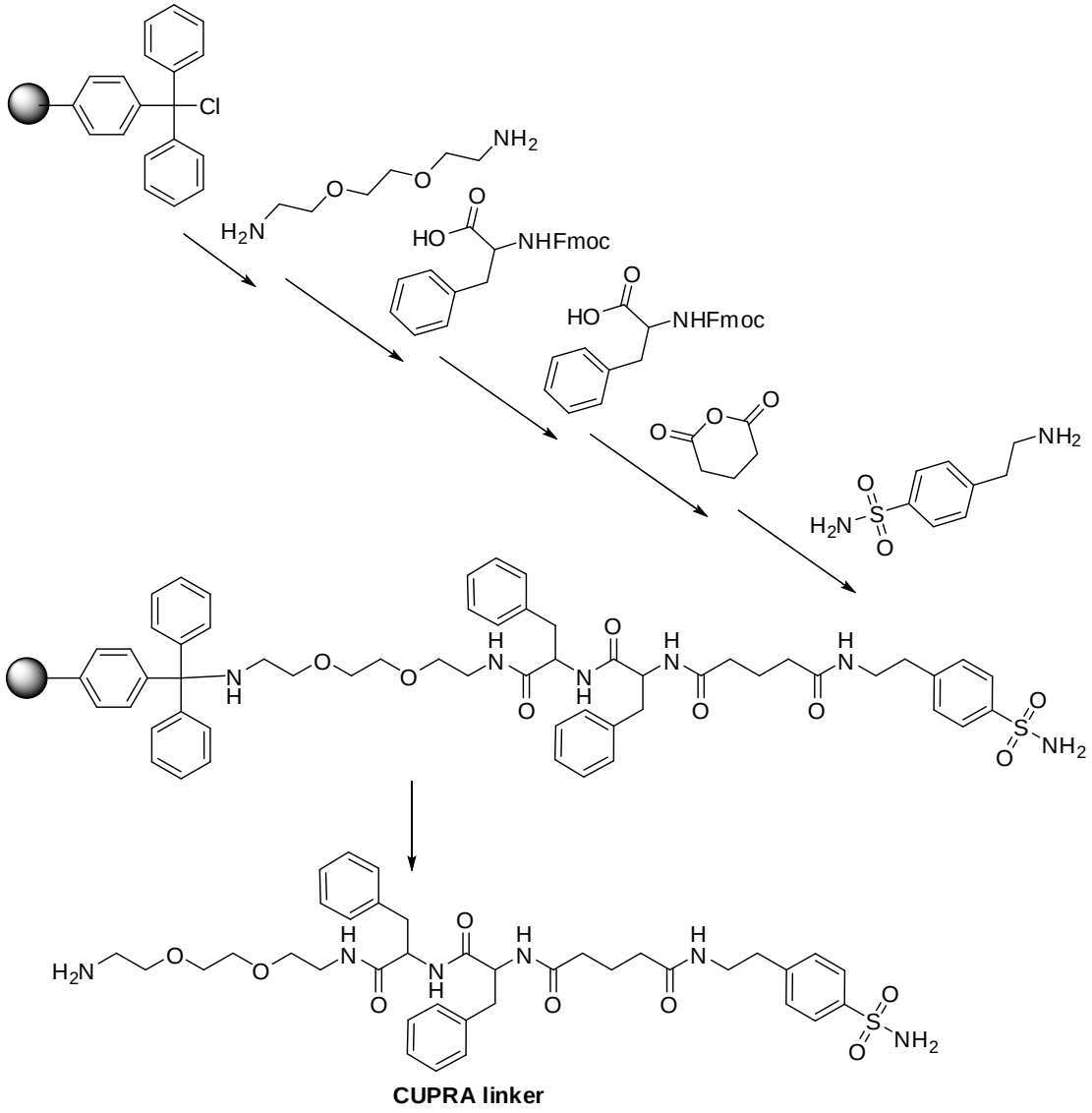
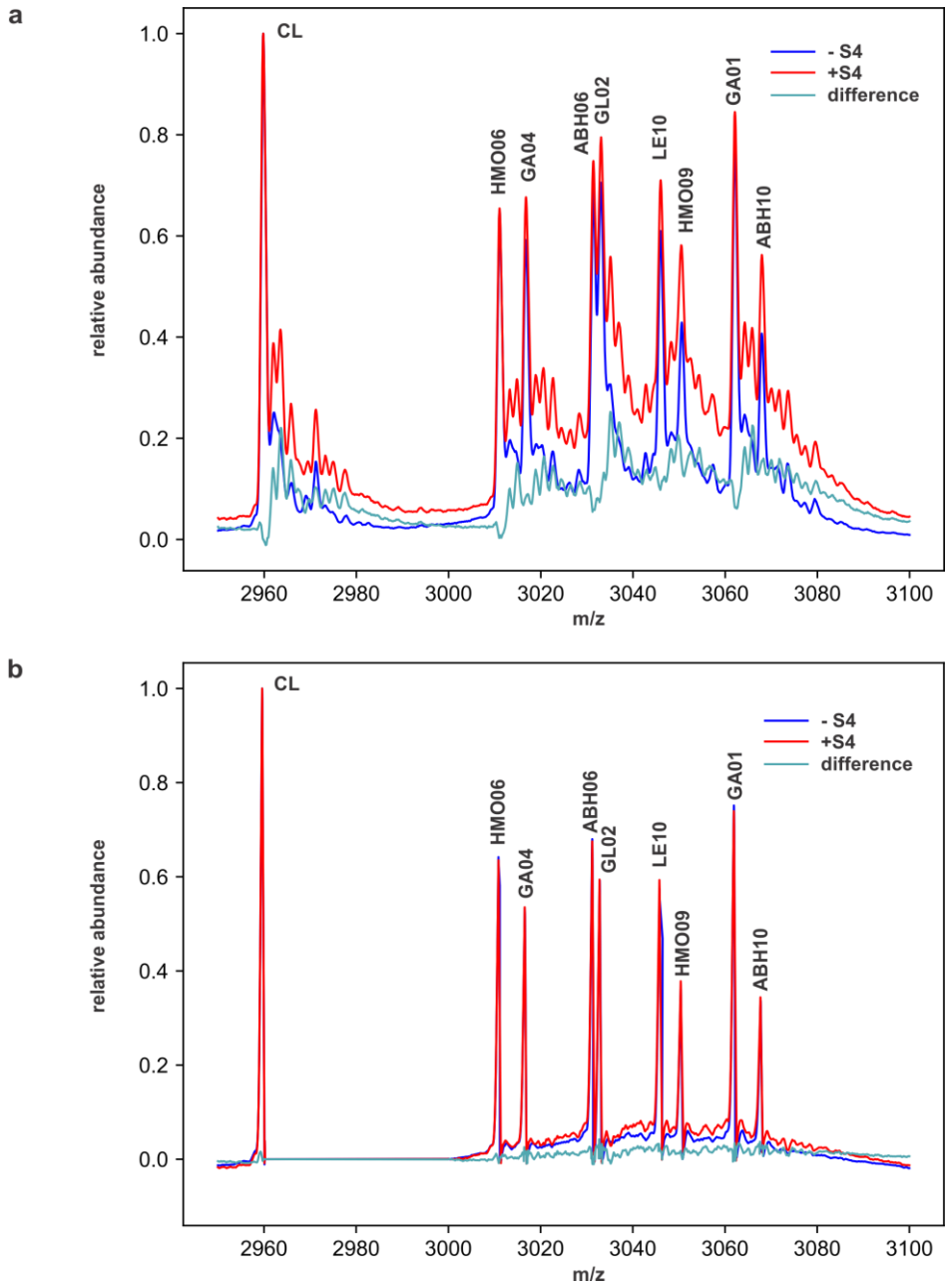
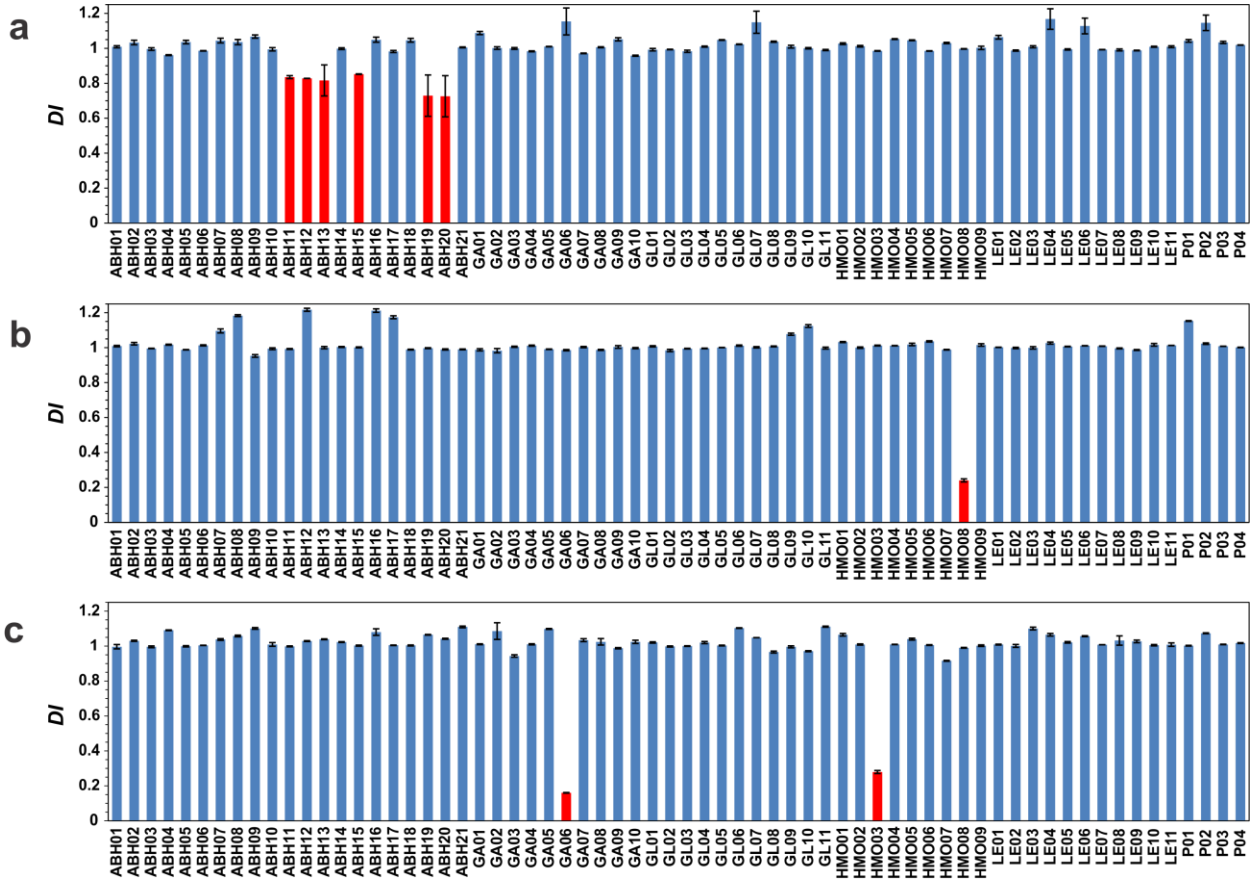
Supplementary Figures



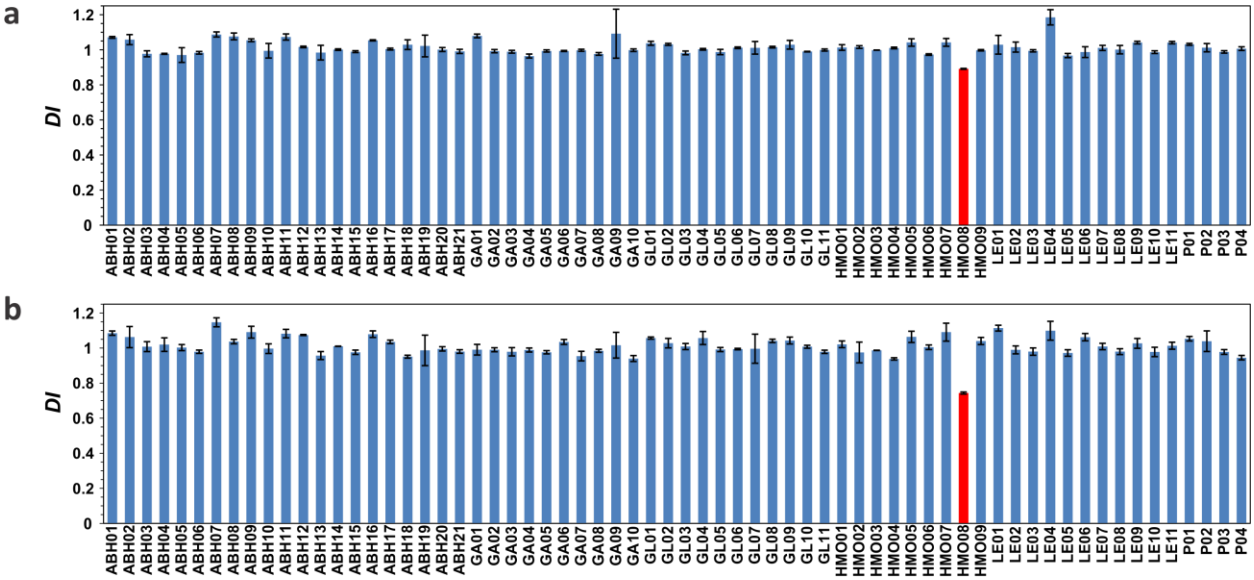
Supplementary Figure 1. Scheme of solid-phase assisted synthesis of CUPRA linker.



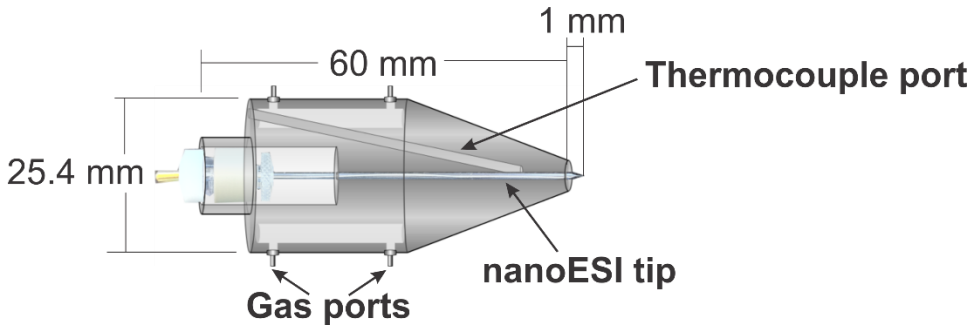
Supplementary Figure 2. Application of SWARM for adduct subtraction from ESI mass spectra. (a) Portion of ESI mass spectrum acquired for an aqueous ammonium acetate (200 mM, pH 7) solution of $^{Uni}P_{proxy}$ (3 μM), a subset of the OS^{mod} library (3 μM each) and CUPRA linker (**CL** 3 μM) in the absence (red trace) and presence (blue trace) of S_4 (50 μM). Cyan trace corresponds to difference between these two mass spectra. (b) Data from (a) after treatment with SWARM.



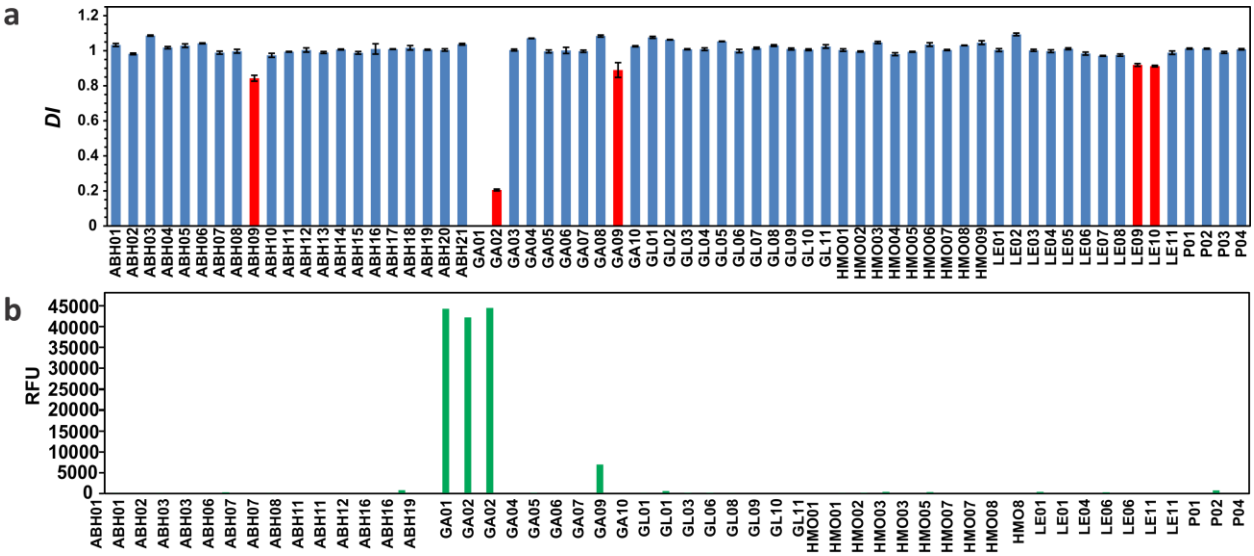
Supplementary Figure 3. CUPRA screening results for CBM51, SNA, and MAA. Depletion index (*DI*) for each OS^{mod} measured in 200 mM aqueous ammonium acetate (pH 7) for: (a) CBM51 (25 μM, 25 ºC), (b) SNA (5 μM, 25 ºC), (c) MAA (50 μM, 25 ºC). Data shown in red indicate ligands identified from screening. Error bars represent standard deviations calculated for n=4 independent experiments.



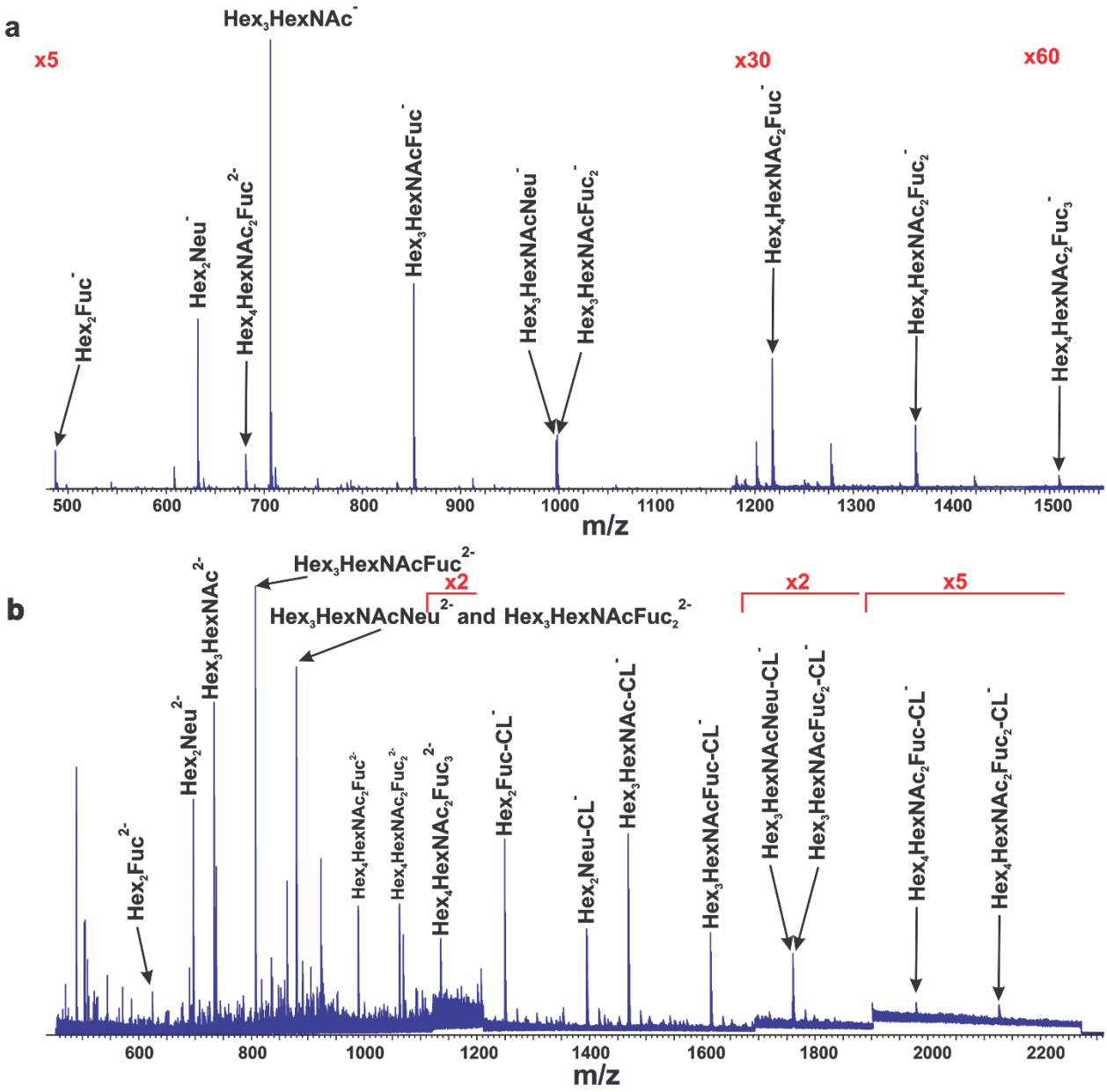
Supplementary Figure 4. CUPRA screening results for siglec-2. Depletion index (*DI*) for each OS^{mod} measured in 200 mM aqueous ammonium acetate (pH 7) for siglec-2 (23 μM) at: (a) 25 ºC and (b) 0 ºC. Data shown in red indicate ligands identified from screening. Error bars represent standard deviations calculated for n=4 independent experiments.



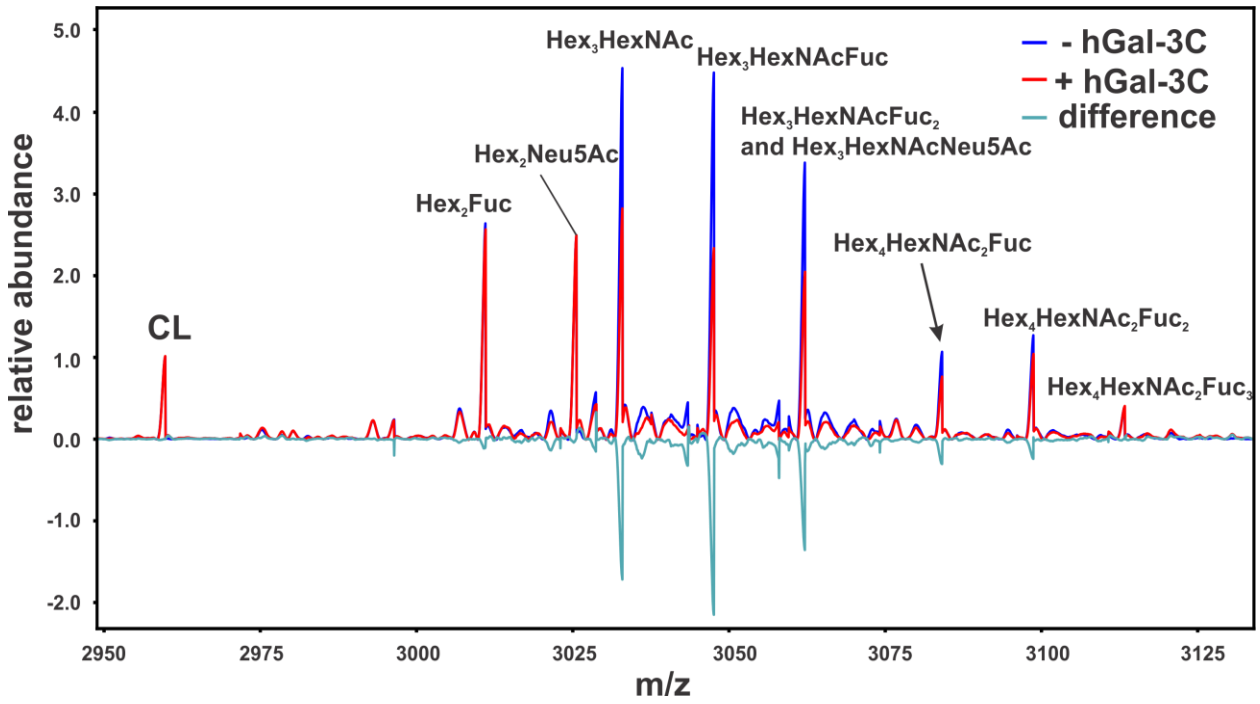
Supplementary Figure 5. Schematic of home-built temperature-controlled nanoESI device. The nanoESI tip is inserted into the central channel of the aluminum device through a small aperture 1.2 mm (i.d.). The tip is positioned such that ~1 mm of the tapered end protrudes from the end of the device. The temperature of the device was regulated by varying the flow rate of nitrogen gas that first passes through a U-tube in contact with a dry ice/ethanol bath and then through two symmetric gas flow channels at the outer edges of the device. The temperature of the device was measured by a thermocouple introduced through the angled channel and placed in proximity to the end of the nanoESI tip.



Supplementary Figure 6. Comparison of variable temperature CUPRA screening results with glycan array data for CTB_5: (a) Depletion index (*DI*) for each OS^{mod} measured in 200 mM aqueous ammonium acetate (pH 7) for CTB_5 (10 µM). Data shown in red indicate ligands identified from screening. Error bars represent standard deviations calculated for n=4 independent experiments. (b) Partial readout in relative fluorescence units (RFU) of CFG array (entry CTB_14501_10ug_v5.0_DATA.xls) screened against CTB_5 (only compounds containing glycans found in CUPRA library are shown). CUPRA codes instead of CFG codes are used for ease of comparison.



Supplementary Figure 7. ESI mass spectra of 200 mM aqueous ammonium acetate (pH 7) solution of 0.04 mg/ml HMO library before (a) and after (b) modification with CUPRA linker (**CL**).



Supplementary Figure 8. Portion of ESI mass spectrum acquired for an aqueous ammonium acetate (200 mM, pH 7) solution of $^{Uni}P_{proxy}$ (3 μM), a subset of the HMO library modified with CUPRA linker (**CL**) (0.08 mg/ml) and CUPRA linker (**CL**, 3 μM) in the absence (red trace) and presence (blue trace) of hGal-3C (40 μM). Cyan trace corresponds to difference between these two mass spectra. All mass spectra were treated with SWARM.

Supplementary Tables

Supplementary Table 1. Structures of CUPRA library components (OS^{mod}); **CL** (CUPRA linker moiety) represents the linker-affinity tag.

OS^{mod}	**Structure**
ABH01	Galβ1-3GlcNAc-CL
ABH02	Galα1-3Galβ1-4GlcNAc-CL
ABH03	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc-CL
ABH04	Fucα1-2Galβ1-4GlcNAcβ1-3Gal-CL
ABH05	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glc-CL
ABH06	Fucα1-2Galβ1-3GalNAcβ1-3Gal-CL
ABH07	GalNAcα1-3(Fucα1-2)Gal-CL
ABH08	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAc-CL
ABH09	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-CL
ABH10	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-CL
ABH11	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAc-CL
ABH12	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-CL
ABH13	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc-CL
ABH14	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-CL
ABH15	GalNAcα1-3(Fucα1-2)Galβ1-4Glc-CL
ABH16	Galα1-3(Fucα1-2)Galβ1-3GlcNAc-CL
ABH17	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal-CL
ABH18	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc-CL

ABH19 Galα1-3(Fucα1-2)Galβ1-4GlcNAc-CL

ABH20 Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal-CL

ABH21 Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Gal-CL

GA01 Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc-CL

GA02 Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc-CL

GA03 Neu5Acα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glc-CL

GA04 GalNAcβ1-4Galβ1-4Glc-CL

GA05 Galβ1-3GalNAcβ1-4Galβ1-4Glc-CL

GA06 Neu5Aca2-8Neu5Aca2-3Galβ1-4Glc-CL

GA07 GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc-CL

GA08 Galβ1-4(Neu5Acα2-3)Galβ1-4Glc-CL

GA09 Galβ1-3GalNAcβ1-4(Neu5Acα2-8Neu5Acα2-3)Galβ1-4Glc-CL

GA10 GalNAcβ1-4(Neu5Acα2-8 Neu5Acα2-3)Galβ1-4Glc-CL

GL01 Galα1-4Galβ1-4GlcNAc-CL

GL02 GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL03 Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL04 GalNAcα1-3GalNAcβ1-3Gal-CL

GL05 GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL06 Galα1-4Galβ1-4Glc-CL

GL07 Neu5Acα2-3Galα-4Galβ-4Glc-CL

GL08 Neu5Acα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL09 Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL10 GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

GL11 Galα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-CL

HMO01 Galβ1-4Glc-CL

HMO02 Galβ1-3GlcNAcβ1-3Galβ1-4Glc-CL

HMO03 Neu5Acα2-3Galβ1-4Glc-CL

HMO04 Fucα1-2Galβ1-4(Fucα1-2)Glc-CL

HMO05 Fucα1-2Galβ1-4Glc-CL

HMO06 Galβ1-4(Fucα1-3)Glc-CL

HMO07 Galβ1-4GlcNAcβ1-3Galβ1-4Glc-CL

HMO08 Neu5Acα2-6Galβ1-4Glc-CL

HMO09 GlcAβ1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-CL

LE01 Galβ1-3(Fucα1-4)GlcNAc-CL

LE02 Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-CL

LE03 Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-CL

LE04 Fucα1-2Galβ1-3(Fucα1-4)GlcNAc-CL

LE05 Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Gal-CL

LE06 Galβ1-4(Fucα1-3)GlcNAc-CL

LE07 Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-CL

LE08 Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-CL

LE09 Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc-CL

LE10 Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal-CL

LE11 Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-CL

P01 Glcα1-4Glc-CL

P02 Glcβ1-4Glc-CL

P03 Galα1-6Glc-CL

P04 Glcα1-6Glcα1-6Glc-CL

Supplementary Table 2. Affinities (K_d) of OS^{mod} for $^{Uni}P_{proxy}$ measured by ESI-MS.[a,b]

OS^{mod}	K_d (μM)	OS^{mod}	K_d (μM)
ABH01	11.9 ± 0.7	**GA01**	10.3 ± 3.5
ABH02	16.4 ± 1.8	**GA10**	14.1 ± 1.9
ABH03	9.9 ± 3.2	**GA02**	18.7 ± 1.2
ABH04	9.2 ± 2.6	**GA03**	5.5 ± 1.7
ABH05	12.7 ± 3.8	**GA04**	10.8 ± 2.4
ABH06	4.1 ± 0.2	**GA05**	7.9 ± 1.0
ABH07	12.1 ± 2.4	**GA06**	26.4 ± 0.8
ABH08	18.4 ± 2.2	**GA07**	6.5 ± 0.3
ABH09	19.4 ± 2.9	**GA08**	12.4 ± 2.6
ABH10	15.8 ± 2.5	**GA09**	32.7 ± 1.2
ABH11	15.2 ± 0.8	**GL01**	10.4 ± 0.4
ABH12	22.4 ± 6.9	**GL02**	7.6 ± 1.5
ABH13	11.5 ± 3.0	**GL03**	7.4 ± 0.7
ABH14	4.7 ± 0.8	**GL04**	8.6 ± 0.9
ABH15	8.6 ± 3.7	**GL05**	6.9 ± 0.2
ABH16	32.9 ± 8.3	**GL06**	4.2 ± 0.8
ABH17	8.8 ± 2.2	**GL07**	14.0 ± 1.3
ABH18	16.0 ± 2.8	**GL08**	10.3 ± 1.3
ABH19	10.6 ± 2.4	**GL09**	18.1 ± 2.1
ABH20	13.0 ± 4.7	**GL10**	4.7 ± 0.4
ABH21	4.1 ± 0.8	**GL11**	7.9 ± 0.7
HMO01	5.1 ± 1.4	**LE04**	48.5 ± 5.0

OS^{mod}	K_d (μM)	OS^{mod}	K_d (μM)
HMO02	6.6 ± 0.7	**LE05**	40.2 ± 13.0
HMO03	4.0 ± 0.7	**LE06**	13.0 ± 4.4
HMO04	6.0 ± 0.2	**LE07**	10.4 ± 2.6
HMO05	12.1 ± 0.9	**LE08**	11.6 ± 4.6
HMO06	7.2 ± 1.2	**LE09**	43.3 ± 12.7
HMO07	2.2 ± 0.5	**LE10**	9.0 ± 2.8
HMO08	14.6 ± 2.1	**LE11**	8.5 ± 3.6
HMO09	10.6 ± 1.4	**P01**	1.7 ± 0.6
LE01	15.9 ± 3.4	**P02**	8.9 ± 1.5
LE02	11.9 ± 4.0	**P03**	6.6 ± 2.0
LE03	8.1 ± 2.7	**P04**	5.9 ± 0.3

a. Errors correspond to one standard deviation. b. Affinities measured in 200 mM aqueous ammonium acetate (pH 7, 25 ºC).

Supplementary Table 3. Affinities (K_d) of OS^{mod} for GBPs measured by CUPRA and affinities of the corresponding OS for the GBPs.[a,b]

GBP	OS^{mod}	T (°C)	K_d (OS^{mod}) (μM)	K_d (OS) (μM)
SNA	**HMO08**	25	0.43 ± 0.01	NA [g]
MAA	**HMO03**	25	15 ± 1	NA [g]
MAA	**GA06**	25	8.3± 0.1	NA [g]
CTB_5	**GA01**	25	0.66 ± 0.01	0.31 ± 0.03 [c]
CTB_5	**GA02**	25	45 ± 4	135 ± 4
CBM51	**ABH11**	25	96 ± 10	14.3 ± 0.8 [d]
CBM51	**ABH12**	25	110 ± 10	38 ± 3
CBM51	**ABH13**	25	170 ± 40	40 ± 8
CBM51	**ABH15**	25	100 ± 10	17.2 ± 0.6 [d]
CBM51	**ABH19**	25	97 ± 18	17.2 ± 1.2 [d]
CBM51	**ABH20**	25	92 ± 12	38 ± 5
siglec-2	**HMO08**	25	110 ± 20	281 ± 10 [e,f]
siglec-2	**HMO08**	0	43 ± 10	NA [g]

a. Errors correspond to one standard deviation. b. Unless otherwise noted, affinities were measured in 200 mM aqueous ammonium acetate (pH 7) by ESI-MS. c. Values taken from Lin et al. *J. Am. Soc. Mass Spectrom.* **25**, 104–110 (2014). d. Values taken from Han et al. *Glycobiology* **27**, 170–180 (2015). e. Measured by ITC in aqueous solution (pH 8.0) of 20 mM Tris and 150 mM NaCl. f. Value taken from Ereño-Orbea, J. et al. *Nat. Commun*. **8**, 764 (2017). g. NA ≡ Not available.

Supplementary Table 4. List of MWs and saccharide compositions of extracted HMOs before and after introduction of CUPRA linker (CL).

HMO	MW (theoretical)	MW (experimental) [a]
Hex_2FucOH	488.17	488.18 ± 0.01
Hex_2Fuc-CL	1250.52	1250.51 ± 0.02
$Hex_2Neu5AcOH$	633.21	633.22 ± 0.01
$Hex_2Neu5Ac$-CL	1395.55	1395.55 ± 0.02
$Hex_3HexNAcOH$	707.25	707.26 ± 0.01
$Hex_3HexNAc$-CL	1469.59	1469.58 ± 0.02
$Hex_3HexNAcFucOH$	853.31	853.32 ± 0.01
$Hex_3HexNAcFuc$-CL	1615.65	1615.64 ± 0.02
$Hex_3HexNAcNeu5AcOH$	998.34	998.35 ± 0.01
$Hex_3HexNAcNeu5Ac$-CL	1760.68	1760.68 ± 0.03
$Hex_3HexNAcFuc_2OH$	999.36	999.37 ± 0.01
$Hex_3HexNAcFuc_2$-CL	1761.71	1761.70 ± 0.03
$Hex_4HexNAc_2FucOH$	1218.44	1218.45 ± 0.01
$Hex_4HexNAc_2Fuc$-CL	1980.78	1980.78 ± 0.03
$Hex_4HexNAc_2Fuc_2OH$	1364.50	1364.52 ± 0.01
$Hex_4HexNAc_2Fuc_2$-CL	2126.84	2126.82 ± 0.03
$Hex_4HexNAc_2Fuc_3OH$	1510.55	1510.56 ± 0.01
$Hex_4HexNAc_2Fuc_3$-CL	2272.90	2272.87 ± 0.04

a. Errors correspond to one standard deviation.

Supplementary Table 5. Apparent concentrations of modified (with CUPRA linker, CL) HMOs estimated from their binding to $^{Uni}P_{proxy}$ measured by ESI-MS and their apparent affinities (K_d, μM) to hGal-3C measured by CUPRA.[a,b]

HMO	Initial concentrations (μM) [c]	K_d (μM)
Hex_2Fuc-CL	9.0 ± 0.1	NB
$Hex_2Neu5Ac$-CL	8.4 ± 0.1	NB
$Hex_3HexNAc$-CL	15 ± 1	31 ± 4
$Hex_3HexNAcFuc$-CL	15 ± 1	2.5 ± 0.5
$Hex_3HexNAcNeu5Ac$-CL[d]	8.8 ± 0.2	20 ± 3
$Hex_3HexNAcFuc_2$-CL[d]	8.8 ± 0.2	20 ± 3
$Hex_4HexNAc_2Fuc$-CL	2.9 ± 0.1	29 ± 3
$Hex_4HexNAc_2Fuc_2$-CL	3.5 ± 0.1	87 ± 5
$Hex_4HexNAc_2Fuc_3$-CL	1.2 ± 0.1	NB

a. Errors correspond to one standard deviation. b. Affinities measured in 200 mM aqueous ammonium acetate (pH 7, 25 ºC). c. Values represent apparent concentrations corresponding to all HMOs of same MW (isomer set). d. Due to the small difference in MW, these two HMO isomer sets were treated as a single set.

Supplementary Table 6. Relative activities of sialylated OS^{mod} substrates measured by time-resolved CUPRA for NEU2 and NEU3 in 200 mM aqueous ammonium acetate (pH 7, 25 ºC).

Substrate	NEU2	NEU3
GA01	0.000	0.008
GA02	0.000	0.000
GA03	0.524	0.551
GA06	0.443	0.298
GA07	0.000	0.009
GA08	0.000	0.034
GA09	0.000	0.247
GA10	0.000	0.292
GL07	0.000	0.462
GL08	0.918	0.523
HMO03	1.000	1.000
HMO08	0.004	0.275
LE08	0.188	0.494