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# Fabrication and application of neoglycolipid arrays in a microtiter plate

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**Abstract**—The work presented herein is a new noncovalent glycoarray assembly method for microplates created by simply mixing together a carbohydrate and a tetradecylamine. α-Mannose was utilized in the model study and product formation was detected by lectin binding. The method can be further extended to array complex carbohydrates.


Mammalian cell surfaces are coated by a layer of carbohydrate molecules attached to lipids and proteins known as the glycocalyx. These often complex and heterogeneous glycans are involved in a diverse array of biological processes including inflammation, metastasis, cell–cell adhesion, and pathogen–cell interactions.[1,2] Identification of the specific carbohydrates involved in these processes is important to better understand cell–cell recognition at the molecular level and to aid in the design of therapeutics and diagnostic tools.

Microarrays are becoming one of the most powerful technologies for the identification and biological evaluation of new drug candidates and targets. However, only very recently have carbohydrate microarrays, or glycoarrays, appeared in the scientific literature as a promising high-throughput methodology for the study of cell–cell recognition processes mediated by carbohydrates.[3–7] And the fabrication strategies for glycoarrays still need to be improved.

In this study, our goal is to develop quite a simple and efficient system to attach neoglycolipids to a microtiter plate to screen the interactions of carbohydrates and proteins.

*Synthesis of lipid-linked saccharides*: The neoglycolipid arrays involve labeling the carbohydrate by reductive amination. The primary amine and the C-1 aldehyde of the reducing sugar react to form a Schiff base, which is reduced to the mixed aryl/aliphatic secondary amine by sodium cyanoborohydride, as shown in Figure 1. It is necessarily emphasized that the reaction must use excess saccharides in order to exhaust tetradecylamine. The yields of reductive amination for α-mannose, *Escherichia coli* O86, O127, and O128-antigens are 85%, 79%, 78%, and 67%, respectively.

The crude lipid-linked saccharides could be purified in microtiter plate (Fig. 2). The method can been further extended to array complex carbohydrates.

*Con A assay for α-mannose*: The FITC-labeled *Canavalia ensiformis* Con A was purchased from Sigma, which has the specificity for α-mannose. Typical fluorescence analysis of FITC-Con A is presented in Figure 3 after subtracting the blank. It shows that the peak intensity increases with the concentration increases of α-mannose (0.2 μmol/L–2mmol/L).

*Application of the neoglycolipid arrays*: We purified different bacteria saccharides from *E. coli*.[8] These preparations included *E. coli* O86-antigen, O127-antigen, O128-antigen, which are shown in Figure 4.

The FITC-labeled human serum IgM also was purchased from Sigma, which has the specificity for *E. coli* antigens. The fluorescence intensity of *E. coli* O86-antigen, O127-antigen, and O128-antigen binding

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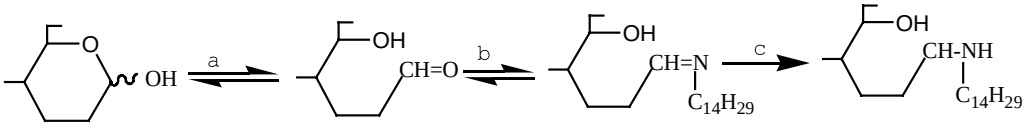


**Figure 1.** Synthetic route of lipid-linked saccharides. Reagents and conditions: (a) $CH_3COOH$ (10%); (b) $C_{14}H_{29}NH_2$; (c) $NaBH_3CN$, DMSO, 37 °C, 20 h.

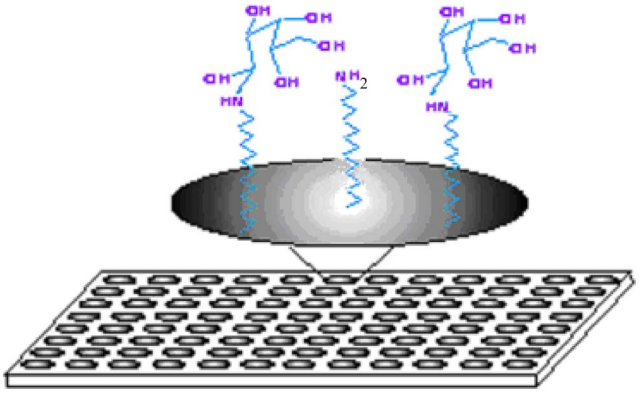

**Figure 2.** The lipid-linked saccharides in a microtiter plate well.

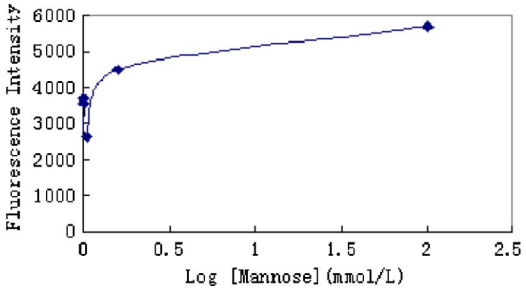


**Figure 3.** Fluorescence analysis of FITC-Con A binding assay for reaction in microtiter plates at different concentrations (0.2 μmol/L–2 mmol/L).

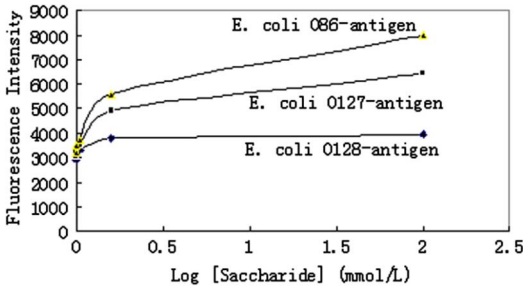


**Figure 5.** The fluorescence intensity of *Escherichia coli* O86-antigen, O127-antigen, and O128-antigen binding with human serum IgM.

with human serum IgM is summarized in Figure 5. It also indicates that the peak intensity increases with the concentration increases of saccharides. It proves that *E. coli* O86-antigen binding IgM is the strongest, *E. coli* O128-antigen is the weakest in the three ones.

The structures of the three carbohydrate-antigens are similar to each other. Noticeably, human blood B trisaccharide epitope on the red blood cell consists of an α-L-Fuc(1 → 2)[α-D-Gal(1 → 3)]β-D-Gal-OMe structure,[9] which is similar to that of the *E. coli* O86 O-antigen.[10] The array assay showed that this epitope structure is possibly responsible for the binding of *E. coli* O86-antigen to the human serum IgM. Compared to the structures *E. coli* O86-antigen and O127-antigen, It reveals that they have a similar backbone, with the only difference in the side chains.

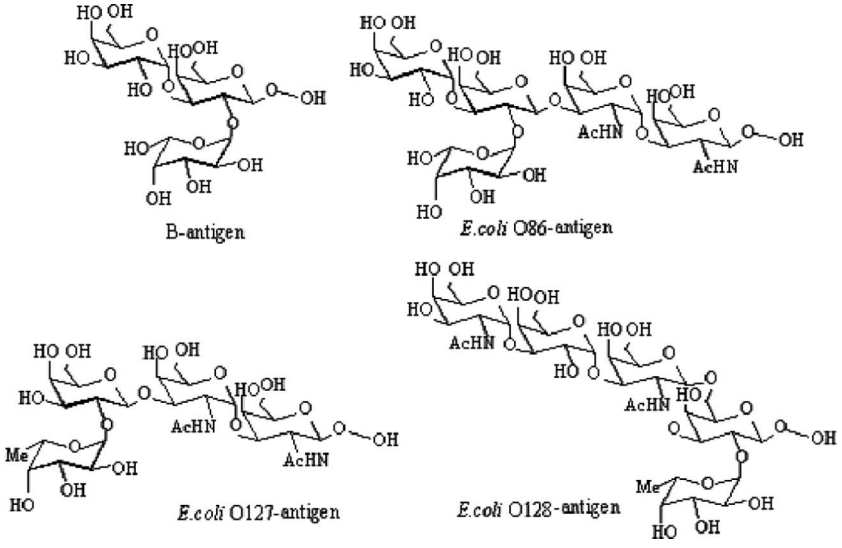


**Figure 4.** The carbohydrate chain structures from human blood group B and *Escherichia coli*.

In summary, this work shows an efficient methodology for the noncovalent assembly of saccharides in microtiter plates and in detecting carbohydrate–protein interactions. Lectin binding studies have proven that this microarray is functional in biological screening and therefore applicable in enzyme-linked immunosorbent assay (ELISA)-type formats. We believe that this array and detection method, together with the efficient methods available for synthesis of other complex saccharides, can become useful for the high-throughput biological evaluation of carbohydrate–protein interactions.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.12.063.

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