Supplementary information

Profiling and Structural Characterization of High Neu5Gc or Sulfate-containing *O*-glycans from Hyla Rabbit Intestinal Mucin

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Table S1. Monosaccharide composition of four fractions purified on a QFF column

Fraction	Monosaccharide composition (molar ratio)								
	Man	GlcNAc	GalNAc	GlcA	GalA	Glc	Gal	Rha	Fuc
RIF4	ND*	13.91	3.43	0.30	0.36	1.00	3.90	0.32	0.89
RIF6	ND*	69.88	52.40	2.70	ND*	1.00	42.86	ND*	6.58
RIF8	ND*	2.50	5.42	0.82	ND*	1.00	ND*	ND*	ND*
RIF10	22.49	2.78	174.08	64.08	ND*	1.00	5.49	0.32	ND*

* "ND"：Not detected.

Table S2. ***O***-glycan structures assigned by ESI-CID-MS/MS in RIF6 and comparison with other species.

m/z	Proposed Structures	Core type	Relative Abundance(%)	Humans	Chickens	Pigs	Mice	Fishes
			Neutral O-glycans					
425.1712^{-}	GlcNAcβ1-3GalNAcol	Core 3	10.42	√	√	×	√	√
530.2014^{-}	Fucα1-2Galβ1-3GalNAcol	Core 1	4.60	×	×	√	√	×
587.2213^{-}	Galβ1-4GlcNAcβ1-3GalNAcol	Core 3	4.30	*	√	√	*	√
733.2773^{-}	Fucα1-2Galβ1-4GlcNAcβ1-3GalNAcol	Core 3	6.15	*	*	√	*	*
			Sulfate-containging O-glycans					
464.1010^{-}	(SO_3^-)4Galβ1-3GalNAcol	Core 1	7.30	×	√	×	×	×
667.1766^{-}	Galβ1-4(SO_3^-)6GlcNAcβ1-3GalNAcol	Core 3	43.19	√	*	*	*	×
708.2025^{-}	(SO_3^-)GlcNAcβ1-6(GlcNAcβ1-3)GalNAcol	Core 4	0.55	×	×	√	×	×
747.1322^{-}	(SO_3^-)Galβ1-4(SO_3^-)6GlcNAcβ1-3GalNAcol	Core 3	0.52	×	×	×	×	×
813.2323^{-}	(SO_3^-)GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcol	Core 1	2.39	*	*	√	*	×
	Fucα1-2Galβ1-4(SO_3^-)GlcNAcβ1-3GalNAcol	Core 3		√	*	√	*	×
870.2523^{-}	Galβ1-4(SO3-)GlcNAcβ1-6(GlcNAcβ1-3)GalNAcol	Core 4	0.61	×	*	*	*	×
975.2650^{-}	Fucα1-2Galβ1-4(SO_3^-)GlcNAcβ1-6(Galβ1-3)GalNAcol	Core 2		*	*	√	√	×
	Galβ1-4(SO_3^-)GlcNAcβ1-6(Fucα1-2Galβ1-3)GalNAcol	Core 2	1.54	*	*	√	*	×

1016.3102$^-$	GlcNAcβ1-3[Fucα1-2Galβ1-4(SO_3^-)GlcNAcβ1-6]GalNAcol	Core 4	0.80	×	×	√	×	×
1032.3019$^-$	(SO_3^-)Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)GalNAcol	Core 4	0.20	×	×	×	*	×
1121.3380$^-$	(SO_3^-)4Galβ1-4(Fucα1-3)GlcNAcβ1-6(Fucα1-2Galβ1-3)GalNAcol	Core 2	0.18	×	×	*	*	×
1178.3591$^-$	(SO_3^-)Galβ1-4GlcNAcβ1-6(Fucα1-2Galβ1-3GlcNAcβ1-3)GalNAcol	Core 4	0.41	×	×	*	*	×
	Neu5Gc-containing O-glycans							
488.1658$^-$	Neu5Gcα2-3Galol	peeling	0.37	×	×	×	×	×
529.1804$^-$	Neu5Gcα2-6GalNAcol	STn	0.34	×	×	×	×	×
691.2303$^-$	Galβ1-3(Neu5Gcα2-6)GalNAcol	Core 1	0.22	×	×	√	×	√
732.2565$^-$	GlcNAcβ1-3(Neu5Gcα2-6)GalNAcol	Core 3	5.16	×	×	√	×	*
836.3022$^-$	Neu5Gcα2-8Neu5Gcα2-6GalNAcol	STn	0.59	×	×	×	×	×
837.3053$^-$	Fucα1-2Galβ1-3(Neu5Gcα2-6)GalNAcol	Core 1	0.18	×	×	×	×	×
894.3061$^-$	Neu5Gcα2-3Galβ1-3(GlcNAcβ1-6)GalNAcol	Core 2	3.39	×	×	√	×	*
1040.3623$^-$	GalNAcβ1-3(Fucα1-2)Galβ1-3(Neu5Gcα2-6)GalNAcol	Core 1	0.39	×	×	×	*	*
	Fucα1-2Galβ1-4GlcNAcβ1-3(Neu5Gcα2-6)GalNAcol	Core 3	0.28	×	×	×	*	*
	Neu5Ac-containing O-Glycans							
675.2558$^-$	Galβ1-3(Neu5Acα2-6)GalNAcol	Core 1	0.27	√	×	√	×	√
	Neu5Acα2-3Galβ1-3GalNAcol	Core 1		√	×	√	√	√
716.2616$^-$	GlcNAcβ1-3(Neu5Acα2-6)GalNAcol	Core 3	0.32	×	×	√	×	√
878.3114$^-$	Neu5Acα2-3Galβ1-3(GlcNAcβ1-6)GalNAcol	Core 2	0.30	√	×	√	*	√
	Mixed acid glycans							

958.2672^{-}	Neu5Acα2-3Galβ1-4(SO_3^-)GlcNAcβ1-3GalNAcol	Core 3	0.34	*	×	×	×	×
974.2620^{-}	Neu5Gcα2-3Galβ1-4(SO_3^-)GlcNAcβ1-3GalNAcol	Core 3	4.53	×	×	√	×	×
982.3572-	Neu5Acα2-3Galβ1-3(NeuGcα2-6)GalNAcol	Core 1	0.17	×	×	×	×	×

'*' means the presence of isomers, '√' means the structures were detected, '×' means the structures were not detected.

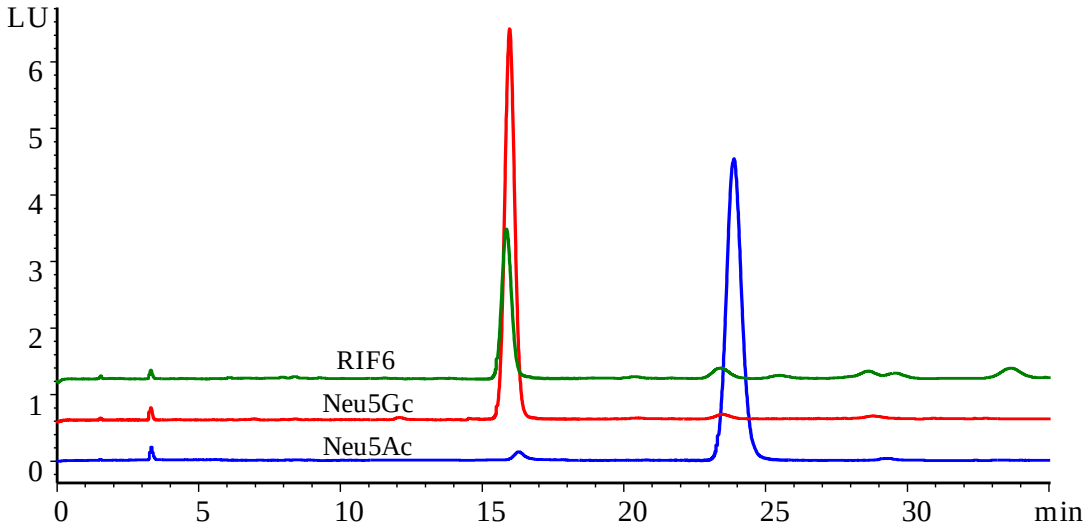
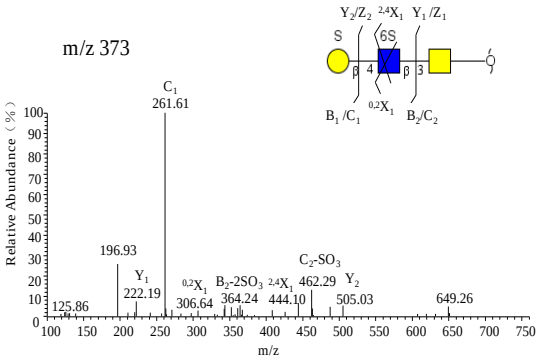
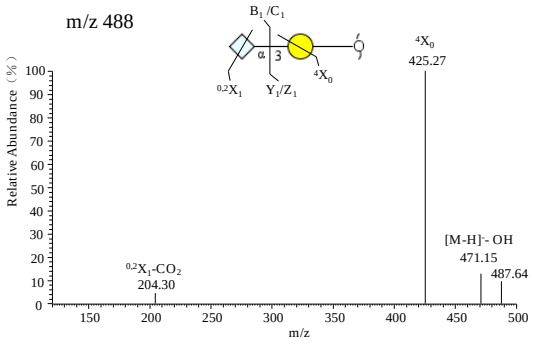
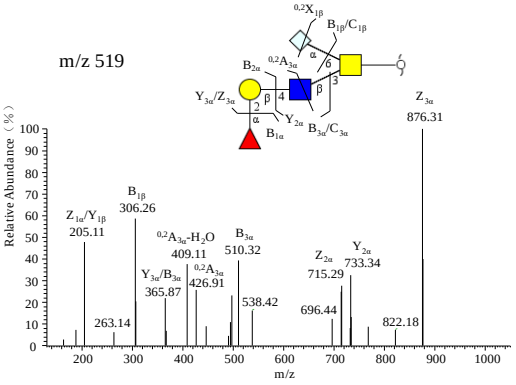
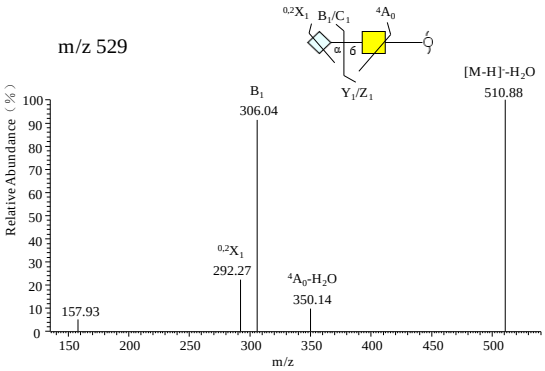


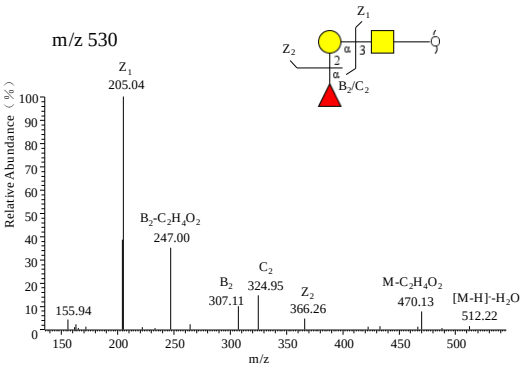
Figure S1. HPLC Chromatogram for sialic acid standards solution (Neu5Ac, Neu5Gc) and sample (RIF6).

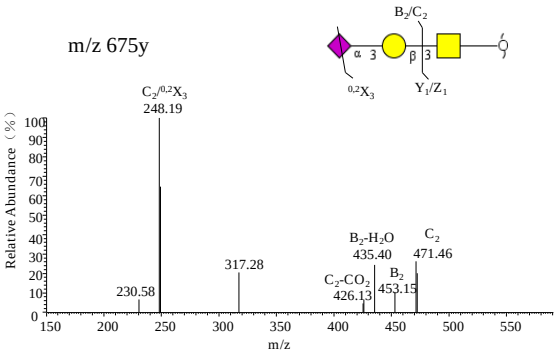

m/z 373
Relative Abundance (%)
m/z
C1 261.61
196.93
Y1 222.19
0,2X1 306.64
B2-2SO3 364.24
2,4X1 444.10
C2-SO3 462.29
Y2 505.03
125.86
649.26

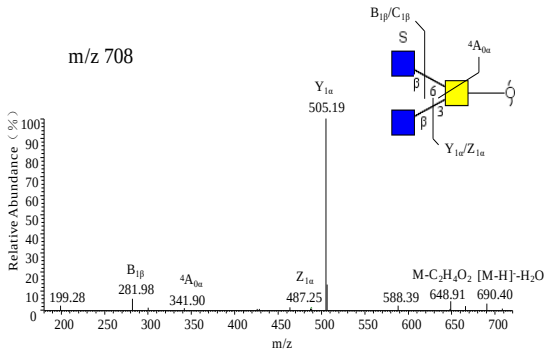

m/z 488
Relative Abundance (%)
m/z
4X0 425.27
0,2X1-CO2 204.30
[M-H]- OH 471.15
487.64

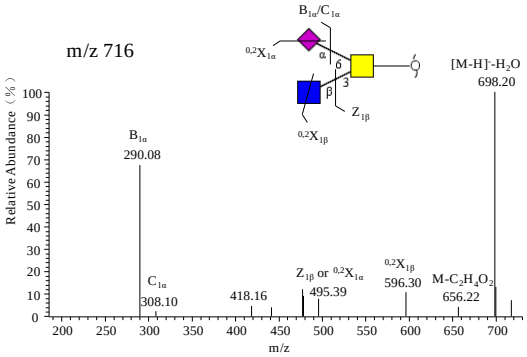

m/z 519
Relative Abundance (%)
m/z
Z3α 876.31
B1β 306.26
Z1α/Y1β 205.11
0,2A3α-H2O 409.11
B3α 510.32
Y3α/B3α 365.87
0,2A3α 426.91
Z2α 715.29
Y2α 733.34
538.42
696.44
263.14
822.18

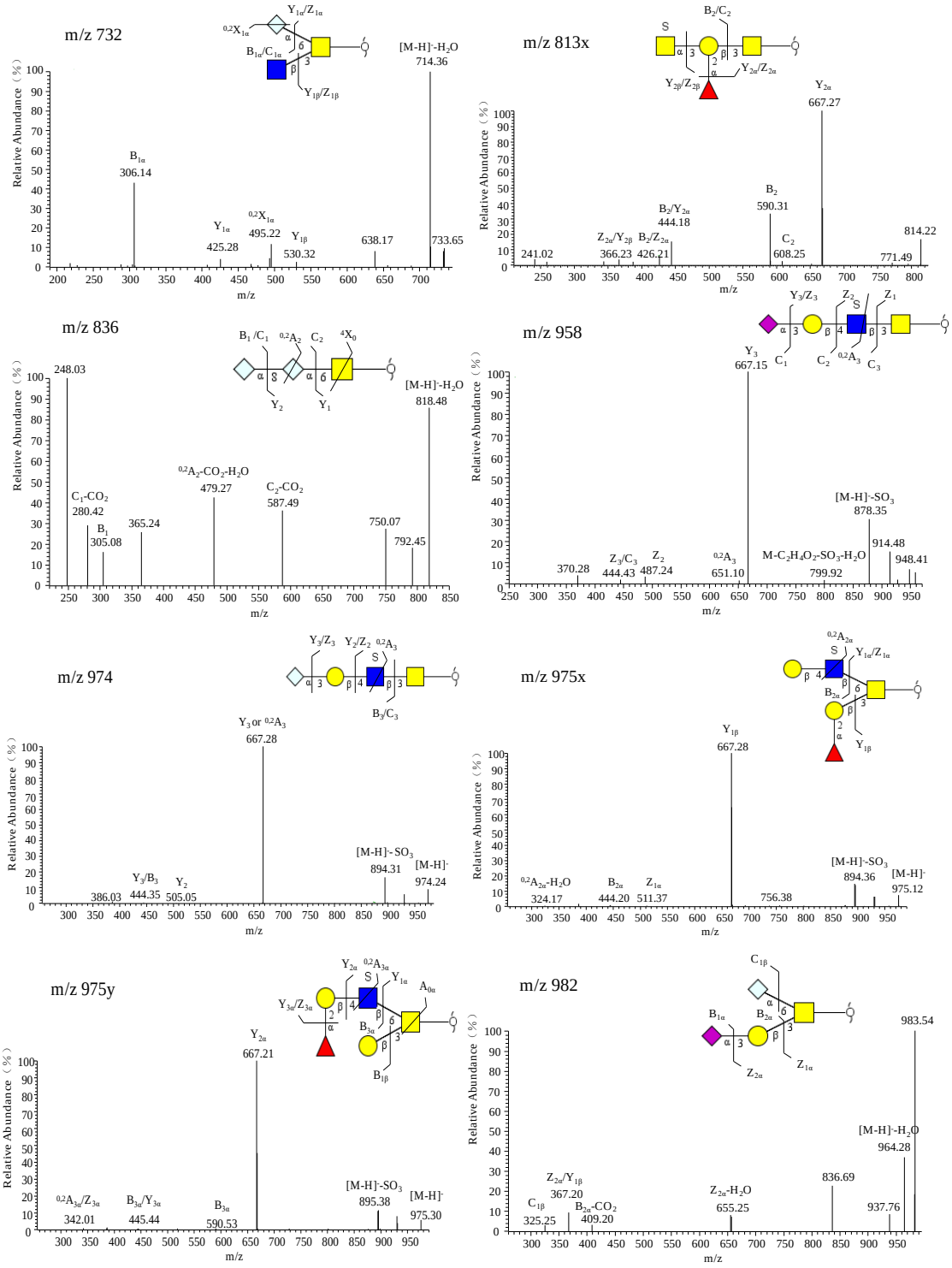

m/z 529
Relative Abundance (%)
m/z
[M-H]-H2O 510.88
B1 306.04
0,2X1 292.27
4A0-H2O 350.14
157.93


m/z 530
Relative Abundance (%)
m/z
Z1 205.04
B2-C2H4O2 247.00
B2 307.11
C2 324.95
Z2 366.26
M-C2H4O2 470.13
[M-H]-H2O 512.22
155.94


m/z 675y
Relative Abundance (%)
m/z
C2/0,2X3 248.19
B2-H2O 435.40
C2 471.46
317.28
C2-CO2 426.13
B2 453.15
230.58


m/z 708
Relative Abundance (%)
m/z
Y1α 505.19
B1β 281.98
4A0α 341.90
Z1α 487.25
M-C2H4O2 588.39
[M-H]-H2O 690.40
199.28
648.91


m/z 716
Relative Abundance (%)
m/z
[M-H]-H2O 698.20
B1α 290.08
C1α 308.10
Z1β or 0,2X1α 495.39
0,2X1β 596.30
M-C2H4O2 656.22
418.16


m/z 732
m/z 813x
m/z 836
m/z 958
m/z 974
m/z 975x
m/z 975y
m/z 982
Relative Abundance (%)
m/z

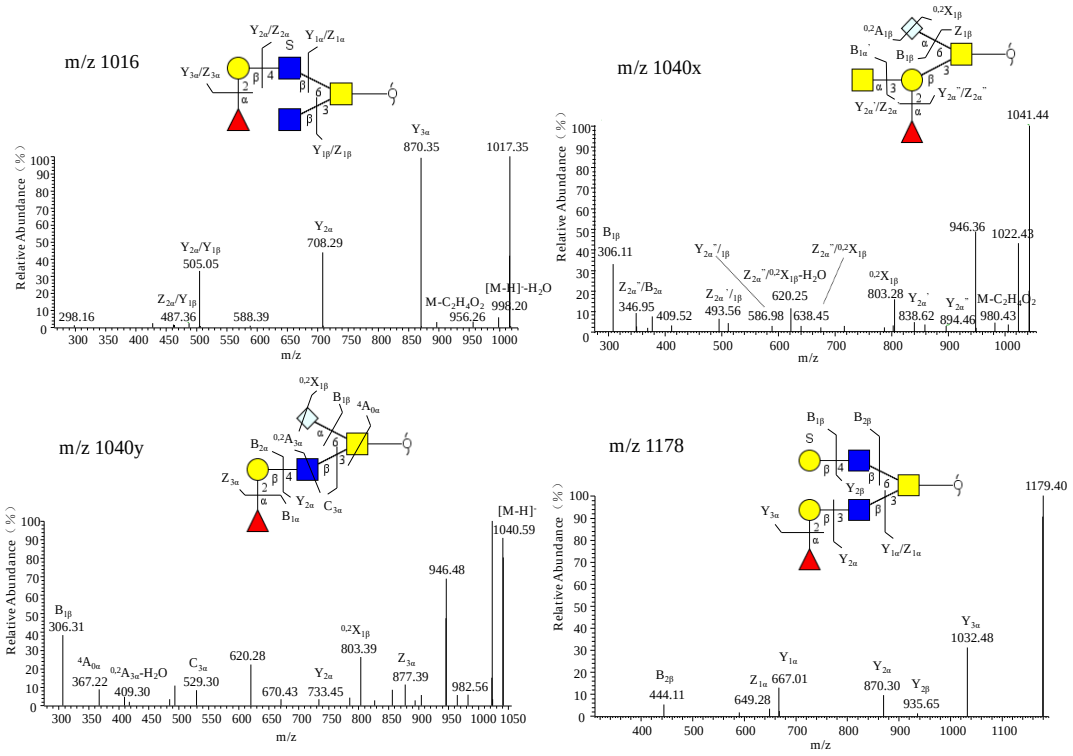


Figure S2. ESI-CID-MS/MS spectra of the remaining ***O***-glycans from RIF6.

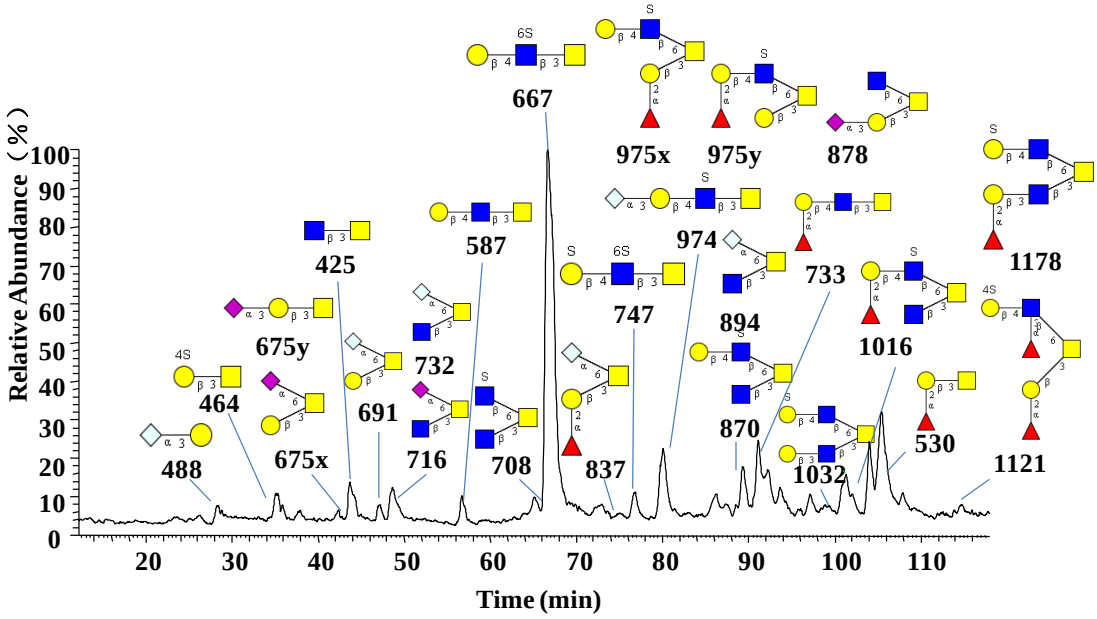


Figure S3. Total ion chromatogram of assigned ***O***-glycan structures of RIF6.