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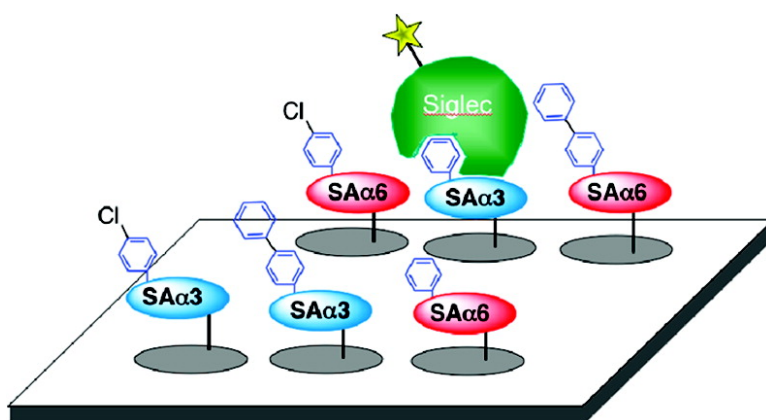
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Sialoside Analogue Arrays for Rapid Identification of High Affinity Siglec Ligands

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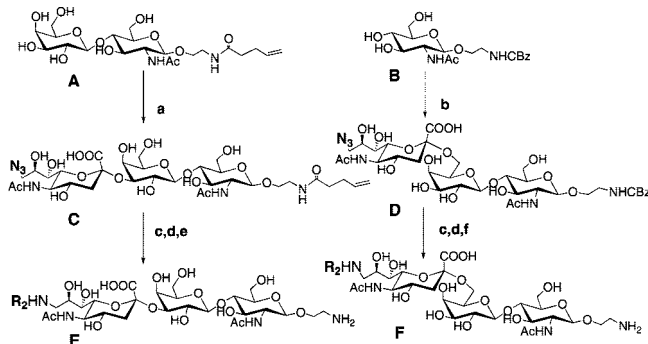Received February 11, 2008; E-mail: jpaulson@scripps.edu

The siglec family of glycan binding proteins recognizes sialic acid containing glycans of glycoproteins and glycolipids as ligands. Most are expressed on various white blood cells that mediate immune function, and one of them, myelin associated glycoprotein (MAG), is expressed on glial cells and functions in myelin−axon interactions.[1] In general, siglecs bind with low intrinsic affinity (0.1−1 mM) to their natural sialoside ligands, and while several exhibit unique specificities, most siglecs bind to sialosides containing the common Neu5Acα2−6Gal and/or Neu5Acα2−3Gal linkages.[2−4]

To investigate the roles of ligand binding in the functions of siglecs, high affinity probes are desired to compete with natural ligands and bind to siglecs on native cells. Several studies have documented the importance of sialic acid substituents (e.g., 9C and 5C positions) for modulating the affinity of siglec binding[5−9] and the utility of high affinity sialoside ligands as biological probes of Siglec-2 (CD22).[10,11] To facilitate development of such ligands for other siglecs, we have implemented a strategy of making a sialoside analogue library compatible with glycan microarray technology[12] to screen for substituents that increase affinity.

The synthesis of sialosides with 9-azido-Neu5Ac opens a route to the facile synthesis of 9-substituted sialic acids. Synthesis of the key azido intermediates (**C** and **D**) with 9-azido-Neu5Acα2−3Gal and 9-azido-Neu5Acα2−6Gal termini was accomplished by enzymatic transfer of 9-azido-Neu5Ac from CMP-9-azido-Neu5Ac[13,14] to an oligosaccharide precursor containing a 4-pentenoyl or CBZ-protected 2-amino ethyl aglycone (Scheme 1). Portions (1−5 mg) of the azido intermediates were reduced to the amine with PPh₃ and acylated in excess from a library of acylchlorides (see Table S1 in Supporting Information). After quenching of excess acylating reagents with water, glycans were deprotected[15,16] to expose the amine (**E** or **F**) for printing onto amino-reactive *N*-hydroxy succinimide (NHS)-activated glass slides (Schott Nexterion, Slide-H).[12] In total, the library comprised 16 α2−3 and 28 α2−6 sialosides.

To determine if the increased affinity of siglecs toward acyl substituents could be detected on a glycan array, we tested the binding of CD22 (Siglec-2) to an array of selected analogues of the α2−6 sialoside **F**. The six compounds included one with a 9-*N*-biphenylcarboxyl (BPC, **15**) substituent previously shown to increase affinity for CD22 by 100-fold.[6,10] The glycans were printed at 10, 2-fold dilutions (10 replicates each). Binding was assessed with a fluorescently labeled recombinant CD22-Fc chimera, an antibody-like molecule containing two CD22 ligand binding domains, that has been demonstrated to bind to glycan microarrays.[12] Binding of CD22 was sialic acid dependent (Figure 1), with

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Scheme 1[a]

[a] Reagents and conditions: (a) ST3Gal and CMP-9-azido-9-deoxy-Neu5Ac; (b) GalT-GalE, UDP-Gal, hST6Gal I, CMP-9-azido-9-deoxy-Neu5Ac; (c) PPh₃, MeOH:TFA:H₂O (4:5:1); (d) acylation, R₂COCl (R₂ = 1−34, see Table S1) (2−4 equiv), base (5 equiv); (e) I₂ (5 equiv), MeOH: H₂O (9:1); (f) Pd/C, H₂.
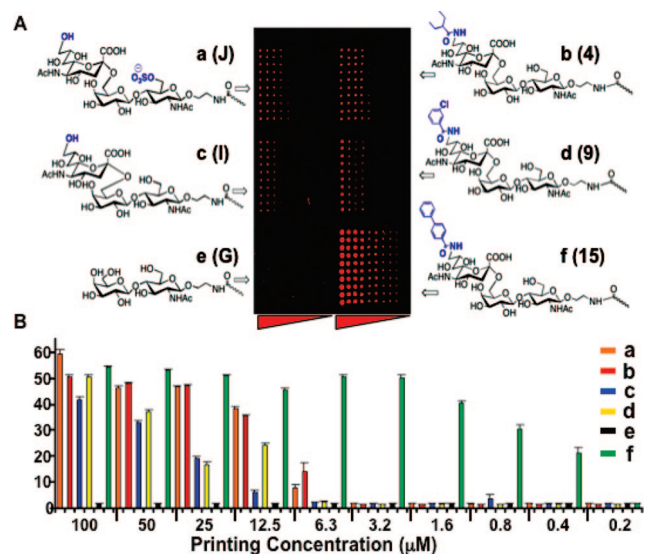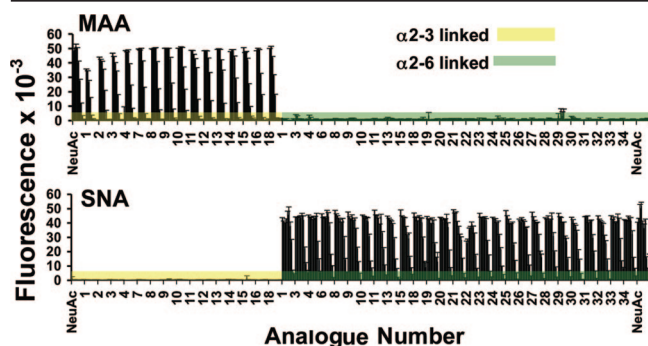

Figure 1. Binding of CD22-Fc chimera to sialoside analogues printed on a glycan array. Sialosides were printed with 10 replicates of 10, 2-fold dilutions from 100 to 0.2 µM (left to right). 9-Acyl substituents of **F** are indicated in parentheses (Table S1). (A) Fluorescent image of the array labeled with a complex of CD22-Fc chimera and secondary FITC-labeled goat antihuman Fc antibody (see Figure 3). (B) Relative fluorescence intensity at each printing concentration is compared for the six compounds.

binding observed to the natural Neu5Ac containing compound (**c**) and increased binding to each of the three 9-acyl-substituted compounds (**b**, **d**, and **f**). Scanning the slide to measure relative fluorescence allowed comparison of binding levels at each printing

Figure 2. Binding of sialoside-specific plant lectins to a sialoside analogue glycan array. Biotinylated lectins, MAA (top) and SNA (bottom) specific for Neu5Acα2−3Gal or Neu5Acα2−6Gal linkages, respectively, were mixed with Alexa-fluor 488 labeled streptavidin, overlaid onto the printed analogue array, washed, and scanned for fluorescence (see Supporting Information). Acyl analogues **1−34** of **E** (yellow) and **F** (green) correspond to acyl groups listed in Table S1. Shown is relative fluorescence of lectins bound to each glycan printed at 5 serial dilutions starting at 100 μM. "NeuAc" labeled lanes are sialosides with unsubstituted Neu5Ac in α2−3 (**H**) and α2−6 (**I**) linkage to *N*-acetyllactosamine.
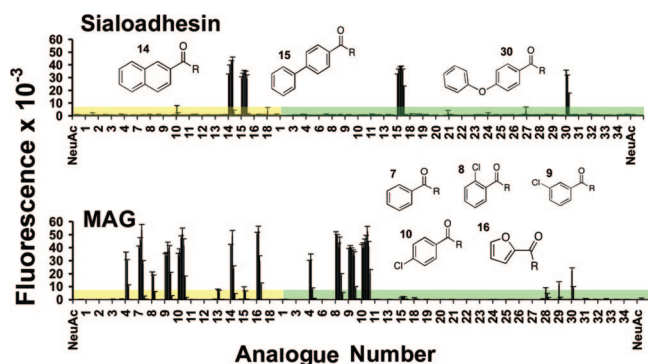
Figure 3. Binding of siglec-Fc chimeras to the sialoside analogue array. Mouse sialoadhesin-Fc chimera (top) and mouse MAG-Fc chimera (bottom) complexed with goat antihuman IgG-FITC (2:1; 50 μg/mL total protein) were overlaid onto the printed analogue array, washed for Figure 2). Numbered acyl analogues attached to compound **E** (yellow) or **F** (green) are found in Table S1.

concentration (Figure 1B). Relative to the natural sialoside (**c**), the 9-BPC substituent (**f**) produced equivalent binding at 64−128-fold lower printing concentration.

The quality of a glycan array containing the full library was assessed using the binding of the plant lectins, Maccia amurensis agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA), specific for α2−3 and α2−6 sialosides, respectively. Fortuitously, binding of neither lectin is blocked by 9-acyl substituents, facilitating their use to assess the printing efficiency. All 9-acyl-substituted **E** and **F** sialosides were recognized comparably by MAA and SNA, respectively (Figure 2).

The full array was then used to assess the specificity of three siglecs, CD22 (Siglec-2), MAG (Siglec-4), and sialoadhesin (Siglec-1). In keeping with its specificity for α2−6 sialosides, CD22 bound only to sialosides in the **F** series (Figure S2). However, none of the *N*-acyl substituents exhibited an affinity equivalent to BPC (**F15**; see Figures 1 and S1).

Surprisingly, Fc chimeras of sialoadhesin and MAG did not bind to sialosides with unsubstituted NeuAc but bound α2−3 (**E**) or α2−6 (**F**) sialosides with selective 9-*N*-acyl substituents in repeated assays (Figure 3). Sialoadhesin exhibited increased affinities to a few bisphenyl analogues of **E** (**14**, **15**), and **F** (**15**, **30**), with the

equivalent binding of the biphenyl substituent **15** to both, demonstrating that the affinity to the acyl substituent is dominant over the sialic acid to galactose linkage. MAG bound to sialosides with other substituents including benzoyl- (**7**) and chlorobenzoyl (**8−10**) analogues of either α2−3 (**E**) or α2−6 (**F**) sialosides. The results are consistent with previous studies evaluating the affinity of MAG toward 9-*N*-acyl substituents on α-methyl-NeuAc.[8]

To confirm that the signals for MAG resulted from increased affinity, a selected series of related sialosides with 9-acyl substituents (**7**, **8**, and **10**) of **E** and **F** were resynthesized and tested for binding by MAG and CD22 (Figure S2). While CD22 bound only the α2−6 sialoside (**F**) series, MAG bound both, with preference for the α2−3 (**E**) series compounds. These compounds were also assessed for inhibitory potency in an ELISA competition assay.[10] Compounds **7E**, **8E**, and **10E** gave IC$_{50}$ values of 4.9, 9.7, and 2.2 μM, respectively, representing 50−200-fold enhancement of affinity over the unsubstituted α2−3 sialoside **H** (IC$_{50}$ of 490 μM; data not shown).

In summary, we demonstrate that a sialoside analogue array allows for the synthesis of sialoside analogues in small quantity for subsequent screening for high affinity ligands of siglecs and other sialoside binding GBPs.

Supporting Information Available: General procedures for preparations of the sialoside analogue library, a complete list of printed analogues, procedures for printing and analysis of GBPs, and analytical data for MAA and SNA plant lectins. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA801052G