Small-Molecule Microarrays: Covalent Attachment and Screening of Alcohol-Containing Small Molecules on Glass Slides

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Screens for small molecules based on either phenotype or binding are contributing to our ability to explore biology with chemistry.[1] Binding-based assays that allow large collections of proteins to be screened against large collections of small molecules may provide a particularly effective means of identifying new biological probes. Previously, microarrays of thiol-containing small molecules have been produced using a Michael addition-based thiol capture reaction. Such microarrays have been used to measure 10,800 binding events involving three different proteins on a single glass slide and in a single experiment.[2] Alcohol-containing (but not thiol-containing) small molecules are readily synthesized by using solid supports having silicon-based linking elements.[3] We sought a method to merge robotic printing with split-pool, diversity-oriented synthesis to produce high-density microarrays of structurally complex and diverse alcohols.[4] Our goal was to print directly alcohol-containing small molecules released from silicon-containing synthesis beads. Here we describe such a method and demonstrate its use in a protein-binding assay.

We found that standard glass slides can be activated for selective reaction with alcohols (Figure 1). Microscope slides were first treated with a H_2SO_4/H_2O_2 solution ("piranha") for 16 h at room temperature. After extensive washing with water, the slides were treated with thionyl chloride and a catalytic amount of DMF in THF for 4 h at room temperature. Surface characterization by X-ray photoelectron spectroscopy (XPS) confirmed the presence of chlorine on the slide.[5,6] To test the ability of these chlorinated slides to capture alcohols released from synthesis beads, we initially used three alcohol-containing small molecules and a bead-linker reagent developed for chemical genetic applications of diversity-oriented synthesis.

A collaborative effort has led to the development of high capacity 500−560 μm polystyrene beads equipped with an all hydrocarbon and silicon linker for the temporary attachment and eventual fluoride-mediated release of synthetic, alcohol-containing compounds.[7] We attached primary alcohol derivatives of a synthetic α-ketoamide (**1**),[8] digoxigenin (**2**), and biotin (**3**) onto the silicon linker-modified beads (Figure 2a). The three derivatives have known protein partners, namely FKBP12,[9] the DI-22 antibody (Sigma), and streptavidin,[10] respectively. After HF−pyridine-mediated release from the beads and subsequent solvent removal, the compounds were dissolved in 5 μL of DMF in individual wells of 96-well plates to give ∼5 mM solutions. A robotic microarrayer was used to spot 1 nL volumes of 5 mM solutions of the compounds (in triplicate) 400 μm apart (average spot diameter of 300 μm) onto the thionyl chloride-activated slides (Figure 2b−e) and the slides were then washed extensively with DMF, THF, 2-propanol, and an aqueous buffer. As shown, when binding was detected separately (Figure 2b−d) or simultaneously (Figure 2e), the recognition of the protein for its ligand was efficient and selective. When the same compounds were printed onto control slides (i.e., not activated with thionyl chloride) no protein−ligand interactions were detected.

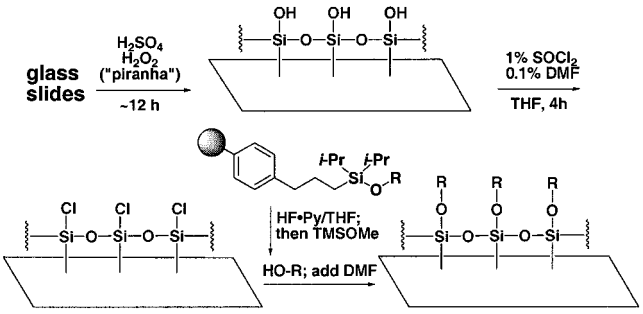

Figure 1. Activation of glass slides and the covalent attachment of alcohols.

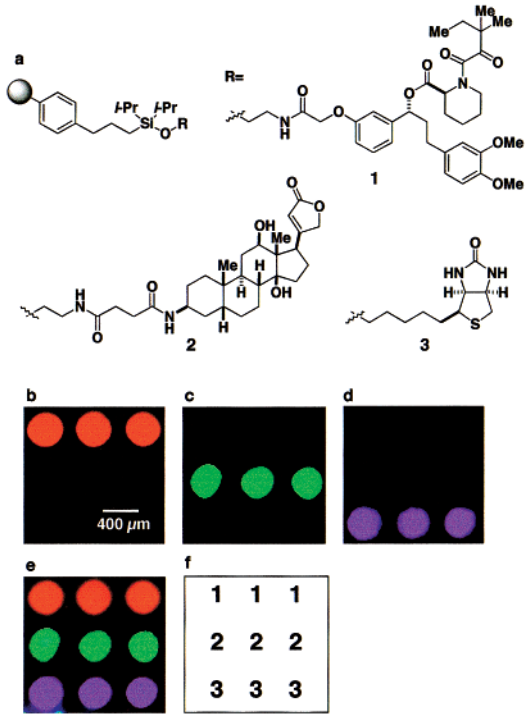

Figure 2. (a) Alcohols attached to 500−560 μm polystyrene resin through a silyl-containing linker. (b−e) A nine spot microarray was printed according to the pattern in 2f and visualized in the following channel(s): (b) Cy5 (false-colored red), (c) Cy3 (false-colored green), (d) FITC (false-colored purple), (e) Cy5, Cy3, and FITC. Average distance between spots = 400 μm; average spot diameter = 300 μm.

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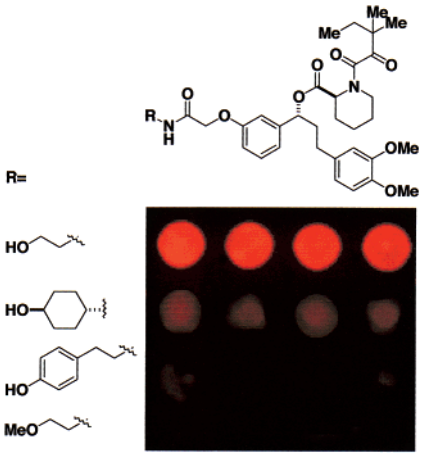

Figure 3. Microarray of primary, secondary, phenolic, and methyl ether derivatives of an FKBP12 ligand. Slides were probed with Cy5-labeled FKBP12 (false-colored red). Average spot diameter = 300 μm.

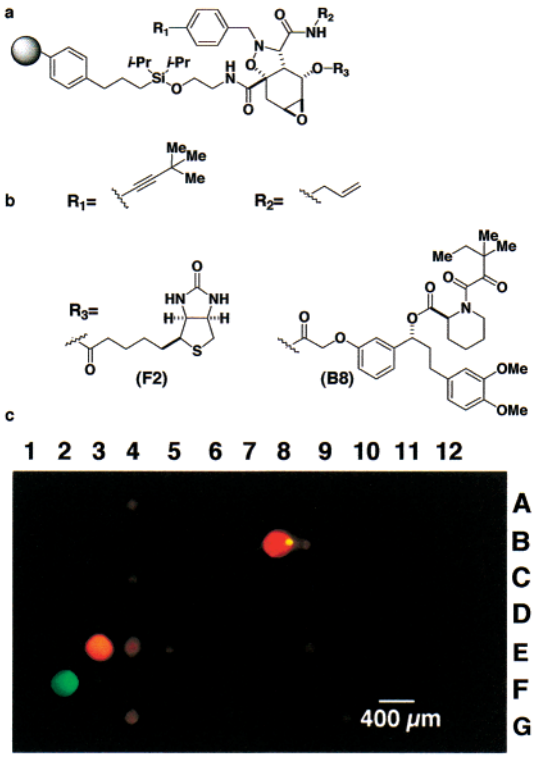

Figure 4. (a) General structure of a small-molecule library, 78 members of which were placed in the wells of a 96-well plate. (b) Structure of two additional "tagged" library members. (c) Alcohol microarray onto which 78 members of the small molecule library and two tagged members were printed. Protein binding detected with Cy5-FKBP12 (false-colored red) and FITC-streptavidin (false-colored green).

Small molecules resulting from diversity-oriented syntheses can contain a wide array of functional groups, including secondary and phenolic hydroxyls. To test the ability of such functionalities to react with the thionyl chloride-activated slides, the synthetic α-ketoamide derivatives shown in Figure 3 were synthesized. A microarray was then printed (1 nL spot volumes in quadruplicate) from wells containing the primary, secondary, phenolic, and methyl ether derivatives at ~5 mM, and probed with Cy5-FKBP12. As shown in Figure 3, the reaction of the primary alcohol is favored, and this bias holds even when the secondary, phenolic, and methyl ether derivatives are arrayed at a concentration 10 times greater than the primary.

As a demonstration of the compatibility of this alcohol-arraying technique with split-pool synthesis, a collection of 78 small molecules derived from such synthesis having the general structure shown in Figure 4a[11] was printed onto glass slides. To this collection were added two members that had been acylated with the synthetic α-ketoamide derivative or biotin (Figure 4b). These "tagged" members were then released from their beads, dissolved in 5 μL of DMF, and placed in known wells of a 96-well plate. After placing the 80 compounds into discrete wells, the entire plate was arrayed onto thionyl chloride/DMF activated slides, which were then probed with the fluorescently labeled proteins, Cy5-FKBP12 and FITC-streptavidin. The results (Figure 4c) show that two spots in the array fluoresce in the Cy5 channel (false-colored red), and another fluoresces in the FITC channel (false-colored green). The positional encoding confirms the result that the compound acylated with the α-ketoamide was spotted in B8, and the compound acylated with biotin was spotted in F2. The spot visible in E3 is an apparent serendipitous and reproducible "hit", and awaits further analysis. Thus, this experiment demonstrates the process of split-pool synthesis, release from the solid support, arraying onto glass slides, and detection/visualization of protein−small molecule binding events.

By arraying alcohols in the manner described, many small molecules derived from solid phase, diversity-oriented syntheses[4] should now be available for high-density protein-binding assays that require exceedingly small quantities of the synthetic compound. Also, the activated slides are both simple and inexpensive to create *en masse*. This method allows thousands of binding assays to be performed with a synthetic compound released from a single synthesis bead.[12] Furthermore, small molecules synthesized by using traditional solution-phase methods can also be immobilized on glass slides by this technique, provided that the molecules contain a hydroxyl group.[13] In initial screens using small-molecule microarrays and diversity-oriented synthesis, we have identified a protein ligand with remarkable specificity as judged by genome-wide expression profiling.[14]

Acknowledgment. We thank Gavin MacBeath for help with the robotic arrayer and for donation of the labeled proteins used in this study. We are also grateful to Ariad Pharmaceuticals and Dr. Kazunori Koide (Harvard) for donations of the FKBP12 ligand AP1497. P.J.H. is an American Cancer Society postdoctoral fellow and K.M.D. and S.L.S. are Research Associate and Investigator, respectively, at the Howard Hughes Medical Institute.

Supporting Information Available: Experimental procedures for the synthesis of compounds, attachment and cleavage from solid support, slide activation, microarraying, and detection of protein−ligand interactions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Although robotic arrayers can print >130 identical microarrays in a single run, if desired the used slides can be stripped of protein and re-probed (see Supporting Information).

(13) In preliminary experiments we have found that activation of the glass slides with $SiCl_4$ allows for covalent attachment of both primary and secondary alcohol containing small molecules.

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