One-Step, Acid-Mediated Method for Modification of Glass Surfaces with *N*-Hydroxysuccinimide Esters and Its Application to the Construction of Microarrays for Studies of Biomolecular Interactions

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Microarray technologies have received considerable attention owing to the fact that they serve as powerful tools for the high-throughput analysis of biomolecular interactions and the identification of bioactive substances that bind to biomolecules. Most of the current methods used to construct microarrays rely on the immobilization of substances on properly derivatized surfaces. Among various functional groups used for this purpose, the *N*-hydroxysuccinimide (NHS) ester group has been largely employed because it can be readily reacted with amine or hydrazide functionalities in substances of interest. However, the NHS ester group is usually introduced onto the surface of a glass slide by employing inconvenient and time-consuming multistep processes. In recent studies, we have developed an efficient, single step method for derivatization of glass surfaces with NHS ester groups that takes advantage of an acid-mediated reaction of NHS ester functionalized dimethallylsilanes with silanols on the glass surface. Conditions for the surface modification procedure that utilize TfOH rather than $Sc(OTf)_3$ were found to be superior. Protein and RNA-binding experiments show that glass surfaces modified by employing this method are suitable for efficient immobilization of various substances that are appended by amine, hydrazide, and alcohol functionalities. The microarrays, generated in this way, are applicable to procedures for rapid analysis of protein−protein, protein−glycan, protein−small molecule, and peptide−RNA interactions, as well as for profiling enzyme activities. The newly developed acid-mediated, glass surface modification method should be generally applicable to the preparation of various functional group-modified surfaces.

INTRODUCTION

Since the discovery of peptide microarrays in 1991 (*1*), technologies based on DNA, peptide, protein, and carbohydrate microarrays have attracted enormous attention as powerful and sensitive tools for genomics, transcriptomics, proteomics, and glycomics research (*2*−*8*). The microarrays are composed of diverse probes that are orderly and densely immobilized on a single chip. Microarray-based technologies have gained prominence over other conventional analytic methods because they can be used to assess simultaneously large numbers of recognition events employing only small amounts of samples. These beneficial features make microarray technologies suitable for the rapid analysis of biomolecular interactions such as those taking place in DNA−RNA/DNA−DNA, peptide−protein, protein−protein, and protein−glycan systems (*9*−*14*). This technology is also applicable to the rapid identification of bioactive molecules that selectively interact with proteins or act as substrates for enzymes (*15*−*18*).

Microarrays are generally prepared by covalent or noncovalent immobilization of (bio)molecules on appropriate solid surfaces. Glass slides are most extensively used as the surface material as a result of ease of manipulation and low cost. Another advantage of glass slides is that they are amenable to applications that involve optical detection of events. Most of the existing modification methods rely on the reaction of piranha solution-treated glass surfaces with alkoxysilane-appended aldehydes, carboxylic acids, or amines (*19, 20*). The resulting slides are directly used for attachment of (bio)molecules, or they are further derivatized with functional groups, such as thiol, maleimide, alkyne, *N*-hydroxysuccinimide (NHS) ester, epoxide, hydrazide, aminooxy, fluoroalkyl, and photoreactive groups, which can be used for attachment of samples (*21*−*35*).

Among the various functionalities used for immobilization on solid surfaces, the NHS ester group has been the most utilized to construct microarrays because it readily reacts with amine or hydrazide functional groups in substances at pH 8−9 (*25*−*27*). However, the NHS ester group is usually introduced onto glass slides by employing multistep modification processes, which often lack efficiency and are time-consuming. As a consequence, the development of simple, facile, and efficient methods for derivatization of glass surfaces with NHS esters is a significant pursuit.

Recently, we developed a procedure for $Sc(OTf)_3$-mediated silylation of hydroxyl groups for the purpose of derivatizing silica surfaces (*36*). In this process, easily prepared and stable methallylsilanes undergo facile reaction with alcohols in the presence of acid catalysts. This catalyst-mediated process has advantages associated with controllability and experimental simplicity. Below, we report the results of a study in which this chemistry has been adapted to an efficient, one-step surface modification method to prepare the NHS ester-derivatized glass slides. The strategy relies on the use of an acid-mediated reaction of an NHS ester functionalized dimethallylsilane with silanols on glass surfaces and avoids the need for laborious surface derivatization. To validate the utility of the surface modification approach, microarrays containing various substances, such as proteins, peptides, sugars, and small molecules, were constructed by printing on NHS ester-coated glass slides, generated by the one-step process. The microarrays were applied to the analysis of carbohydrate−protein, protein−protein, RNA−peptide, and

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small molecule−protein interactions. The results of these studies are described and discussed below.

MATERIALS AND METHODS

General. All chemical and biochemical products were of analytical grade and purchased from commercial suppliers: lectins from Vector Laboratories (Peterborough, U.K.) and Sigma-Aldrich (St. Louis, MO); protein G from *Streptococcus* sp. and goat anti-mouse FITC-labeled IgG from Sigma-Aldrich; Cy3-streptavidin from Amersham Pharmacia biotech (Piscataway, NJ); β-1,4-galactosyltransferase (β-1,4-GalT) and UDP-Gal from Calbiochem (Gibbstown, NJ); carbohydrates from Sigma-Aldrich, Calbiochem (Gibbstown, NJ), TCI Corp. (Tokyo, Japan), and Acros (Morris Plains, NJ); anti-dextran antibody from Stemcell Technologies Inc. (Vancouver, BC, Canada); microscope glass slide from Corning (Lowell, MA); Dy547-RRE from Thermo Fisher Scientific (Lafayette, CO). The solutions of probes were printed on the surface with a MicroSys 5100 from Cartesian Technologies Inc. (Irvine, CA). Microarrays probed with fluorescent dye labeled proteins and RNA were scanned with an ArrayWoRx biochip reader from Applied Precision (Northwest Issaquah, WA).

Synthesis of 3-Chloropropyldimethallylmethylsilane (2a). A solution of $H_2PtCl_6 \cdot xH_2O$ (10.4 mg, 0.02 mmol) in 2-propanol (0.2 mL) and dichloromethylsilane (26 mL, 250 mmol) was added in a round-bottom flask, and the mixture was stirred until it became homogeneous. Diethyl ether (10 mL) was added into the reaction vessel, and the reaction temperature was raised to 40 °C by an oil bath. After dropwise addition of allyl chloride (20 mL, 250 mmol), the mixture was stirred for 6 h at 80 °C. After the reaction, all volatiles were evaporated under the reduced pressure. The crude product, 3-chloropropyldichloromethylsilane, was used in the next step without further purification.

The crude 3-chloropropyldichloromethylsilane was added dropwise into methallylmagnesium chloride (1.1 equiv) solution in 300 mL of THF at 0 °C, and the reaction mixture was stirred for 6 h at 0 °C. After the reaction, saturated aqueous NH_4Cl was added to the reaction mixture, and the mixture was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$ and filtered through Celite pad. The crude mixture was purified by flash column chromatography (*n*-hexane; $R_f = 0.6$) to give **2a** in 70% yield (40 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.57 (d, $J = 30.7$ Hz, 4 H), 3.50 (t, $J = 13.9$ Hz, 2 H), 1.86−1.76 (m, 2 H), 1.73 (s, 6 H), 1.60 (s, 4 H), 0.73−0.68 (m, 2 H), 0.09 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.3, 109.3, 48.1, 27.7, 25.8, 25.6, 11.9, 4.2; IR spectrum (neat) 3083, 2966, 2914, 1644, 1450, 1748, 1378, 1280, 1168, 871, 840 cm^{-1}. Anal. Calcd for $C_{12}H_{23}ClSi$: C, 62.43; H, 10.04. Found: C, 62.48; H, 9.93.

Synthesis of 3-Azidopropyldimethallylmethylsilane (3a). 3-Chloropropyldimethallylmethylsilane (**2a**, 10 g, 43.2 mmol) and sodium azide (5.63 g, 86.58 mmol) was added to 100 mL of DMF and stirred for 4 h at 80 °C. After the reaction, saturated aqueous NH_4Cl solution was added. The solution was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$ and filtered through Celite pad. The crude mixture was purified by flash column chromatography (*n*-hexane/ethyl acetate = 10:1, $R_f = 0.6$) to give **3a** in 92% yield (9.5 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.57 (d, $J = 28.5$ Hz, 4 H), 3.23 (t, $J = 7.0$ Hz, 2 H), 1.78 (s, 6 H), 1.77−1.60 (m, 2 H), 1.58 (s, 4 H), 0.66−0.60 (m, 2 H), 0.07 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.3, 109.3, 54.7, 25.8, 25.6, 23.8, 11.4, 4.2; IR spectrum (neat) 3079, 2971, 2922, 2876, 2102, 1748, 1636, 1459, 1378, 1282, 1255, 1170, 873, 846 cm^{-1}. Anal. Calcd for $C_{12}H_{23}N_3Si$: C, 60.71; H, 9.76; N, 17.70. Found: C, 60.68; H, 9.72; N, 16.78.

Synthesis of 11-Chloroundecyldimethallylmethylsilane (2b). A solution of $H_2PtCl_6 \cdot xH_2O$ (10.4 mg, 0.02 mmol) in 2-propanol (0.2 mL) and dichloromethylsilane (5 mL, 48 mmol) was added in a round-bottom flask, and the mixture was stirred until it became homogeneous. Diethyl ether (10 mL) was added into the reaction vessel, and the reaction temperature was raised to 40 °C by an oil bath. After dropwise addition of 11-chloroundec-1-ene (10 g, 52.98 mmol), the mixture was stirred for 6 h at 80 °C. After the reaction, unreacted 11-chloroundec-1-ene was removed by distillation. The remaining crude product, dichloro-11-chloroundecylmethylsilane, was used in the next step without further purification.

The crude dichloro-11-chloroundecylmethylsilane was added dropwise into methallylmagnesium chloride (1.1 equiv) solution in 300 mL of THF at 0 °C, and the reaction mixture was stirred for 6 h at 0 °C. After the reaction, saturated aqueous NH_4Cl was added to the reaction mixture, and the mixture was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$ and filtered through Celite pad. The crude mixture was purified by flash column chromatography (*n*-hexane; $R_f = 0.8$) to give **2b** in 65% yield (10.71 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.74 (d, $J = 26.6$ Hz, 4 H), 3.70 (t, $J = 6.74$ Hz, 2 H), 2.04−1.91 (m, 8 H), 1.64−1.48 (m, 18 H), 0.77−0.74 (m, 2 H), 0.25 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.4, 108.9, 45.0, 33.9, 32.9, 29.7, 29.6, 29.4, 29.1, 27.1, 26.3, 25.9, 25.5, 23.9, 14.3, 4.2; IR spectrum (neat) 3073, 2924, 2853, 1636, 1448, 1279, 1250, 870 cm^{-1}. Anal. Calcd for $C_{20}H_{39}ClSi$: C, 70.02; H, 11.46. Found: C, 70.08; H, 11.79.

Synthesis of 11-Azidoundecyldimethallylmethylsilane (3b). 11-Chloroundecyldimethallylsilane (**2b**, 5.5 g, 16.7 mmol) and sodium azide (2.18 g, 33.5 mmol) were added to 100 mL of DMF and stirred for 4 h at 80 °C. After the reaction, saturated aqueous NH_4Cl solution was added. The solution was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$ and filtered through Celite pad. The crude mixture was purified by flash column chromatography (*n*-hexane/ethyl acetate = 10:1, $R_f = 0.6$) to give **3b** in 78% yield (4.56 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.51 (d, $J = 27.3$ Hz, 4 H), 3.22 (t, $J = 6.9$ Hz, 2 H), 1.68 (s, 6 H), 1.58 (m, 6 H), 1.24 (s, 18 H), 0.54 (t, $J = 7.8$ Hz, 2 H), 0.01 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 143.7, 108.9, 51.7, 33.9, 30.0, 29.8, 29.5, 29.4, 29.1, 26.9, 25.9, 25.6, 23.9, 14.3, −4.3; IR spectrum (neat) 3072, 2921, 2851, 2094, 1636, 1454, 1373, 1279, 1250, 1166, 998, 971, 868, 839, 722 cm^{-1}. Anal. Calcd for $C_{20}H_{39}N_3Si$: C, 68.71; H, 11.24; N, 12.02. Found: C, 68.60; H, 12.15; N, 12.15.

Synthesis of 2,5-Dioxopyrrolidin-1-yl-1-(3-(methylbis(2-methylallyl)silyl)propyl)-1*H*-1,2,3-triazole 4-Carboxylate (1a). A solution of propiolic acid (2.1 mL, 34.0 mmol), *N*-hydroxysuccinimide (4.0 g, 34.8 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (8.6 g, 41.7 mmol) in 80 mL of dimethoxyethane was stirred for 18 h at room temperature. After the reaction, the mixture was filtered through Celite pad to remove dicyclohexylurea, and all volatiles were evaporated under the reduced pressure. The crude product, 2,5-dioxopyrrolidin-1-yl propiolate, was used in the next step without further purification. To a solution of crude 2,5-dioxopyrrolidin-1-yl propiolate in 50 mL of THF was added $CuSO_4 \cdot 5H_2O$ (0.43 g, 1.74 mmol), sodium ascorbate (0.69 g, 3.48 mmol) in 50 mL of H_2O, and 3-azidopropylmethyldimethallylsilane (**3a**, 4.13 g, 17.38 mmol). The reaction mixture was stirred for 4 h at room temperature. After the reaction, saturated aqueous NH_4Cl was added, and the resulting mixture was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$ and filtered through Celite pad, then purified by flash column chromatography (*n*-hexane/ethyl acetate = 1:1, $R_f = 0.3$) to give **1a** in 60% yield (4.21 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.33

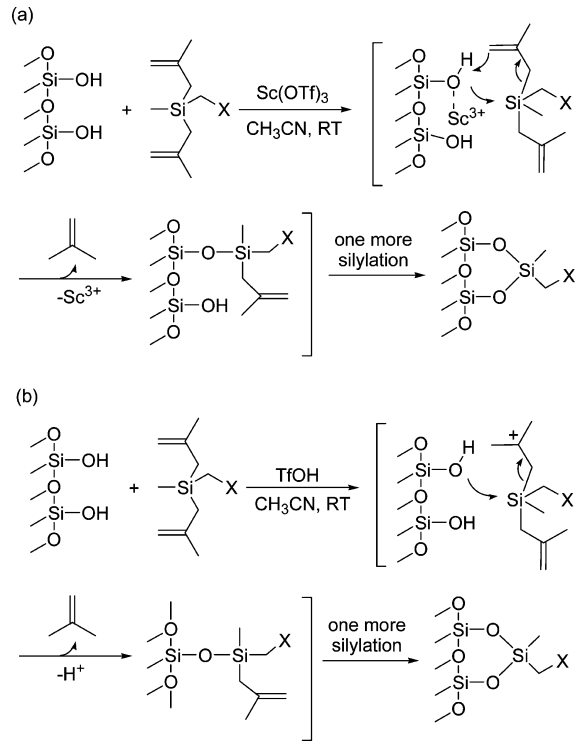
(s, 1 H), 4.56 (d, $J = 16.5$ Hz, 4 H), 4.44 (t, $J = 4.8$ Hz, 2 H), 2.93 (s, 4 H), 1.98 (m, 2 H), 1.70 (s, 6 H), 1.59 (s, 4 H), 0.59 (m, 4 H), 0.08 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 169.3, 155.9, 143.0, 129.3, 54.0, 25.8, 25.6, 25.5, 25.2, 10.9, 4.3; IR spectrum (neat) 3149, 3071, 2935, 2252, 1815, 1793, 1742, 1636, 1535, 1466, 1450, 1437, 1374, 1278, 1254, 1202, 1168, 1074, 958, 907, 839, 732, 649 cm^{-1}; HR-MS (TOF) calcd for $C_{19}H_{28}N_4O_4SiNa$ ($M + Na^+$) 427.1778, found 427.1774. Anal. Calcd for $C_{12}H_{23}N_3Si$: C, 56.41; H, 6.98; N, 13.85. Found: C, 56.94; H, 6.96; N, 13.09.

Synthesis of 2,5-Dioxopyrrolidin-1-yl 1-(11-(methylbis(2-methylallyl)silyl)undecyl)-1*H*-1,2,3-triazole 4-Carboxylate (1b). A solution of propiolic acid (2.1 mL, 34.0 mmol), *N*-hydroxysuccinimide (4.0 g, 34.8 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (8.6 g, 41.7 mmol) in 80 mL of dimethoxyethane was stirred for 18 h at room temperature. After the reaction, the mixture was filtered through a Celite pad to remove dicyclohexylurea, and all volatiles were evaporated. The crude product, 2,5-dioxopyrrolidin-1-yl propiolate, was used in the next step without further purification. To a solution of crude 2,5-dioxopyrrolidin-1-yl-propiolate in 50 mL of THF was added 11-azidoundecyldimethallylmethylsilane (6.08 g, 17.38 mmol) and catalyst mixture of $CuSO_4 \cdot 5H_2O$ (0.43 g, 1.74 mmol) and sodium ascorbate (0.69 g, 3.48 mmol) in 50 mL of H_2O, and the reaction mixture was stirred for 4 h at room temperature. After the reaction, saturated aqueous NH_4Cl was added, and the mixture was extracted with diethyl ether three times. The collected organic layers were dried over anhydrous $MgSO_4$, then filtered through a Celite pad and purified by flash column chromatography (*n*-hexane/ethyl acetate = 1:1, $R_f = 0.3$) to give **1b** in 50% yield (4.2 g): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.24 (s, 1 H), 4.56 (d, $J = 28.6$ Hz, 2 H), 4.45 (t, $J = 7.2$ Hz, 2 H), 2.91 (s, 4 H), 1.98−1.93 (m, 2 H), 1.71 (s, 3 H), 1.57 (s, 2 H), 0.56−0.53 (m, 2 H), 0.03 (s, 3 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 169.0, 155.9, 143.7, 134.9, 129.1, 108.8, 51.1, 33.8, 30.2, 29.6, 29.5, 29.4, 29.1, 26.5, 25.9, 25.8, 25.5, 23.9, 14.3, 4.2; IR spectrum (neat) 3515, 3347, 3137, 3073, 2920, 2853, 1811, 1785, 1636, 1536, 1465, 1370, 1202, 1078, 870 cm^{-1}; HR-MS (TOF) calcd for $C_{27}H_{44}N_4O_4SiNa$ ($M + Na^+$) 539.3030, found 539.3032. Anal. Calcd for $C_{27}H_{44}N_4O_4Si$: C, 62.76; H, 8.58; N, 10.84. Found: C, 62.32; H, 8.69; N, 10.69.

Preparation of NHS Ester-Functionalized Glass Slides. The microscope slides (2.5 cm × 7.5 cm) were immersed in a "piranha" solution (a mixture of 70% volume of concentrated sulfuric acid and 30% volume of a 30% hydrogen peroxide solution) for 12 h at room temperature. (*Caution:* Piranha solution should be handled with extreme caution because of its high oxidizing power and risk of explosion. Experimenters should wear protective clothes and safety goggles.) The cleaned glass slides were carefully removed from the piranha solution, washed extensively with deionized water, and dried by purging with argon gas. The cleaned slides were kept in a custom slide reaction vessel containing a solution of **1a** or **1b** (3% or 6%) in the presence of $Sc(OTf)_3$ (0.1 equiv) or TfOH (0.1 equiv) as a acid catalyst in 1:1 toluene/acetonitrile for 8 h. The reaction vessel was closed to prevent evaporation of solvent during reaction. After washing with acetonitrile three times, the slides were dried by purging with argon gas. The derivatized slides can be stored at room temperature in a desiccator for several weeks.

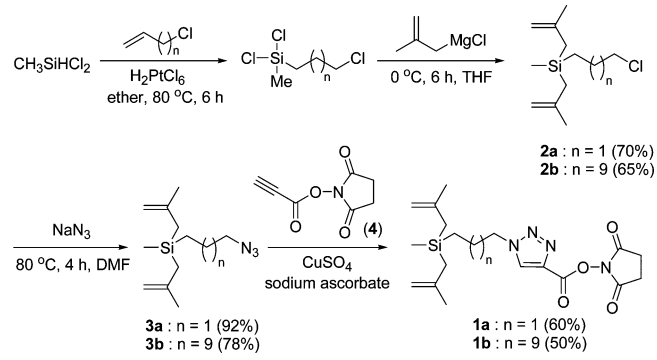
Printing of Samples on the NHS-Ester Functionalized Glass Surface. Carbohydrates, small molecules, proteins, and peptides were dissolved in 50 mM sodium phosphate buffer (pH 8.0) containing 40% glycerol. A solution of samples (1 nL) from a 384-well plate was printed in predetermined places on the NHS-ester functionalized glass slide with a distance of 270 μm between the centers of adjacent spots. After completion

Scheme 1. Mechanism for Acid-Mediated Silylation of Silanols on a Glass Surface[a]



[a] (a) $Sc(OTf)_3$ and (b) TfOH are used as acid catalysts.

Scheme 2. Preparation of NHS-Ester Functionalized Dimethallylsilanes 1a and 1b



of printing, the slide was placed into a humidity chamber (60%) at room temperature for 3−5 h. The spotted slide was divided into several blocks by using a compartmentalized plastic film that was coated with adhesive on one side (thickness: 0.1−0.2 mm) to avoid cross-contamination. The slide was washed with PBS (pH 7.4) containing 0.1% Tween 20, and a solution possessing 1% 2-aminoethanol in 50 mM sodium phosphate buffer (pH 8.0) was dropped in the blocks to remove unreacted NHS esters on the surface. After incubation for 0.5 h, the slide was washed with PBS (pH 7.4) containing 0.1% Tween 20, rinsed with water, and dried by purging with argon gas.

Detection of (Bio)Molecular Interactions. The printed slides were probed with fluorescent dye-labeled proteins (1−20 μg/mL) in PBS (pH 7.4) containing 0.1% Tween 20 or Dy547-RRE RNA (10 μM) in 20 mM HEPES (pH 7.4) containing 1 mM $MgCl_2$, 5 mM KCl, and 140 mM NaCl for 1 h at room temperature. For ConA binding, $MnCl_2$ and $CaCl_2$ were added at final concentrations of 1 mM. The unbound proteins and RRE were then removed by washing with the same buffer and rinsed with water. After drying by purging with argon gas, the slide treated with fluorescent dye-labeled proteins and RRE was

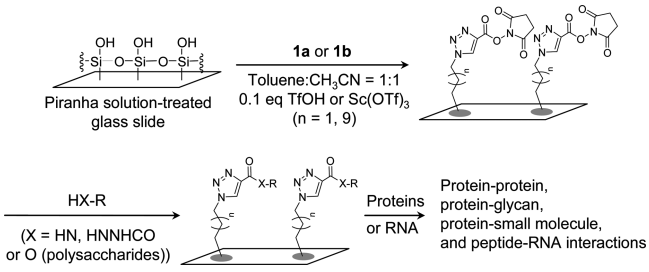


Figure 1. Fabrication of microarrays using NHS-ester derivatized glass slides and its application in studies of (bio)molecular interactions.

scanned by using an ArrayWoRx scanner. The slide probed with unlabeled antibody was further treated with FITC-labeled goat IgG and then scanned by using a microarray scanner. Fluorescence intensity was analyzed using ImaGene 6.1 software from BioDiscovery.

Enzymatic Glycosylation on Microarrays by *β*-1,4-GalT. A solution of β-1,4-galactosyltransferase (1 mU, a final concentration of 0.5 μM), $MnCl_2$ (10 mM), and UDP-Gal (1 mM) in 50 mM HEPES buffer (pH 7.5) was dropped into each block on the spotted slide, and the slide was placed in a temperature- and humidity-controlled incubator (80% humidity) at 37 °C for 3 h. The slide was washed with PBS buffer containing 0.1% Tween 20 (3 × 3 min). After drying by purging with Ar gas, the slide was probed with Cy3-RCA_{120} at room temperature for 1 h. The unbound protein was removed with PBS buffer containing 0.1% Tween 20 (3 × 3 min) by gentle shaking and rinsed with water. After drying by purging with Ar gas, the slide was scanned by using an ArrayWorx microarray scanner.

RESULTS AND DISCUSSION

The identification of efficient reactions that can be used for derivatization is a prerequisite for developing a single step method to introduce NHS esters on glass surfaces as part of the construction of microarrays. We hypothesized that Si−O−Si bond formation, using moisture-stable silylating reagents, could be employed to carry out a one-step glass surface modification process in which NHS esters are introduced. Pertinent to this proposal are recent observations we have made in studies of $Sc(OTf)_3$-mediated silylation reactions of silanols (*36*). We envisaged that NHS ester-containing methallylsilanes could be utilized for this purpose owing to their ready preparation, high stability, and the ease with which they react with alcohols in the presence of acid catalysts.

A mechanism for glass surface modification through an acid-mediated silylation of silanol groups is shown in Scheme 1. NHS-ester functionalized dimethallylsilanes are reacted with glass surface silanols at room temperature in the presence of $Sc(OTf)_3$ or trifluoromethanesulfonic acid (TfOH) as catalysts. Two different tether lengths (**1a** for C3 and **1b** for C11) were created in order to examine the effects on surface modification efficiency and subsequent protein binding. NHS-functionalized dimethallylsilanes **1a** and **1b**, employed in this methodology, were prepared on a large scale by the route delineated in Scheme 2. Briefly, the pathway involves conversion of dichloromethylsilane to the azide-containing dimethallylsilanes **3a** (C3 tether)

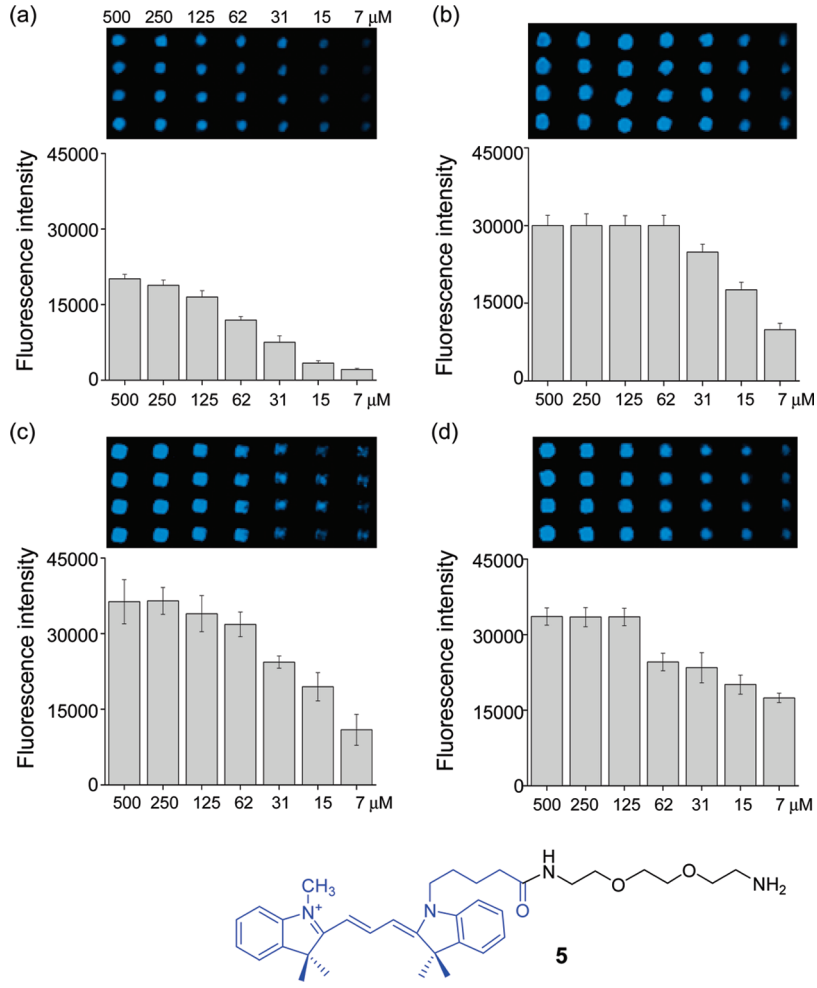


Figure 2. Immobilization of amine-linked Cy3 (**5**) (7−500 μM) on NHS-ester coated glass slides, prepared by using (a) 3% **1a** and 0.1 equiv of $Sc(OTf)_3$, (b) 3% **1a** and 0.1 equiv of TfOH, (c) 6% **1a** and 0.1 equiv of TfOH, and (d) 3% **1b** and 0.1 equiv of TfOH.

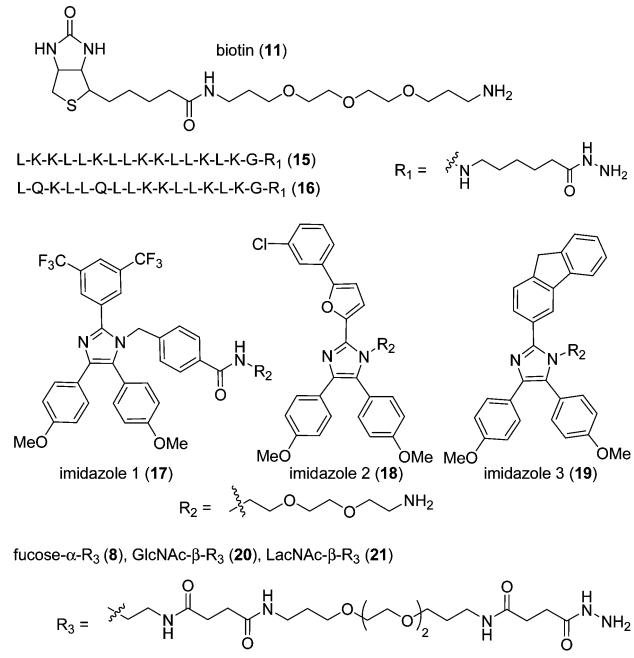
and **3b** (C11 tether) via sequential platinum-catalyzed hydrosilylation, Grignard reaction with methallyl magnesium chloride, and substitution of Cl for N_3. Finally, copper(I)-catalyzed [2 + 3] cycloaddition of **3a** and **3b** with NHS propiolate ester (**4**), generated in situ from *N*-hydroxysuccinimide and propiolic acid under *N*,*N*′-dicyclohexylcarbodiimide (DCC), was used to produce **1a** and **1b**.

Microarrays were prepared by treating glass slides with **1a** or **1b** in a 1:1 mixture of toluene and acetonitrile in the presence of 0.1 equiv of $Sc(OTf)_3$ or TfOH, followed by printing with amine-, hydrazide-, or hydroxy-conjugated (bio)molecules on the NHS ester derivatized surfaces (Figure 1). The constructed microarrays were probed by using fluorophore-conjugated proteins or RNA for analysis of biomolecular interactions.

Initially, optimized surface derivatization conditions were explored using amine-conjugated Cy3 (**5**). The piranha solution-treated slide was incubated with dimethallylsilanes **1a** (3% or 6%) or **1b** (3%) in the presence of 0.1 equiv of $Sc(OTf)_3$ or TfOH for 8 h at room temperature. Various concentrations (7−500 μM) of **5** in 50 mM sodium phosphate buffer (pH 8.0) containing 40% glycerol were printed on the NHS ester-derivatized substrates by using a robotic pin-type microarrayer (*37*). The extent of probe immobilization was determined by measuring the fluorescence intensities of Cy3 in spots on the surfaces. It was found that the fluorescence intensities of spots on microarrays, fabricated using 0.1 equiv of $Sc(OTf)_3$, were reproducibly lower than those generated by using 0.1 equiv of TfOH (Figure 2a,b) as the catalyst. This finding shows that TfOH is a more efficient catalyst than $Sc(OTf)_3$ for the key Si−O−Si bond forming reaction. However, an increase in the amount of **1a** used in the modification reaction from 3% to 6% led to a slight improvement in the surface derivatization efficiency (Figure 2b,c). Interestingly, the length of tether (**1a** versus **1b**) has little effect on the surface modification efficiency (Figure 2b,d).

To further probe this surface modification/microarray methodology, various concentrations of proteins, carbohydrates, and small molecule samples (protein G, fucose, biotin, and mannan in 50 mM sodium phosphate buffer (pH 8.0) containing 40% glycerol, Figure 3) were printed on NHS ester coated slides, prepared by treatment with **1a** (3% or 6%) or **1b** (3%) in the presence of 0.1 equiv of $Sc(OTf)_3$ or TfOH. The microspotted slides were kept in a humidity chamber at room temperature for 3−5 h and were divided into several blocks by using an incubation blocker to avoid cross-contamination. Probing the microarrays with Cy5-*Aleuria aurantia* (Cy5-AA, a fucose-binding lectin), Cy3-streptavidin, and FITC-ConA (an α-mannose/α-glucose binding lectin) shows that the microarrays prepared using TfOH exhibit higher fluorescence intensities than those obtained using $Sc(OTf)_3$ (Figure 4a). It was also found that both the amount of **1a** (3% or 6%) used in the surface derivatization process and the length of tether (**1a** versus **1b**) have little influence on the surface derivatization efficiency and protein binding (Figure 4b,c). Therefore, surface derivatization conditions using 3% **1a** and 0.1 equiv of TfOH were used throughout the remainder of this study.

Prior to developing applications of this surface derivatization procedure for the fabrication of microarrays, the number of molecules immobilized on the NHS ester coated surface prepared with 3% **1a** and 0.1 equiv of TfOH was determined. To this end, various concentrations (7−500 μM) of **5** were printed on the NHS ester slide and the fluorescence intensities of the printed spots were measured before and after washing. The maximum density of the molecule immobilized on the surface was determined to be $(2-8) \times 10^{15}$ molecules/cm^2 (see Supporting Information), which is a little higher than that for an array prepared by using the conventional multistep approach (*33, 38*).



6: protein G, **7:** fetuin, **8:** fucose-CONHNH$_2$, **9:** mannan, **10:** dextran, **11:** biotin-NH$_2$, **12:** proteinase K, **13:** collagenase, **14:** insulin, **15:** peptide 1-CONHNH$_2$ **16:** peptide 2-CONHNH$_2$, **17:** imidazole 1-NH$_2$, **18:** imidazole 2-NH$_2$, **19:** imidazole 3-NH$_2$, **20:** GlcNAc-CONHNH$_2$, **21:** LacNAc-CONHNH$_2$, **22:** asialo-fetuin.

Figure 3. Structures of substances used for immobilization on NHS-ester derivatized glass surfaces.

This result shows that glass surfaces are more efficiently derivatized with NHS esters when the single step process is employed.

To demonstrate the utility of the new surface derivatization method, various concentrations of the 17 substances shown in Figure 3 (1 nL in 50 mM sodium phosphate buffer (pH 8.0) containing 40% glycerol) were printed in duplicate on a NHS ester-derivatized glass slide, prepared by using 3% **1a** and 0.1 equiv of TfOH. The microarrays were divided into blocks in order to examine several biomolecular interactions with a single microarray. The substances used for printing are comprised of proteins (**6**, **7**, **12**−**14**, **22**), peptides (**15**, **16**), simple carbohydrates (**8**, **20**, **21**), polysaccharides (**9**, **10**), and small molecules (**11**, **17**−**19**). In the microarray, proteins and small molecules (*39, 40*) are attached to the surface via amide bonds formed between amines and NHS esters on the surface. Peptides (*41*) and simple carbohydrates are immobilized on the surface through reactions of hydrazides with NHS esters (*28*). However, polysaccharides are attached to the surface via ester bonds between alcohols and NHS esters.

Glycan−protein interactions that play a critical role in a variety of physiological and pathological processes (*42*) were investigated initially by probing with fluorophore-labeled carbohydrate-binding proteins (five lectins and one antibody). Fluorescence data, obtained after incubating the microarrays with carbohydrate-binding proteins, are shown in Figure 5a−f. The findings show that Cy5-AA and FITC-ConA interact with fucose (**8**) and mannan (**9**), respectively (Figure 5a,b). The microarrays treated with sialic acid-binding proteins, such as Cy5-*Sambucus nigra* lectin (SNA, a α-2,6-linked sialic acid binding protein) and Cy3-*Maackia amurensis* lectin II (MAL II, a α-2,3-linked sialic acid binding protein), exhibit binding to fetuin (**7**), which is a glycoprotein containing NeuNAcα2,3Gal and NeuNAcα2,6Gal residues (Figure 5c,d) (*43*). However, these lectins do not interact with asialofetuin (**22**), which lacks sialic acid residues (*44*). In addition, the binding specificity of a carbohydrate-

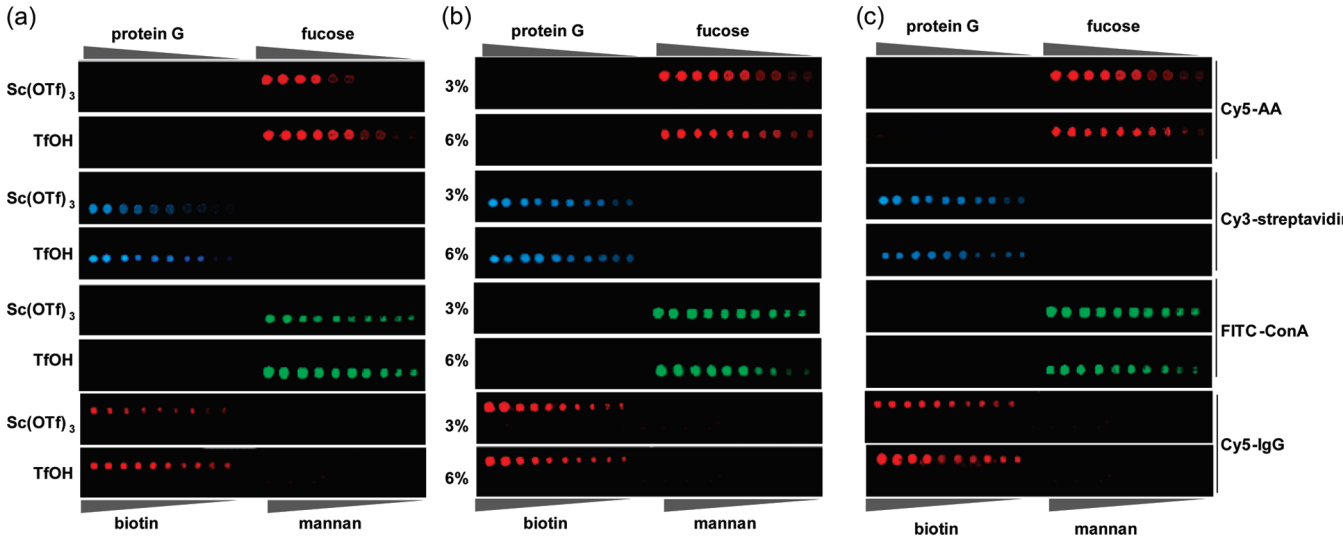


Figure 4. Immobilization of various concentrations of protein G (**6**) (15, 7.5, 3.75, 1.88, 0.94 μM), mannan (**9**) (10, 5.0, 2.5, 1.25, 0.65 mg/mL), fucose (**8**), and biotin (**11**) (2.0, 1.0, 0.5, 0.25, 0.125 mM) on the NHS-ester coated glass surface prepared under (a) 3% **1a** in the presence of 0.1 equiv of $Sc(OTf)_3$ or TfOH, (b) 3% or 6% **1a** in the presence of 0.1 equiv of TfOH, (c) 3% **1a** or 3% **1b** in the presence of 0.1 equiv of TfOH. The prepared microarrays were probed with Cy5-AA, Cy3-streptavidin, FITC-ConA, and Cy5-IgG (see Supporting Information for quantitative fluorescence intensity data).

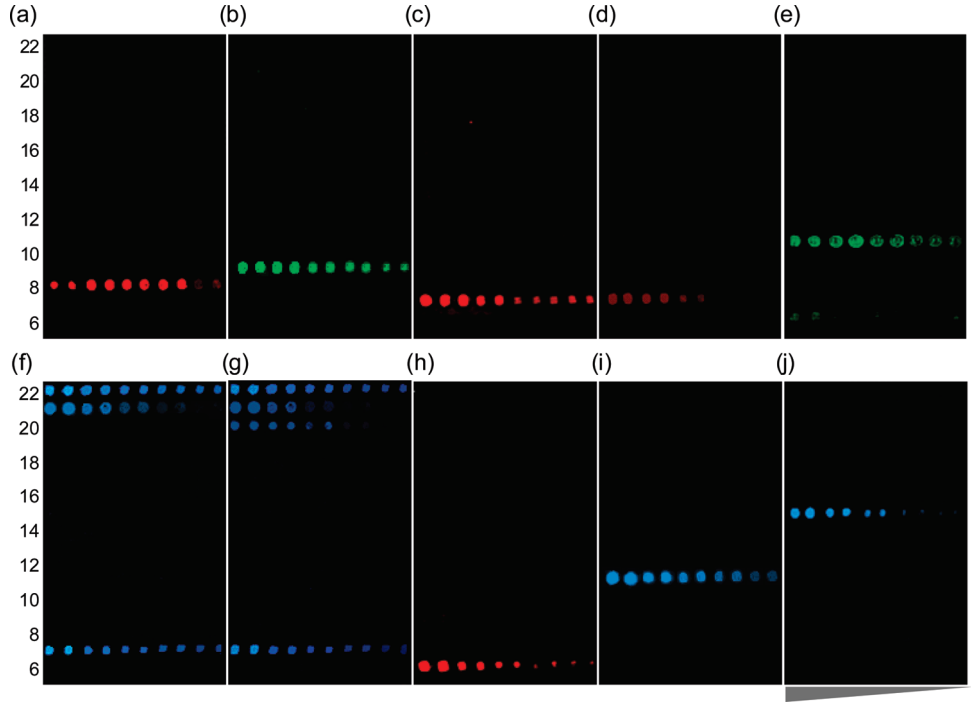


Figure 5. Fluorescence images of microarrays containing 17 substances (**6**−**22**) after probing with (a) Cy5-AA, (b) FITC-ConA, (c) Cy5-SNA, (d) Cy3-MAL II, (e) dextran-antibody followed by incubation with FITC-labeled anti-antibody, (f) Cy3-RCA_{120}, (g) Cy3-RCA_{120} after enzymatic reaction with β-1,4-GalT, (h) Cy5-IgG, (i) Cy3-streptavidin, and (j) Dy547-RRE. Concentrations of samples used for immobilization: proteins (**6**, **7**, **12**, **13**, **14**, **22**) 15, 7.5, 3.75, 1.88, 0.94 μM; polysaccharides (**9**, **10**) 10, 5.0, 2.5, 1.25, 0.65 mg/mL; small molecules, peptides and simple carbohydrates (**8**, **11**, **15**−**21**) 2.0, 1.0, 0.5, 0.25, 0.125 mM. See Supporting Information for quantitative fluorescence intensity data.

binding antibody was analyzed by using the microarrays. The results of experiments, in which the microarrays were probed with anti-dextran antibody followed by treatment with FITC-labeled goat IgG, show that anti-dextran antibody recognizes dextran (**10**) (Figure 5e). Since goat FITC-IgG was used as a secondary antibody to detect anti-dextran antibody, the spots containing protein G (**6**) also exhibit fluorescence.

The binding specificity of a galactose binding protein Cy3-*Ricinus communis* agglutinin 120 (Cy3-RCA_{120}) was determined by using the microarrays. The results indicate that RCA_{120} binds to galactose-containing substances, such as fetuin (**7**), LacNAc (**21**), and asialofetuin (**22**) (Figure 5f). Next, in order to profile β-1,4-GalT activity, the microarrays were incubated with 1 mU β-1,4-GalT (final concentration of 0.5 μM), 10 mM Mn^{2+}, and 1 mM UDP-Gal in 50 mM HEPES buffer (pH 7.5) at 37 °C. After a 3 h incubation period, the microarray slides were probed with Cy3-RCA_{120} in order to detect galactose moieties transferred by β-1,4-GalT (*18, 45*). Notably, even when washing with hot SDS solution under sonication is not employed, a step that is normally (*18, 45*) employed to reduce background fluorescence signals, the arrays do not display high background fluorescence signals. Probing the enzyme-treated microarrays

with Cy3-RCA_{120} leads to fluorescence patterns that show the conversion of GlcNAc to LacNAc catalyzed by this enzyme. The observations made in this effort demonstrate that the microarrays prepared by the new acid-mediated, one-step modification method can be effectively employed to assess carbohydrate-binding properties of proteins and to profile enzyme activities.

Attention next turned to the question of whether or not the fabricated microarrays could be used to study protein−protein and protein−small molecule interactions. For this purpose, the microarrays were incubated with IgG and streptavidin. The spots containing protein G (**6**) and biotin (**11**) were found to display fluorescence after being subjected to the respective IgG and streptavidin probes, respectively (Figure 5h,i). Finally, the microarrays were used to identify peptides or small molecules that interact with hairpin RNA, a motif often targeted by therapeutic agents (*46, 47*). For these studies, microarrays were treated with 10 μM 5′-Dy547-RRE (the HIV-1 Rev response element; see Supporting Information for its structure), a substance that forms a hairpin secondary structure that interacts with its natural partner the Rev peptide (*48*). The results show that the hairpin RRE RNA binds to the amphiphilic peptide **15**. However, peptide **16**, in which two lysines at the 2 and 6 positions are replaced with glutamines, is not recognized by RRE. This finding is consistent with studies that show that the 2- and 6-lysines are important elements for RRE recognition (*49*). The observations made in the RNA−peptide interaction studies demonstrate that the new microarrays can be applied in efforts aimed at the discovery of peptide-based drugs that target hairpin RNA.

CONCLUSIONS

The investigation described above has led to the development of a one-step, simple, and efficient method for the surface derivatization of glass as part of procedures for the construction of microarrays. The technique utilizes acid-mediated reactions of NHS ester functionalized dimethallylsilanes with the silanols present on glass surfaces. The surface modification process that employs TfOH as an acid catalyst was found to be superior to the one using $Sc(OTf)_3$. The results of protein and RNA-binding investigations show that glass surfaces modified by using this method are suitable for efficient immobilization of various substances, including proteins, peptides, carbohydrates, and small molecules, which are appended by amine, hydrazide, and alcohol functionalities. Microarrays, generated in this manner, are applicable to the rapid analysis of protein−protein, protein−glycan, protein−small molecule, and peptide−RNA interactions and profiling enzyme activities. The acid-mediated glass surface modification procedure developed in this effort should be applicable to the preparation of various functional group-modified surfaces that are utilized in the construction of various types of microarrays.

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Supporting Information Available: Measurement of the density of an immobilized molecule and quantitative analysis of fluorescence intensity of microarrays probed with biomolecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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