

Supplementary information

Isomeric Separation of Permethylated Glycans by Extra-Long Reversed-Phase Liquid Chromatography (RPLC)-MS/MS

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Figure S1. EICs of permethylated *N*-glycan standards with the composition of HexNAc₄Hex₅NeuAc₁, analyzed using the 500 mm PepMap C18 column with total elution time of (A) 120 min, (B) 180 min, and (C) 200 min. Symbols: ■, N-acetylglucosamine (GlcNAc); ●, Galactose (Gal); ▼, Fucose (Fuc); ●, Mannose (Man); ●, Glucose (Glc); ◆, N-acetylneuraminic acid (NeuAc/Sialic Acid).

Figure S2. (A) TIC of *N*-glycan sample derived from bovine fetuin. (B-D) EICs of permethylated *N*-glycans with sialic acid linkage isomers with the compositions of (B) HexNAc₅Hex₆NeuAc₂, (C) HexNAc₅Hex₆NeuAc₃, and (D) HexNAc₅Hex₆NeuAc₄. (E-G) Distributions of isomers from NMR (blue) and LC/MS (orange). Symbols: see **Figure S1**.

Figure S3. TIC and EICs of permethylated *N*-glycans with high mannose structures which were derived from ribonuclease B. Symbols: see **Figure S1**.

Figure S4. TIC and EICs of permethylated *N*-glycans with sialic acid linkage isomers which were released from human blood serum. Symbols: see **Figure S1**.

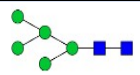
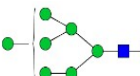
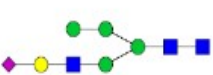
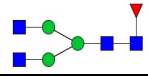

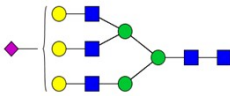


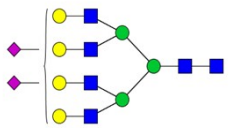
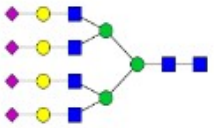
Figure S5. TIC and EICs of permethylated glycans which were released from breast cancer cell line MDA-MB-231BR. Symbols: see **Figure S1**.

Figure S6. TIC and EICs of permethylated glycans which were released from brain cancer cell line CRL-1620. Symbols: see **Figure S1**.

Table S1. Run-to-run variations in 500mm PepMap C18 column from glycans derived from bovine fetuin. (n=3)

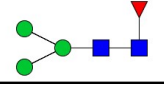
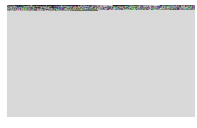
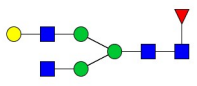
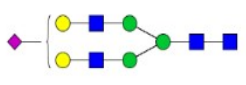
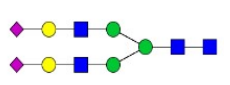
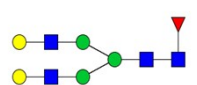
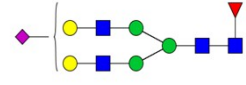
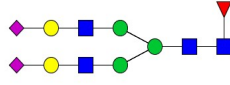
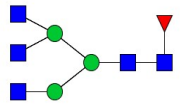
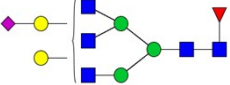
Structure	Average RT(minutes)	Average FWHM(minutes)
	89.8±0.1	0.62±0.02
	91.4±0.1	0.62±0.03
	99.5±0.1	0.75±0.04
	101.0±0.1	0.80±0.01
	102.9±0.1	0.95±0.06
	104.0±0.1	0.91±0.08
	106.5±0.1	0.83±0.02
	109.0±0.1	0.82±0.08
	110.0±0.1	0.87±0.03
	111.8±0.1	0.80±0.01
	113.2±0.1	0.82±0.05
	115.0±0.2	0.85±0.01
	117.7±0.2	1.05±0.01
	120.4±0.1	1.04±0.03
	125.9±0.2	0.86±0.01
	128.3±0.1	0.98±0.02
	132.6±0.1	0.97±0.03

Table S2. Run-to-run variations in 500mm PepMap C18 column from glycans derived from

Structure	Average RT(minutes)	Average FWHM(minutes)
	85.9±0.1	0.71±0.01
	114.6±0.1	0.96±0.03
	105.0±0.1	1.17±0.02
	107.8±0.2	1.06±0.04
	86.5±0.1	0.74±0.01
	104.2±0.2	1.20±0.01
	106.8±0.1	1.12±0.07
	111.0±0.1	1.10±0.05
	117.6±0.1	1.22±0.07
	122.9±0.1	1.38±0.03
	125.9±0.1	1.22±0.03
	130.4±0.3	1.78±0.03
	131.8±0.2	1.50±0.02
	133.6±0.2	1.27±0.06
	134.2±0.2	1.34±0.04
	136.6±0.1	1.24±0.04
	140.2±0.1	1.25±0.06
	135.7±0.1	1.37±0.06
	138.1±0.2	1.24±0.04
	157.6±0.2	1.27±0.08
	159.9±0.2	1.18±0.03
	162.5±0.2	1.19±0.08

human blood serum. (n=3)

Table S3. Run-to-run variations in 500mm PepMap C18 column from glycans derived from

Structure	Average RT(minutes)	Average FWHM(minutes)
	79.9±0.1	0.74±0.01
	87.1±0.1	0.83±0.04
	92.6±0.1	1.05±0.01
	93.6±0.1	0.88±0.01
	93.6±0.1	1.28±0.04
	101.2±0.1	0.78±0.02
	113.2±0.1	0.92±0.02
	115.6±0.1	0.86±0.01
	125.9±0.2	1.08±0.01
	127.9±0.2	1.00±0.01
	130.4±0.3	0.92±0.01
	132.2±0.2	0.79±0.01
	100.6±0.1	0.75±0.04
	108.8±0.1	0.77±0.02
	123.3±0.1	0.84±0.01
	126.2±0.1	0.91±0.01
	135.7±0.1	1.21±0.01
	138.2±0.1	1.08±0.02
	140.7±0.1	0.83±0.02
	92.8±0.1	0.95±0.02
	95.4±0.1	0.94±0.04
	101.1±0.1	1.07±0.02
	137.7±0.2	1.35±0.07
	141.4±0.2	1.18±0.01

cultured cells. (n=3)

Table S4. Day-to-day variations in 500mm PepMap C18 column from glycans derived from bovine fetuin. (n=3)






Structure	Average RT(minutes)	Average FWHM(minutes)
	89.5±0.3	0.66±0.02
	91.2±0.3	0.71±0.05
	99.3±0.3	0.74±0.01
	100.8±0.3	0.78±0.02
	102.7±0.3	1.1±0.1
	104.1±0.4	0.91±0.04
	106.3±0.3	0.86±0.01
	108.8±0.3	0.89±0.07
	109.7±0.3	0.95±0.03
	111.5±0.4	0.85±0.01
	113.0±0.3	0.72±0.08
	114.7±0.4	0.89±0.02
	117.5±0.4	1.07±0.02
	120.2±0.3	1.21±0.03
	125.7±0.4	0.82±0.01
	128.0±0.4	0.94±0.01
	132.3±0.3	1.01±0.06

Table S5. Month-to-month variations in 500mm PepMap C18 column from glycans derived from bovine fetuin. (n=3)

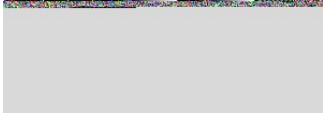




Structure	Average RT(minutes)	Average FWHM(minutes)
	89.2±0.6	0.68±0.01
	91.0±0.6	0.72±0.04
	98.9±0.8	0.78±0.06
	100.4±0.7	0.78±0.02
	102.3±0.7	1.1±0.1
	103.7±0.9	0.91±0.02
	105.9±0.8	0.87±0.03
	108.4±0.8	0.8±0.1
	109±1	0.8±0.1
	111±1	0.89±0.03
	113±1	0.82±0.03
	114±1	0.91±0.09
	117±1	1.18±0.01
	119.8±0.8	1.2±0.2
	125±1	0.86±0.06
	127.6±0.9	0.99±0.06
	131.9±0.9	1.02±0.08

Table S6. *N*-glycans with isomeric structures extracted from cultured cell lines. The mass accuracy of each identified structure and the retention time of isomeric peaks are provided. (n=3)


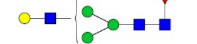

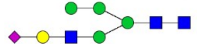

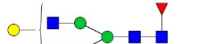
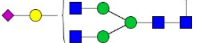
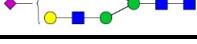

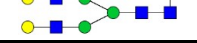
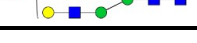
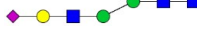

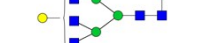

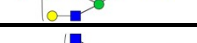
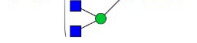
Glycan Structure	Theoretical <i>m/z</i>	Observed <i>m/z</i>	Mass Accuracy (ppm)	Retention Time (min)
	988.5204	988.5242	3.84	128.1±0.1
				131.2±0.1
	894.9782	894.9849	7.54	93.0±0.1
				101.0±0.1
	1075.5650	1075.5734	7.81	108.9±0.1
				110.4±0.1
				112.0±0.1
	1090.5703	1090.5791	8.07	114.0±0.1
				116.6±0.1
	915.4914	915.4976	6.77	87.0±0.1
				92.5±0.1
				93.6±0.1
	1017.5413	1017.5472	5.80	93.5±0.1
				101.2±0.1
	799.0881	799.0945	8.05	115.9±0.1
				118.8±0.1
	809.0916	809.0972	6.92	113.1±0.1
				115.6±0.1
	929.4828	929.4871	4.59	126.4±0.1
				128.4±0.2
				130.9±0.1
	1119.5912	1119.5983	6.34	100.5±0.1
				108.7±0.1
	1300.1774	1300.1882	8.31	123.3±0.1
				126.1±0.1
	1480.7642	1480.7747	7.09	135.7±0.1
				138.2±0.1
				140.7±0.1
	1038.0546	1038.0607	5.92	92.8±0.1
				95.4±0.1
				100.9±0.2
	1140.1045	1140.1121	6.71	117.5±0.1
				118.8±0.1
	1079.2249	1079.2329	7.44	134.8±0.2
				137.8±0.1
	1016.8634	1016.8702	6.72	129.6±0.1
				131.9±0.1
				123.6±0.1
	962.5056	962.5123	6.96	125.2±0.1
				127.7±0.1

Figure S1

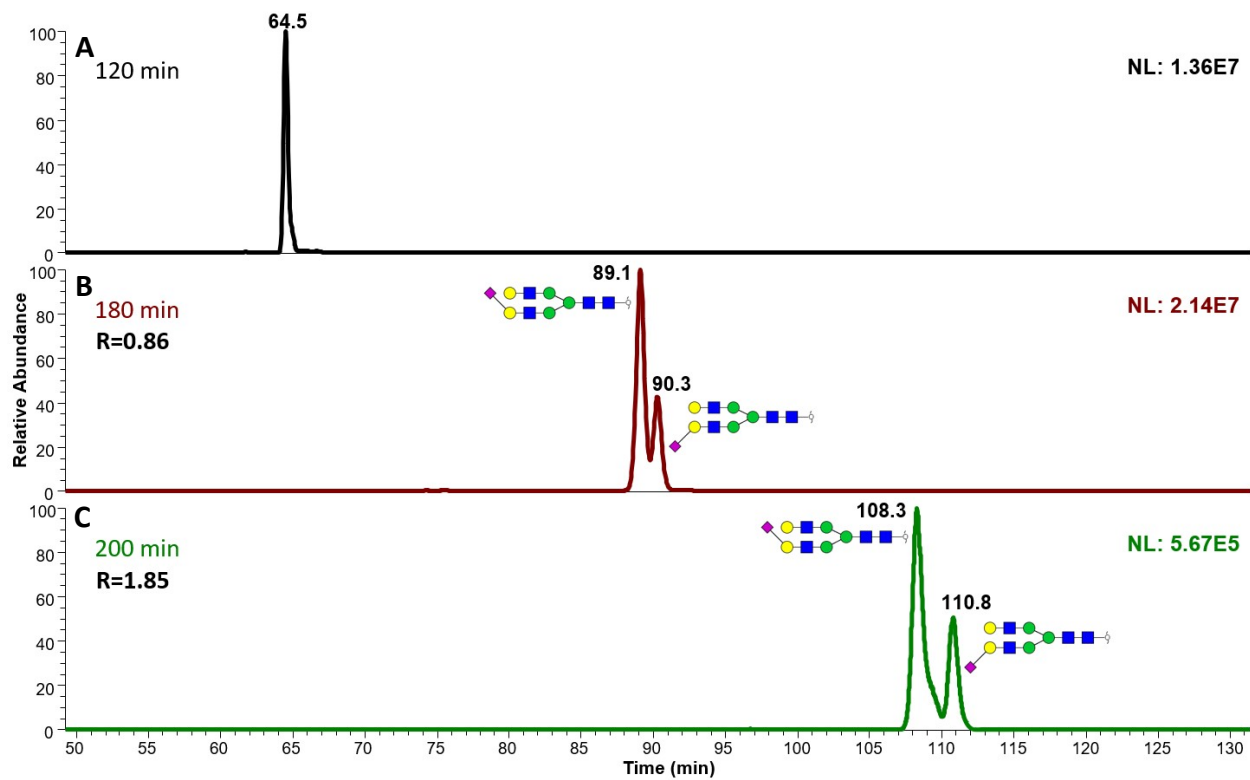


Figure S2

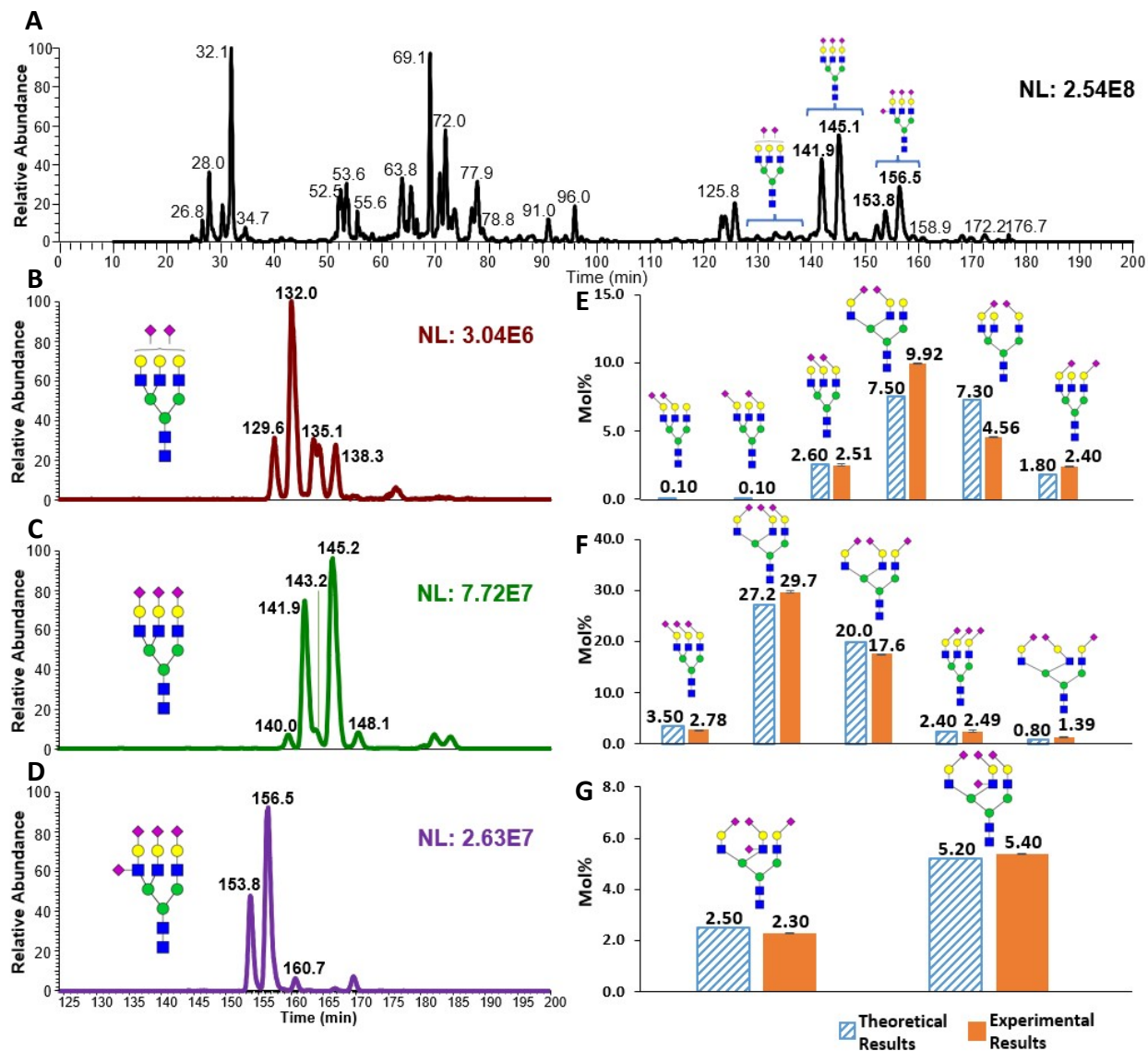


Figure S3

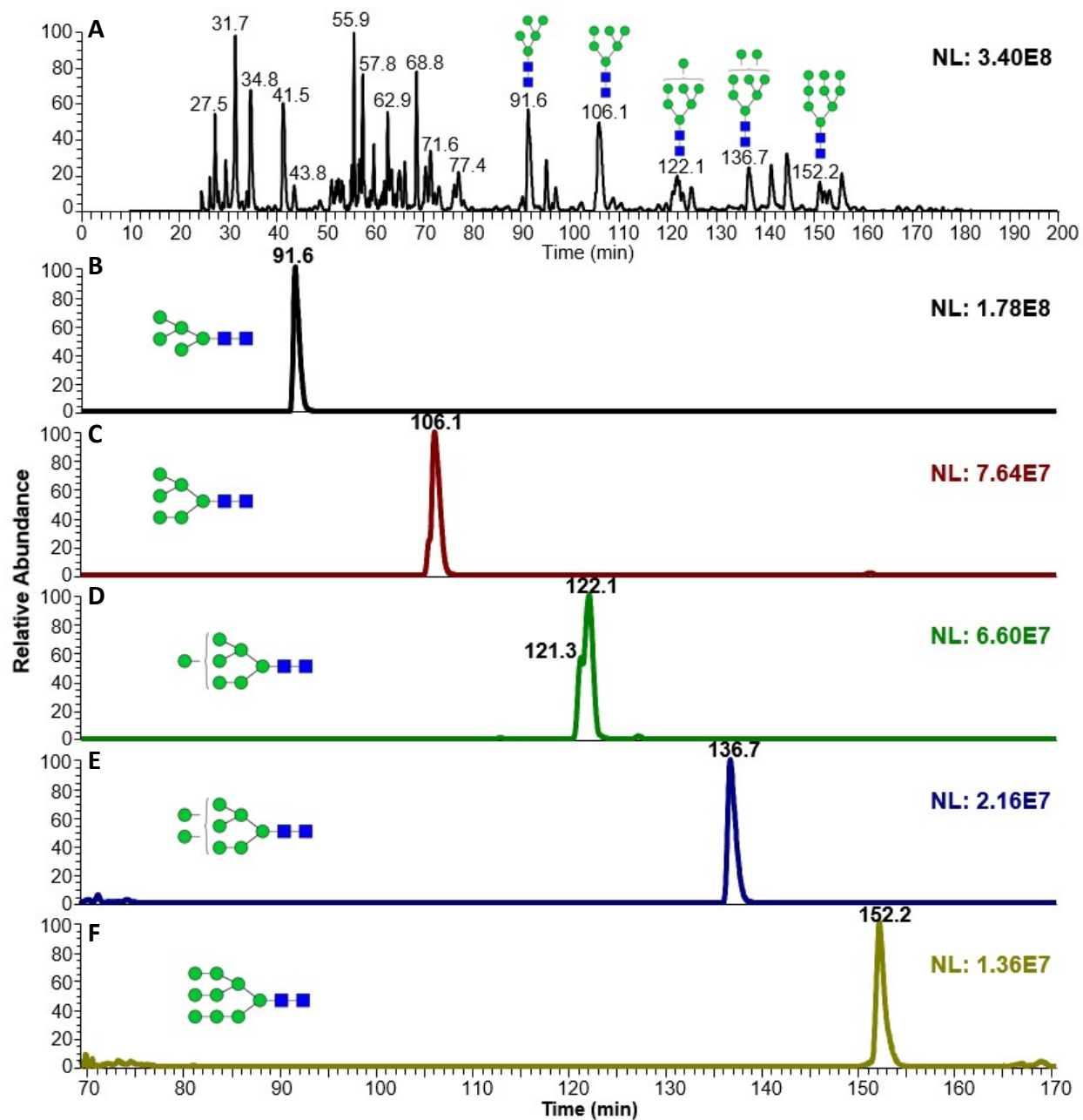


Figure S4

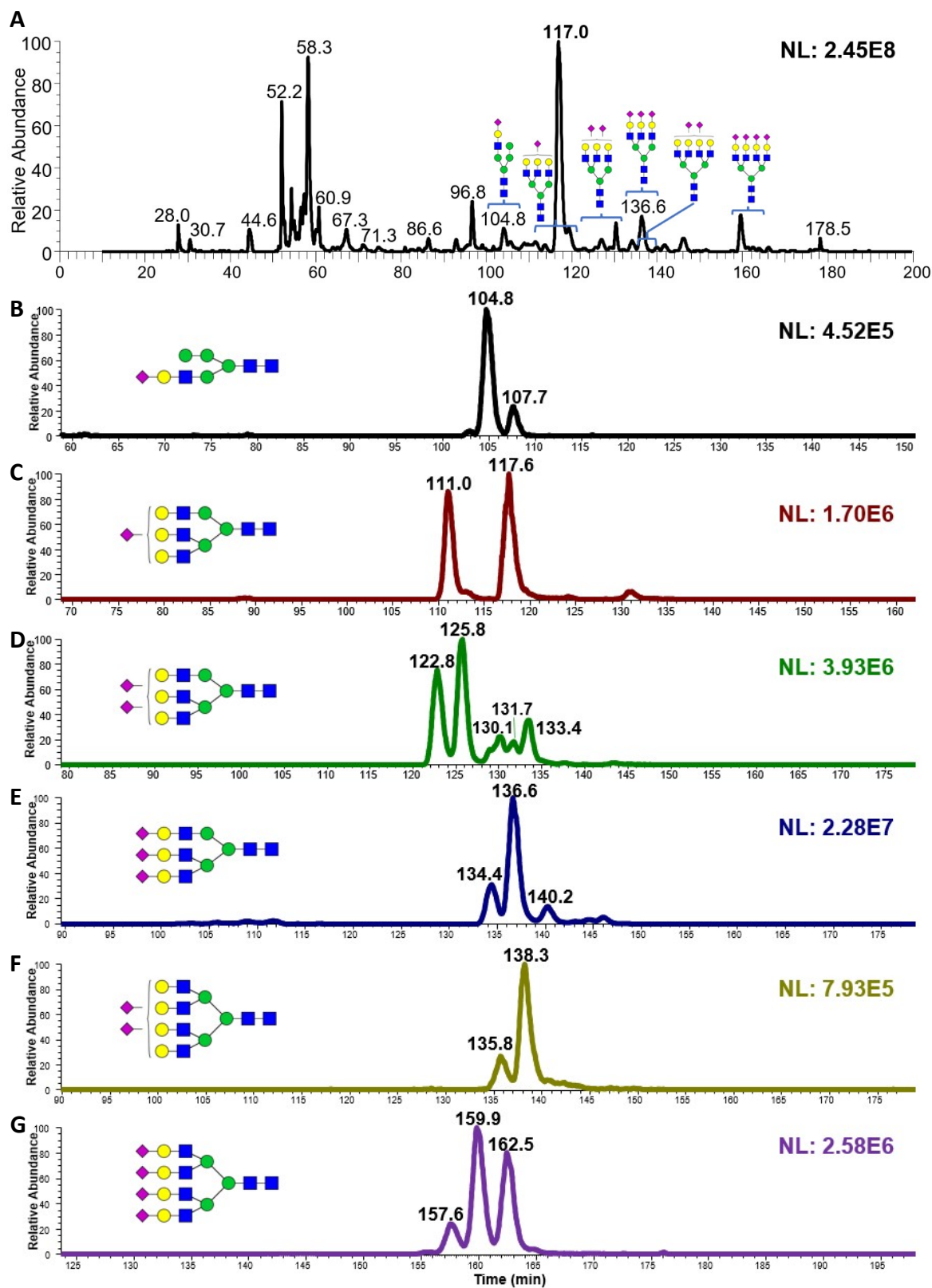


Figure S5

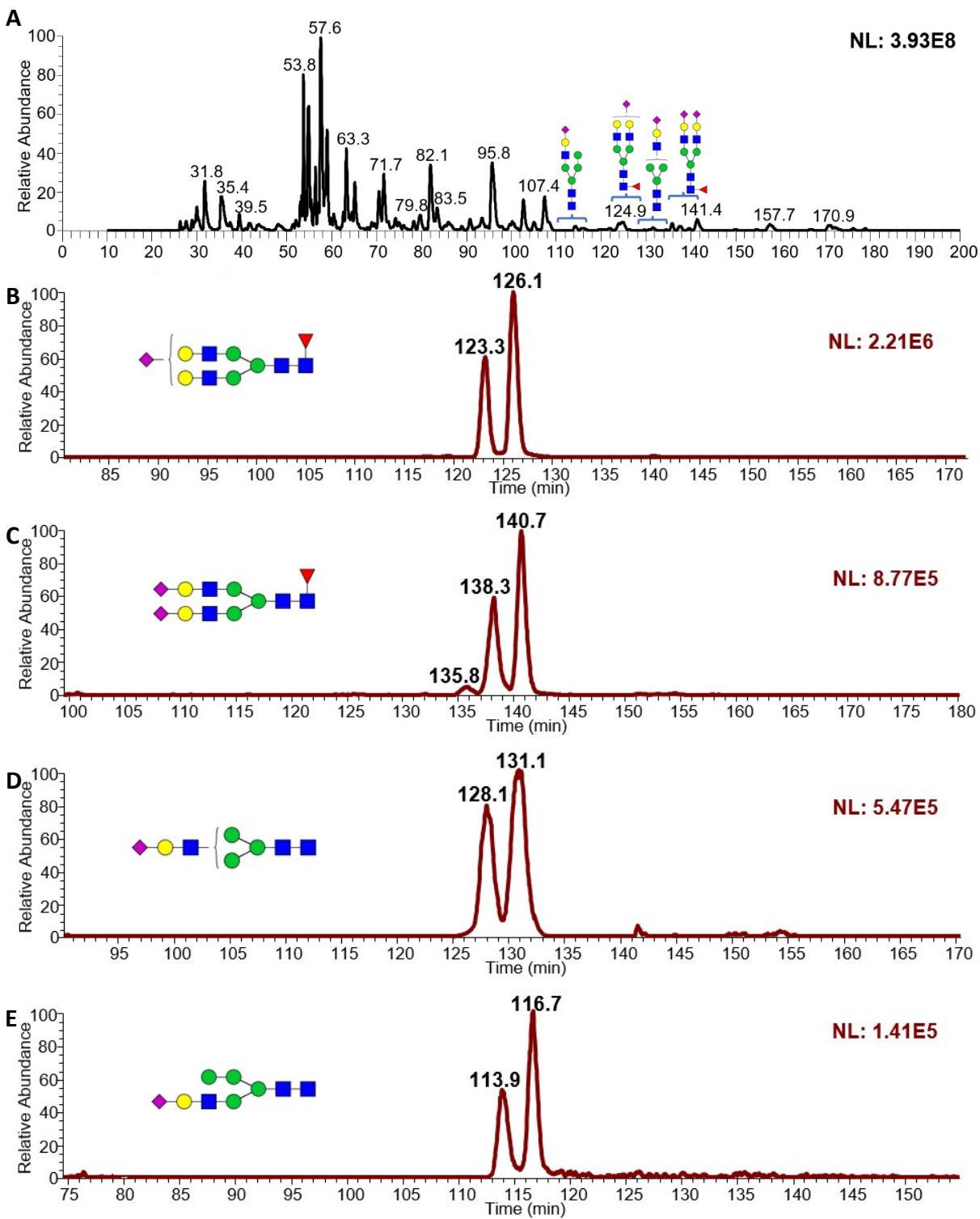


Figure S6

