Supplementary mass spectrometric data to Figure 2

Fraction No.	[M-H] _{obs}	[M-H] _{calc}	Adduct	Assignment
1	1508	1508	DHB	Hex5HexNAc2
	1670	1670	DHB	Hex ₆ HexNAc ₂
	1735	1736	DHB	Fuc ₁ Hex ₃ HexNAc ₄
	1831	1832	DHB	Hex ₇ HexNAc ₂
	1898	1898	DHB	Fuc ₁ Hex ₄ HexNAc ₄
	1915	1914	DHB	Hex₅HexNAc₄
	1994	1994	DHB	Hex ₈ HexNAc ₂
	2061	2060	DHB	Fuc, Hex-HexNAc4
	2280	2279	DHB	Hex ₆ HexNAc ₅
	2426	2425	DHB	Fuc, Hex HexNAc
	2636	2636		FuciHex-HexNAc
	3156	3155	DHB	Fuc, Hex, HexNAc,
2	2050	2051	DIID	Neu Ac. Hey. Hey. NAc.
	2000	2051		NeuAc, Fuc, Hey, HeyNAc, *
	2190	2197		NouCo Fuo Hoy HoyNAo
	2212	2213		NeuOcirucinex5nexnAc4
	2416	2416		$NeuAc_1Hex_6HexNAc_5$
	2562	2562		$NeuAc_1Fuc_1Hex_6HexNAc_5$
	2577	2578		NeuGc ₁ Fuc ₁ Hex ₆ HexNAc ₅
	2781	2781		$NeuAc_1Hex_7HexNAc_6$
	2927	2927		$NeuAc_1Fuc_1Hex_7HexNAc_6$
	2944	2943		NeuGc ₁ Fuc ₁ Hex ₇ HexNAc ₆
	3292	3292		NeuAc ₁ Fuc ₁ Hex ₈ HexNAc ₇
3	2927	2927		NeuAc1Fuc1Hex7HexNAc6
	3072	3072		NeuAc ₂ Hex ₇ HexNAc ₆
	3218	3218		NeuAc2Fuc1Hex7HexNAc6*
	3234	3234		NeuAc1NeuGc1Fuc1Hex7HexNAc6
	3583	3583		NeuAc ₂ Fuc ₁ Hex ₈ HexNAc ₇
4	1595	1596		Phos ₁ Hex ₆ HexNAc ₂ *
	2050	2051		NeuAc1Hex5HexNAc4
	2342	2342		NeuAc ₂ Hex ₅ HexNAc ₄ *
	2416	2416		NeuAc1Hex6HexNAc5
	2488	2488		NeuAc ₂ Fuc ₁ Hex ₅ HexNAc ₄ *
	2504	2504		NeuAc ₁ NeuGc ₁ Fuc ₁ Hex ₅ HexNAc ₄
	2707	2707		NeuAc2Hex6HexNAc5*
	2853	2853		NeuAc ₂ Fuc ₁ Hex ₆ HexNAc ₅
	2869	2869		NeuGc, NeuAc, Fuc, Hex/Hex/Ac.
	3072	3072		NeuAc ₂ Hex ₇ Hex _{NAc}
	3218	3218		Neu AczFuc Hey-HeyNAcz*
5	3584	3583		Neu Ac-Fuc-Hey-Hey-NAc-
	3720	3728		Neu Ac Hey Hey NAc
	3975	3974		NouA a Fua Hay HayNA a *
	2801	2800		NeuCo Neu Ao Euo Hey Hey NAC
6 7 8	2019	3890		New A - Eve Here HereNA -
	3218	3218		$NeuAc_2Fuc_1Hex_7Hex_NAc_6$
	3363	3363		NeuAc ₃ Hex ₇ HexNAc ₆
	3509	3509		NeuAc ₃ Fuc ₁ Hex ₇ HexNAc ₆ *
	3526	3525		$NeuGc_1NeuAc_2Fuc_1Hex_7HexNAc_6$
	2707	2707		$NeuAc_2Hex_6HexNAc_5$
	2999	2998		NeuAc ₃ Hex ₆ HexNAc ₅ *
	3145	3144		NeuAc ₃ Fuc ₁ Hex ₆ HexNAc ₅
	2633	2633		NeuAc ₃ Hex ₅ HexNAc ₄
	2998	2998		NeuAc ₃ Hex ₆ HexNAc ₅
	4531	4531		NeuAc ₄ Fuc ₁ Hex ₉ HexNAc ₈
9	3000	2998		NeuAc ₃ Hex ₆ HexNAc ₅
	4166	4166		NeuAc ₄ Fuc ₁ Hex ₈ HexNAc ₇ *
10	3510	3509		NeuAc ₃ Fuc ₁ Hex ₇ HexNAc ₆
	3654	3654		NeuAc ₄ Hex ₇ HexNAc ₆
	3800	3800		NeuAc ₄ Fuc ₁ Hex ₇ HexNAc ₆ *
	3816	3816		NeuGc ₁ NeuAc ₃ Fuc ₁ Hex ₇ HexNAc ₄
11	2998	2998		NeuAc ₂ Hex ₆ Hex ₈ NAc ₅
	3289	3289		NeuAc.Hex.HexNAc.*
	3306	3305		NeuGo NeuAco Hex/Hex/Aco
	2230	2200		

The peak fractions 1-11 collected from the ion exchange chromatography of 2-aminobenzamide-labeled Nglycans shown in **Fig. 2A** were analyzed by MALDI-TOF MS in negative polarity linear mode. The assignment of glycan compositions is based on comparison of the observed monoisotopic masses [M-H]⁻_{obs} with monoisotopic masses calculated from N-linked oligosaccharide structures commonly found on glycoproteins expressed in wild-type Chinese hamster ovary cells ([M-H]⁻_{calc}; NeuAc, N-acetylneuraminic acid; NeuGc, Nglycolylneuraminic acid; Fuc, fucose; Hex, hexose; HexNAc, N-acetylhexosamine). The major glycan species present in each peak fraction as revealed by MALDI-TOF MS (which are schematically shown in **Fig. 2B**) are highlighted by bold letters and asterisks designate glycans whose number of terminal negatively charged substituents was confirmed by MALDI-TOF/TOF tandem mass spectrometry. Most of the neutral N-glycans contained in fraction No. 1 were found as negatively charged, non-covalent matrix (2,5-dihydrobenzoic acid; DHB) adducts of the type [M+DHB-H]⁻ as frequently observed for neutral oligosaccharides composed of more than four monosaccharides with underivatized OH groups (Mele and Malpezzi, 2000).

Mele, A. and Malpezzi, L. (2000) Noncovalent association phenomena of 2,5-dihydroxybenzoic acid with cyclic and linear oligosaccharides. A matrix-assisted laser desorption/ionization time-of-flight mass spectrometric and X-ray crystallographic study. J Am Soc Mass Spectrom, 11, 228-236.