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Assembly of sugars on polystyrene plates: a new facile microarray fabrication technique

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Abstract—The work presented herein is a new noncovalent glycoarray assembly method for microplates created by simply mixing together an isocyanate-containing C_{14}-hydrocarbon and an amine-containing carbohydrate. 2-Aminoethyl-β-D-galactopyranoside (**1**) was utilized in model studies and product formation was detected by both ESI-MS and lectin binding. The method has been further extended to array complex carbohydrates.

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.[1–8] Glycoarray development has been hindered first by synthesis of carbohydrate libraries and second by development of orthogonal attachment strategies that are critical to their efficient display. With the advent of high-throughput oligosaccharide synthesis, this leaves the bottleneck with the development of the actual glycoarray technology.[9,10]

Carbohydrates alone show very low retention to polystyrene plates due to their high polarity.[3,11] As such, a variety of attachment strategies have been developed including covalent attachment through Michael addition and Diels–Alder reaction and noncovalent attachment through glycolipid application where the saccharide is linked to a C_{14}-hydrocarbon to favor their retention in polystyrene wells.[1,2,5,6] In order to directly link library synthesis with oligosaccharide display, techniques allowing for capture of an orthogonal group to the surface for direct analysis with minimal intermediate treatment have to be developed. We have recently applied the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction between azides and alkynes to glycoarray assembly.[1] Our continuous efforts toward elucidation of the function of complex carbohydrates in a rapid manner prompted us to investigate alternative methods for the preparation of carbohydrate arrays.

Though complex oligosaccharides have been displayed effectively through cyclic triazole formation and are stable under the most organic reaction conditions, oxygen, and water,[1] we are interested in new coupling strategies to increase applicability. In oligosaccharide chemical synthesis, the terminal step is usually the removal of protecting groups such as esters and ethers. While the azido group sustains ester deprotection, the reductive conditions commonly employed to remove benzyl ethers (among the most common protecting groups in carbohydrate chemistry) also reduce azides to amines. Although amines can be easily reconverted into azides and methods other than reductive cleavage are available for the deprotection of benzyl groups, we decided to directly exploit the amino group for the fabrication of a noncovalent carbohydrate array.[12–14]

While amide formation is surely an excellent ligation method, we thought to exploit the reagent-free urea formation between isocyanates and amines, a very well known process in the gluing industry.[15] Therefore, amino-sugars were reacted with a C_{14}-isocyanate to form the highly stable urea functionalized with a suitably long hydrocarbon chain necessary for noncovalent adsorption to the microtiter plate. This reaction was first

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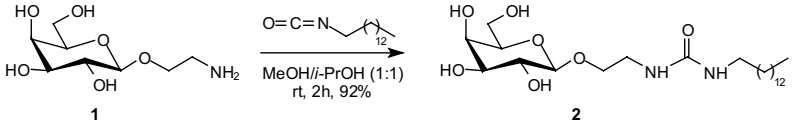
performed in solution on a relatively large scale (2 mmol) using 2-aminoethyl-β-D-galactopyranoside (**1**) as a model. Amino-sugar **1** was reacted with commercially available tetradecyl isocyanate to form the urea derivative **2** in 92% yield and high purity (Scheme 1).[16]

This transformation is clean, quick, and coupling reagent-free. By simply mixing the two reactants together, the urea derivative is obtained in very high yield as a unique product. This reaction is not moisture or air sensitive and product formation occurs at rt. Therefore, these conditions are ideal for microscale application. Indeed, we ran this reaction in the microtiter plate[17] by mixing together the two reactants in MeOH/isopropanol (1:1) and placing the plate in a ventilated fume hood for evaporation of solvents.

The course of the reaction was followed by electrospray ionization-mass spectrometry (ESI-MS). Figure 1C shows ESI of the starting amine **1** (224, $M+H^+$). After 2 h reaction with tetradecyl isocyanate, ESI (Fig. 1D) shows disappearance of the starting material and formation of the galactosyl-urea derivative **2** (463, $M+H^+$; 485, $M+Na^+$) together with formation of tetradecylamine (214, $M+H^+$) derived from hydrolysis of excess of tetradecyl isocyanate. When the reaction was complete, traces of solvents were removed by placing the plate in a dessicator under vacuum.

In addition, it was important to confirm that this array is stable under repeated aqueous washings. Indeed, when wells were washed with milli-Q water (3×100 μL), retention of galactosyl-urea **2** in the plate was observed by both ESI-MS (Fig. 1E) and biological assay (Fig. 1B). A variety of lectins (carbohydrate-recognizing proteins) have been previously applied to the analysis of noncovalent arrays including *Ricinus communis* (Gal), *Tetragonolobus purpureas* (Fuc), and *Sambucus nigra* (NeuAc).[1,2] While the presence of galactose was again being analyzed, a less toxic lectin than *R. communis* was



Scheme 1.

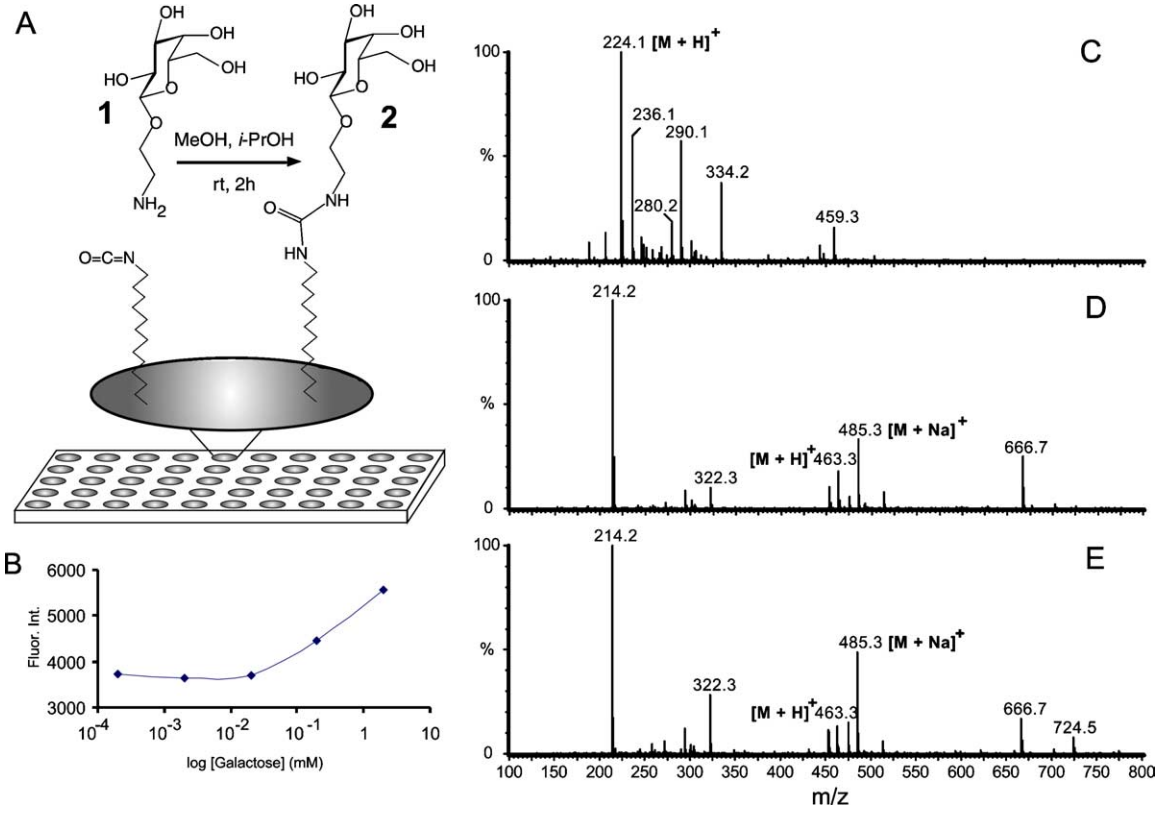


Figure 1. (A) Formation of compound **2** in the microtiter plate well (scale 2 mM). (B) Fluorescence analysis of lectin binding assay with *S. japonica* for reaction in microtiter plates at different concentrations (0.2 μM–2 mM). ESI-MS of starting amine **1** (C) before reaction in plate, (D) after 2 h reaction, and (E) after three washings with milli-Q water.

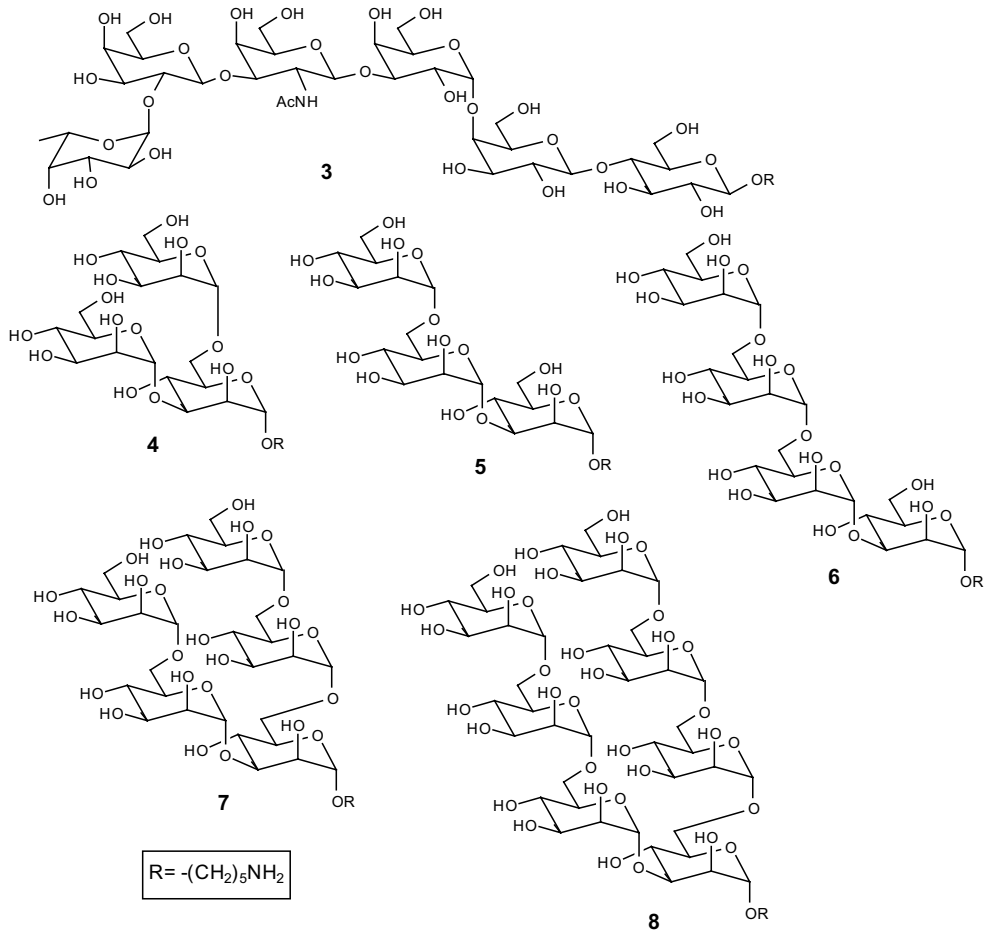


Figure 2. Oligosaccharides displayed in microtiter plate via urea formation with tetradecyl isocyanate.

desired. Therefore, a galactose-specific lectin from *Sophora japonica* was selected.[18] Observation of the fluorescein-labeled lectin over a range of concentrations was readily detectable (from 0.2 μM to 2 mM). Also, the lectin responded in a concentration dependent manner to the galactose as would be expected. This indicates that this method is indeed highly applicable to ELISA-type lectin assays.

Various complex carbohydrates were also displayed in a microtiter plate following this procedure. Cancer antigen Globo-H (**3**) and gp120 oligomannose type sugars **4**–**8** (Fig. 2) were displayed via the urea bond linkage and product formation in all cases was confirmed with ESI-MS.[19–21] The formation of a large array of oligosaccharides following this newly developed method and the biological screening with different lectins is currently under investigation.

In summary, this work shows a new and efficient methodology for the noncovalent assembly of saccharides in microtiter plates. Lectin binding studies have proven that this microarray is functional in biological screening and therefore applicable in ELISA-type formats. We believe that this new array, together with the efficient methods available for synthesis of complex oligosaccharides, can become useful for the high-throughput biological evaluation of carbohydrate–protein and carbohydrate–carbohydrate interactions.[9,10]

References and notes

1. Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 14397–14402.
2. Bryan, M. C.; Plettenburg, O.; Sears, P.; Rabuka, D.; Wacowich-Sgarbi, S.; Wong, C.-H. *Chem. Biol.* **2002**, *9*, 713–720.
3. Wang, D.; Liu, S.; Trummer, B. J.; Deng, C.; Wang, A. *Nature Biotech.* **2002**, *20*, 275–281.
4. Fukui, S.; Feitzi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nature Biotech.* **2002**, *20*, 1011–1017.
5. Park, S.; Shin, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 3180–3182.
6. Houseman, B. T.; Mrksich, M. *Chem. Biol.* **2002**, *9*, 443–454.
7. Mellet, C. O.; Fernandez, J. M. G. *ChemBioChem* **2002**, *3*, 819–822.
8. Adams, E. W.; Ueberfeld, J.; Ratner, D. M.; O'Keefe, B. R.; Walt, D. R.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 5317–5320.
9. Sears, P.; Wong, C.-H. *Science* **2001**, *291*, 2344–2350.
10. Seeberger, P. H.; Haase, W.-C. *Chem. Rev.* **2000**, *100*, 4349–4393.
11. Lee, Y. C.; Lee, R. T. Recognition of Carbohydrates in Biological Systems, Part A: General Procedures. In

Methods in Enzymology; Abelson, J. N., Simons, M. I., Eds.; Academic: New York, 2003; Vol. 362, p 625.
12. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029–6032.
13. Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773–10778.
14. Birch, A. J.; Rao, G. S. *Adv. Org. Chem.* **1972**, *8*, 1–65.
15. Diphenylmethane diisocyanate (MDI) and Toluene diisocyanate (TDI) are two common, widely used, isocyanate based glues.
16. Compound **2**: mp 151–152 °C (from Et_2O). 1H NMR (CD_3OD): 4.13 (d, 1H, $J = 7.45$ Hz), 3.73 (m, 2H), 3.68 (d, 1H, $J = 7.45$ Hz), 3.64 (m, 2H), 3.43 (m, 2H), 3.38 (dd, 1H, $J = 10.08$ Hz, $J = 3.50$ Hz), 3.24 (m, 2H), 3.00 (t, 2H, $J = 7.01$ Hz), 1.37 (m, 2H), 1.20 (s, 22H), 0.81 (t, 3H, $J = 7.01$ Hz). 13C NMR (CD_3OD): 161.27, 105.44, 76.81, 74.88, 72.57, 71.18, 70.33, 62.69, 41.41, 41.01, 33.08, 31.33, 30.80, 30.77, 30.52, 30.48, 27.99, 23.73, 14.45. HR-MALDI-FTMS: calcd for $C_{23}H_{46}N_2O_7Na$ $[M+Na]^+$, 485.3197; found, 485.3202.
17. Protocol for array formation: tetradecyl isocyanate (40 μL of a freshly prepared 4 mM solution in isopropanol, 2 equiv) was added, in microtiter plate, to a solution of the arrayed carbohydrates (2 mM solution in MeOH, 40 μL). The plate was placed in a fume hood for 2 h to allow for evaporation of solvent and then transferred for ca. 15 min in a dessicator connected with a vacuum pump. Washings with milli-Q water (3×100 μL) afforded the carbohydrate microarray ready for biological screening.
18. McPherson, A.; Hankins, C. N.; Shannon, L. *J. Biol. Chem.* **1987**, *262*, 1791–1794.
19. Burkhart, F.; Zhang, Z.; Wacowich-Sgarbi, S.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2001**, *40*, 1274–1277.
20. Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science* **2003**, *300*, 2065–2071.
21. ESI-MS of oligosaccharides **3**–**8**, after urea formation in microtiter plate (most abundant signals): compound **3**: $C_{58}H_{105}N_3O_{31}Na$ $[M+Na]^+$, 1362.6; compound **4**: $C_{38}H_{72}N_2O_{17}Na$ $[M+Na]^+$, 851.5, $C_{38}H_{72}N_2O_{17}Cl$ $[M+Cl]^-$, 863.3; compound **5**: $C_{38}H_{72}N_2O_{17}Na$ $[M+Na]^+$, 851.5, $C_{38}H_{73}N_2O_{17}Cl$ $[M-H]^-$, 827.3; compound **6**: $C_{44}H_{83}N_2O_{22}$ $[M-H]^-$, 989.4, $C_{44}H_{82}N_2O_{22}Cl$ $[M+Cl]^-$, 1025.3; compound **7**: $C_{50}H_{93}N_2O_{27}$ $[M-H]^-$, 1151.4; compound **8**: $C_{62}H_{113}N_2O_{37}$ $[M-H]^-$, 1475.7.