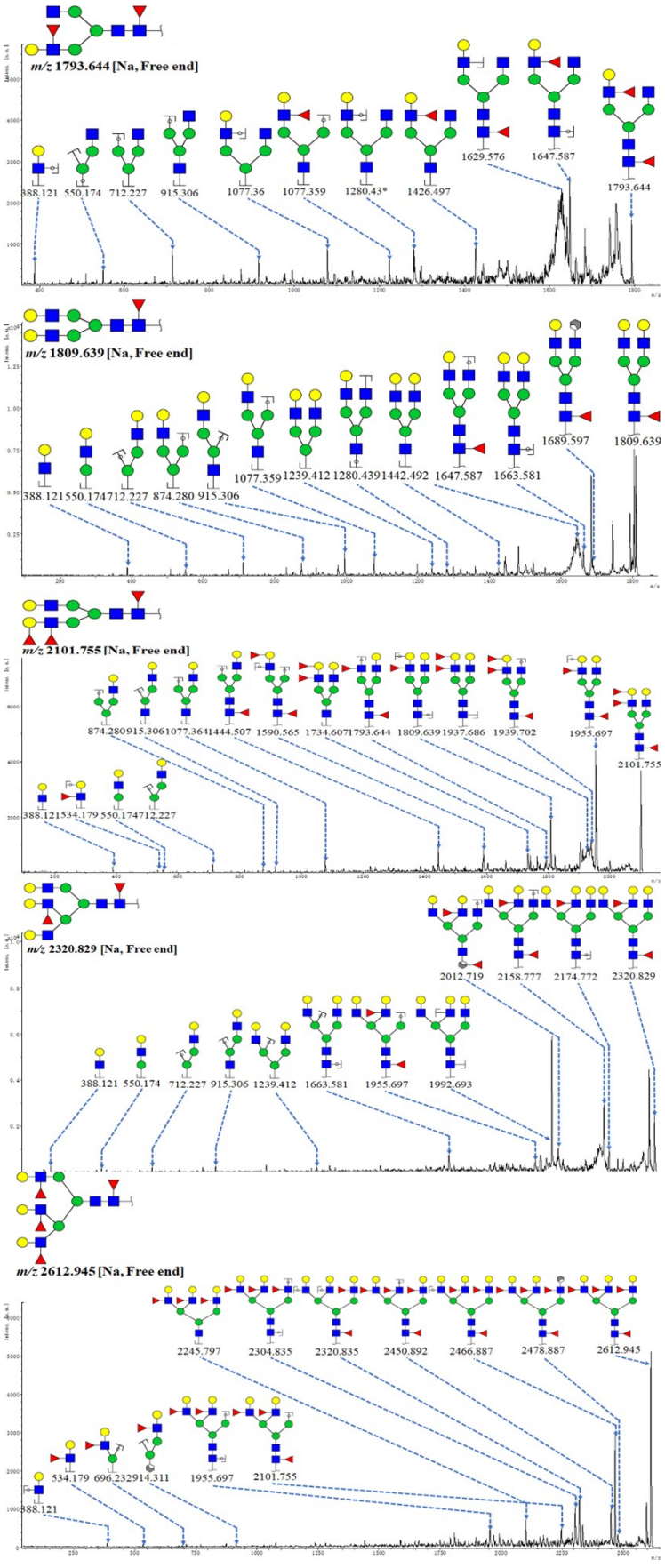


Figure S1

Table. S1 The normalized fluorescence intensities from HV and patients with T2DM in discovery and validation cohort by the lectin microarray analysis based on data of 37 lectins[a]

Lectin	Specificity	Discovery cohort		Validation cohort	
		HV	T2DM	HV	T2DM
Jacalin	Galβ1-3GalNAcα-Ser/Thr(T), GlcNAcβ1-3-GalNAcα-Ser/Thr(Core3), sialyl-T(ST)	0.071 (0.051-0.090)	0.030 (0.026-0.033)	0.066 (0.046-0.086)	0.033 (0.030-0.037)
ECA	Galβ-1,4GlcNAc (type II), Galβ1-3GlcNAc (type I)	0.017 (0.012-0.021)	0.022 (0.018-0.025)	0.019 (0.013-0.025)	0.024 (0.022-0.027)
HHL	High-Mannose, Manα1-3Man, Manα1-6Man, Man5-GlcNAc2-Asn	0.027 (0.022-0.033)	0.019 (0.017-0.022)	0.029 (0.022-0.036)	0.021 (0.019-0.024)
WFA	Terminating in GalNAcα/β1-3/6Gal	0.027 (0.022-0.033)	0.025 (0.018-0.031)	0.029 (0.023-0.034)	0.023 (0.016-0.030)
GSL-II	GlcNAc and agalactosylated tri/tetra antennary glycans	0.021 (0.015-0.027)	0.038 (0.033-0.044)	0.021 (0.016-0.025)	0.034 (0.031-0.037)
MAL-II	Siaα2-3Galβ1-4Glc(NAc)/Glc, Siaα2-3Gal, Siaα2-3, Siaα2-3GalNAc	0.017 (0.013-0.021)	0.015 (0.012-0.018)	0.012 (0.010-0.015)	0.019 (0.017-0.021)
PHA-E	Bisecting GlcNAc, biantennary complex-type N-glycan	0.029 (0.023-0.036)	0.019 (0.017-0.022)	0.025 (0.019-0.031)	0.018 (0.016-0.019)
PTL-I	GalNAc, GalNAcα-1,3Gal, GalNAcα-1,3Galβ-1,3/4Glc	0.009 (0.006-0.012)	0.010 (0.006-0.013)	0.008 (0.006-0.011)	0.009 (0.007-0.011)
SJA	Terminal in GalNAc and Gal, anti-A and anti-B human blood group	0.010 (0.007-0.012)	0.011 (0.008-0.015)	0.010 (0.008-0.012)	0.011 (0.009-0.014)
PNA	Galβ1-3GalNAcα-Ser/Thr(T)	0.020 (0.016-0.025)	0.025 (0.021-0.028)	0.020 (0.015-0.025)	0.028 (0.024-0.031)

EEL	Galα1-3(Fucα1-2)Gal (blood group B antigen)	0.028 (0.024-0.031)	0.021 (0.017-0.025)	0.028 (0.023-0.033)	0.039 (0.035-0.042)
AAL	Fucα1-6 GlcNAc(core fucose), Fucα1-3(Galβ1-4)GlcNAc	0.052 (0.039-0.065)	0.027 (0.024-0.029)	0.053 (0.040-0.067)	0.035 (0.032-0.038)
LTL	Fucα1-3Galβ1-4GlcNAc, Fucα1-anti-H blood group specificity	0.014 (0.010-0.018)	0.016 (0.013-0.019)	0.015 (0.012-0.018)	0.015 (0.012-0.017)
MPL	Galβ1-3GalNAc, GalNAc	0.017 (0.014-0.020)	0.017 (0.013-0.020)	0.015 (0.013-0.018)	0.021 (0.019-0.023)
LEL	Galβ-1,4GlcNAc(LacNAc) and poly LacNAc	0.012 (0.008-0.015)	0.063 (0.053-0.072)	0.012 (0.009-0.015)	0.057 (0.053-0.062)
GSL-I	αGalNAc, αGal, anti-A and B	0.016 (0.012-0.019)	0.021 (0.017-0.025)	0.014 (0.011-0.016)	0.025 (0.022-0.028)
DBA	αGalNAc, Tn antigen, GalNAcα1-3((Fucα1-2))Gal (blood group A antigen)	0.016 (0.013-0.019)	0.020 (0.018-0.023)	0.023 (0.017-0.028)	0.017 (0.015-0.020)
LCA	Fucα-1,6GlcNAc,α-D-Man, α-D-Glc	0.064 (0.051-0.078)	0.104 (0.083-0.126)	0.073 (0.056-0.090)	0.063 (0.048-0.077)
RCA120	β-Gal, Galβ1-3GlcNAc (type I)	0.042 (0.029-0.054)	0.015 (0.010-0.020)	0.043 (0.030-0.056)	0.016 (0.012-0.019)
STL	Trimers and tetramers of GlcNAc, core (GlcNAc) of N-glycan	0.045 (0.038-0.052)	0.023 (0.017-0.029)	0.052 (0.039-0.066)	0.015 (0.011-0.018)
BS-I	α-Gal, α-GalNAc, Galα-1,3Gal, Galα-1,6Glc	0.016 (0.010-0.021)	0.013 (0.011-0.015)	0.014 (0.009-0.020)	0.013 (0.010-0.016)
ConA	High-Mannose, Manα1-6(Manα1-3)Man, αMannose, αGlc	0.055 (0.047-0.064)	0.078 (0.068-0.088)	0.049 (0.041-0.057)	0.075 (0.068-0.083)
PTL-II	Gal, blood group H, T-antigen	0.014 (0.010-0.018)	0.015 (0.013-0.018)	0.015 (0.011-0.020)	0.018 (0.016-0.020)

DSA	$(GlcNAc)_{2-4}$	0.024 (0.019-0.029)	0.011 (0.007-0.015)	0.022 (0.018-0.026)	0.008 (0.005-0.011)
SBA	□α- or β-linked terminal GalNAc, (GalNAc)n, GalNAcα1-3Gal, blood-group A	0.018 (0.013-0.024)	0.012 (0.011-0.013)	0.016 (0.012-0.020)	0.016 (0.015-0.018)
VVA	Terminal GalNAc, GalNAcα-Ser/Thr(Tn), GalNAcα1-3Gal	0.032 (0.026-0.038)	0.075 (0.063-0.088)	0.034 (0.028-0.040)	0.083 (0.069-0.096)
NPA	High-Mannose, Manα1-6Man	0.015 (0.011-0.019)	0.018 (0.014-0.021)	0.016 (0.011-0.020)	0.020 (0.017-0.023)
PSA	α-fucose, α-D-Man, α-D-Glc	0.042 (0.019-0.064)	0.020 (0.016-0.025)	0.027 (0.013-0.041)	0.015 (0.013-0.018)
ACA	Galβ1-3GalNAcα-Ser/Thr (T antigen), sialyl-T(ST) tissue staining patterns are markedly different than those obtained with either PNA or Jacalin	0.043 (0.036-0.049)	0.040 (0.037-0.042)	0.048 (0.041-0.055)	0.051 (0.045-0.056)
WGA	Multivalent Sia and $(GlcNAc)_n$	0.057 (0.037-0.078)	0.046 (0.038-0.054)	0.062 (0.036-0.087)	0.039 (0.033-0.046)
UEA-I	Fucα1-2Galβ1-4Glc(NAc)	0.029 (0.023-0.036)	0.017 (0.014-0.020)	0.036 (0.026-0.046)	0.021 (0.015-0.026)
PWM	$(GlcNAc)_n$ and polyLacNAc	0.026 (0.021-0.031)	0.024 (0.020-0.028)	0.021 (0.018-0.025)	0.032 (0.029-0.035)
MAL-I	Galβ-1,4GlcNAc(LacNAc)	0.009 (0.006-0.011)	0.019 (0.016-0.022)	0.009 (0.007-0.011)	0.016 (0.014-0.018)
GNA	Manα1-3Man	0.023 (0.020-0.027)	0.014 (0.011-0.017)	0.024 (0.017-0.030)	0.013 (0.012-0.015)
BPL	Galβ1-3GalNAc, Terminal GalNAc	0.016 (0.012-0.020)	0.030 (0.026-0.033)	0.013 (0.010-0.016)	0.025 (0.020-0.030)
PHA-E+L	Bisecting GlcNAc, bi-antennary N-glycans, tri- and tetra-antennary complex-type N-glycan	0.011 (0.008-0.014)	0.004 (0.003-0.006)	0.010 (0.007-0.012)	0.006 (0.004-0.008)

SNA	Sia2-6Gal/GalNAc	0.016 (0.013- 0.019)	0.024 (0.022-0.027)	0.017 (0.014-0.020)	0.027 (0.024-0.030)

[a] The normalization fluorescence intensities of each lectin represented as mean with 95% confidence interval.

Table. S2 Altered glycopattern of salivary glycoproteins between HV and T2DM patients in validation cohort based on data of lectins giving significant differences[a]

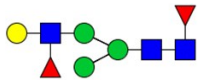
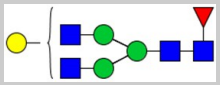
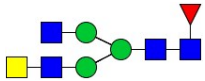
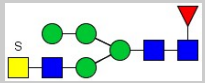
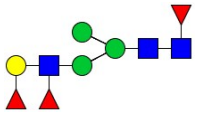
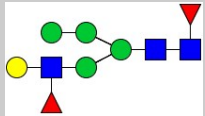
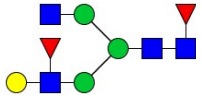
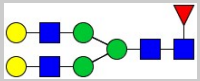
Category	Lectin	Specificity	HV	T2DM	Foldchange[b]
LacNAc	LEL	Galβ-1,4GlcNAc(LacNAc) and poly LacNAc	0.012 (0.009-0.015)	0.057 (0.053-0.062)	4.845***
Terminal GalNAc	VVA	Terminal GalNAc, GalNAcα-Ser/Thr(Tn), GalNAcα1-3Gal	0.034 (0.028-0.040)	0.083 (0.069-0.096)	2.445***
T/ sialyl T antigen	Jacalin	Galβ1-3GalNAcα-Ser/Thr(T), GlcNAcβ1-3-GalNAcα-Ser/Thr(Core3), sialyl-T(ST)	0.066 (0.046-0.086)	0.033 (0.030-0.037)	0.491**
Gal and GalNAc	RCA120	β-Gal, Galβ1-3GlcNAc (type I)	0.043 (0.030-0.056)	0.016 (0.012-0.019)	0.362***
Oligomers of GlcNAc	STL	Trimers and tetramers of GlcNAc, core (GlcNAc) of N-glycan	0.052 (0.039-0.066)	0.015 (0.011-0.018)	0.366***
	DSA	$(GlcNAc)_{2-4}$	0.022 (0.018-0.026)	0.008 (0.005-0.011)	0.281***

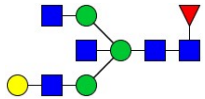
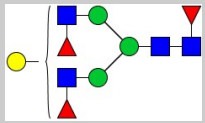
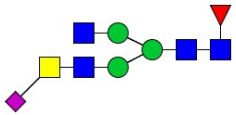
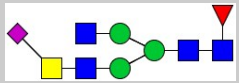
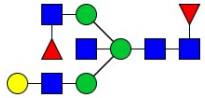
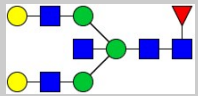
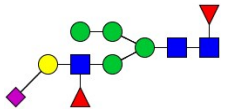
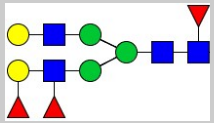
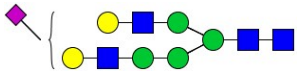
[a] The normalization fluorescence intensities of each lectin represented as mean with 95% confidence interval.

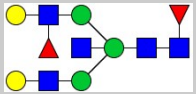
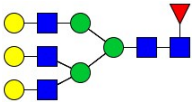
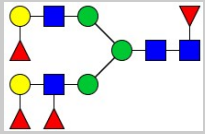
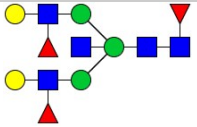
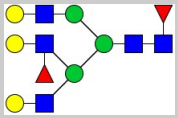
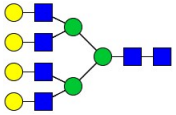
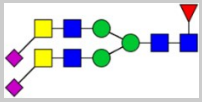
b: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

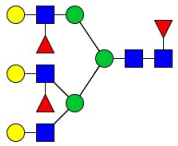
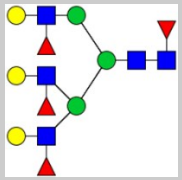
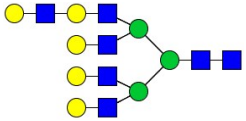
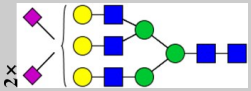
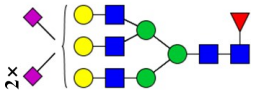
Table. S3 Proposed structures for N-glycan signals detected in isolated salivary glycoproteins from HV and patients with T2DM

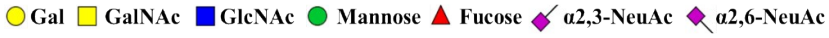
No	Calculated m/z	Ion type	Predicted components	Proposed structure[a]	N-glycan	Relative intensities of N-glycans	
						HV(%)	T2DM(%)

1	1590.565	Na	Hex4HexNAc3dHex2		15.567	24.536
2	1647.586	Na	Hex4HexNAc4dHex1		ND	14.490
3	1688.613	Na	Hex3HexNAc5dHex1		ND	14.926
4	1705.561	H	Hex4HexNAc4S1dHex1		15.074	ND
5	1736.623	Na	Hex4HexNAc3dHex3		23.095	11.651
6	1752.618	Na	Hex5HexNAc3dHex2		ND	9.530
7	1793.644	Na	Hex4HexNAc4dHex2		30.949	14.696
8	1809.639	Na	Hex5HexNAc4dHex1		ND	31.281

9	1850.666	Na	Hex4HexNAc5dHex1		ND	10.832
10	1939.702	Na	Hex4HexNAc4dHex3		30.112	9.331
11	1956.742	H	Hex3HexNAc5(α2,3)NeuAc1dHex1		42.020	69.623
12	1984.773	H	Hex3HexNAc5(α2,6)NeuAc1dHex1		ND	11.422
13	1996.724	Na	Hex4HexNAc5dHex2		ND	8.431
14	2012.719	Na	Hex5HexNAc5dHex1		ND	13.422
15	2020.747	H	Hex5HexNAc3(α2,3)NeuAc1dHex2		10.758	ND
16	2101.755	Na	Hex5HexNAc4dHex3		100.000	100.000
17	2121.795	H	Hex6HexNAc4(α2,6)NeuAc1		11.190	ND

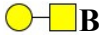
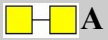
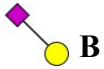
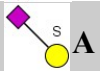
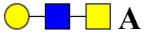
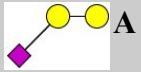
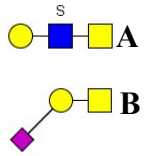
18	2158.777	Na	Hex5HexNAc5dHex2		10.909	13.713
19	2174.771	Na	Hex6HexNAc5dHex1		ND	12.953
20	2247.813	Na	Hex5HexNAc4dHex4		63.345	53.662
21	2304.834	Na	Hex5HexNAc5dHex3		12.414	11.644
22	2320.829	Na	Hex5HexNAc5dHex3		11.847	19.707
23	2393.846	Na	Hex7HexNAc6		29.823	27.223
24	2449.933	H	Hex3HexNAc6(α2,6)NeuAc2dHex1		6.555	4.749

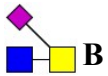
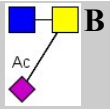
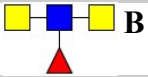
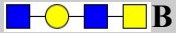
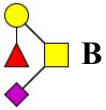
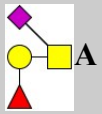
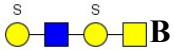
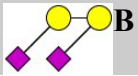
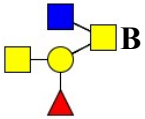
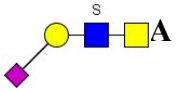
25	2466.887	Na	Hex6HexNAc5dHex3		19.021	24.646
26	2612.945	Na	Hex6HexNAc5dHex4		20.156	26.956
27	2758.978	Na	Hex8HexNAc7		12.692	18.502
28	2905.097	H	Hex6HexNAc5(α2,3)NeuAc2(α2,6)NeuAc1		10.725	13.175
29	3051.155	H	Hex6HexNAc5(α2,3)NeuAc2(α2,6)NeuAc1dHex1		ND	6.360

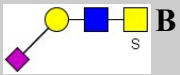
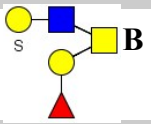
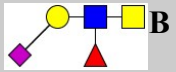
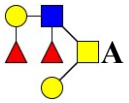
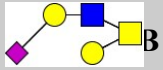
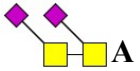
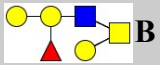
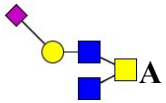


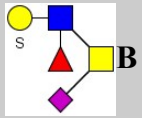
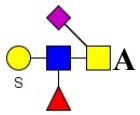
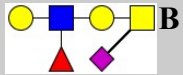
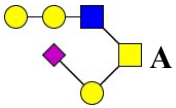
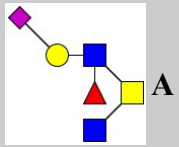
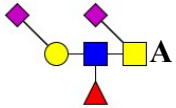
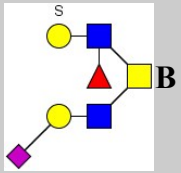
[a] Proposed glycan structure, monosaccharides were represented according to MS-tools from the GlycoWorkbench software.

Table. S4 Proposed structures for O-glycan signals detected in isolated salivary glycoproteins from HV and patients with T2DM

No	Calculated m/z	Ion type	Predicted components	Proposed N-glycan structure[a]	Relative intensities of O-glycans	
					HV (%)	T2DM (%)
1	478.132	Na	Hex1HexNAc1		7.518	ND
2	505.158	Na	HexNAc2		38.668	41.527
3	571.213	H	Hex1(α2,6)NeuAc1		17.696	36.336
4	598.240	H	HexNAc1(α2,3)NeuAc1		6.739	12.676
5	612.240	H	HexNAc1(α2,3)NeuAc1		100.000	12.171
6	637.170	H	Hex1(α2,6)NeuAc1S1		7.257	ND
7	645.229	H	Hex1HexNAc2		11.320	ND
8	713.217	Na	Hex2(α2,3)NeuAc1		11.103	16.792
9	725.186	H	Hex1HexNAc2S1		ND	7.485

10	768.243	Na	Hex1HexNAc1(α2,3)NeuAc1		11.986	35.333
11	837.301	Na	HexNAc2(α2,6)NeuAc1		12.408	18.595
12	851.281	Na	Ac1HexNAc(α2,3)2NeuAc1		16.717	15.088
13	868.296	Na	HexNAc3dHex1		6.422	13.063
14	884.291	Na	Hex1HexNAc3		64.770	100.000
15	914.301	Na	Hex1HexNAc1(α2,3)NeuAc1 dHex1		7.561	12.735
16	928.332	Na	Hex1HexNAc1(α2,6)NeuAc1 dHex1		7.224	10.339
17	981.196	H	Hex2HexNAc2S2		5.656	8.5186
18	995.346	H	Hex2(α2,3)NeuAc2		50.821	48.588
19	1008.367	H	Hex1HexNAc3dHex1		11.611	19.463
20	1024.362	H	Hex2HexNAc3		21.473	18.375
						

21	1037.280	Na	Hex1HexNAc2(α2,3)NeuAc1S1		ND	4.102
22	1051.280	Na	Hex1HexNAc2(α2,3)NeuAc1S1		50.187	78.874
23	1069.279	Na	Hex2HexNAc2S1dHex1		ND	5.179
24	1095.399	H	Hex1HexNAc2(α2,3)NeuAc1dHex1		7.251	5.179
25	1121.380	Na	Hex2HexNAc2dHex2		5.500	11.368
26	1133.376	Na	Hex2HexNAc2(α2,3)NeuAc1		ND	5.897
27	1141.443	Na	HexNAc2(α2,6)NeuAc2		6.677	5.068
28	1151.375	Na	Hex3HexNAc2dHex1		6.670	7.576
29	1166.451	H	Hex1HexNAc3(α2,6)NeuAc1		57.772	91.382

30	1197.377	Na	Hex1HexNAc2(α2,3)NeuAc1 S1dHex1		11.009	12.500
31	1211.369	Na	Hex1HexNAc2(α2,6)NeuAc1 S1dHex1		5.500	9.609
32	1279.434	Na	Hex2HexNAc2(α2,3)NeuAc1 dHex1		8.642	24.486
33	1309.459	Na	Hex3HexNAc2(α2,3)NeuAc1		6.621	11.573
34	1334.491	Na	Hex1HexNAc3(α2,6)NeuAc1 dHex1		ND	18.719
35	1449.554	Na	Hex1HexNAc2(α2,6)NeuAc2 dHex1		ND	16.547
36	1562.470	Na	Hex2HexNAc3(α2,3)NeuAc1 S1dHex1		ND	4.915

Gal GalNAc GlcNAc Mannose Fucose α2,3-NeuAc α2,6-NeuAc

[a] Proposed glycan structure, monosaccharides were represented according to MS-tools from the GlycoWorkbench software. In O-glycans, the reducing end A or B was residue of -CH_2COOH from serine or residue of -$CH(CH_3)COOH$ from threonine.