A Visual Tagging Process for Mix and Sort Combinatorial Chemistry

Joseph W. Guiles,* Carolina L. Lanter, and Ralph A. Rivero

Solid-phase synthesis (SPS) is an integral part of the combinatorial chemistry technology that is emanating from academic and industrial institutions worldwide.[1] The extension of solid-phase peptide synthesis[2] for the generation of small druglike molecules is an area of tremendous research interest.[3] Also this extension is performed rapidly, provides samples of good purity, and is amenable to robotics. These characteristics of SPS have been exploited for the rapid generation of large numbers of compounds as discrete samples or mixtures. Currently, major differences between utilizing discrete compounds versus mixtures exist, as was recently pointed out by Berk and Chapman.[4] The major differential aspect is the impact that synthesis and screening speed have on the rate at which one obtains information (e.g. development of structure – activity relationships). To date this is a distinguishing factor that determines at which step in the drug-discovery process either of these two approaches is applied.

A comparison of speed characteristics for combinatorial techniques as they apply to their roles in the drug-discovery process is depicted in Table 1. The ability to obtain modest quantities of compound (e.g. >10 mg) with a reliable purity makes parallel synthesis (entry 1) the method of choice for meeting the needs of lead optimization. Recent technological advances with robotics have transformed parallel synthesis into a "higher throughput" process. This improved ability is generally recognized as alleviating the tedium of multiple pipetting routines normally done by hand. However, the throughput capacity of this method is limited by the size of the parallel array platform, and therefore only marginal increases in throughput have been realized. In considering other techniques, chemically encoded mix and split (entry 4) provides a moderate information retrieval while maintaining a fast speed of library synthesis.[5] An additional chemical encoding technique makes use of covalently linked dyes for tagging and decoding by the absorption or fluorescence spectrum of the resin bead.[6] Lastly, the mix and sort technique (entry 5) that utilizes microreactors provides an avenue for moderate speed of library production and an expedient delivery of information.[7] These factors make this technique appealing from the standpoint of being useful for both lead identification and optimization. This led us to investigate alternative traceless encoding techniques. We describe here a noncovalent, color-coding strategy for performing mix and sort combinatorial chemistry.

Table 1. Combinatorial techniques and their characteristics.

Entry	Technique	Single substance/ mixture	Speed of library synthesis	SAR retrieval	Utility
1	parallel synthesis	single	slow	fast	lead optimization
2	parallel arrayed mixture[4]	mixture	moderate	moderate	lead identification
3	mix and split	mixture	fast	slow	lead identification
4	chemically encoded mix and split	mixture	fast	moderate	lead identification
5	mix and sort	single	moderate	fast	lead identification and optimization

Recent reports have exploited the use of noncovalent tagging strategies in conjunction with microreactors or porous containers (PCs).[7] The primary advantages of these techniques are the chemical inertness of the tags and the convenience of deciphering the encoded information (no additional tagging and untagging chemistry is required). However, this technology does necessitate computer tracking (a possible economic issue), and the current sizes of the tags consume a moderate amount of space inside the container (a possible yield issue). As a means to address these two problems, smaller and more economical tags were sought. Our objective was to utilize this variation of traceless encoded mix and sort for the generation of moderately sized three-dimensional libraries.

The process utilizes two sets of simple color codes: color-coded glass beads and color-coded container caps. The color codes are used after the mixing steps to sort into the matrix of a 96-well plate (Figure 1). The colored glass beads are chemically inert, whereas the colored polypropylene caps and the PCs are compatible with a wide variety of synthetic reagents.[8] In addition to the cost effectiveness of this method, very little internal container space is yielded to the tag, routinely allowing 10 – 15 mg of compound to be obtained. We consider this quantity to be the minimum amount necessary to meet the needs of lead identification and optimization.

This technique allows for two mix and split steps followed by sorting into a parallel array (Figure 1). The library is typically of an X-Y-Z format. The X and Y subunits are each assigned a set of eight bead colors and a set of twelve cap colors, and Z is added globally for each group of 96 wells. The size of the library is contingent upon the number of Z subunits available. With 20 Z subunits—that is, when 20 differently substituted reagents are attached in the third step of the process—20 separate 96-well plates, or 1920 compounds, are generated. Repeating the process for different sets of X and Y would of course lead to larger libraries. It is possible to

[*] Dr. J. W. Guiles,[+] C. L. Lanter
The R. W. Johnson Pharmaceutical Research Institute
1000 Route 202, P.O. Box 300
Raritan, NJ 08869 (USA)
Fax: (+1) 908-526-6469
Dr. R. A. Rivero
The R. W. Johnson Pharmaceutical Research Institute
Spring House, PA (USA)
[+] Current address:
Hoechst Marion Roussel, Inc.
Route 202 – 206, P.O. Box 6800
Bridgewater, NJ 08807-0800 (USA)
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E-mail: joseph.guiles@hmrag.com

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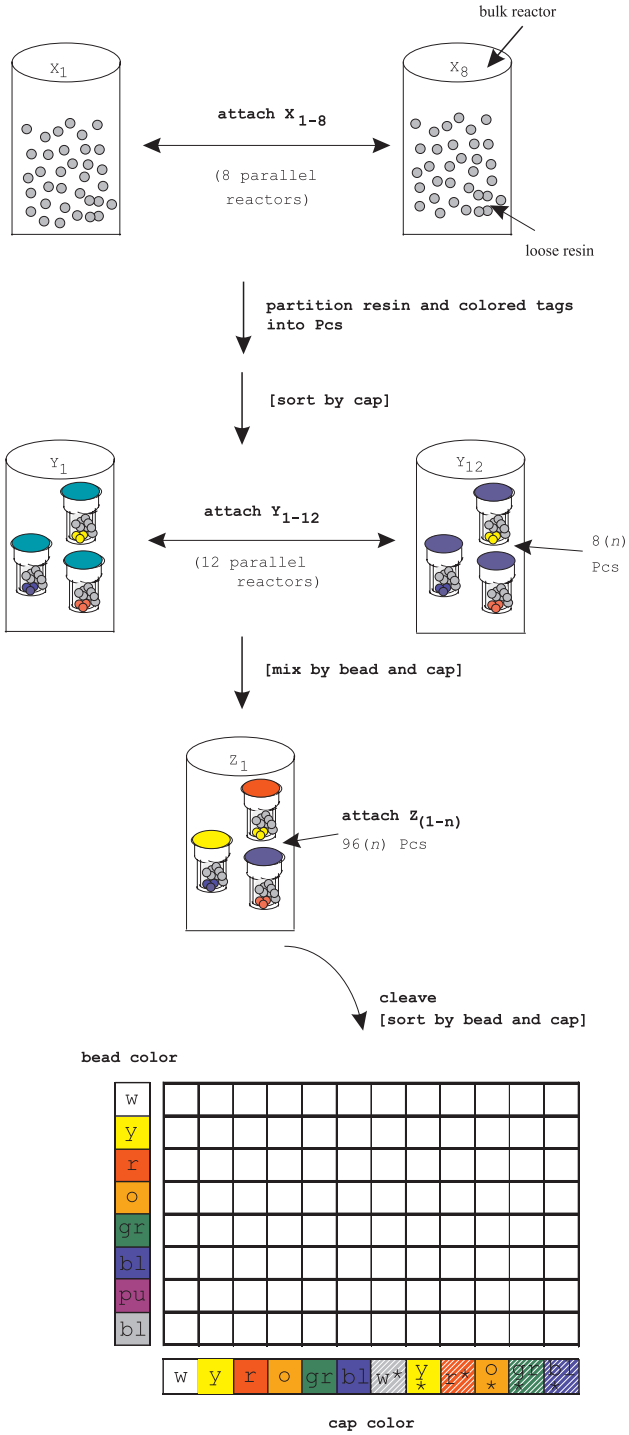
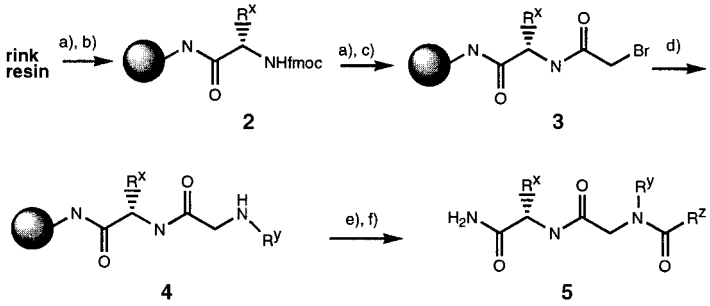


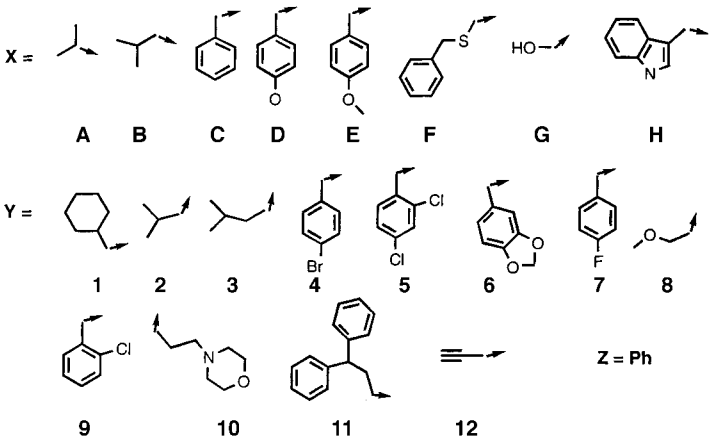
Figure 1. Mix and sort protocol for coding into porous containers.

construct W-X-Y-Z or larger libraries by attaching the additional subunits once the PCs have been sorted into the 96-well plate matrix. However, for these larger dimension libraries beyond the X-Y-Z format, the speed of handling 96 × Z plates for the additional synthetic steps becomes an issue. As depicted in Figure 1, the technique has facilitated the construction of moderately sized three-dimensional libraries.

To exemplify this technique, the synthesis of a library of N-substituted glycine-capped dipeptides is described (Scheme 1). The construction of pseudopeptides or peptoids[3e] was chosen owing to the reproducibility of the synthetic method and the availability of the subunits. The library was arrayed as eight X subunits, twelve Y subunits, and one Z subunit (corresponding to the groups R^x, R^y, and R^z (Schemes 1 and 2), and constituting the rows, columns, and



Scheme 1. Synthesis of pseudopeptides: a) piperidine/DMF (80/20); b) R^x–fmoc, DIC, 1M in CH_2Cl_2; c) bromoacetic acid, DIC, 1M in CH_2Cl_2; d) R^y–NH_2, 1M in CH_2Cl_2; e) PhCOCl, DIEA, 1M in CH_2Cl_2; f) CH_2Cl_2/TFA/TES (8.5/1.5/0.5). DIC = Diisopropylcarbodiimide, DIEA = *N*,*N*-diisopropylethylamine, DMF = dimethylformamide, fmoc = 9-fluorenylmethoxycarbonyl, TES = 2-[tris(hydroxymethyl)methylamino]ethanesulfonic acid, TFA = trifluoroacetic acid.



Scheme 2. Substituents R^x, R^y, and R^z in **2**–**5**.

number, respectively, of 96-well plates). Rink resin (6.8 g, meshed at 100–200 μm)[9] was used to attach the eight fmoc-protected amino acids. The resins derived from the same X subunit in the bulk reactors were then partitioned equally (ca. 35 mmol) into twelve different PCs. A small sample of individual colored beads was then added, coding for the X subunit, and the containers were sealed with the twelve possible different colored caps.[10] After this splitting operation was completed for all eight batches, the 96 PCs were segregated by cap color into twelve reaction vessels. At this point there were eight PCs in each vessel representing all eight X subunits. The fmoc group was then removed, and the twelve Y subunits were attached with standard peptoid chemistry.

After this process was complete, a mixing step combined the 96 unique samples into one vessel,[11] to which the Z subunit was then attached. The 96 samples from each

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container are individually sorted into the wells of a polypropylene-fritted 96-well plate according to their bead and cap color, and subsequently treated with a cleavage reagent. The cleavage reagent was then collected by filtration under a slight vacuum into a second nonporous 96-well plate. The individual removable wells of this plate were preweighed before collection of the cleavage solution. Alternatively, we also utilized glass test tubes for this procedure. The tubes were preweighed, and a single porous container was added to each tube followed by the cleavage solution (1 mL). After approximately 20 minutes the porous container was removed above the liquid level and rinsed with a small aliquot of CH_2Cl_2. The volatile components were subsequently removed under vacuum, and the residue was treated with ether. The resulting solid products were isolated in a range of 9.1 – 15.6 mg with an overall average yield of 83.5 % for the six-step sequence. The purity of each sample is excellent, and the analytical data are consistent with the structures assigned for all samples.[12]

We have applied this inert two color encoding strategy for the rapid construction of moderately sized, discrete sample libraries. It presents a simple, cost-effective method for performing mix and sort combinatorial chemistry that does not rely upon robotics, computer monitoring controls, or spectroscopic techniques. The versatility of this technology may be applied not only for the identification of lead structures but also for their subsequent optimization.

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[11] A glass-fritted vessel with a screw cap or an amber jar with a screw cap is routinely used.

[12] HPLC analysis was performed on a Hewlett-Packard ODS Hypersil column (5 μM, 4 × 125 mm) with monitoring at 220 nm. Elution was carried out with a solvent mixture A/B (A = 0.1 % TFA in water, B = 0.1 % TFA in acetonitrile); the amount of A was increased from 20 to 80 % over 20 min. A mass spectrum in the positive-ionization mode was recorded for all samples on a Hewlett-Packard electrospray mass spectrometer; selected ES-MS data: *m*/*z*: **A1** 374 [373], **B2** 348 [347], **C3** 396 [395], **D4** 510, 512 [510], **E7** 464 [463], **E9** 480 [479], **F7** 480 [479], **F10** 499 [498], **G5** 424 [424], **G11** 460 [459], **H10** 492 [491], **C12** 364 [363].

Ligand Self-Recognition in the Self-Assembly of a $[\{Cu(L)\}_2]^{2+}$ Complex: The Role of Chirality**

M. Athar Masood, Eric J. Enemark, and T. Daniel P. Stack*

A large interest in the assembly of discrete molecular architectures from smaller molecules has developed in recent years with the realization that these assemblies may show interesting chemical, physical, and mechanical properties.[1] Examples of metal-assisted self-assembly of independent ligands to form helices,[2–8] grids,[9] knots,[10, 11] cylinders,[12] platonic shapes,[13, 14] and circular helicates[15] quantitatively with labile metals represent impressive feats of molecular design and assembly.[3, 16–19] Many forms of supramolecular complexes are intrinsically chiral, even if the individual

[*] Prof. T. D. P. Stack, Dr. M. A. Masood, E. J. Enemark
Department of Chemistry, Stanford University
Stanford, CA 94305-5080 (USA)
Fax: (+1) 650-725-0259
E-mail: stack@leland.stanford.edu

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