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Photolithographic Synthesis of Peptoids

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Synthetic molecules capable of binding proteins with high specificity and affinity are potentially valuable reagents for chemical genetics studies,[1,2] the construction of protein-detecting microarrays[3,4] and a variety of other applications. Since protein-binding small molecules generally cannot be designed, they are almost always discovered by screening combinatorial libraries or compound collections. A significant development in this regard has been the development of small molecule microarrays.[5] These are made by first creating a "one compound, one bead" library by split and pool techniques,[6] sorting the beads into individual wells of microtiter plates, releasing the compounds from the beads, then using a robotic pin spotter to attach the compounds covalently to chemically modified glass slides in a defined array.[7] The array can then be screened by incubation with a labeled protein under suitable conditions. This protocol has important advantages over screening bead-based libraries directly. Since very small amounts of compound are spotted onto a slide, it allows a library made on high-capacity beads to be replicated hundreds or thousands of times and also avoids a number of technical difficulties associated with screening on beads.[8] However, spotted small-molecule microarrays are usually derived from encoded libraries of beads to allow the identification of each compound on the array. Many current encoding methods are expensive and require specialized equipment and infrastructure.[9−12]

One way to avoid encoding would be to synthesize a small molecule array in situ in a spatially defined manner. Low-density arrays of small molecules have been made using the SPOT synthesis technique.[13] However, the only significant application of this approach to high-density arrays has been the photolithographic synthesis of DNA oligonucleotide arrays, which is practiced on an industrial scale.[14] There has been a single report of the photolithographic synthesis of a peptide array,[15] but this approach has not been developed further, in part because of the expense and logistical difficulties associated with using 20 or more photo-unmaskable monomers. In this communication, we show that arrays of peptoids (oligomers of N-substituted glycines) can be made by photolithography using a single photolabile synthon. Peptoid libraries have been shown by us[16] and others[17−19] to be rich sources of protein-binding agents, suggesting that high-density peptoid arrays created by photolithography will represent powerful tools for protein ligand discovery.

Peptoids are almost ideal molecules for combinatorial chemistry due to their ease of synthesis by the "sub-monomer" route developed by Zuckermann and colleagues.[20] In this process, an amine-functionalized surface is acylated with an activated derivative of 2-bromoacetate. The resultant α-bromo amide is then treated with an excess of a primary amine, resulting in displacement of the bromide and creation of a peptoid monomer. This cycle is repeated as necessary to create a peptoid oligomer (Figure 1A). Hundreds of commercially available primary amines can be used as diversity elements in the synthesis of peptoid libraries. To adapt this chemistry for the photolithographic synthesis of arrays, a light-dependent process must be inserted into the synthetic scheme. Thus, the four-step cycle shown in Figure 1B was developed. First, glycolic acid protected with a light-sensitive MeNPOC group[21] was coupled to an amine-modified surface. The hydroxyl group was unmasked by UV irradiation and then activated with tosyl chloride. Finally, the tosylate was displaced with a primary amine to complete the construction of a monomer unit. This chemistry should allow the spatially addressable synthesis of peptoids on an array by photolithography, since hydroxyl group unmasking, activation, and amine displacement will occur only at addresses that have been irradiated with UV light.

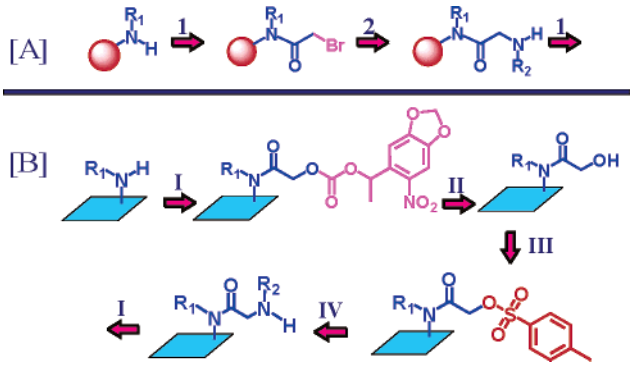

Figure 1. [A] Two-step cycle of sub-monomer synthesis of a peptoid. (1) Bromoacetic acid is coupled to the N-terminal amine of peptoid. (2) The bromide is displaced by a primary amine. [B] Four-step cycle for the photolithographic synthesis of a peptoid. (I). MeNPOC-glycolic acid is coupled to the N-terminal amine of a peptoid chain. (II). The light-sensitive MeNPOC is removed by UV irradiation at 365 nm. (III). The newly exposed hydroxyl group is activated with Tosyl-chloride. (IV). The tosylate is displaced with a primary amine.

To evaluate the efficacy of the critical photomediated deprotection in an array format, MeNPOC-protected glycolic acid was coupled to a chemically modified glass microscope slide through ester bond formation.[22] Specific rectangular regions of this slide were irradiated with UV light (365 nm, 2 mW/cm^2) for different times and then treated with Cy3-phosphoramidite to affix a fluorescent label to all exposed hydroxyl groups. The correlation between fluorescent intensities of these areas and their exposure times under UV light was used to estimate the time required to achieve maximum deprotection of MeNPOC groups (See Figure 2). Optimal conditions for all of the other steps in Figure 1B were developed on Rink amide resin, with the expectation that similar results would be observed on glass slides with similar surface chemistry. Coupling yields are at least 85%.

To assemble all of the chemistry together and test its utility for the creation of an array, the experiment, shown in Figure 3, was conducted. Starting from a clean glass slide, the following reactions were carried out: (1) tosylation of the hydroxyl group, (2) displacement of the tosylate with 2-methoxylethylamine, and (3)

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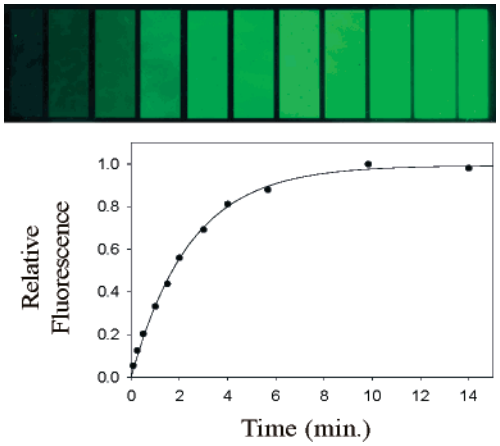

Figure 2. Time-dependent deprotection of MeNPOC groups on a glass slide. The upper image shows the fluorescent intensities of different areas on the slide where MeNPOC-groups were photodeprotected for different times and then labeled with Cy3-phosphoramidite. The bottom is the correlation between the fluorescent intensities of these areas and the time of UV irradiation. This curve indicates that maximum removal of the MeNPOC group is achieved after 10 min of exposure to 365 nm UV light at 2 mW/cm^2.

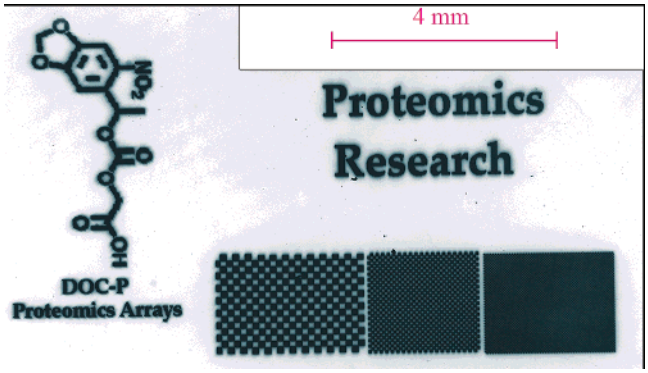

Figure 3. Peptoid array synthesized by digital photolithography. The image was recorded with excitation at 350 nm and emission at 460 nm. The dark regions of the array (letters, structure, etc.) represent features on which the Dabsyl-containing amine was added. The EDANS-containing peptoid monomer should cover the entire slide. Dabsyl-mediated quenching of EDANS fluorescence produces the image. Scale in red is added afterward for illustration purposes.

coupling of MeNPOC-protected glycolic acid. This was expected to provide a uniform layer of MeNPOC-protected hydroxyl groups on the slide. Using digital photolithography,[23−26] the MeNPOC groups were selectively deprotected at specified positions to form a predesigned pattern. The slide was then subjected to the four-step cycle shown in Figure 1B, using the primary amine *N*-(2-aminoethyl)-4-[[4-(dimethylamino)phenyl]azo]-benzenesulfonamide (Dabsyl-amine). Last, the entire slide was irradiated with UV light to remove all remaining MeNPOC groups. An additional peptoid monomer was then added using the Figure 1B chemistry, but with the amine 5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS). EDANS and Dabsyl are a pair of molecules that is frequently used in fluorescent-quenching experiments. The EDANS-containing monomer should coat the slide uniformly, but its fluorescence should be quenched at positions where the Dabsyl-containing unit was constructed in the first cycle of synthesis. As expected, when the slide was scanned, the image obtained corresponded to that designed, showing much more intense fluorescence in regions lacking the Dabsyl-containing monomer (Figure 3).

This experiment validates that the chemistry shown in Figure 1B is able to support the creation of a defined array of peptoids. Since essentially the same instrument used here has been employed to synthesize high-density arrays of DNA oligonucleotides,[26] we anticipate that spatially defined arrays of up to 100 000 peptoids can be constructed using the technology described here. The photolithographic synthesis of these large libraries of peptoids constructed from a variety of amines is underway and will be reported in due course. We anticipate that these small-molecule arrays will be of considerable utility in the isolation of protein-binding agents.

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Supporting Information Available: Synthesis of compounds and detailed experimental protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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