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Covalent Display of Oligosaccharide Arrays in Microtiter Plates

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A significant challenge in glycobiology is the development of carbohydrate arrays (glycoarrays) for the large-scale high-throughput analysis of sugar−receptor interactions.[1−6] Unlike arrays for other biomolecules such as peptides, proteins, and oligonucleotides, glycoarrays have just begun to appear in the literature. We have recently reported the noncovalent attachment of complex oligosaccharides to the surface of microtiter plates through lipid tethers.[7] This strategy has since been further improved to avoid the difficult handling of glycolipids and has been applied to the capture of saccharides containing azides and amines to a long (C14) and functionalized hydrophobic tether in microtiter plates for direct noncovalent display.[7−9] Our current interest is to develop a more stable, reusable, and easy-to-characterize covalent array for the high-throughput screening of complex carbohydrates.

As mentioned above, azide-containing saccharides have been successfully captured to lipid alkynes in microtiter plates via the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction for high-throughput screening in situ[8,10,11] Because azide-containing saccharides can be easily prepared[8] or created from their amine-containing precursors,[12,13] we again apply this strategy for the capture of azide-containing saccharides to solid phase. To conjugate the sugars to the functionalized microtiter plate surface, a cross-linker was developed for orthogonal attachment to the surface with a terminal alkyne for conjugation to the saccharide. For covalent attachment to solid phase, a divergent strategy was applied to both amine- and NHS-functionalized surfaces as shown in Figure 1.

In addition to a terminal alkyne for capture of azide-containing saccharides, we wanted to introduce a cleavage site in the linker to facilitate the analysis of the covalent array by standard techniques such as mass spectrometry (MS) and NMR. Among the various options, we selected a disulfide bond, which is stable to many biological applications but can be easily cleaved through reduction to release the thiol.[14−18] In addition, it can be oxidized to further improve stability. Therefore, linkers **1** and **2** (Figure 1) were synthesized for the covalent attachment of azide-containing saccharides to solid phase. Linker **1** was attached to the NHS-coated surface under basic conditions to give the alkyne-functionalized surface. To use amine-coated surfaces, thioisocyanate (**2**) was generated from amine **1**. Prior to capture of the azide-containing saccharide to the linker on the surface, attachment of the linker was verified via mass spectrometry (MS).

After incubation of linkers **1** and **2**, surfaces were repeatedly washed with H_2O and treated with dithiothreitol (DTT) to reduce the disulfide bonds and release the alkyne.[14−17] Cleavage was then monitored directly by sonic spray ionization (SSI) and electrospray ionization (ESI) MS, which not only verified the presence of the linker but also showed low background upon DTT treatment. Capture of azide-containing saccharides was then accomplished in an additional step (Figure 2).

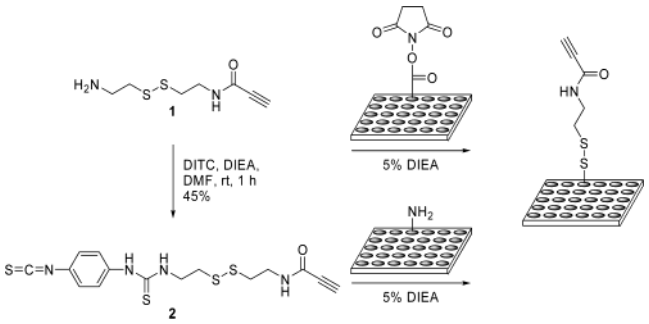

Figure 1. Cleavable linkers **1** and **2** can be attached to either NHS- or amine-coated surfaces to give the alkyne-functionalized surface.

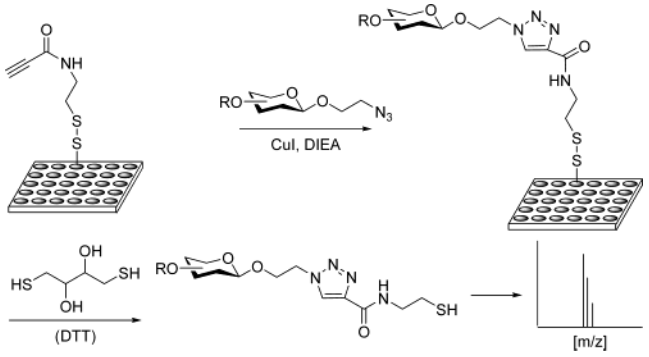

Figure 2. Triazole formation and cleavage by DTT for MS analysis.

Functionalized plates displaying the activated alkynes were treated with the azide-containing sugars in the presence of CuI.[8] The efficiency of this method was then monitored over time using DTT cleavage, with the resulting thiol being directly analyzed by MS. This attachment strategy was conducive to submicromolar concentrations and was successfully applied to the covalent attachment of saccharides as shown in Table 1.[8,19,20] As an example, the MS analysis of arrayed Globo-H, a breast cancer antigen, after cleavage from the solid support is shown in Figure 3A.

To characterize the biological applicability of this display method, lectin-binding studies were performed. Two lectins (sugar-recognizing protein) were used to study the bound carbohydrates: *Lotus tetragonolobus* lectin (LTL), which recognizes α-l-fucose,[21] and *Erythrina cristagalli* (EC) which recognizes galactose.[22] Both lectins were assayed successfully with the simple monosaccharides **3** and **5**. Globo-H (**23**) was also recognized in a concentration-dependent manner giving a K_d value of 22.8 μM. This value correlates well

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Table 1. Azide-Containing Saccharides Covalently Attached to Microtiter Plates

R = -$(CH_2)_2N_3$
R' = -$(CH_2)_5N_3$

3. Fuc-OR
4. Glc-OR
5. Gal-OR
6. Galβ1,3GlcNAc-OR
7. Galβ1,4GlcNAc-OR
8. GlcNAcβ1,3Galβ1,4GlcNAc-OR
9. Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-OR
10. NeuAcα2,3Galβ1,4Glc-OR
11. NeuAcα2,3Galβ1,3GlcNAc-OR
12. NeuAcα2,3Galβ1,4GlcNAc-OR
13. NeuAcα2,6Galβ1,4Glc-OR
14. NeuAcα2,6Galβ1,4GlcNAc-OR
15. NeuAcOHα2,3Galβ1,3GlcNAc-OR
16. NeuAcOHα2,3Galβ1,4GlcNAc-OR
17. Manα1,2Manα1,3Man-OR'
18. Manα1,2Manα1,2Manα1,3Man-OR'
19. Manα1,3[Manα1,6]Man-OR'
20. Manα1,2Manα1,3[Manα1,2Manα1,6]Man-OR'
21. Manα1,2Manα1,2Manα1,3[Manα1,2Manα1,2Manα1,6]Man-OR'
22. Manα1,2Manα1,2Manα1,3[Manα1,2Manα1,3(Manα1,2Manα1,2Manα1,6)]Man-OR'
23. Fucβ1,2Galβ1,3GalNacβ1,3Galα1,4Galβ1,4Glc-OR'

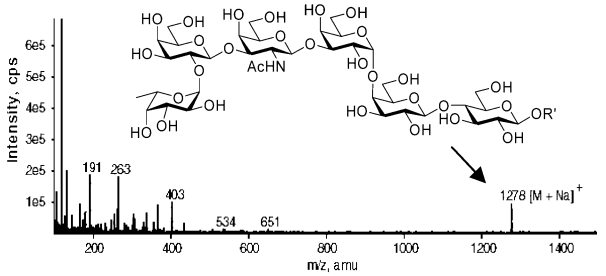

Figure 3. Electrospray ionization of compound **23** (Globo-H) cleaved from the polystyrene well surface by DTT reduction of disulfide bond.

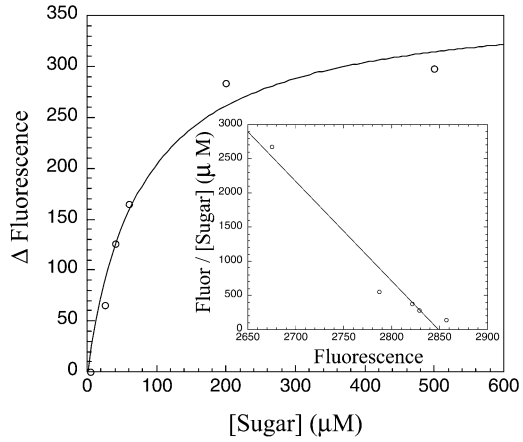

Figure 4. Recognition of 2G12 by oligomannose **20**.

with previously determined dissociation constants for (1,2)-linked fucose derivatives with LTL.[23]

This array strategy was also used to study the interaction of 2G12, a broadly neutralizing antibody that recognizes oligomannose residues on envelope protein gp120 of HIV-1.[23] The 2G12 antibody forms a novel domain-swapped dimer that interacts with a dense cluster of oligomannose moieties ($Man_9GlcNAc_2$) on the surface of HIV gp120 with four independent binding sites allowing for multivalent binding to the carbohydrates. We have defined the specificity of the antibody−carbohydrate interaction and identified **18** and **20** as the optimal epitope with micromolar affinity to 2G12,[19] and this result was further confirmed with the glycoarray approach.

Oligomannose **20** displayed in a microtiter plate with different density was further analyzed (Figure 4), and the K_d for **20** was determined to be 0.1 μM (K_d for **18**, **21**, and **22** = 0.1, 0.7, and 1.0 μM respectively). This significant enhancement of binding (>10,000-fold) for **20** compared to the previous solution-phase assay[19] is hypothesized to be a function of a multivalent interaction, which is under further investigation in our laboratory.

In summary, this work shows a new and efficient methodology for the covalent array of saccharides on microtiter plates. The use of a cleavable linker allows characterization and quantitative analysis of the array. Moreover, binding studies have proven that this microarray is functional in biological screening and therefore applicable in ELISA-type formats. The chemistry can be applied to other surfaces such as glass slides. We believe that this new covalent array, together with the one-pot automated synthesis of complex oligosaccharides, can become useful for the high-throughput biological evaluation of carbohydrate−protein interaction.[24]

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Supporting Information Available: Synthesis of linkers **1** and **2**, azide-containing saccharides, and assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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