

SUPPLEMENTAL DATA

Modulation of Cellular Adhesion by Glycoengineering

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Adhesion on P-Selectin coated plate

Before Wash

Replicate #	Untreated	2a	3a	4a	5a
1	28593	30275	29618	33156	40212
2	31761	25923	33193	32822	42740
3	38144	23252	34228	35635	42827
4	36894	23596	36561	37684	43220
MEAN	33848.00	25761.50	33400.00	34824.25	42249.75
STD DEV	4461.10	3234.44	2888.13	2282.36	1374.45
STD DEV (%)	13.18	12.56	8.65	6.55	3.25
MEAN - BKGRD (lane 11)	33848.00	25761.50	33400.00	34824.25	42249.75

After Wash

Replicate #	Untreated	2a	3a	4a	5a
1	5304	6660	4419	3705	3366
2	10537	6058	2526	5718	1251
3	11526	5754	4203	6897	2896
4	15291	6003	4764	8391	5034
MEAN	10664.50	6118.75	3978.00	6177.75	3136.75
STD DEV	4118.98	384.31	995.19	1978.35	1556.29
STD DEV (%)	38.62	6.28	25.02	32.02	49.61
MEAN - BKGRD	10664.50	6118.75	3978.00	6177.75	3136.75

Average % Adhesion

Replicate #	Untreated	2a	3a	4a	5a
1	0.185	0.220	0.149	0.112	0.084
2	0.332	0.234	0.076	0.174	0.029
3	0.302	0.247	0.123	0.194	0.068
4	0.414	0.254	0.130	0.223	0.116
MEAN	0.308	0.239	0.120	0.176	0.074
STD DEV	0.095	0.015	0.031	0.047	0.036
STD DEV (%)	9.476	1.526	3.105	4.696	3.624
MEAN*100	30.847	23.889	11.960	17.554	7.427

Data table for Student's t-test

Sx - BKGRD	0.308	23.889	11.960	17.554	7.427
n	4	4	4	4	4
mean (xbar) - BKGRD	0.077	5.972	2.990	4.389	1.857
Sx ^2	0.408	0.229	0.060	0.130	0.026
(Sx)^2	0.095	0.057	0.014	0.031	0.006
(Σx)^2/n	0.024	0.014	0.004	0.008	0.001
Sd ^2	0.384	0.215	0.057	0.122	0.025
s^2	0.128	0.072	0.019	0.041	0.008

Student's t-test (controls in lanes 1 to 5)

vs. lane 1

σd^2 (comp to lane 1)	0.064	0.050	0.037	0.042	0.034
SQRT(σd^2)= σd	0.253	0.223	0.192	0.205	0.184
t (vs. lane 1)	0.000	26.397	15.207	20.997	9.646

P	F	0.001	0.001	0.001	0.001
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vs. lane 2

σd^2 (comp to lane 2)	0.050	0.036	0.023	0.028	0.020
SQRT(σd^2)= σd	0.223	0.189	0.150	0.168	0.141
t (vs. lane 2)	46.083	0.000	158.253	38.886	501.411

P	0.001	F	0.001	0.001	0.001
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vs. lane 3

σd^2 (comp to lane 3)	0.037	0.023	0.009	0.015	0.007
SQRT(σd^2)= σd	0.192	0.150	0.097	0.122	0.082
t (vs. lane 3)	22.771	41.670	0.000	34.342	138.075

P	0.001	0.001	F	0.001	0.001
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vs. lane 4

σd^2 (comp to lane 4)	0.042	0.028	0.015	0.020	0.012
SQRT(σd^2)= σd	0.205	0.168	0.122	0.143	0.111
t (vs. lane 4)	33.704	22.128	74.217	0.000	308.472

P	0.001	0.001	0.001	F	0.001
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vs. lane 5

σd^2 (comp to lane 5)	0.483	1.957	1.212	1.561	0.928
SQRT(σd^2)= σd	0.695	1.399	1.101	1.250	0.964
t (vs. lane 5)	13.911	57.506	60.139	62.171	0.000

Legend
F = Failed

P	0.001	0.001	0.001	0.001	F
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Table S1. Typical data table for adhesion experiments. Data are shown for adhesion to P-selectin. “Before Wash” and “After Wash” values are direct reads from the plate reader. The “Average % adhesion” was calculated as described under 'Cell Adhesion Assays' (EXPERIMENTAL PROCEDURES). See also Figure 4.

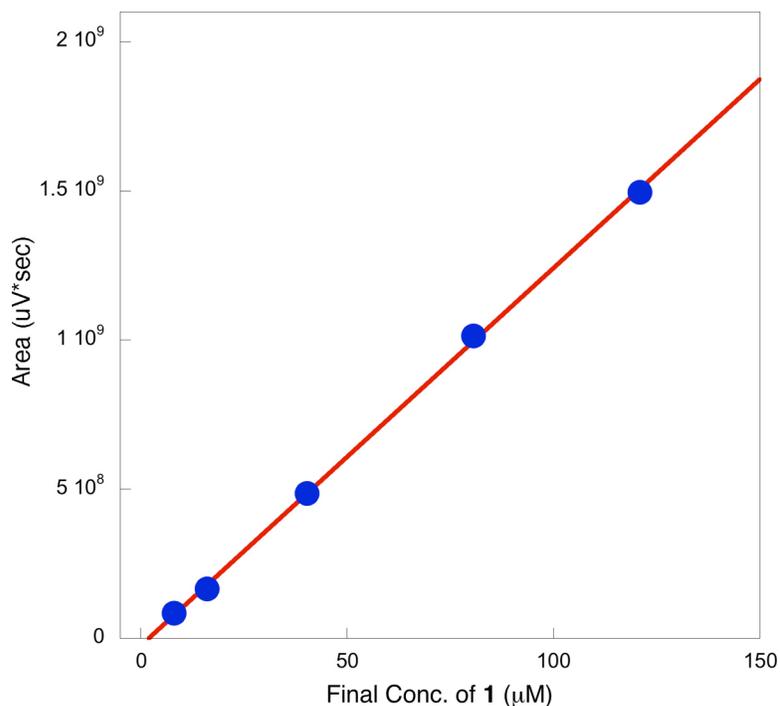


Figure S1. Sialic acid DMB labeling. Standard curve for quantification of DMB-labeled sialic acids using fluorescence detection by HPLC ($\lambda_{\text{detection, em}} = 448 \text{ nm}$). See also Table 1.

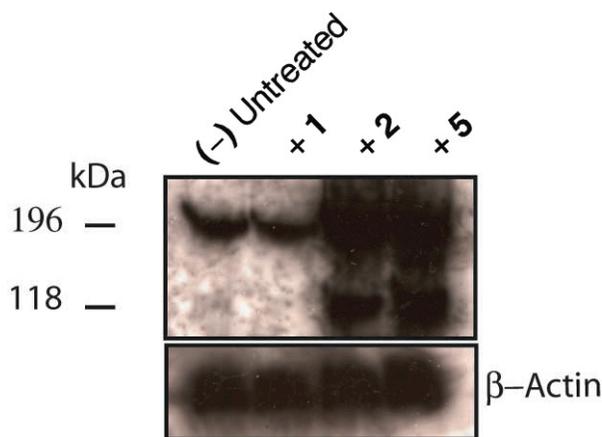


Figure S2. Expression of sialyl-Lewis^x on CD162 in HL60 cells treated with modified sialic acid derivatives. Representative Western blot of HL60 cells incubated with compounds **1a**, **2a**, and **5a** for 3 days. Cell lysates were separated on SDS-PAGE and analyzed with the sialyl-Lewis^x-specific monoclonal antibody KM93 ($n = 2$) and with a β -actin specific antibody (Affinity BioReagents MA1-744, clone mAbGEa). CD162 appears as both monomer (100-120 kDa) and dimer (220-240 kDa) in SDS-PAGE (Horstkorte, M., Rau, K., Reutter, W., Nohring, S., and Lucka, L. (2004). Increased expression of the selectin ligand sialyl-Lewis(x) by biochemical engineering of sialic acids. *Exp. Cell Res.* 295, 549-554). See also Figure 4.