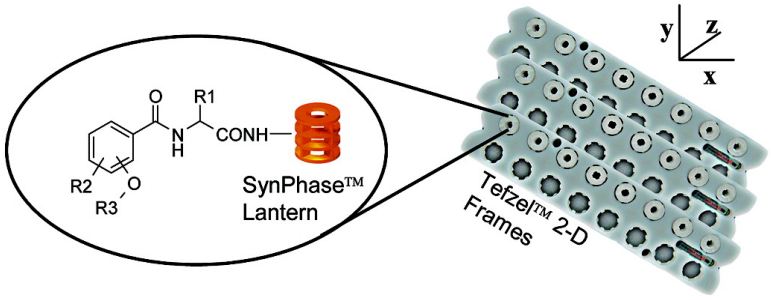
JOURNAL OF combinatorial CHEMISTRY

Article

2D and 3D Spatially Addressed Arrays for High-Throughput Automated Synthesis of Combinatorial Libraries

Marcel Patek, Pavel Safar, Martin Smrc#ina, Eric Wegrzyniak, Kirsten Bjergarde, Aleksandra Weichsel, and Peter Strop

J. Comb. Chem., **2004**, 6 (1), 43-49• DOI: 10.1021/cc0300311 • Publication Date (Web): 30 October 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Articles

2D and 3D Spatially Addressed Arrays for High-Throughput Automated Synthesis of Combinatorial Libraries

Marcel Patek,* Pavel Safar, Martin Smrčina, Eric Wegrzyniak, Kirsten Bjergarde, Aleksandra Weichsel, and Peter Strop

Selectide−Combinatorial Technology Center of Aventis Pharmaceuticals, Inc., 1580 East Hanley Boulevard, Tucson, Arizona 85737

Received March 28, 2003

One of the key elements in the drug discovery process is the use of automation to synthesize libraries of compounds for biological screening. The "split-and-mix" approaches in combinatorial chemistry have been recognized as extremely powerful techniques to access large numbers of compounds, while requiring only few reaction steps. However, the need for effective encoding/deconvolution strategies and demands for larger amounts of compounds have somewhat limited the use of these techniques in the pharmaceutical industry. In this paper, we describe a concept of directed sort and combine synthesis with spatially arranged arrays of macroscopic supports. Such a concept attempts to balance the number of reaction steps, the confidence in compound identity, and the quantity of synthesized compounds. Using three-dimensional arrays of frames each containing a two-dimensional array of macroscopic solid supports, we have conceptualized and developed a modular semiautomated system with a capacity of up to 100 000 compounds per batch. Modularity of this system enables flexibility either to produce large diverse combinatorial libraries or to synthesize more focused smaller libraries, both as single compounds in 12−15 μmol quantities. This method using sortable and spatially addressed arrays is exemplified by the synthesis of a 15 360 compound library.

Introduction

Combinatorial chemistry and related high-throughput synthesis technologies have become essential parts of most contemporary processes for discovery of new biologically active molecules. Efficient output of new molecular entities depends not only on library design but also on the technological level of automation, synthesis flexibility, and compound logistics.[1] Development of new processes for generation of compound arrays thus plays an important role in evolving drug discovery technologies. To date, two main strategies for synthesis of combinatorial libraries have emerged, spatially addressable and pooling strategies.

Multipin[2] and Lantern arrays,[3] photolitography,[4] SPOT techniques on membrane segments or stacks of membrane disks,[5] and synthesis in syringes[6] or in 96 well plates[7] are examples of the former strategy. The latter strategy includes approaches such as the random split-mix technique,[8] DCR "tea bag" synthesis,[9] the MPS "tea bag" method,[10] directed sorting,[11] and necklace coding.[12] Their distinction is based merely on a method used to synthesize and identify each library compound. Spatially addressable arrays allow the *x*/*y*/*z* coordinates of the "reactor" or solid support to be related to the structure (usually supplemented by a label), while split-mix approaches require an encoding, tracking, or deconvolution method to retrieve information about a structure or a sequence of chemical manipulations. Intuitively, split and mix methods are synthetically more efficient and adaptable to different reaction conditions and chemistries, whereas spatially addressable methods require preservation of *x*/*y*/*z* segregation throughout the synthesis, thus rendering them less flexible.

At the beginning of 1998, one of our objectives was to enable production of individual compounds in multimilligram quantities, while maintaining our capability to synthesize larger compound arrays. Such a task required an alternative method, which would allow broad synthesis flexibility, provide larger compound quantities, and provide clear indications of compound identity. Manipulation of spatially arranged plastic frames charged with a macroscopic solid support seemed a viable approach fulfilling our major requirements. Because of their convenient shape, high enough loading, and consistency of grafted polystyrene layer, Mimotopes SynPhase Lanterns were chosen as the macroscopic solid support.

Frame Concept. The concept of spatially arranged arrays is based on two-dimensional (2D) frames, which have macroscopic solid-phase supports (SynPhase L-series Lanterns) arranged in an orderly X-Y array. Each frame has

* To whom correspondence should be addressed. Tel: 520-544-5850. Fax: 520-575-8283. E-mail: marcel.patek@aventis.com.

10.1021/cc0300311 CCC: $27.50
Published on Web 10/30/2003

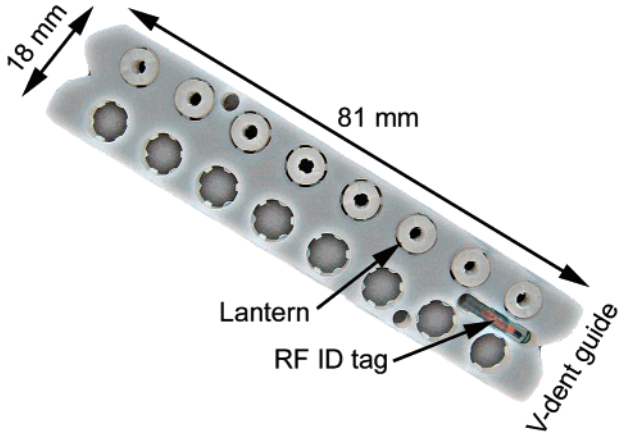
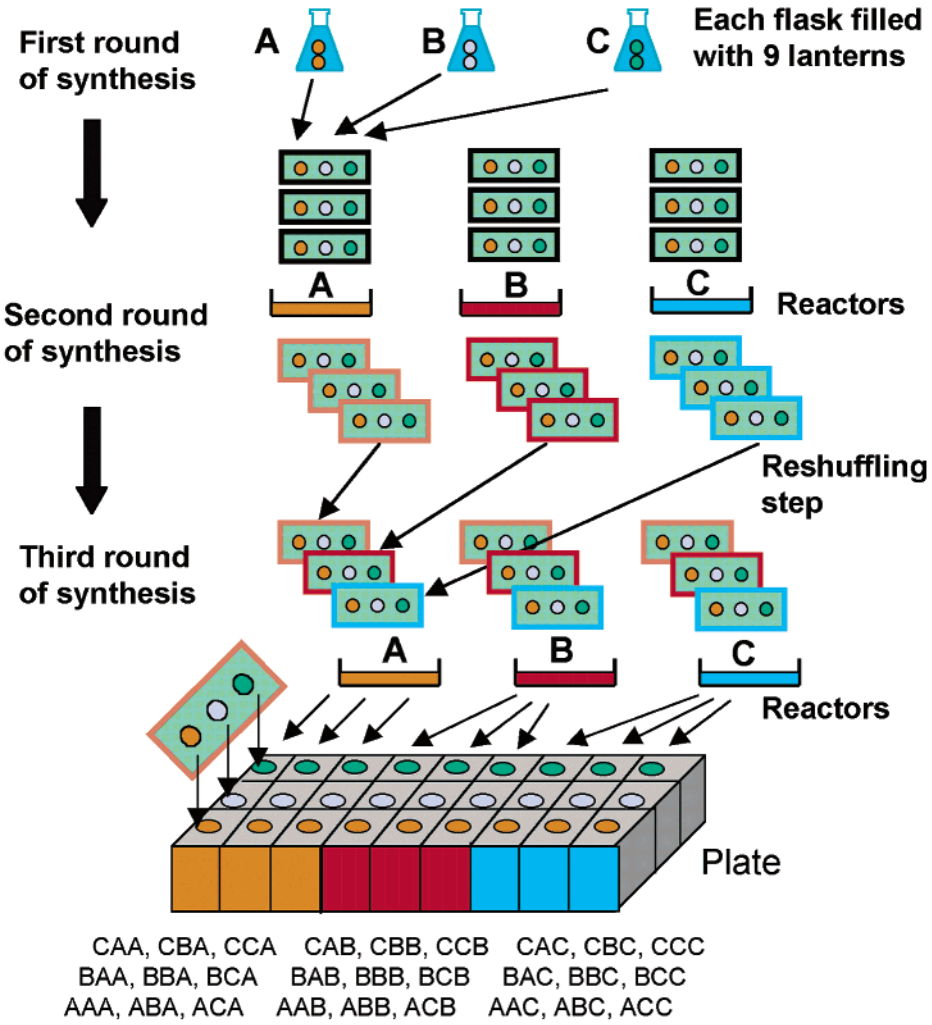


Figure 1. Details of the half-filled Tefzel Frame.

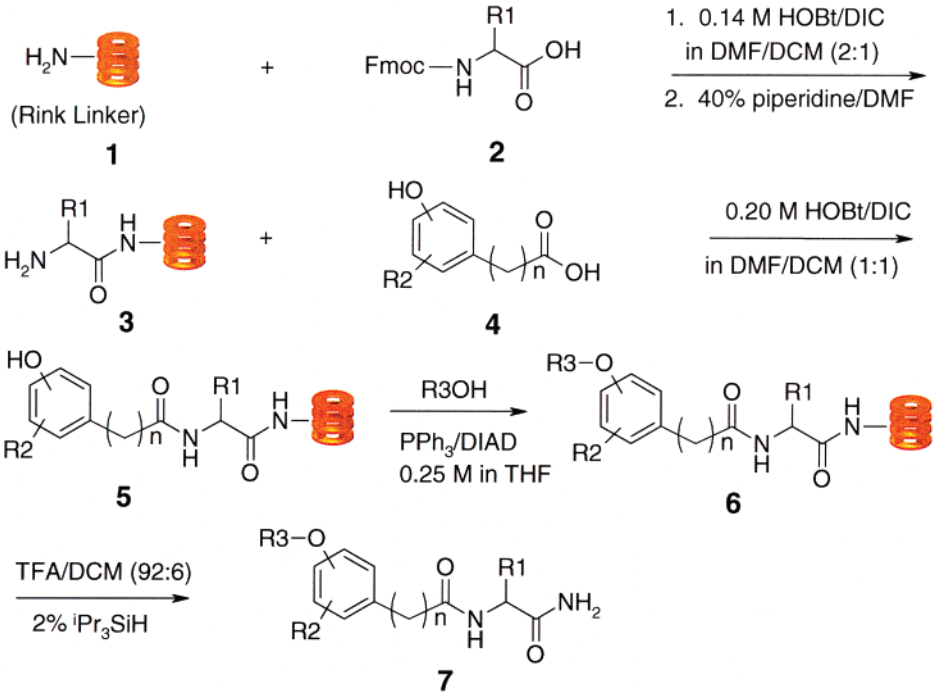
multiple holes arranged in a two by eight array with Lanterns friction fitted into these holes to temporary hold them to the frame during synthesis (Figure 1). Initially, to examine the effect of frame composition on chemistry performance of friction fitted Lanterns, five different materials were tested. Three polyethylene frames of different polymer density, polypropylene, and Tefzel frames were exposed to a variety of solvents (toluene, 1,2-dichloroethane, THF, dioxane, DMF, EtOAc, and AcOH) at different temperatures (−20 to 120 °C). Tefzel and polypropylene showed the best chemical and mechanical stability and the least shape distortion that might interfere later in various automated operations. Additional features of frames include six distancing knobs to prevent close contact of frames in three-dimensional (3D) stacks (not shown in Figure 1), two V shape dents for accurate positioning in reactor cassettes, and 45° chamfer on the top to make spatial orientation unambiguous. To enable independent secondary tracking of frames, we also included a groove into the frame design to accommodate a RF microchip.

The use of 2D frames within a 3D spatial arrangement is illustrated on example of hypothetical library of $3 \times 3 \times 3$ inputs = 27 compounds (Scheme 1). Twenty-seven Lanterns, nine in each flask, are first reacted with a single reagent **R1**{*A*−*C*} and then placed into nine frames, such that three different Lanterns are in each frame. In the next step, groups of three frames are placed into reactors, each having a different reagent **R2**{*A*−*C*}. After the groups are removed from the reactors, one frame is taken from each stack, forming a new stack regroup of all nine frames. The three new groups of frames are then immersed into three reactors, each reactor having a different reagent **R3**{*A*−*C*}. After final synthesis step and washing, Lanterns are detached from the frames and placed into a labeled cleavage plate. Spatial

Scheme 1. Synthesis of a Hypothetical Library of $3 \times 3 \times 3$ Inputs



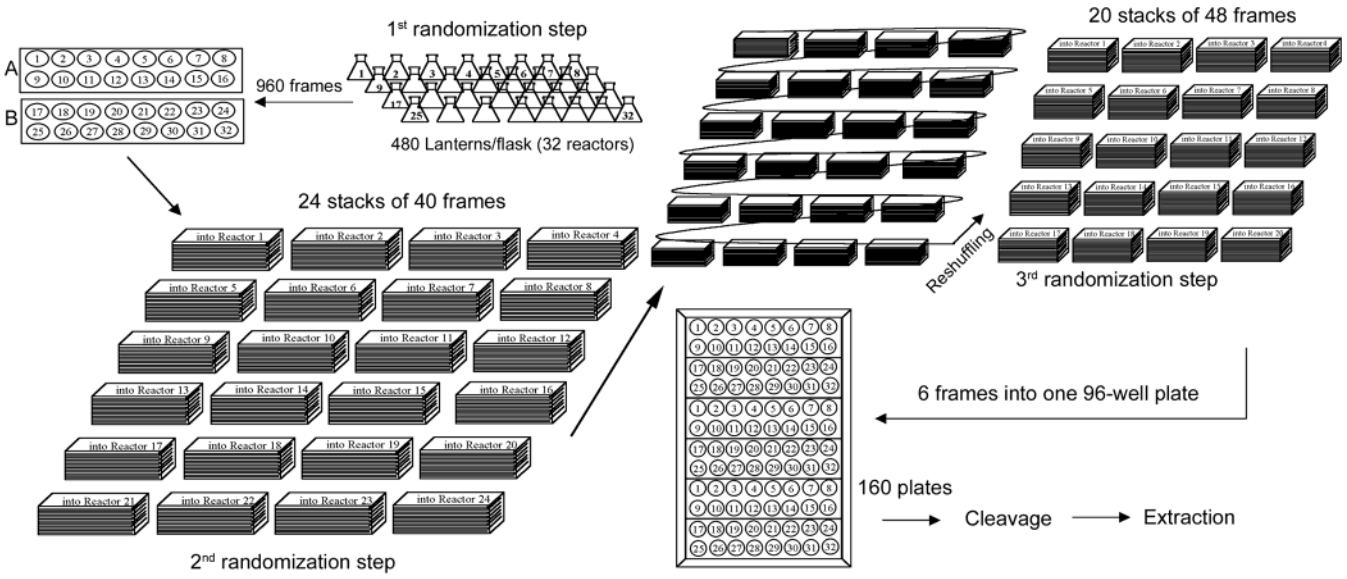
Scheme 2. Synthesis of O-Alkylarylcarboxamides



address is thus mapped onto a hypothetical 27 well plate. Cleavage from Lanterns would provide 27 unique compounds, where identity of each one can be deduced from the recorded path of the particular frame. It should be noted that for a library of three randomization inputs, only one redistribution process needs to take place.

Library Synthesis. To demonstrate the "frame sort and combine" concept in practice, we approached our next objective—a "proof of principle" library. On the basis of our previous experience with synthesis of O-alkylarylcarboxamides,[13] we decided to target a complete permutational library of 32 × 24 × 20 inputs = 15 360 compounds (Scheme 2). Because this paper primarily focuses on the technical aspects of the synthesis, only general descriptions will be provided for each synthesis step. Attachment of the Rink amide linker to Lanterns was done under standard coupling conditions using 0.12 M HOBt/DIC in a mixture of CH_2Cl_2/DMF (4:1). After the Fmoc group (40% piperidine/DMF) was removed, initial loading of **1** was assessed on a sample of 15 Lanterns by Fmoc reading (UV active piperidine−dibenzofulvene adduct).[14] The loading level of Lanterns (14.3 μmol/Lantern) was in good agreement with the substitution declared by the manufacturer (15.0 μmol/Lantern). The first reaction step, attachment of 32 Fmoc-amino acids **2**, was performed under standard coupling conditions using 0.14 M HOBt/DIC in a mixture of CH_2Cl_2/DMF (1:2) using 1000 mL glass containers equipped with a frit at the bottom. In each batch, 480 Lanterns were reacted with one Fmoc-amino acid. Deprotection of the Fmoc group with 40% piperidine/DMF (5 + 60 min) provided 32 batches of 480 Lanterns **3** with R1 input attached (Scheme 3). Fmoc reading from Lantern samples (13.5−14.3 μmol) indicated nearly quantitative attachment of the amino acids **R1**{*1*−*32*}. All Lanterns were then manually placed into 960 frames such that each two frames (A and B) contained 16 different Lanterns (frame A, R1 = 1−16; frame B, R1 = 17−32). Frames were then grouped into 24 stacks, each containing 40 frames in a two by 20 array.[15] Frame stacks were then placed into reactors (rectangular plastic Nalgene bottles), each assigned to have a different reagent **R2**{*1*−*24*}, and reacted with 24 hydroxyaromatic acids **4** under standard coupling conditions using 0.20 M HOBt/DIC in a mixture of CH_2Cl_2/DMF (1:1). After multiple washes (DMF, THF, and CH_2Cl_2) and sufficient drying, stacks of frames were removed from reactors and arranged in 24 physically separated groups ready for the reshuffling step. The presence of all frames was confirmed at this stage by rescanning the RF tags in each stack and comparing them with original records in the database. Reshuffling followed a simple translation pattern: each pair of frames (A and B) on the top of each stack was moved to the bottom of the newly formed stack. This process was repeated until all frames were reshuffled into 20 new stacks, each containing 48 frames in a two by 24 array.[16] Frame stacks were then placed into 20 reactors (glass bottles), each assigned to have a different reagent **R3**{*1*−*20*}, and reacted with 20 alcohols under Mitsunobu conditions using 0.25 M PPh_3/diisopropyl azodicaboxylate (DIAD) in THF. To ensure complete alkylation, this reaction step was repeated after 16 h with the same reagents. The frames in each reactor were then washed sequentially with THF, DMF, CH_2Cl_2, MeOH, and CH_2Cl_2, dried, and finally removed. As illustrated in Scheme 3, six frames from each ordered stack were positioned on the top of a 96 deep well plate and friction fitted Lanterns were pushed out into corresponding 96 wells. Spatial address was thus mapped onto each 96 well plate, which allowed us to identify the chemical history of each compound from the recorded path of the particular frame. Cleavage of the products **7** from the Lanterns was effected with 95% TFA/CH_2Cl_2/^{i}Pr$_3$SiH (92:6:2) for 2 h. The cleavage solution was transferred into another set of 96 well plates while Lanterns were repeatedly treated with the same cleavage solution. After the combined cleavage solution was

Scheme 3. Synthesis of a Combinatorial Library of 15 360 Compounds



evaporated using a Genevac high-speed evaporator, the reaction products were extracted into shallow titer plates using THF and acetonitrile.[17] Library analysis was done with the following sampling protocol: each building block in each randomization was analyzed in multiple redundancies (R1, 14−18 times; R2, 19−22 times; and R3, 23−26 times) in a total number of 488 unique samples. Through the whole library, 96% of analyzed wells had expected molecular weight.[18] Eighty-one percent of all samples contained the expected compound as the major component with a purity of over 50% (by UV at 220 nm). Sixty-six percent of all analyzed wells contained expected compounds with a purity of 75% or better.[19] Quantity was determined by ^{1}H NMR with 2,5-dimethylfuran as an internal standard. The average yield based on a sample of 15 compounds was 12.6 μmol per compound. The structural identity of these compounds was further confirmed by ^{1}H NMR, ^{13}C NMR, FTIR, and MS.

Modular Semiautomated System. To automate the tedious processes of Lantern loading, sorting, tracking, and unloading, a modular system for Lantern/frame handling (LFH) has been conceptualized and further developed in collaboration with researchers at Gem City Engineering Co.[20] A first functional noncommercial prototype was then engineered and built in Gem City Engineering Co. facilities. The system consists of three major modules: the Lantern Load Station, Frame Assembly Station, and Lantern Unload Station (Figure 2). In short, the LFH system first distributes batches of Lanterns (R1) into tube cassettes and then loads Lanterns into frames and later assembles frames in stainless steel frame cassettes. For a specific reaction (e.g., R2), the frame cassettes are manually unloaded from the system and then loaded into chemical reactors. After the synthesis step, the frame cassettes are reloaded into the LFH for a shuffling process to redistribute the frame into different arrays. They are then again loaded into different chemical reactors for another randomization step (R3). When the reaction step is complete, the frame cassettes are loaded back into the LFH to unload the Lanterns from the frames into the 96 well plates. More specifically, the Lantern Loading Station enables assemblage of different batches of Lanterns (**R1**{*A*−*Z*}) in tube cassettes. It consists of the X-Y positioning table equipped with tube damping devices (stores up to 23 tubes), Lantern feeding device (bowl feeder) equipped with a Lantern counter, Lantern purging device, and RFID reader/decoder. When all of the tubes are filled with the Lanterns (different kind in different tube; **R1**{*A*−*Z*} patternlike array), all tube cassettes are removed and brought to the Frame Assembly Station. The main part of this module is the Assembly Dial Table, which has four sets of locating holes that are framelike configured. Each set of holes is equipped with a load plunger, which pushes Lanterns into the frames. Empty frames are taken from frame cassettes on one conveyor belt and once filled with Lanterns, they are moved to frame cassettes on the second conveyor belt. The same conveyor belts are used during reshuffling and as part of the Lantern Unload Station. After the synthesis is finished, the operator loads the required number of plates (up to 100) on a third conveyor belt and initializes the Lantern Unload device, which ejects Lanterns from the frames to the plates. Library configuration, location of frames, cassettes, and tubes are stored in Microsoft Access database. Most of the interactions with the system are through a PC with a touch-screen monitor. The PC integrates the vision and tracking systems and communicates with other control elements using Ethernet or serial communication protocols. Additional features include industry standard safeguards that ensure safe operation in an unattended mode. Regarding the capacity specification, the LFH system can accommodate up to 110 592 Lanterns (6912 frames) per run in a 48 × 48 × 48 format.

Conclusions

In conclusion, we have introduced the concept of a directed sort and combine synthesis with spatially arranged arrays of macroscopic supports. The library synthesis of O-alkylaryl-carboxamides has been achieved in the frame format, thus demonstrating the applicability of the frame Lantern concept

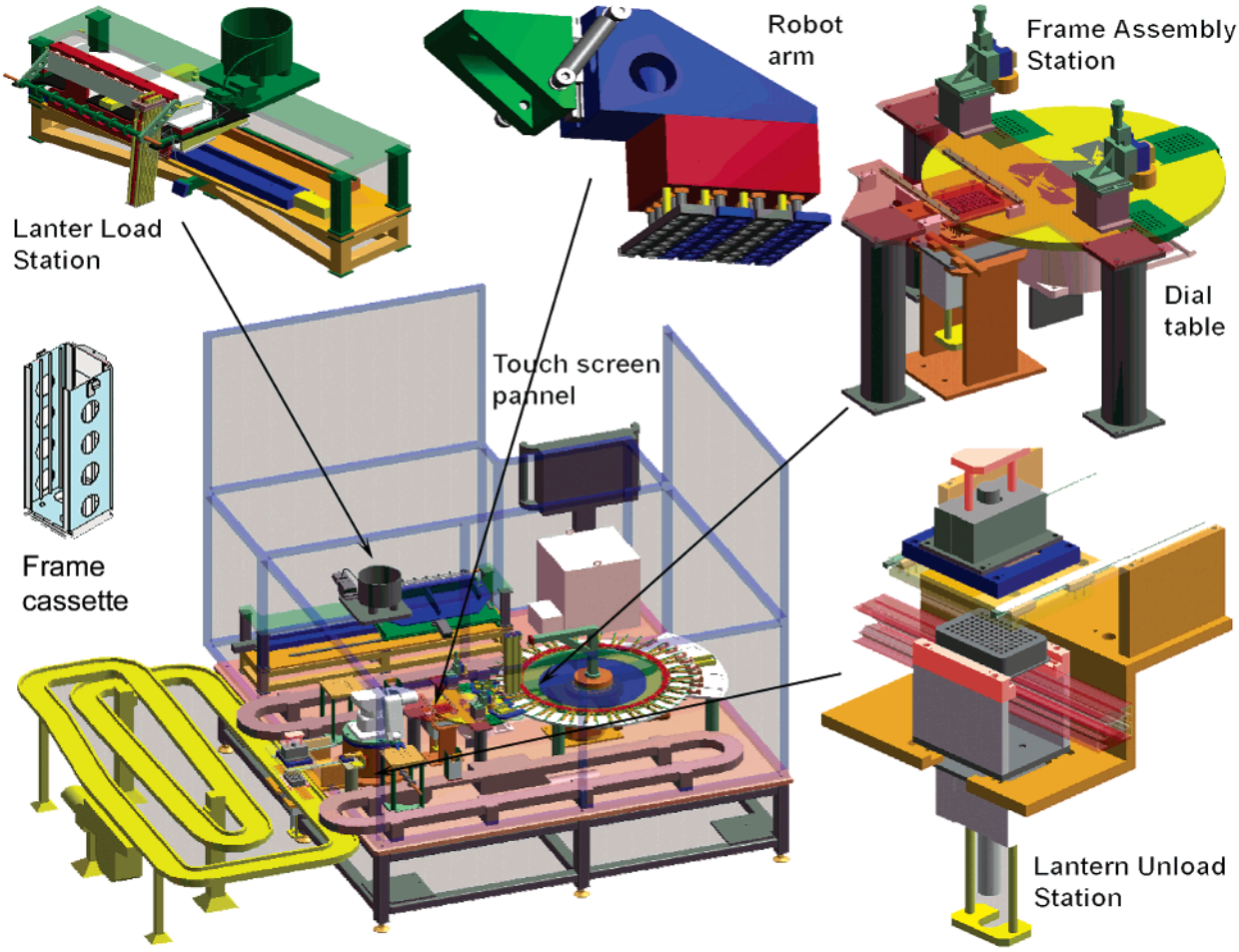


Figure 2. Semiautomated Lantern Frame Handler.

to the solid-phase combinatorial synthesis. The limitations of this methodology include restriction on number of R1s to multiples of 16 (unless several copies of a compound are desired). Also, because of the array character of this concept, synthesis of noncombinatorial matrixes is quite restricted. A significant advantage of the described method is its flexibility, allowing modular integration into a robotic system or, if preferred, an inexpensive standalone manual mode in a laboratory setting. Additional benefits of the method are that larger amounts of compound can be synthesized in parallel on a variety of other macroscopic supports. Such a concept of using spatially arrayed macroscopic supports thus offers another attractive tool to solid-phase chemists working in the field of combinatorial chemistry.

Experimental Section

General. Commercial reagents were used as received without additional purification. Unless otherwise noted, solvents were of the reagent grade available from commercial sources and used without further purification. Synphase SP-PS-AMM Lanterns (L-series, substitution 15 μmol/Lantern), Stems, and Stemholders were purchased from Mimotopes, Clayton, Victoria, Australia. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, and are referenced to an internal standard of tetramethylsilane.

Rink Amide Linker Attachment. Lanterns were first treated with a solution of 10% NEt_3 in 4:1 CH_2Cl_2/DMF (2 $\times$ 30 min) to release a free base from the TFA salt. The solution was then decanted, and the Lanterns were washed with 4:1 CH_2Cl_2/DMF (6 $\times$ 15 min) and CH_2Cl_2 (6 $\times$ 15 min). A solution of Rink linker (16.7 g, 30.0 mmol), HOBt$\cdot$ H_2O (5.51 g, 36.0 mmol), and DIC (3.78 g, 30.0 mmol) in 4:1 CH_2Cl_2/DMF (250 mL) was added to a flask containing the above prepared Lanterns (1000 pieces, 15 mmol). After it was shaken at room temperature overnight, the reaction mixture was decanted and the Lanterns were washed with DMF (3 $\times$ 15 min), 4:1 CH_2Cl_2/DMF (3 $\times$ 15 min), and CH_2Cl_2 (3 $\times$ 15 min). The loading level of 14.3 μmol/Lantern was determined by Fmoc reading (UV active piperidine$-$dibenzofulvene adduct)[14] after Fmoc deprotection with 40% piperidine in DMF (5 + 60 min). The final Lantern wash was done as follows: DMF (3 $\times$ 15 min), 4:1 CH_2Cl_2/DMF (3 $\times$ 15 min), and CH_2Cl_2 (3 $\times$ 15 min). Washed Lanterns **1** were then dried in vacuo and stored in plastic bottles.

General Method for Fmoc$-$Amino Acid Coupling (R1). Solutions of 32 Fmoc-amino acids **2** (21.5 mmol), HOBt$\cdot$ H_2O (3.85 g, 25.2 mmol), and DIC (2.71 g, 21.5 mmol) in 1:2 CH_2Cl_2/DMF (150 mL) were added to the Lanterns (500 pieces, 7.15 mmol) placed in a glass container (1000 mL), and the mixture was agitated by a stream of nitrogen at room temperature overnight. The reaction mixture was decanted, and the Lanterns were washed with DMF (3 $\times$ 15 min), 4:1 CH_2Cl_2/DMF (3 $\times$ 15 min), and CH_2Cl_2 (3 $\times$ 15 min). The

loading level of 13.5−14.3 μmol/Lantern was determined by Fmoc reading (UV active piperidine−dibenzofulvene adduct)[14] after Fmoc deprotection with 40% piperidine in DMF (5 + 60 min). The final Lantern wash was done as follows: DMF (2 × 15 min), 4:1 CH_2Cl_2/DMF (2 × 15 min), and CH_2Cl_2 (2 × 15 min). Washed Lanterns **3** were then dried in vacuo and stored in plastic bottles.

General Method for Hydroxyaromatic Acid Coupling (R2). Stock solutions (370 mL, 1:1 CH_2Cl_2/DMF) of 24 hydroxyaromatic acids **4** (72 mmol/acid), HOBt·H_2O (11.93 g, 78 mmol), and DIC (9.07 g, 72 mmol) were added to the Lanterns charged in frames (640 pieces, 9.00 mmol), and the mixture was slowly shaken in rectangular plastic Nalgene bottles at room temperature overnight. The reaction mixture was decanted, and the frames with Lanterns were washed with DMF (2 × 10 min), 1:1 CH_2Cl_2/DMF (2 × 10 min), and THF (1 × 10 min). To minimize overacylation products, Lanterns were treated with 1 M $NHEt_2$/THF (400 mL) for 4 h at room temperature and then thoroughly washed with THF (5 × 10 min) and CH_2Cl_2 (2 × 10 min).

General Method for Mitsunobu O-Alkylation (R3). To 20 glass bottles (900 mL) containing Lanterns (768 pieces, 10.8 mmol) in frames and a mixture of 20 alcohols (125.0 mmol/alcohol) and PPh_3 (32.75 g, 125.0 mmol) was added dry THF (500 mL) at room temperature. The bottles were cooled to −20 °C, and a stock solution of DIAD (15.78 g, 125.0 mmol) was added slowly while the temperature was kept below −10 °C. After the addition, the bottles were placed on shaker and slowly shaken while warming to room temperature. After they were shaken for 16 h, the Lanterns were washed with THF (2 × 10 min) and CH_2Cl_2 (2 × 10 min) and Mitsunobu alkylation was repeated under the same conditions using twice as less concentrated reagents (62 mmol). To completely remove PPh_3 and $POPh_3$, the final Lantern wash was done as follows: THF (2 × 10 min), CH_2Cl_2 (2 × 10 min), DMF (2 × 15 min), CH_2Cl_2 (2 × 10 min), MeOH (2 × 10 min), CH_2Cl_2 (16 h), DMF (5 h), CH_2Cl_2 (2 × 15 min), and CH_2Cl_2 (16 h). Washed Lanterns **6** were then let dry in a fume hood for several days.

Product Cleavage from Lanters. To unload Lanterns from the frames, six frames were placed on the top of one deep 96 well plate and pushed to fall on the bottom of a 96 well plate. Loose Lanterns in wells were then briefly treated with CH_2Cl_2 (500 μL) and transferred onto 96 Stems attached in 160 Stemholders. A solution of TFA/CH_2Cl_2/^{i}Pr_3SiH (92:6:2, 500 μL) was then distributed into each well, and the mixture was shaken for 2 h at room temperature. After the Lanterns were removed, the cleavage solution was concentrated in a Genevac HT-12 Atlas Evaporator. To ensure maximum recovery of synthesized products, the cleavage procedure was repeated once more using the same protocol.

Acknowledgment. We thank Daniel Schirlin, Gary Flynn, Pavel Rychetsky, and James Connelly for many valuable discussions during the conceptual as well as the later stages of the project. Magda Stankova, Viktor Nikolaev, and Shelly Wade are thanked for skillful technical assistance during chemistry validation and compound analysis. We are also grateful to N. Joe Maeji and Firas Rasoul from Polymerat Pty Ltd., Brisbane, Australia, for conceptual input into the design of frames. Yakov Kaplan, David Barton, Bob Scherrer, and Carl Graves from Gem City Engineering, Inc. are thanked for excellent technical solutions, design, and construction of the final LFH system.

Supporting Information Available. More detailed snapshots of the final Lantern Frame System with brief descriptions for each module, ^{1}H NMR spectra of crude compounds used in the library quantification, and a complete list of analyzed compounds with representative examples of LC/MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Cargill, J. F.; Lebl, M. *Curr. Opin. Chem. Biol.* **1997**, *1*, 67−71. (c) Fennirini, H. *Combinatorial Chemistry*; Oxford University Press: New York, 2000.

(2) Geysen, H. M.; Meloen, R. H.; Barteling, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3998−4002.

(3) Krchnak, V. *Methods Mol. Biol.* **2002**, *201*, 61−75.

(4) (a) Fodor, S. P.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767−773. (b) Gruber, S. M.; Yu-Yang, P.; Fodor, S. P. A. In *Peptides: Chemistry, Structure and Biology (Proceedings of the Twelfth American Peptide Symposium)*; Rivier, J. E., Smith, J., Eds.; Escom: Leiden, 1992; pp 489−491.

(5) (a) Frank, R. *Tetrahedron* **1992**, *48*, 9217−9232. (b) Frank, R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 425−430 and references therein. (c) Wenschuh, H.; Volkmer-Engert, R.; Schmidt, M.; Schulz, M.; Schneider-Mergener, J.; Reineke, U. *Biopolymers* **2000**, *55*, 188−206. (d) Scharn, D.; Wenschuh, H.; Reineke, U.; Schneider-Mergener, J.; Germeroth, L. *J. Comb. Chem.* **2000**, *2*, 361−369.

(6) Krchnak, V.; Vagner, *J. Pept. Res.* **1990**, *3*, 182−193.

(7) Schnorrenberg, G.; Gerhardt, H. *Tetrahedron* **1989**, *45*, 7759−7764.

(8) (a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *14th International Congress Biochemistry*, Prague, Czechoslovakia, 1988; p 47. (b) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487−493. (c) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84. (d) Lam, K. S.; Lebl, M.; Krchnak, V. *Chem. Rev.* **1997**, 4111−448.

(9) (a) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84−86. (b) Houghten, R. A.; Pinilla, C.; Appel, J. R.; Blondelle, S. E.; Dooley, C. T.; Eichler, J.; Nefzi, A.; Ostresh, J. M. *J. Med. Chem.* **1999**, *42*, 3743−3778. (c) Zhang, S. D.; Liu, G.; Xia, S. Q.; Wu, P.; Zhang, L. *J. Comb. Chem.* **2002**, *4*, 131−137.

(10) Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131−5135.

(11) (a) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787−10788. (b) Nicolaou, K. C.; Xiao, X. Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289−2291.

(12) (a) Smith, J. M.; Krchnak, V. *Tetrahedron Lett.* **1999**, *40*, 7633−7636. (b) Furka, A.; Christensen, J. W.; Healy, E.; Tanner, H. R.; Saneii, H. J. *J. Comb. Chem.* **2000**, *2*, 220−223.

(13) Krchnak, V.; Flegelova, Z.; Weichsel, A. S.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 6193−6196.

(14) For review, see Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214.

(15) The position of frames within the stack is not important at this step.
(16) The relative location of frames within the stack is now important for final compound tracking.
(17) Depending on the presence of moisture as well as on the nature of the amino acid **R1**{*1*−*32*}, partial hydrolysis of the carboxamides **7** to the corresponding acids was observed.
(18) The remaining 4% of the expected compounds was not detected by LC/MS analysis. Spatial address was thus successfully maintained and correctly mapped into final 96 well plates. These results therefore validate the presented concept for the synthesis of larger combinatorial libraries.
(19) These results are in accordance with the intended purity profile and correlate closely with performance of R1−R2−R3 building blocks during the chemistry development. Such a purity profile reflects the presence of desired building blocks while it fully meets our internal purity standards for combinatorial libraries (75% of the library compounds to have purities greater than 50%, multiple products are separable on HPLC, and side products are reproducibly formed).
(20) http://www.gemcity.com.

CC0300311