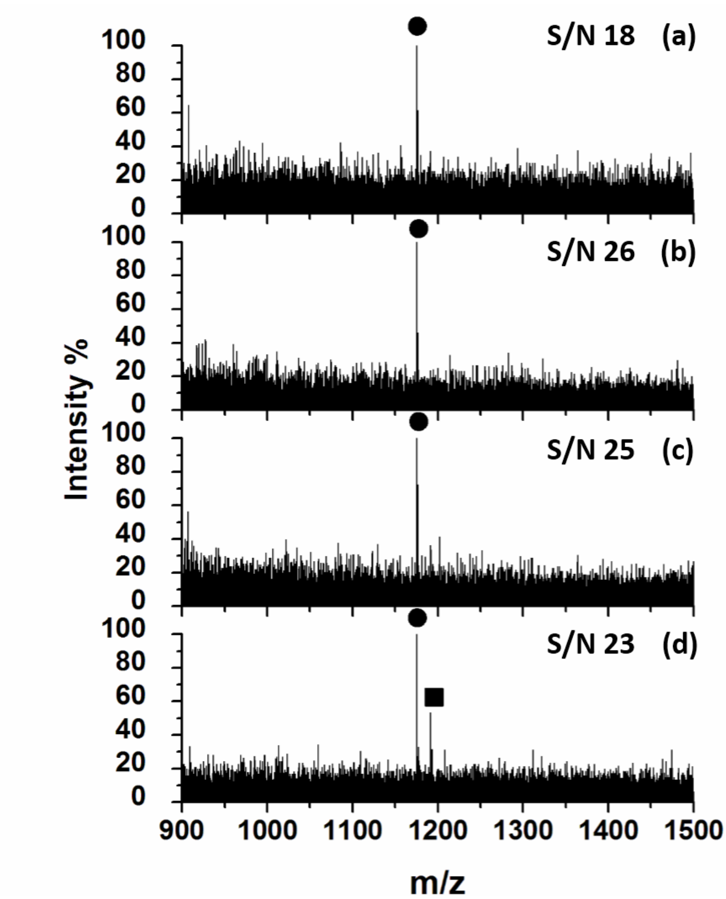


Figure S5. MALDI mass spectra of DP7 with DHB matrix prepared by different dropping methods (a) thin layer method (b) thin layer method using acetonitrile recrystallized DHB (c) dried droplet method (d) double-layer method. ● denotes $[DP7+Na]^+$ signals. ■ denotes $[DP7+K]^+$ signal.

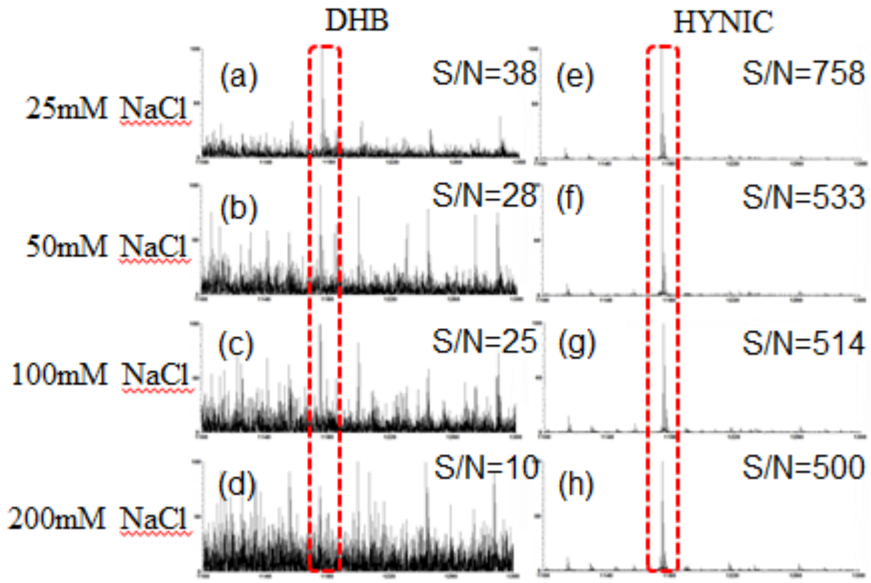


Figure S6. Salt tolerance of DHB matrix and HYNIC matrix. Mass spectra of 1 pmol DP7 containing different concentration of NaCl with (a-d) DHB and (e-h) HYNIC. The signal of DP7 was circled by the red dashed line.

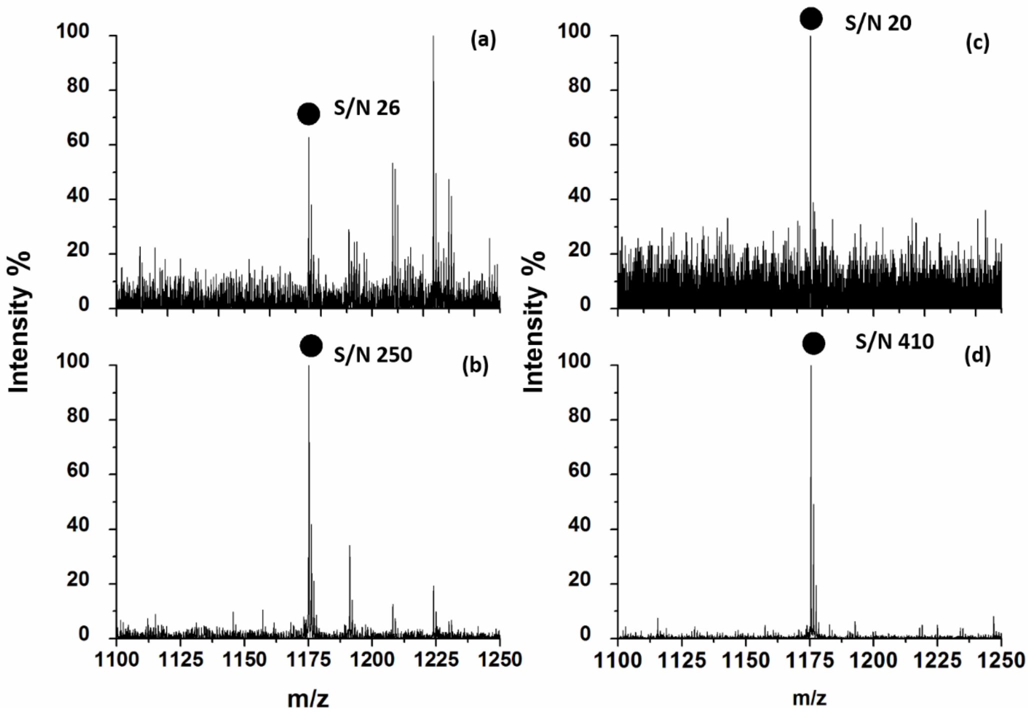


Figure S7. Complex salt buffer tolerance of DHB matrix and HYNIC matrix. MALDI mass spectra of 10 pmol DP7 disolved in diluted cell lysis solution (140 mM urea and 40 mM sulfocarbamide, left panel) using matrix (a) DHB and (b) HYNIC as well as in 50 mM ammonium bicarbonate (right panel) using matrix (c) DHB and (d) HYNIC. ● denotes $[DP7+Na]^+$ signals.

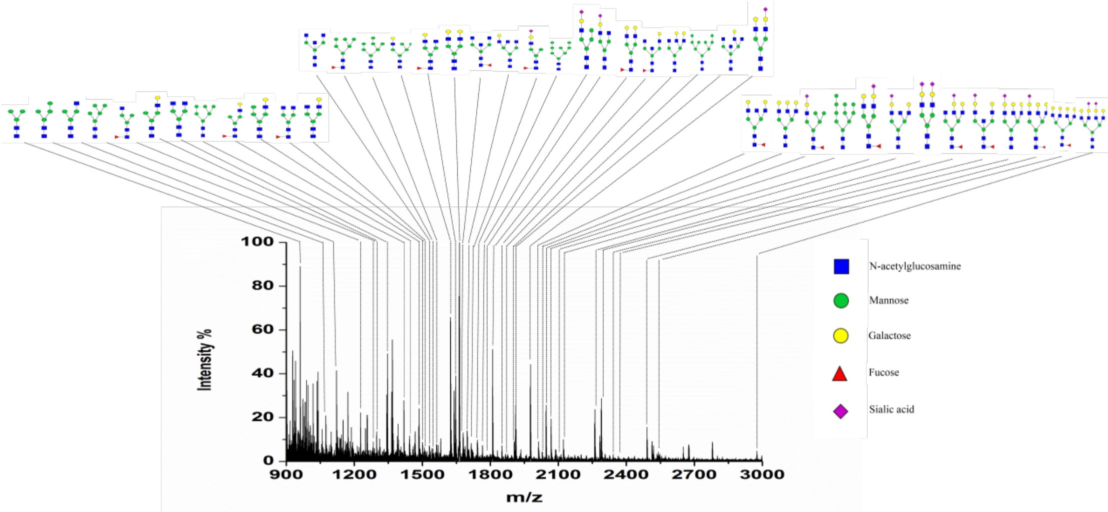


Figure S8. MALDI-TOF-MS spectrum of N-glycans from human serum using HYNIC as matrix. More than 40 glycan signals were successfully detected and their detailed structure are summarized in Table S4.

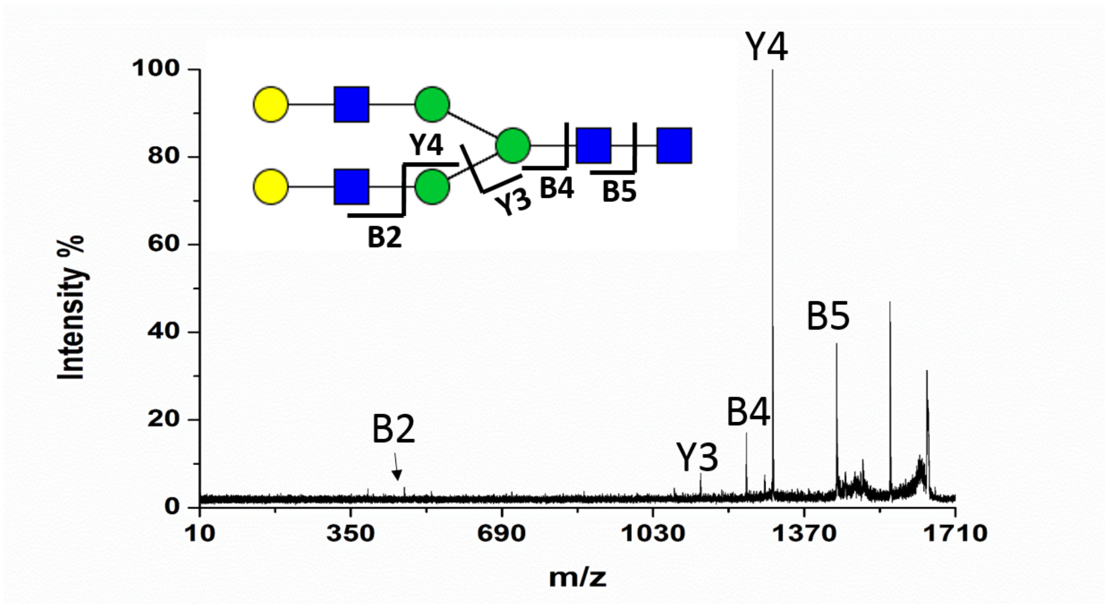


Figure S9.Tandem mass spectrum of the glycan (m/z 1663.6) from serum.

Table S1. Comparison of S/N for DP7 of different loading amount using DHB and HYNIC matrix by MALDI-TOF-MS analysis, respectively. The value came from the average results of 3 parallel tests.

Loading amount per well	DHB	HYNIC
1 pmol	30	260
10 fmol	-	70
100 amol	-	26
1 amol	-	10

Table S2. Exact m/z values for $[M+Na]^+$ and $[M+K]^+$ of Dextran 1000.

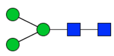
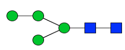
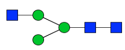
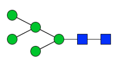
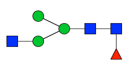
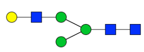
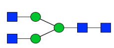
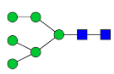
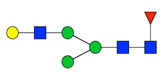
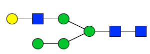
N	DHB		HYNIC	
	$[M+Na]^+$	$[M+K]^+$	$[M+Na]^+$	$[M+K]^+$
3	527.2	-	527.2	543.2
4	689.3	705.3	689.3	705.2
5	851.3	867.4	851.4	867.3
6	1013.4	1029.4	1013.5	1029.4
7	1175.5	1191.5	1175.5	1191.5
8	1337.6	1353.6	1337.6	1353.6
9	1499.7	-	1499.7	1515.7
10	1661.8	-	1661.8	1677.7
11	-	-	1823.9	1839.8
12	-	-	1985.9	2001.9
13	-	-	2148.0	2164.0
14	-	-	2310.1	2326.1
15	-	-	2472.2	-

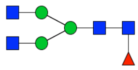
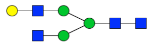
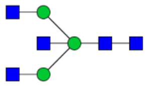
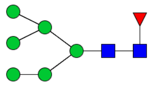
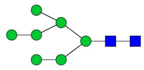
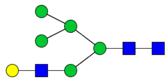
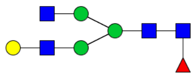
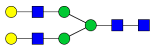
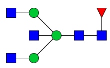
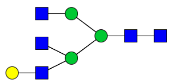
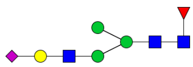
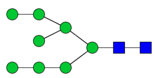
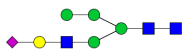
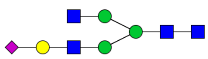
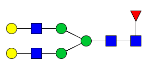
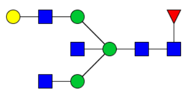
N represent the number of degree of polymerizations

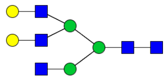
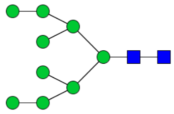
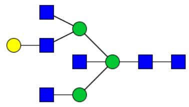
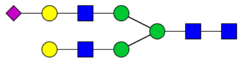
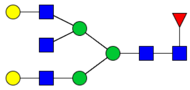
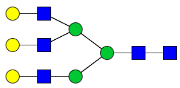
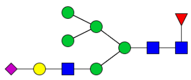
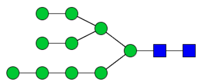
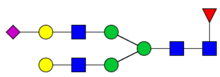
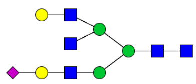
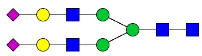
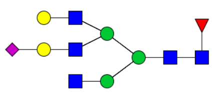
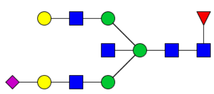
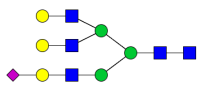
Table S3. m/z value and fragmentation type of DP7 observed in Figure 5.

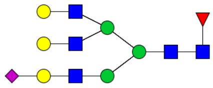
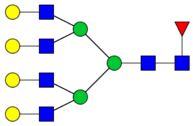
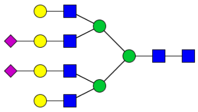
Number	Observed m/z value	Fragmentation type
1	231.2	$C^{1,5}X_{Glc}$
2	257.2	$B^{0,3}X_{Glc}$; $B^{1,4}X_{Glc}$
3	393.3	$C^{1,5}X_{Glc}$
4	467.4	$C^{0,4}X_{Glc}$; $C^{1,3}X_{Glc}$; $C^{2,4}X_{Glc}$
5	555.4	$C^{1,5}X_{Glc}$
6	585.2	$C^{0,2}X_{Glc}$
7	717.5	$C^{1,5}X_{Glc}$
8	747.2	$C^{2,5}X_{Glc}$
9	879.5	$C^{1,5}X_{Glc}$

Table S4. The glycans released and purified from human serum using HYNIC as matrix

No.	m/z	Glycan Structure	
1	932.9		Ref 1
2	1096.5		Ref 1
3	1135.4		Ref 1
4	1256.4		Ref 2
5	1283.5		Ref 1
6	1297.5		Ref 2
7	1337.8		Ref 2
8	1418.5		Ref 1
9	1443.6		Ref 1
10	1459.5		Ref 1

11	1485.6		Ref 2
12	1502.5		Ref 1
13	1541.6		Ref 3
14	1564.8		Ref 2
15	1580.6		Ref 3
16	1623.8		Ref 1
17	1646.6		Ref 4
18	1662.6		Ref 1
19	1688.6		Ref 1
20	1704.6		Ref 1
21	1736.7		Ref 1
22	1742.8		Ref 1
23	1750.7		Ref 1
24	1791.6		Ref 1
25	1809.6		Ref 1
26	1850.7		Ref 1

27	1865.7		Ref 4
28	1904.7		Ref 2
29	1909.7		Ref 3
30	1954.9		Ref 1
31	2011.8		Ref 1
32	2027.7		Ref 1
33	2056.8		Ref 3
34	2067.1		Ref 1
35	2122.7		Ref 3
36	2158.8		Ref 1
37	2288.7		Ref 3
38	2304.8		Ref 1
39	2324.8		Ref 3
40	2340.8		Ref 3

41	2490.3		Ref 3
42	2540.3		Ref 1
43	2974.4		Ref 2

Ref 1.Annotation of a Serum N-Glycan Library for Rapid Identification of Structure
Ref 2.The development of retrosynthetic glycan libraries to profile and classify the human serum N-linked glycome
Ref 3. Facile Preparation of Ordered Mesoporous Silica–Carbon Composite Nanoparticles for Glycan Enrichment
Ref 4. Glycan Analysis by Reversible Reaction to Hydrazide Beads and Mass Spectrometry