Electronic Supplementary Information

Microwave-assisted Kochetkov amination followed by permanent charge derivatization: A facile strategy for glycomics

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Experimental section

Materials and chemicals

α-Cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), anhydrous DMSO, tris(2,4,6-trimethoxyphenyl)phosphine, bromoacetic acid N-hydroxysuccinimide ester, ribonuclease B from bovine pancreas (RNase B), albumin from chicken egg white (ovalbumin), asialofetuin from fetal calf serum Type I (A-fetuin) were obtained from Sigma-Aldrich (St. Louis, MO). PGC cartridges were obtained from Alltech Associates, Inc. (Deerfield, IL). Peptide-N-glycosidase F (PNGase F) was from Roche Diagnostics (Indianapolis IN). Methanol, acetonitrile (MeCN) and trifluoroacetic acid (TFA) were purchased from Burdick & Jackson (Muskegon, MI).

TMPP-Ac-OSu was prepared by reacting tris(2,4,6-trimethoxyphenyl)phosphine with bromoacetic acid N-hydroxysuccinimide ester in toluene for 30 min at room temperature. Working concentration at 10 mM of TMPP-Ac-OSu was prepared in anhydrous DMSO and can be stored at 4 ºC for at least 3 months without degradation.

All aqueous solutions were prepared using water purified with a Milli-Q purification system (Millipore, Bedford, MA).

N-glycan preparation

Glycoproteins (0.1 mg) were dissolved in 50 µL sodium phosphate (20 mM pH 7.5) containing 0.2% SDS and 0.1 M DTT and denatured at 100ºC for 10 min. After cooling, 10% NP-40 (12 µL) was added. The reaction mixture was incubated with PNGase F (10 units) for 24 h at 37ºC. The samples were then boiled for 5 min to stop the reaction the released oligosaccharides were purified using PGC cartridges.

PGC cartridges were washed with 3.0 mL of 80% (v/v) MeCN containing 0.1% TFA followed by 3.0 mL water. The oligosaccharides released by PNGase F were loaded on PGCs and then washed with water (3.0 mL) to remove buffer and salts.

Oligosaccharides were eluted with 25% MeCN in 0.1% TFA and dried for further analysis or processing.

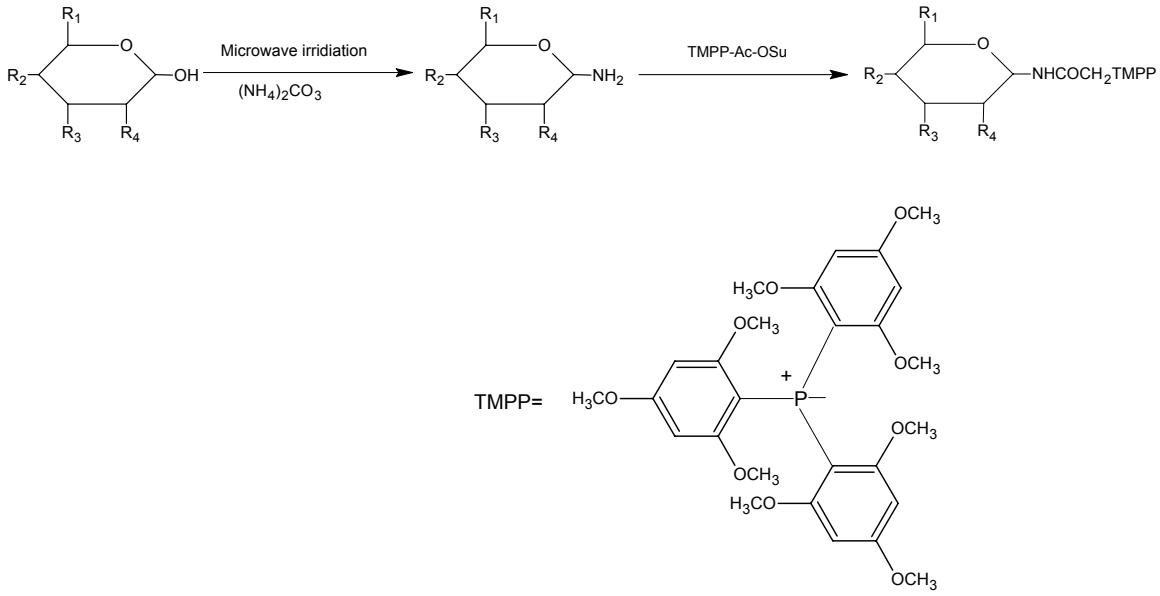
Glycan derivatization

Dried oligosaccharides were dissolved in 100 μL of saturated ammonium carbonate DMSO solution and incubate at 45 ºC for 30 min under microwave irradiation (CEM Discovery system, Matthews, NC, USA). The output of microwave power was set as 40 W. After microwave irradiation, 20 μL of 10 mM TMPP-Ac-OSu DMSO solution and 10 μL DIEA were added. The mixtures were vortexed and then stand at room temperature for 60 min, followed by the purification with cellulose beads.

MALDI-TOF MS

Mass spectra were acquired using 4800 MALDI-TOF/TOF (Applied Biosystems/MDS Sciex, Concord, Canada) equipped with an Nd:YAG laser with 355 nm wavelength of <500 ps pulse and 200 Hz repetition rate. The spectrometer was operated in the positive reflectron mode. The spectra were accumulated by 1000 laser shots.

The samples were loaded onto MALDI target in 1 μL water and mixed with 1 μL fleshly made matrices and allowed to dry in a gentle stream of air.



Scheme S1.

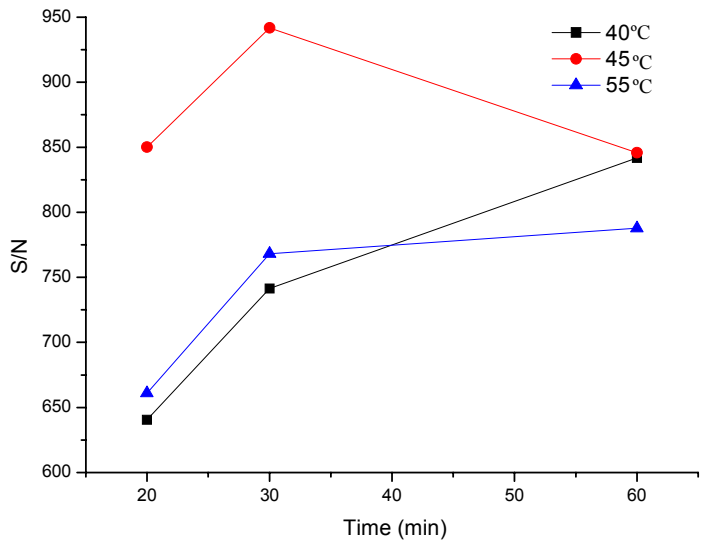


Figure S1. Effect of microwave irradiation temperature and reaction time on the S/N ratios of TMPP-Ac-maltoheptaose.

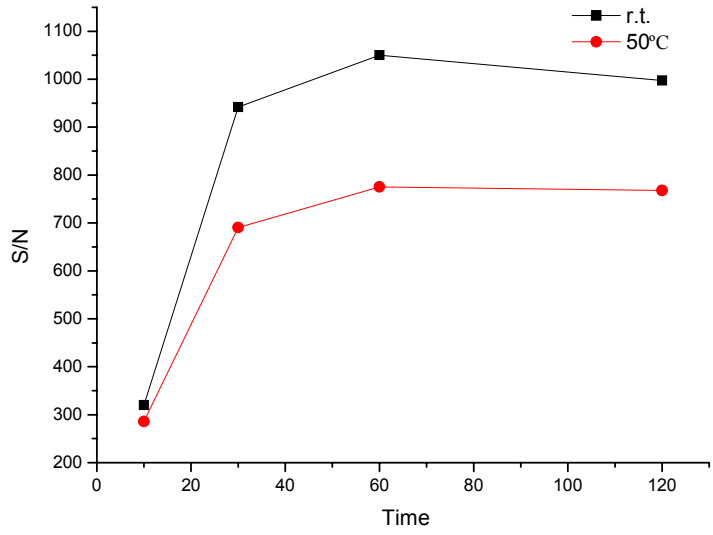


Figure S2. Effect of temperature and reaction time of TMPP-OSu derivatization on S/N ratios of TMPP-Ac-maltoheptaose.

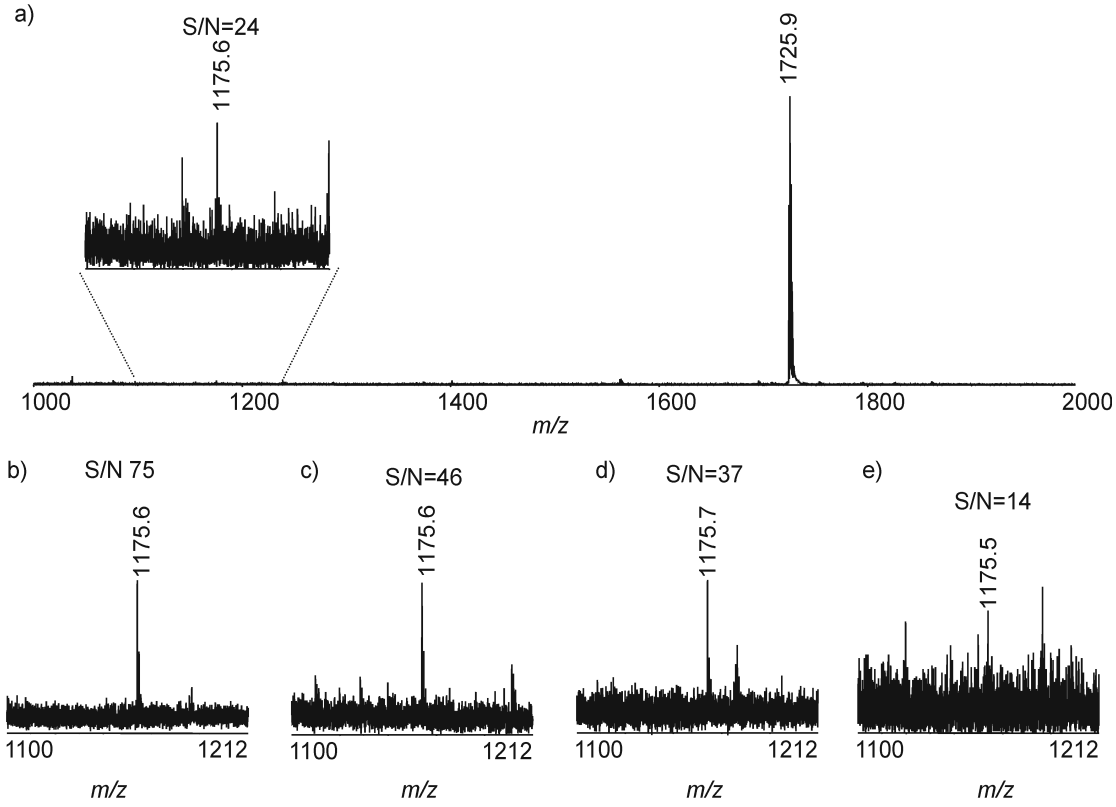


Figure S3. MALDI-TOF spectra of derivatized and underivatized maltoheptaose samples with different sample loadings. (a). TMPP-derivatized, 1 pmol equivalent to maltoheptaose ; native maltoheptaose: (b) 400 fmol; (c) 300 fmol; (d) 200 fmol; (e) 100 fmol. All MS spectra were acquired with DHB as a matrix. By comparing the S/N ratios of the ions at *m/z* 1175.6 in each MS spectrum, we obtained the derivatization efficiency in the range of 80-90%.

Table S1. Detected glycoforms in the N-glycan sample released from chicken ovalbumin

No#	Native oligosaccharides (Da)		TMPP-Ac-derivatives (Da)		Chemical compositions
	Calc.	Obs.	Calc.	Obs.	
1	933.1	-	1482.5	1482.6	$Hex_3HexNAc_2$
2	1095.4	-	1644.6	1644.6	$Hex_4HexNAc_2$
3	1136.4	-	1685.6	1685.7	$Hex_3HexNAc_3$
4	1257.4	1257.5	1806.6	1806.7	$Hex_5HexNAc_2$
5	1298.5	-	1847.7	1847.7	$Hex_4HexNAc_3$
6	1339.5	1339.5	1888.7	1888.7	$Hex_3HexNAc_4$
7	1419.5	1419.5	1968.7	1968.7	$Hex_6HexNAc_2$
8	1460.5	-	2009.7	2009.7	$Hex_5HexNAc_3$
9	1501.5	1501.5	2050.7	2050.8	$Hex_4HexNAc_4$
10	1542.6	1543.6	2091.8	2091.8	$Hex_3HexNAc_5$
11	1581.5	-	2130.7	2130.8	$Hex_7HexNAc_2$
12	1622.6	-	2171.8	2171.8	$Hex_6HexNAc_3$
13	1663.6	1663.6	2212.8	2212.8	$Hex_5HexNAc_4$
14	1704.6	1704.6	2253.8	2253.9	$Hex_4HexNAc_5$
15	1745.6	1745.6	2294.8	2294.9	$Hex_3HexNAc_6$
16	1866.7	1866.6	2415.9	2415.9	$Hex_5HexNAc_5$
17	1907.7	-	2456.9	2457.0	$Hex_4HexNAc_6$
18	1948.7	1948.7	2497.9	2498.0	$Hex_3HexNAc_7$
19	2028.7	-	2577.9	2578.0	$Hex_6HexNAc_5$
20	2069.7	-	2618.9	2619.0	$Hex_5HexNAc_6$
21	2110.8	-	2660.0	2660.0	$Hex_4HexNAc_7$
22	2151.8	2151.7	2701.0	2701.1	$Hex_3HexNAc_8$
23	2272.8	-	2822.0	2822.1	$Hex_5HexNAc_7$
24	2313.9	-	2863.1	2863.1	$Hex_4HexNAc_8$
25	2475.9	-	3025.1	3025.2	$Hex_5HexNAc_8$