Supplementary Information for

Comprehensive quali-quantitative profiling of neutral and sialylated *O*-glycome by mass spectrometry based on oligosaccharide metabolic engineering and isotopic labeling

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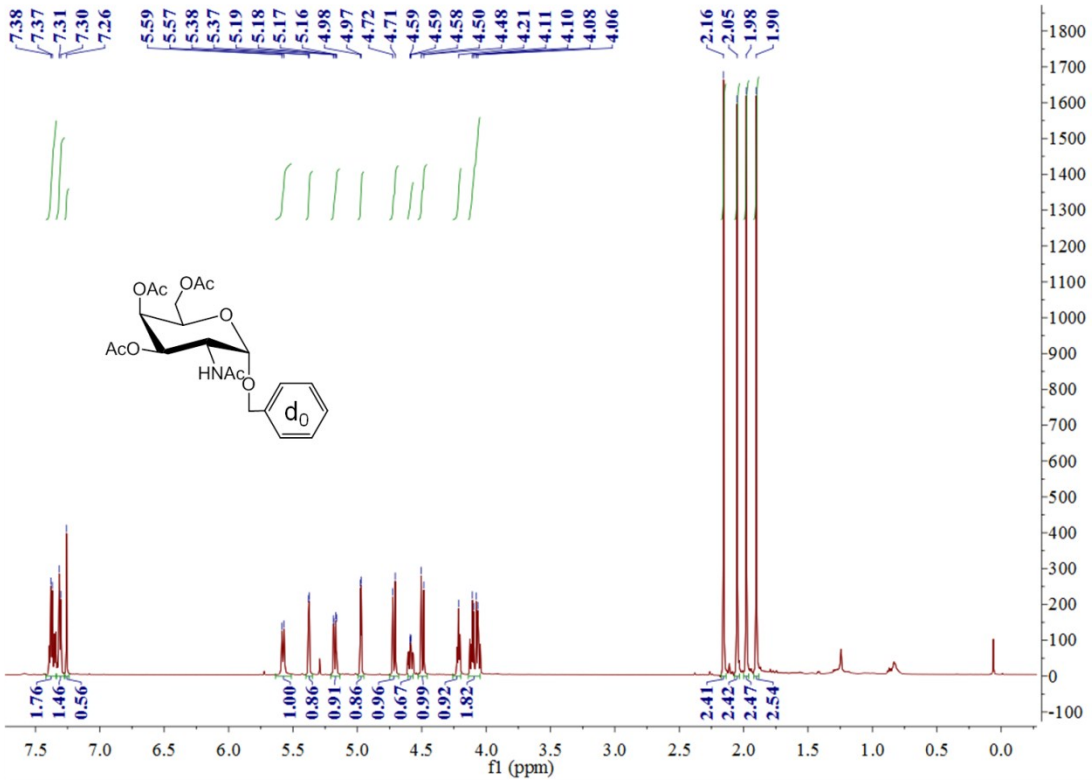


Fig.S 1A ^{1}H NMR spectra of $Ac_3GalNAc$-α-Bn^{d0}

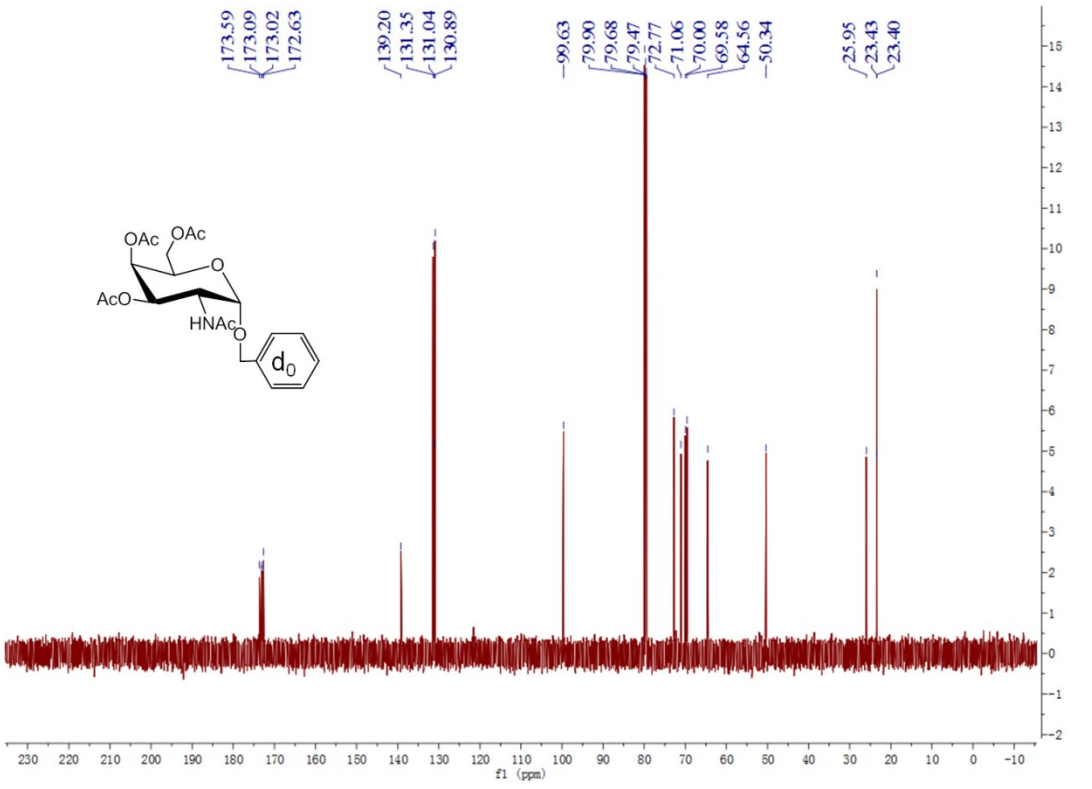


Fig.S 1B ^{13}C NMR spectra of $Ac_3GalNAc$-α-Bn^{d0}

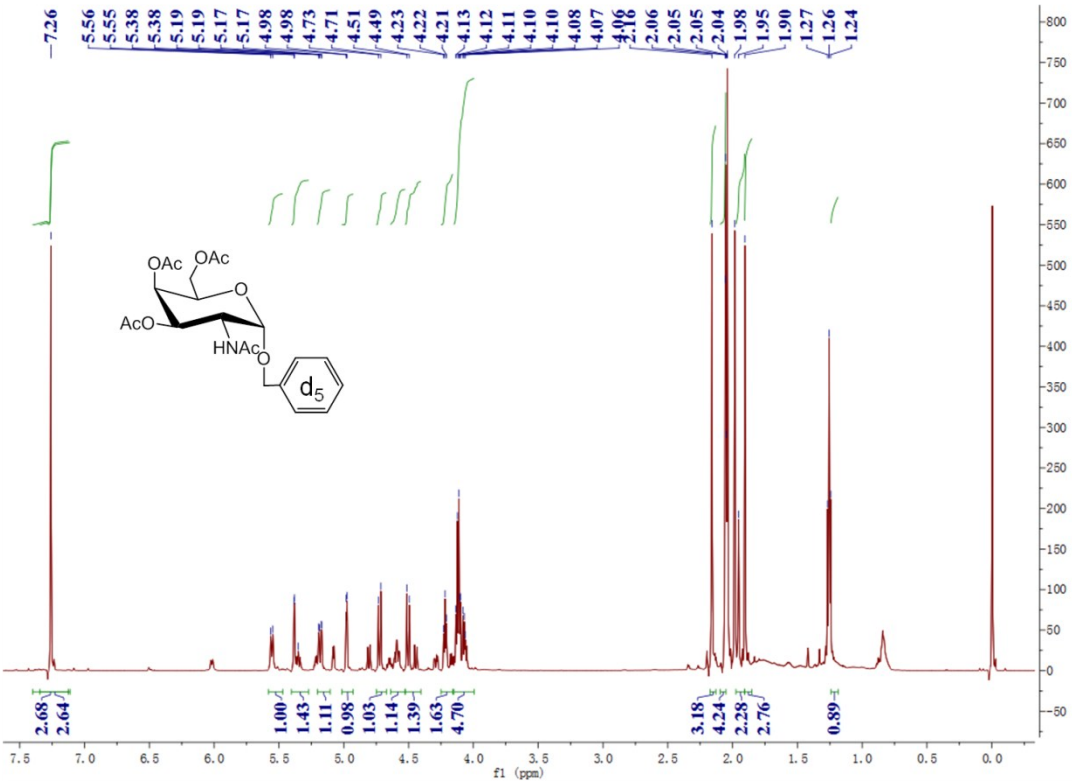


Fig.**S 1C** ^{1}H NMR spectra of Ac_3GalNAc-α-Bn^{d5}

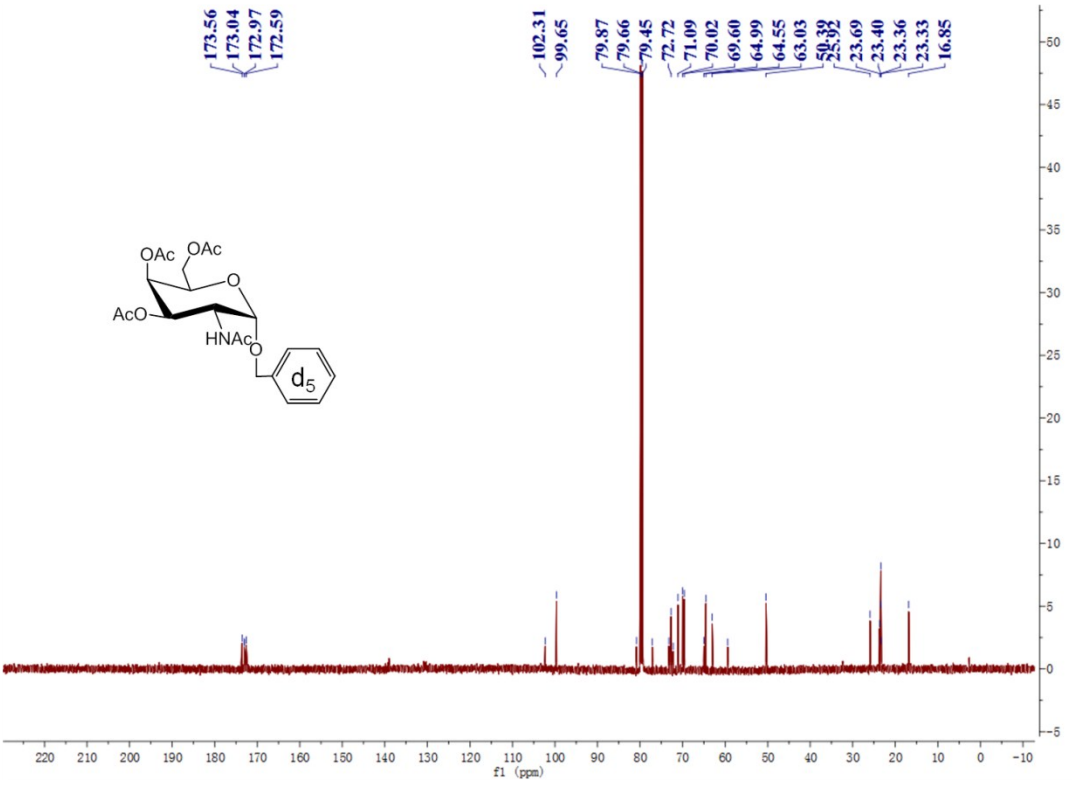


Fig.**S 1D** ^{13}C NMR spectra of Ac_3GalNAc-α-Bn^{d5}

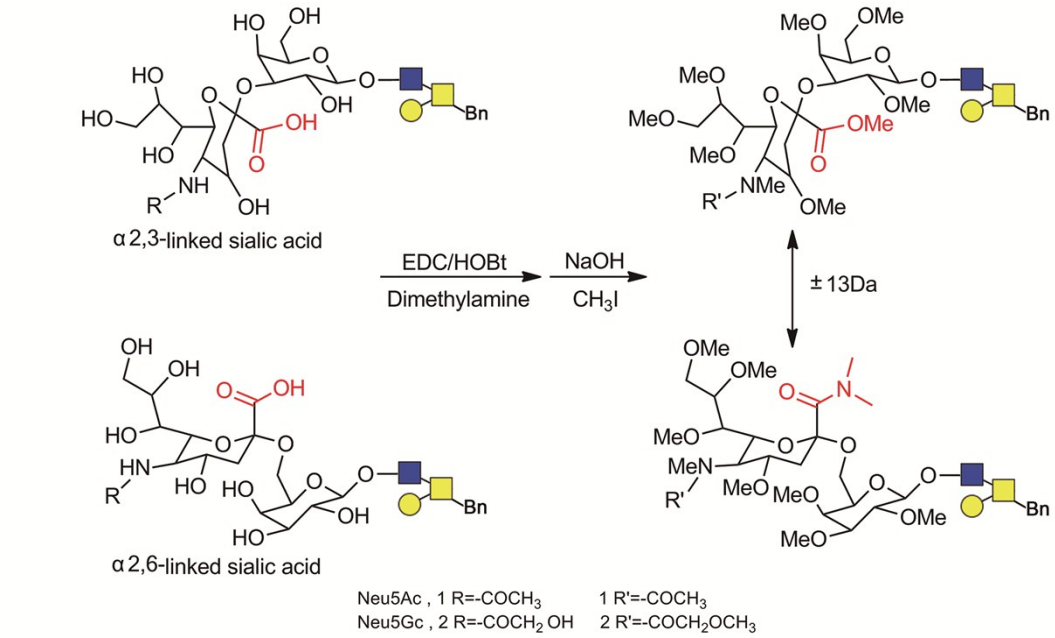


Fig.S 2 Reaction scheme for sialic acid specific derivatization of Bn-*O*-glycans with terminal sialic acids

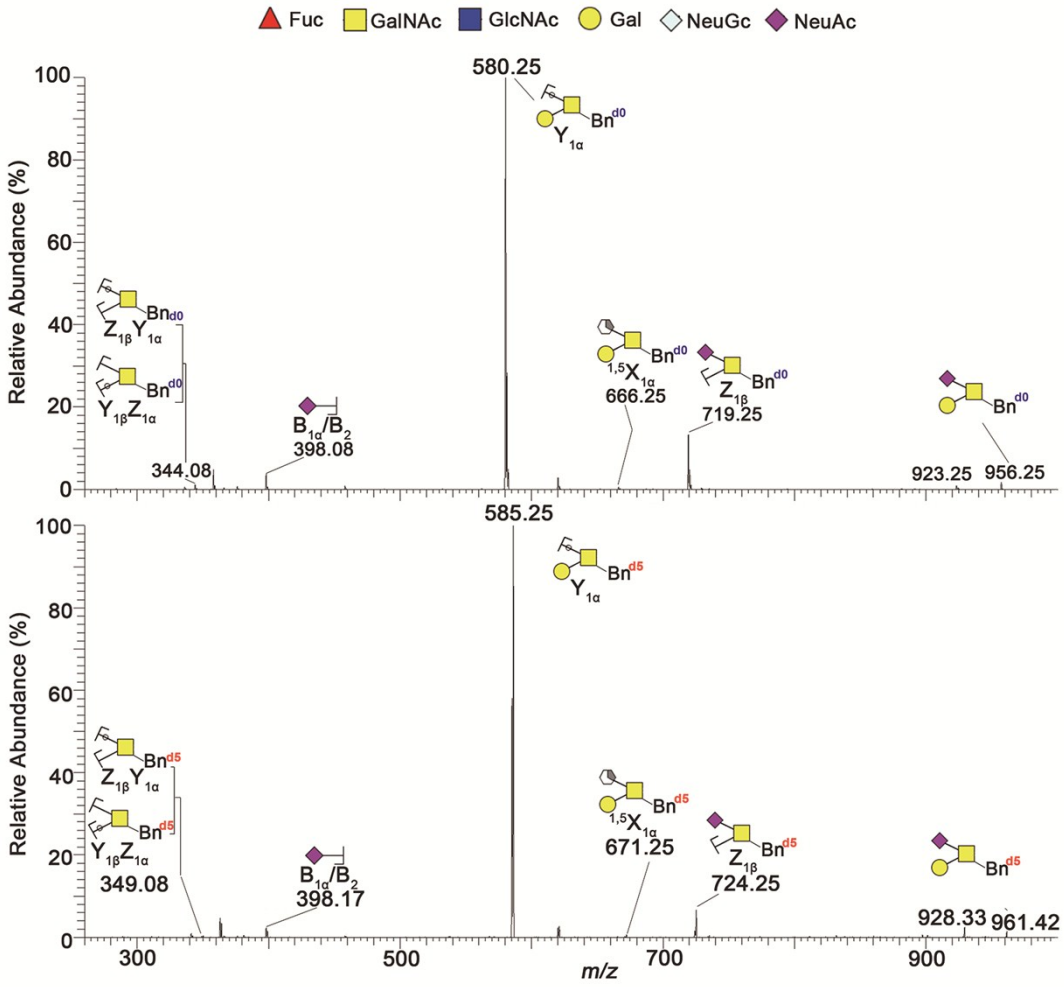


Fig.S 3A Comparative sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 955.25/960.33 was fragmented by CID, respectively.

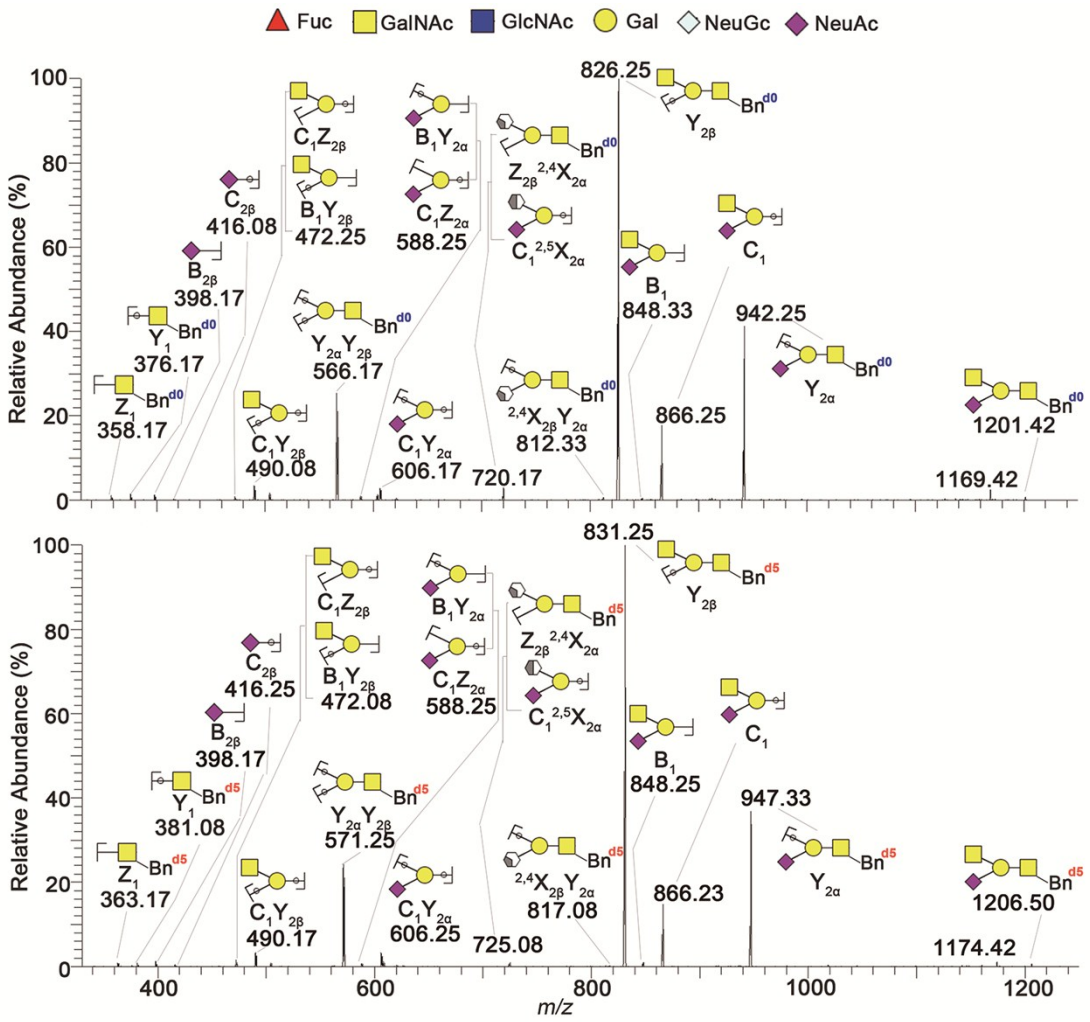


Fig.S 3B Comparative sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1200.33/1205.33 was fragmented by CID, respectively.

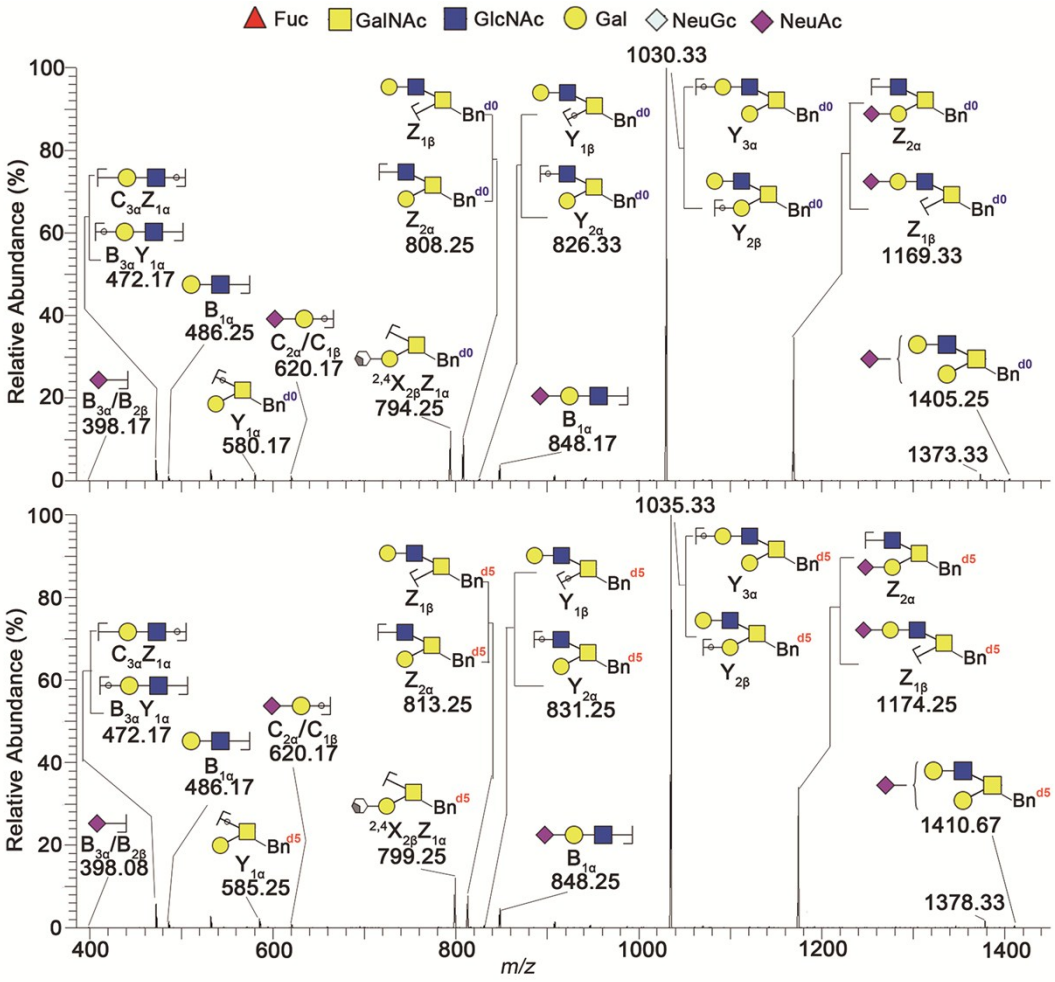


Fig.S 3C Comparative sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1404.42/1409.42 was fragmented by CID, respectively.

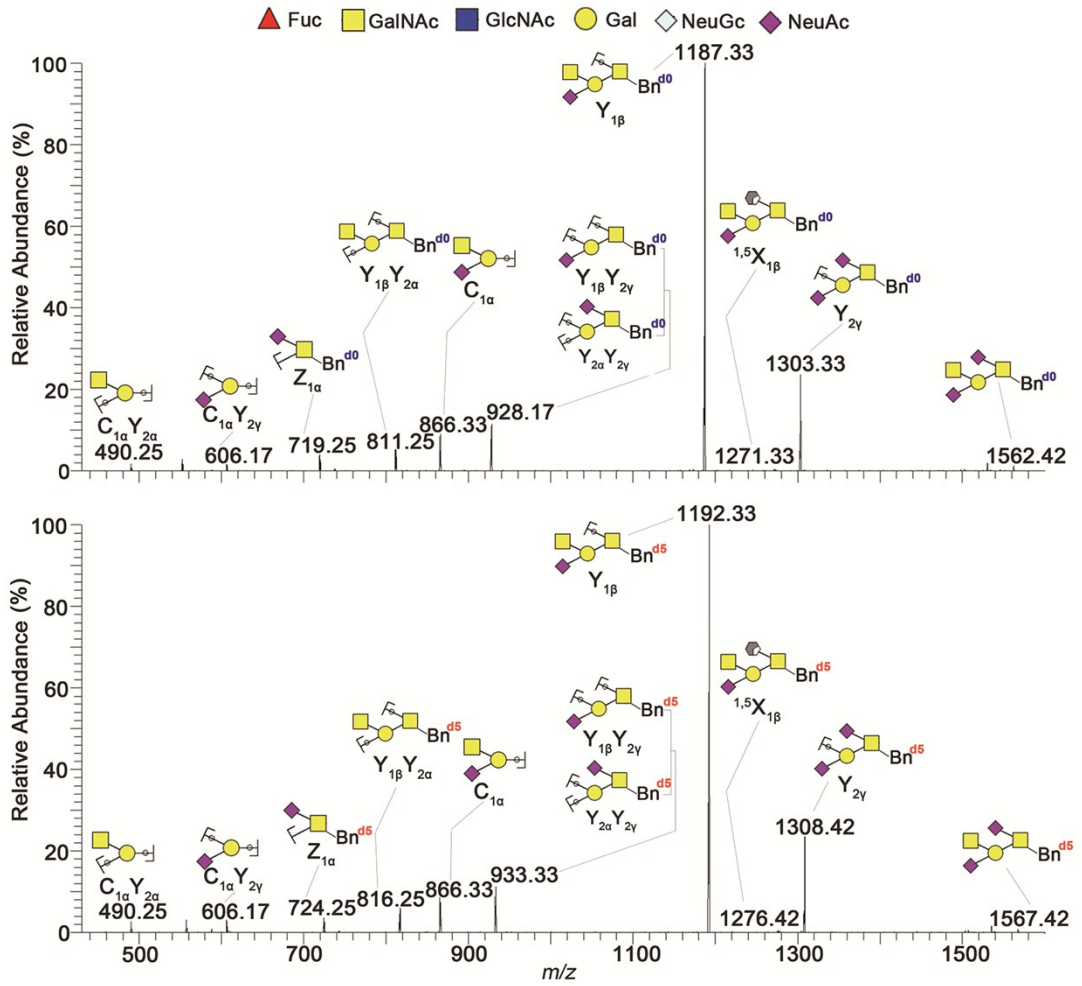


Fig.**S 3D** Comparative sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1561.33/1566.42 was fragmented by CID, respectively.

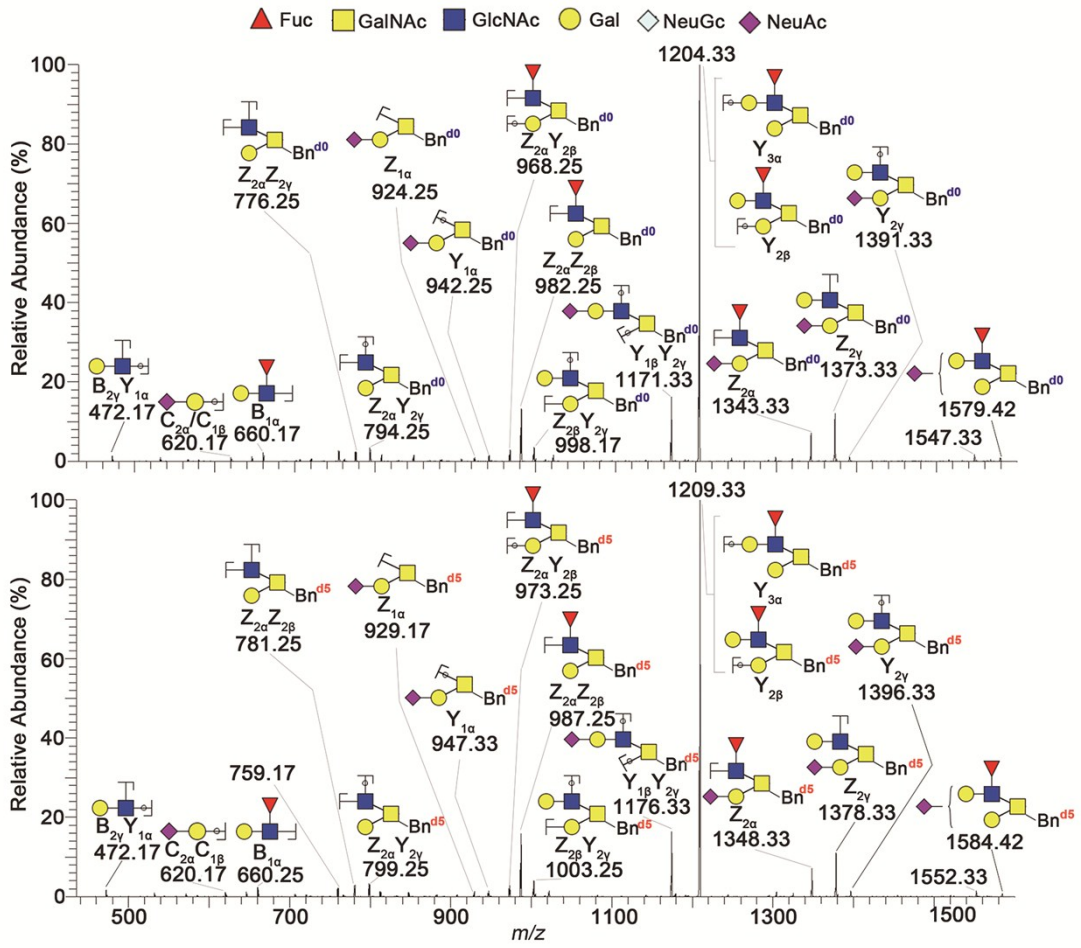


Fig.**S 3E** Sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1578.17/1583.25 was fragmented by CID, respectively.

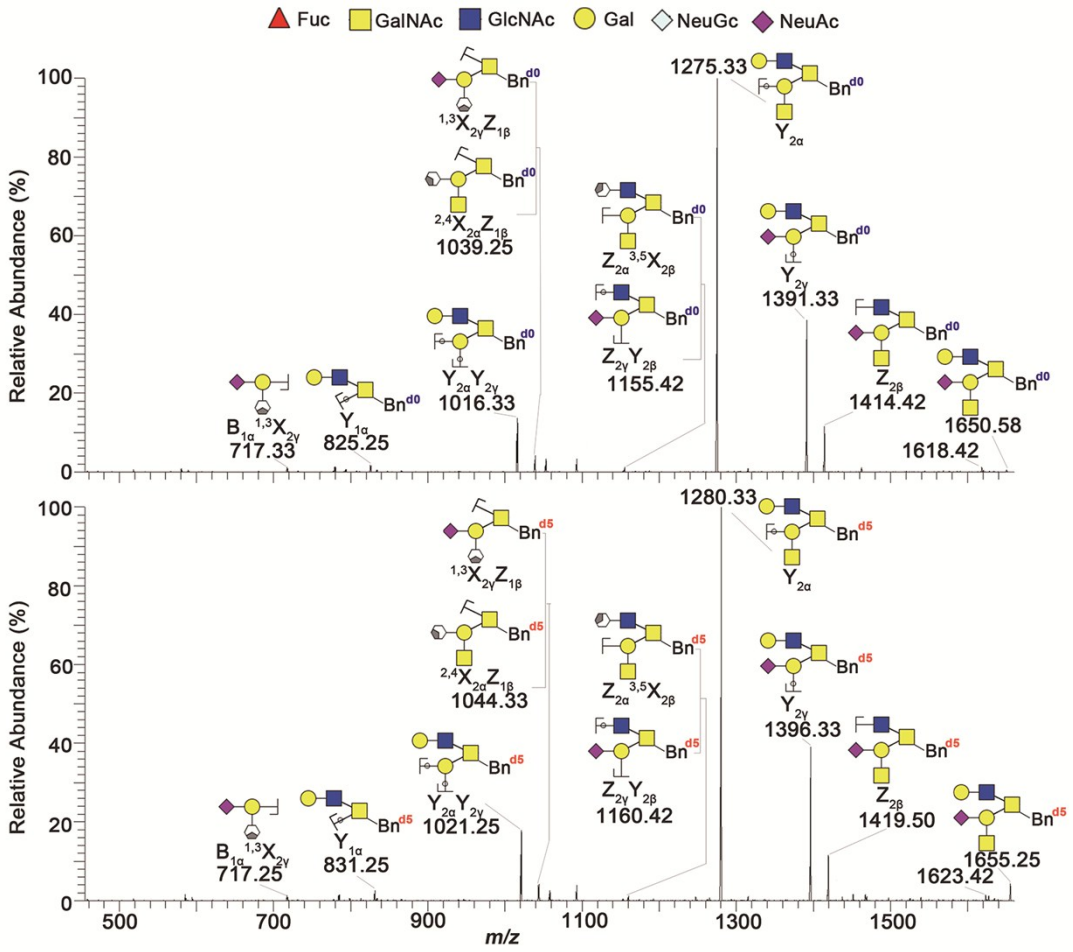


Fig.**S 3F** Sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1649.25/1654.42 was fragmented by CID, respectively.

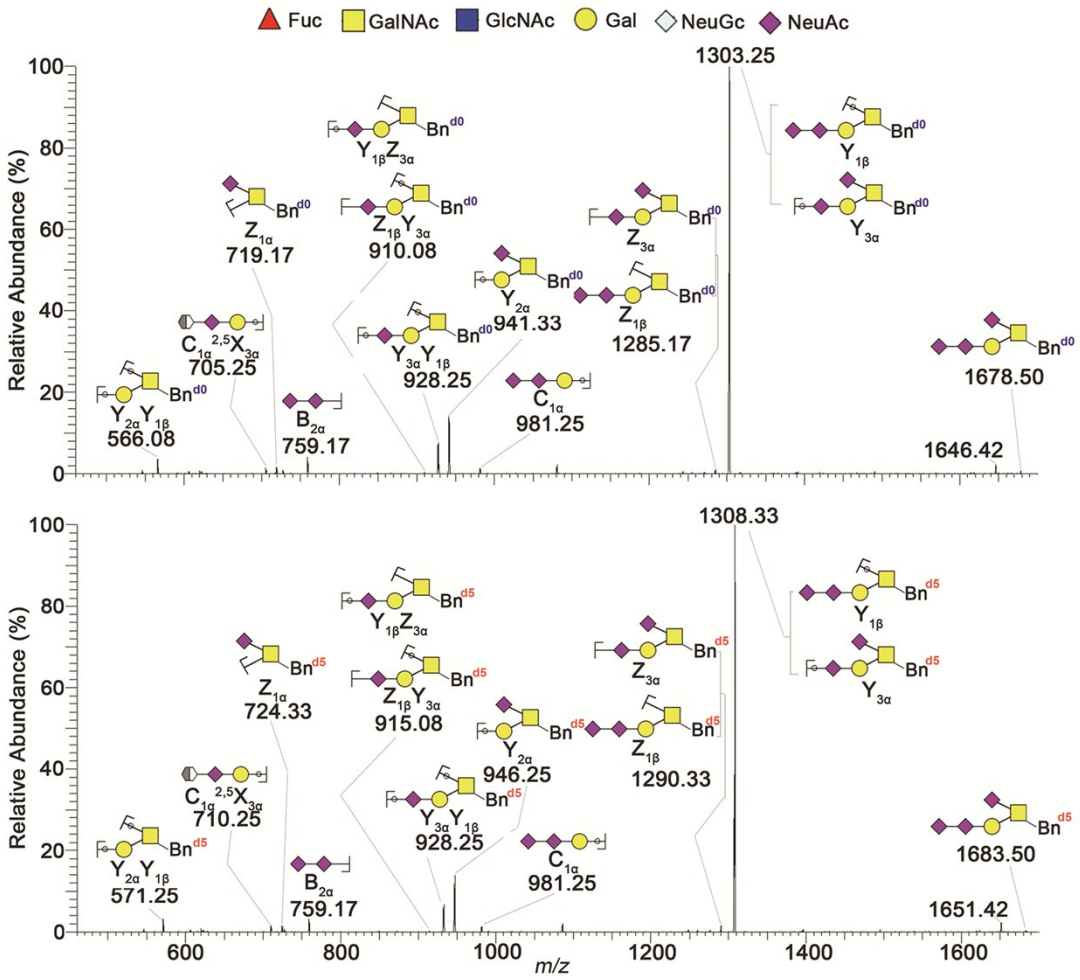


Fig.**S 3G** Sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1677.42/1682.33 was fragmented by CID, respectively.

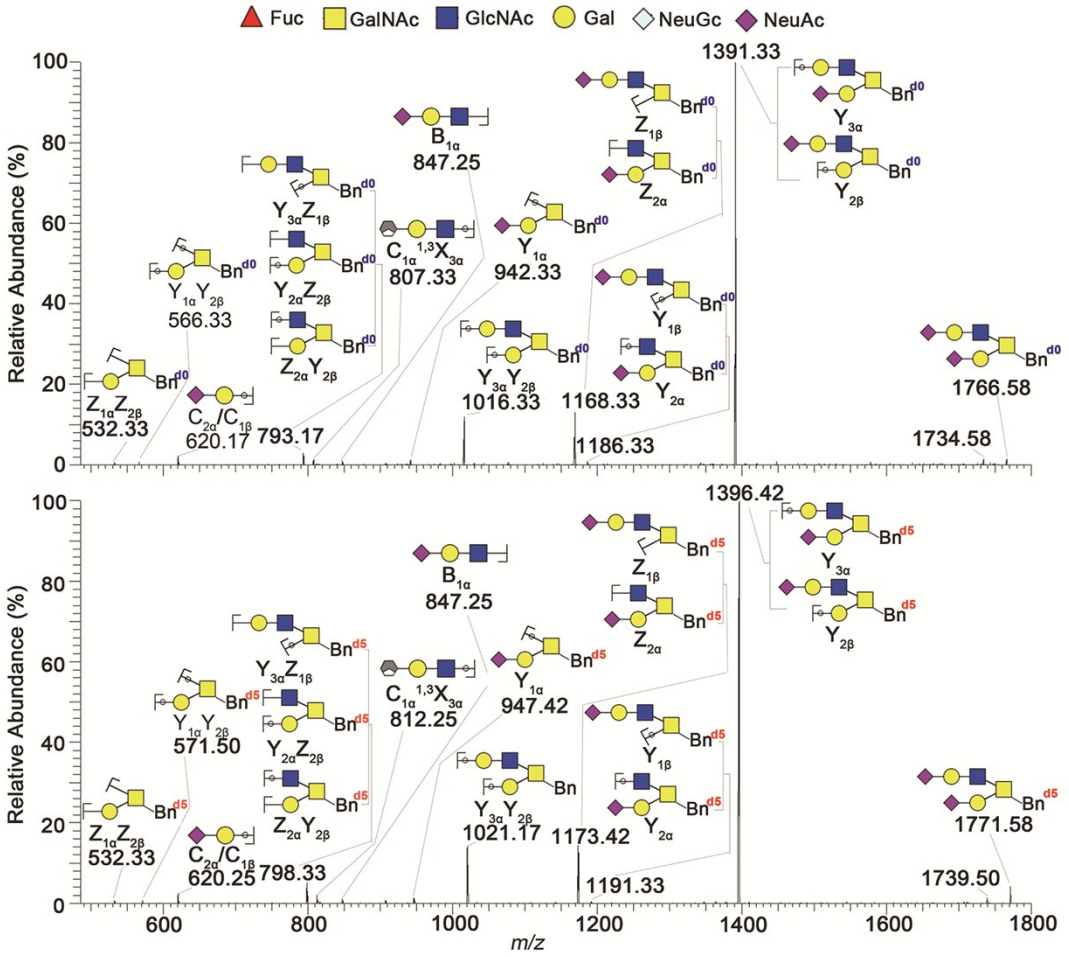


Fig.S 3H Sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1765.42/1770.50 was fragmented by CID, respectively.

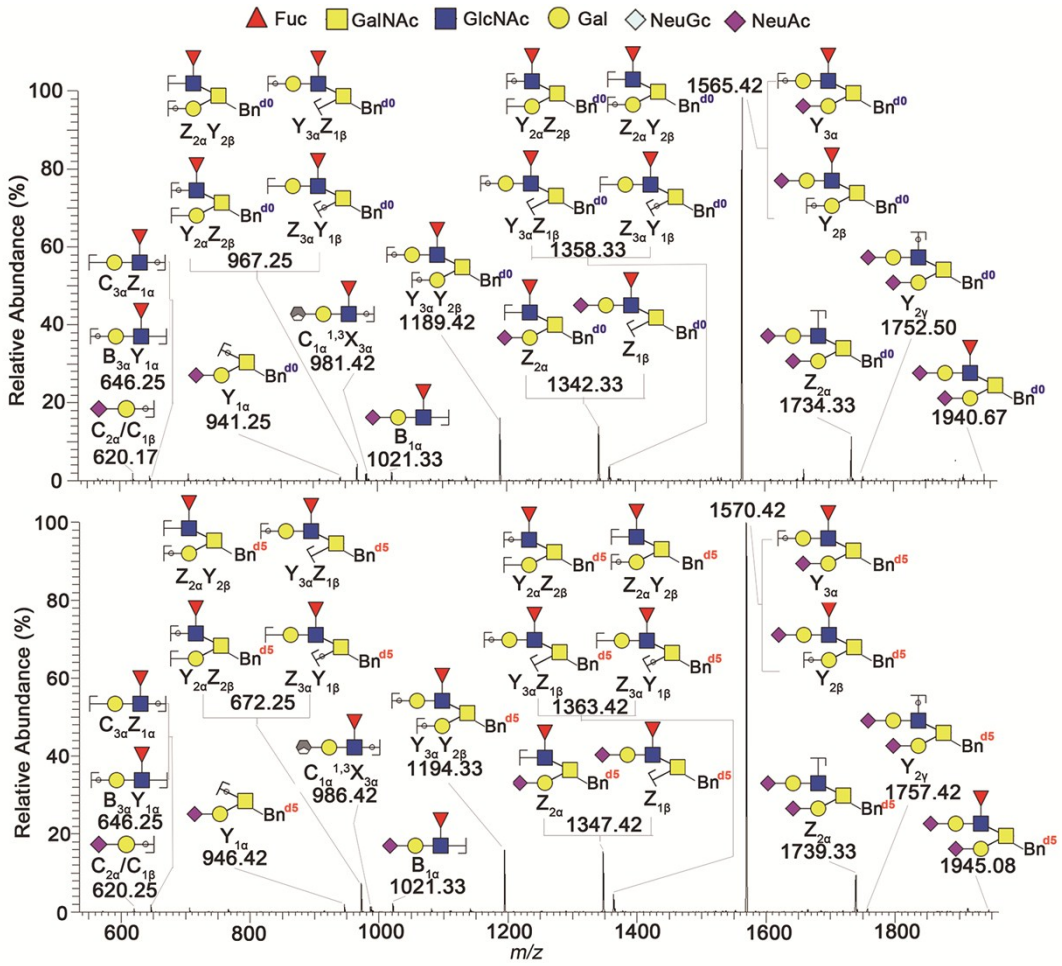


Fig.S 3I Sequencing of d0- and d5-Bn-labeled *O*-glycans from SMMC-7721 by ESI-MS/MS. Peaks at *m/z* 1939.33/1944.50 was fragmented by CID, respectively.

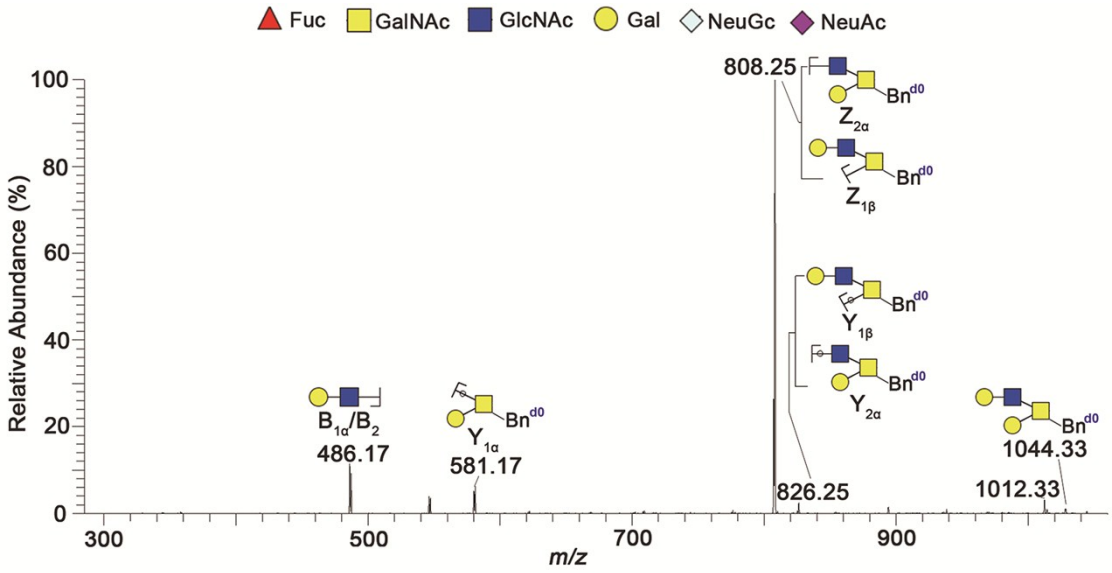


Fig.S 4A Sequencing of d0-Bn-labeled *O*-glycans from L02 by ESI-MS/MS. Peaks at *m/z* 1043.33 was fragmented by CID.

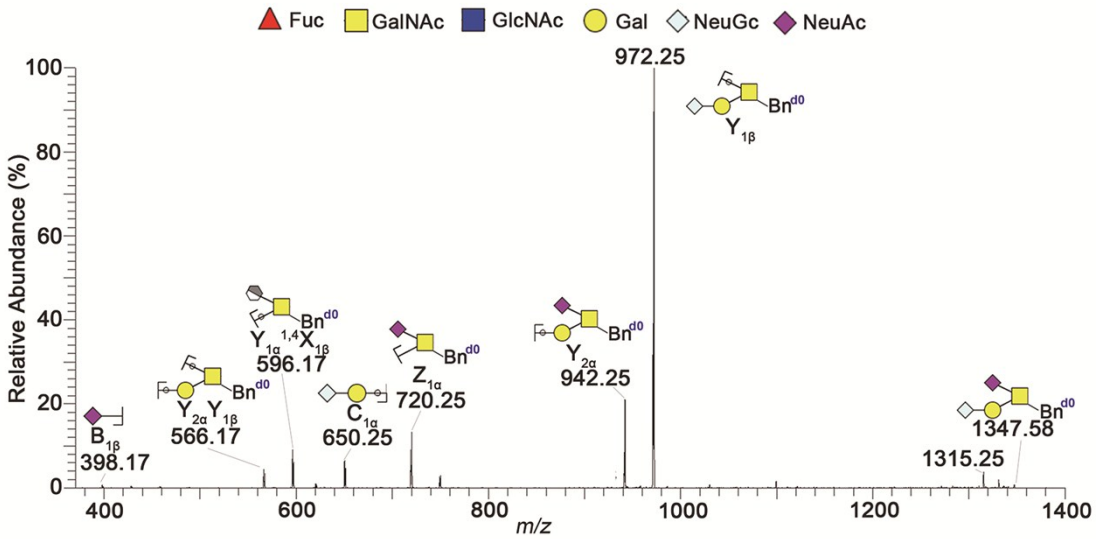


Fig.**S 4B** Sequencing of d0-Bn-labeled *O*-glycans from L02 by ESI-MS/MS. Peaks at *m/z* 1346.33 was fragmented by CID.

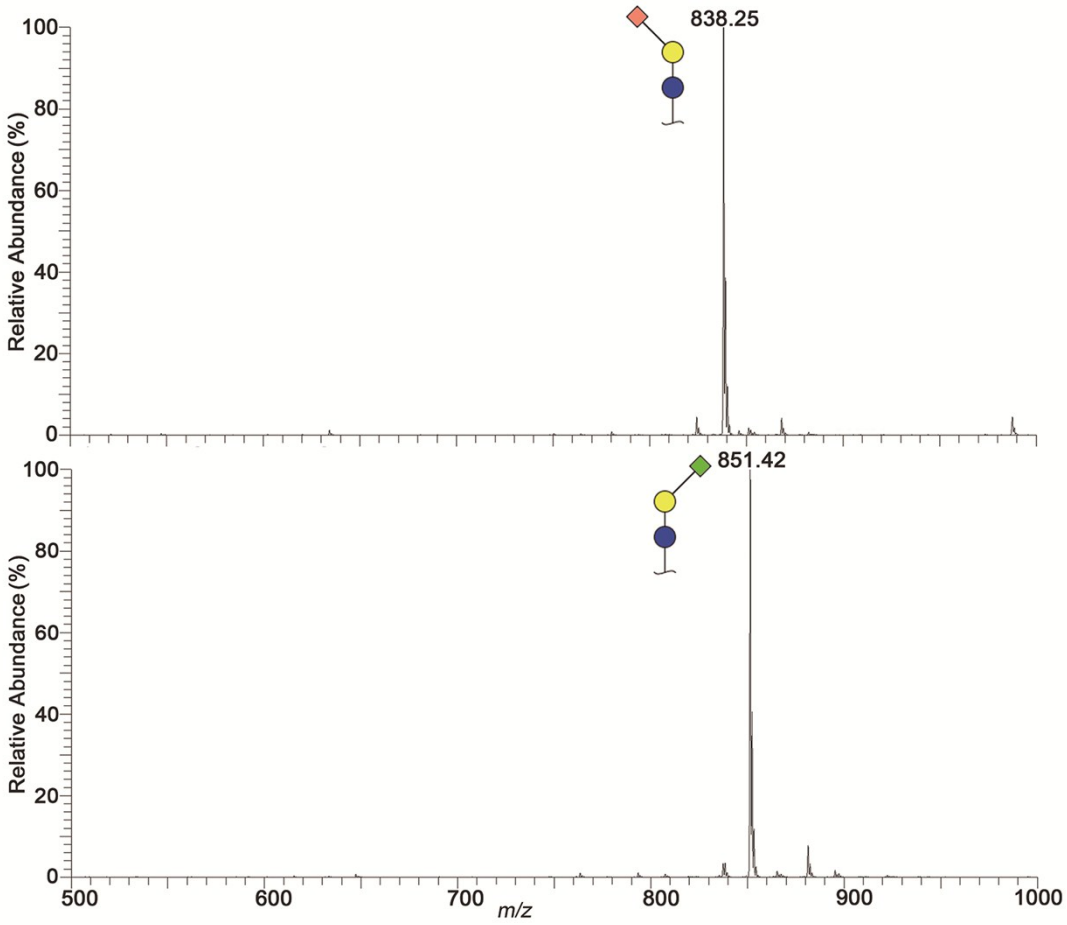
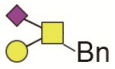
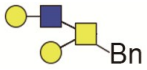
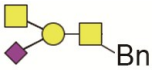
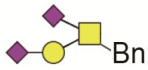
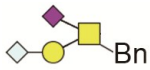
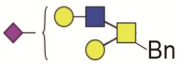
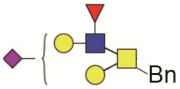
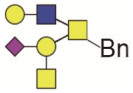
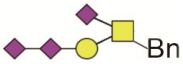
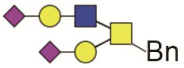
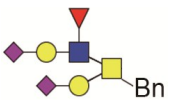


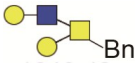
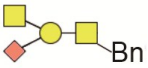
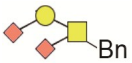
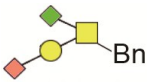
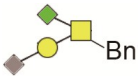
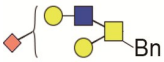
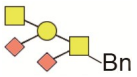
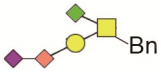
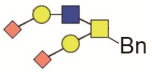
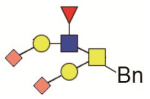
Fig.S 5 ESI-MS spectra of α 2,3/α 2,6-Neu5Ac-Lac when α 2,6-Neu5Ac-Lac after dimethylamidated. Reaction condition, 1 h, 60°C, in the presence of EDC + HOBt+dimethylamine in DMSO. The α 2,3-Neu5Ac-Lac were lactonized. Permethylation was conducted for the dimethylamidated α 2,6-Neu5Ac-Lac and the lactonized α 2,3-Neu5Ac-Lac. With the two-step derivatization, a mass difference of 13 Da between α 2,3-sialic acid and α 2,6-sialic acid can be obtained.

Table S1. Comprehensive quali-quantitative profiling of Bn-*O*-glycome in L02 and SMMC-7721 cells by MS.

No.	Glycan composition	Permethylation of Bn-*O*-glycans		
		m/z ([M+Na]$^+$)	Intensity ratios[a] (Mean±SD, n=3)	Proposed structure
1	N1H1S1	955.25 (960.33)	1.26±0.11	
2	N2H2	1043.33	-	
3	N2H1S1	1200.33 (1205.33)	0.29±0.01	
4	N1H1S2	1316.33 (1321.33)	0.70±0.01	
5	N1H1S1G1	1346.33	-	
6	N2H2S1	1404.42 (1409.42)	2.72±0.63	
7	N2H1S2	(1566.42)	-	
8	N2H2F1S1	1578.17 (1583.25)	0.88±0.23	
9	N3H2S1	(1654.42)	-	
10	N1H1S3	(1682.33)	-	
11	N2H2S2	1765.42 (1770.50)	0.96±0.08	
12	N2H2F1S2	1939.33 (1944.50)	0.31±0.07	

Structure formulas: yellow square, N-acetylgalactosamine (GalNAc, N); blue square, N-acetylglucosamine (GlcNAc, N); yellow circle, galactose (Gal, H); red triangle, fucose (Fuc, F); purple diamond, sialic acid (Neu5Ac, S); white gray diamond, sialic acid (Neu5Gc, G). [a] Intensity ratios of Bn^{d0}-*O*-glycans to Bn^{d5}-*O*-glycans (n=3). – means *O*-glycans were detected only in L02 or SMMC-7721 cells.

Table S2. Comprehensive quali-quantitative descrimination linkage specificity of Bn-*O*-glycans with/without sialic acids ends in L02 and SMMC-7721 cells.

No.	Glycan composition	Two-step derivatization of Bn-*O*-glycans		Proposed structure
		m/z ([M+Na]$^+$)	Intensity ratios[a] (Mean±SD, n=3)	
1	N1H1S1	955.25 (960.33)	0.81±0.06	
		968,33 (973.42)	1.19±0.09	
2	N2H2	1043.42	-	
3	N2H1S1	1200.50 (1205.42)	0.38±0.10	
4	N1H1S2	1316.42 (1321.42)	1.35±0.14	
		1329.42 (1334.42)	0.84±0.01	
5	N1H1S1G1	1359.33	-	
6	N2H2S1	1404.42 (1409.42)	2.32±0.30	
7	N2H1S2	(1566.50)	-	
		(1579.50)	-	
8	N2H2F1S1	-	-	-
9	N3H2S1	(1654.42)	-	
10	N1H1S3	(1695.50)	-	
11	N2H2S2	1765.58 (1770.58)	1.07±0.14	
12	N2H2F1S2	1939.67 (1944.67)	0.81±0.17	

Structure formulas: yellow square, N-acetylgalactosamine (GalNAc); blue square, N-acetylglucosamine (GlcNAc);

yellow circle, galactose (Gal); red triangle, fucose (Fuc); gray diamond, α2,3-Neu5Gc; pink diamond, α2,3-Neu5Ac; green diamond, α2,6-Neu5Ac; purple diamond, α2,8-Neu5Ac. [a] Intensity ratios of Bn^{d0}-*O*-glycans to Bn^{d5}-*O*-glycans (n=3). – means *O*-glycans were detected only in L02 or SMMC-7721 cells.