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Microchip Encoded Combinatorial Libraries: Generation of a Spatially Encoded Library from a Pool Synthesis

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Abstract. An encoding method which provides ready access to the structure of individual compounds in a combinatorial library of small organic molecules has been developed. Glass-encased microchips each of which contain a unique binary encoded ID which can be scanned and recorded using radiofrequency (RF) were added to individual tea bags containing polystyrene polymer functionalized with a *Wang* linker. The tea bags were subjected to a three-step synthesis. At each stage of the synthesis, the microchips were RF-scanned and the unique ID's were recorded. After the synthesis was complete, each tea bag was introduced to individual wells of a microtiter plate and the products were deblocked from the polymer. The histogram of the ID for each well was then used to assign the structure of every product in the library. A library of 64 compounds was thus synthesized using a pooled compound strategy, affording a positionally encoded discrete library.

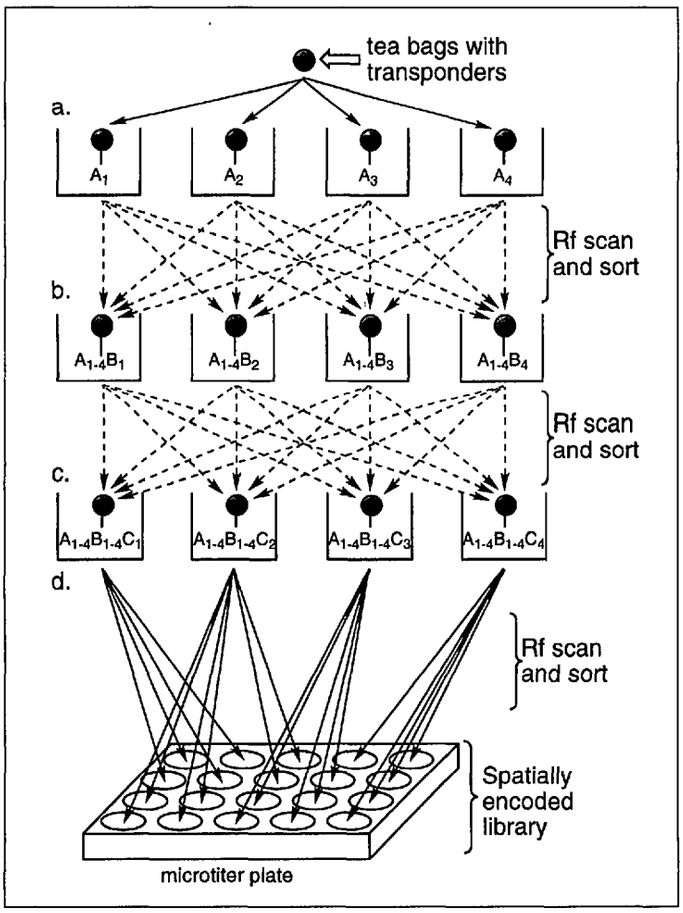

Fig. 1. a–c) *Three-step linear synthesis of a pooled microchip encoded library of 64 compounds;* d) *conversion to a discrete positionally encoded array*

The combinatorial synthesis of libraries of small organic molecules represents a powerful tool for the identification of biologically active compounds with potential therapeutic value [1]. One approach to the generation of libraries on solid support involves the parallel synthesis of an array of spatially separate [2] or spatially addressable [3] compounds. These are generated as discrete products (single compounds) whose structural identity can be derived from their particular location in the reaction array. A second strategy involves the generation of compound mixtures or pools, generally using a 'split synthesis' approach [4]. This method requires a deconvolution process in which the component of interest must be identified from the compound mixture and its structure elucidated [5]. Chemical tagging techniques have been used to encode the structure of each of the components of a pool in order to facilitate the identification of selected members of the library [6]. Introduction, removal, and decoding of chemical tags can comprise a large portion of the effort to generate and screen the library. Described herein is a new encoding method using resin-associated radiofrequency (RF) transponders which takes advantage of the efficiency of pooled synthesis yet converts the mixtures into easily deconvoluted positionally encoded libraries.

Introduction of encoded chemical tags at each cycle of a 'split synthesis' represents a WRITE function in which information relating to chemical structure is *written* to the resin. Encoding can also be achieved by a READ function if there is a unique identifier associated with each resin bead in the library which may be *read*. This latter strategy removes the write steps needed to encode a library. In the strategy described herein, commercially available RF transponders [7] commonly used for laboratory animal tagging [8] were chosen to tag each compound in the library. These transponders are pre-encoded with a unique ID, and are glass-encased and thus stable to most solvents and reagents. They are reusable and have been submitted to reactions from –78 to 100°. The transponders can be scanned [9] directly through standard laboratory glassware, even while immersed in a solvent. The RF

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signal emitted is multidirectional, requiring no specific alignment of the transponder to a detector. In order to associate resin with unique ID's, polypropylene mesh 'tea bags' containing *Wang* benzylhydroxy-polystyrene resin [10] and a single transponder per bag were used.

The basic procedure for carrying out the synthesis was similar to the 'split synthesis' method (*Fig. 1*). A library containing 64 members was generated from a linear three-step synthesis (steps A, B, and C) with four inputs (1–4) per step. The tea bags containing the functionalized resin and a single transponder per bag were partitioned into four individual reaction vessels and subjected to reaction with inputs A. The mixture of bags (16/flask) were then removed, washed of excess reagents, scanned, and sorted for the second step of the synthesis (inputs B). Scanning involved passing a single transponder at a time near an RF detector, where the unique ID was recorded on a computer. This process was repeated for the B inputs. After the parallel C input cycle was completed, the bags were sorted to 64 individual wells of a 96-well microtiter plate format, and the products were cleaved from the resin. Following removal of the tea bags, a single compound per well format of the discrete library of 64 unique products was obtained. A histogram of the reaction sequence for each unique ID provides the unequivocal structure of the expected single product in each well [11]. Unlike the 'split synthesis', the use of tea bags means that the scale of synthesis is not limited to the load of individual resin beads.

Our synthesis of a 64-compound library uses a linear three-step synthesis with an intermediate four-component condensation. Four blocked amino acids were coupled to *Wang* benzylhydroxy resin and the amine was deprotected to afford **1** (*Scheme*). After a READ function and apportionment to the appropriate reaction vessels, an *Ugi* four-component condensation with four different aldehydes resulted in **2** in which the *p*-hydroxyphenylacetic acid and benzyl isocyanide inputs were kept constant. Subsequent recording of ID's and sorting provided the four sets of tea-bag mixtures necessary for the acylation reactions leading to **3**. The mixtures of bags were then sorted individually to the microtiter plate format and compounds were removed from the resin with TFA. Evacuation of excess solvent in a vacuum oven provided the products **4**. The structure of the inputs for the three-step sequence are shown in *Fig. 2*. Analysis of the products in the library indicated that all

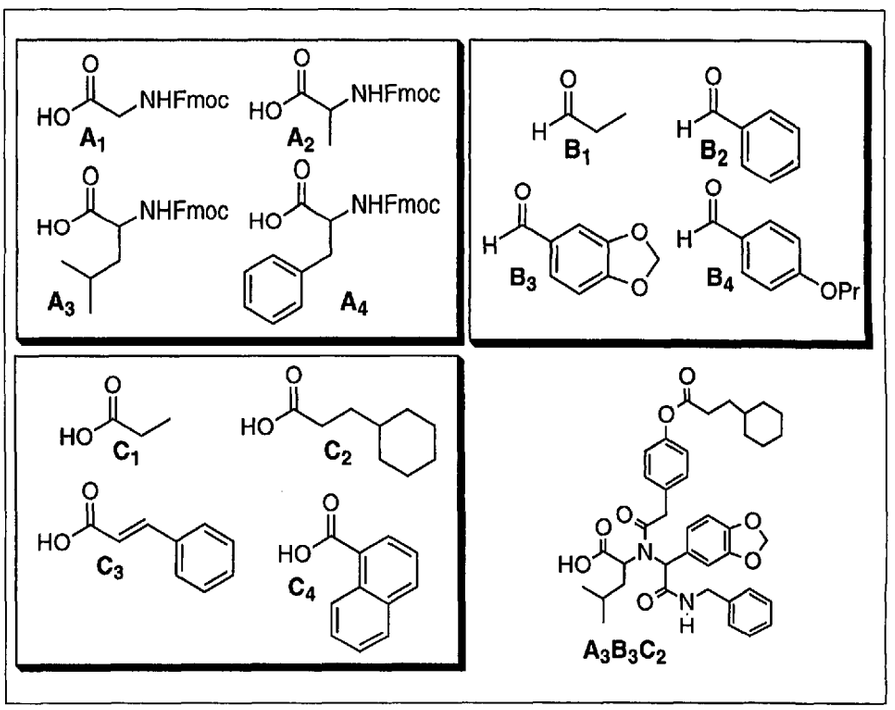
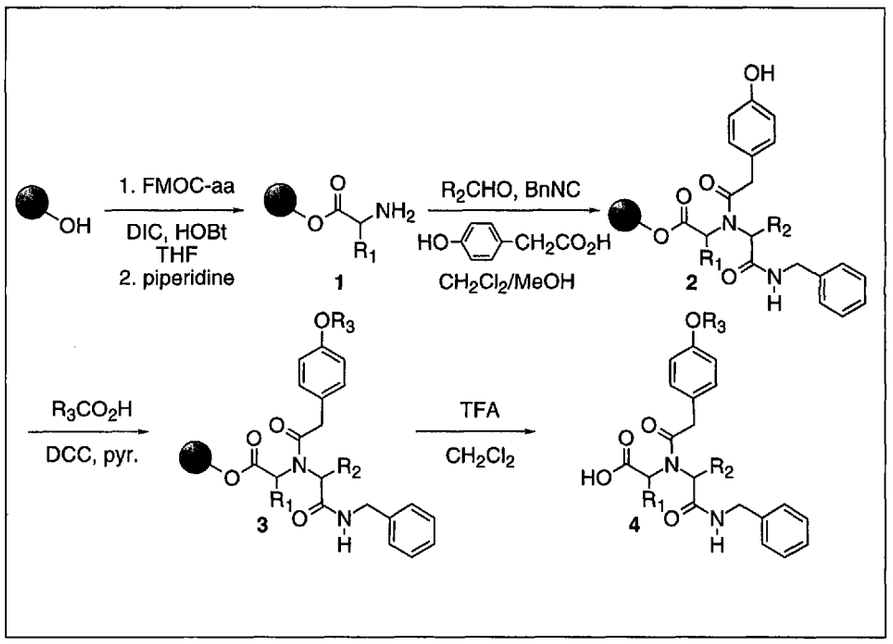


Fig. 2. a) *Structure of chemical inputs for each step in the reaction sequence shown in the* Scheme; b) *structure of library product* $A_3B_3C_2$

Scheme



expected products were generated with an average yield of 53% (8–14 mg) [12].

The end products of a microchip encoded library are single compounds (per well) whose structure is effortlessly decoded from the histogram of each transponder ID. This 'decoding' is a facile process which can be software-driven. The 'read and sort' strategy does not require separate reactions to introduce tags and avoids potential incompatibility issues associated with the library synthesis. It eliminates the need for a biological screen-directed deconvolution of a mixture of compounds and the necessity for the chemical or biochemical analysis of the code. A mixing process to achieve a statistical distribution of beads is unnecessary because the scanning step provides the sorting information for the synthesis of all structures. Thus, the total number of tea bags required is equal to the total theoret-

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ical number of compounds which are to be made in the particular library. Because the final product of this library is a discrete array, biological screening of individual compounds of known structure can provide an SAR profile of the complete library. The development of READ/WRITE strategies which further improve the efficiency of this approach are currently underway and will be reported in due course.

Experimental Procedure

The tea bags were apportioned and scanned as described in text and *Fig. 1. a*) Each of the four FMOC-amino acids (4.59 mmol), DIC (4.59 mmol), and HOBt (3.44 mmol) were dissolved in THF (10 ml) individually and stirred for 20 min, filtered, and the filtrate added to 1.275 g of *Wang* resin (*Advanced Chemtech*, 0.9 mmol/g) in tea bags containing the transponders (16/flask). After 18 h of gentle stirring, the resin was washed with DMF, MeOH, and CH_2Cl_2. The tea bags were immersed in 20% piperidine/DMF for 30 min and then rinsed with MeOH and CH_2Cl_2, then scanned and sorted to four new reaction vessels. *b*) To each reaction vessel was added 15 ml of MeOH/CH_2Cl_2 (1:1), one of four aldehydes (15 mmol), *p*-hydroxyphenylacetic acid (15 mmol), and benzyl isocyanide (15 mmol). The resin was gently stirred for 12 h, then rinsed repeatedly with MeOH and CH_2Cl_2 and sorted to four new reaction vessels. *c*) To each of the reaction vessels was added the acid (14 mmol) followed by DCC (15 mmol) in 15 ml of pyridine. After 18 h, the resin was rinsed with DMF (50°), then MeOH and CH_2Cl_2. *d*) The tea bags were scanned and sorted into individual wells of a polypropylene microtiter plate (96 well, 2 ml/well). A soln. (1 ml) of 20% TFA/CH_2Cl_2 was added to each well and let stand for 20 min. The tea bags were removed and rinsed with CH_2Cl_2 and the solvents stripped *i.v.*

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[8] Implantable Micro Identification (IMI) systems, Bio Medic Data Sytems, Inc. Maywood, N.J. Transponders were scanned using a DAS-4004 Pocket scanner and the ID's can be downloaded to a Mac or PC.

[9] Transponders were scanned using a DAS-4004 Pocket scanner.

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[11] Tea bags can be combined such that compound mixtures of known composition can be screened.

[12] Yields were obtained from the weight of individual products in library after removal from resin. They are based on initial loading of polymer as described by manufacturer and represent the overall yield for the five steps shown in the *Scheme*. Low-resolution electrospray MS was obtained for all compounds. Full characterization (HRMS, ^{13}C- and ^1H-NMR, IR) of several members ($A_1B_4C_1$, $A_4B_1C_3$) was obtained: $A_1B_1C_1$, 50%; $A_1B_1C_2$, 45%; $A_1B_1C_3$, 49%; $A_1B_1C_4$, 39%; $A_1B_2C_1$, 55%; $A_1B_2C_2$, 53%; $A_1B_2C_3$, 64%; $A_1B_2C_4$, 76%; $A_1B_3C_1$, 49%; $A_1B_3C_2$, 51%; $A_1B_3C_3$, 61%; $A_1B_3C_4$, 67%; $A_1B_4C_1$, 50%; $A_1B_4C_2$, 50%; $A_1B_4C_3$, 57%; $A_1B_4C_4$, 40%; $A_2B_1C_1$, 44%, $A_2B_1C_2$, 44%; $A_2B_1C_3$, 52%; $A_2B_1C_4$, 56%; $A_2B_2C_1$, 40%, $A_2B_2C_2$, 43%, $A_2B_2C_3$, 52%; $A_2B_2C_4$, 55%; $A_2B_3C_1$, 43%, $A_2B_3C_2$, 36%; $A_2B_3C_3$, 42%; $A_2B_3C_4$, 48%; $A_2B_4C_1$, 40%; $A_2B_4C_2$, 37%; $A_2B_4C_3$, 44%; $A_2B_4C_4$, 57%; $A_3B_1C_1$, 65%; $A_3B_1C_2$, 68%; $A_3B_1C_3$, 73%; $A_3B_1C_4$, 60%; $A_3B_2C_1$, 30%; $A_3B_2C_2$, 64%; $A_3B_2C_3$, 69%; $A_3B_2C_4$, 76%; $A_3B_3C_1$, 54%; $A_3B_3C_2$, 55%; $A_3B_3C_3$, 58%; $A_3B_3C_4$, 30%; $A_3B_4C_1$, 55%; $A_3B_4C_2$, 58%; $A_3B_4C_3$, 64%; $A_3B_4C_4$, 66%; $A_4B_1C_1$, 61%; $A_4B_1C_2$, 61%; $A_4B_1C_3$, 71%; $A_4B_1C_4$, 72%; $A_4B_2C_1$, 55%; $A_4B_2C_2$, 56%; $A_4B_2C_3$, 57%; $A_4B_2C_4$, 61%; $A_4B_3C_1$, 47%; $A_4B_3C_2$, 46%; $A_4B_3C_3$, 47%; $A_4B_3C_4$, 54%; $A_4B_4C_1$, 55%; $A_4B_4C_2$, 49%; $A_4B_4C_3$, 59%; $A_4B_4C_4$, 45%.

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The Solid-Phase Synthesis of Complex Small Molecules

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The design, synthesis, and evaluation of small-molecule libraries currently being performed in our laboratory is overviewed. We consider a number of factors in the selection of a compound class for library synthesis. One strategy that we have employed is to select 'privileged' structures, where the display of different functionality upon the structure has previously provided a number of potent and specific drugs or candidates towards different therapeutic targets. The first class of 'privileged' structures that we focused on were the 1,4-benzodiazepines, one of the most prescribed classes of orally active drugs that target a wide range of different receptors and enzymes. Other examples include libraries based upon tropane, prostaglandin, and tricyclic frameworks. An alternative strategy that we have employed is to design compound classes based on important biological recognition motifs. One example where we have applied this strategy is the synthesis of libraries of mimetics of β-turns, which play a key role in molecular recognition events in biological systems. A second example is the

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