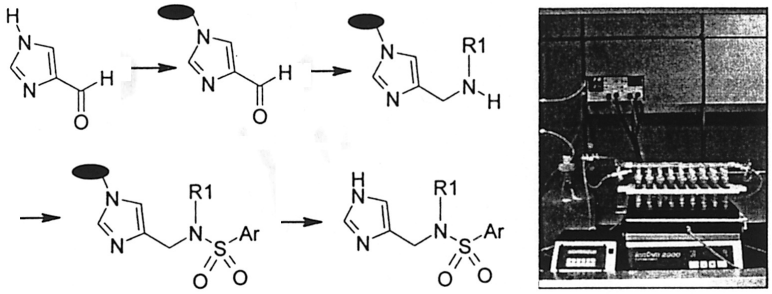
JOURNAL OF combinatorial CHEMISTRY

Article

A Versatile and Inexpensive Apparatus for Rapid Parallel Synthesis on Solid Support: Description and Synthesis Illustration

Ashis K. Saha, Li Liu, and Richard L. Simoneaux

J. Comb. Chem., **2001**, 3 (2), 181-188• DOI: 10.1021/cc0000794 • Publication Date (Web): 16 February 2001



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

A Versatile and Inexpensive Apparatus for Rapid Parallel Synthesis on Solid Support: Description and Synthesis Illustration

Ashis K. Saha,* Li Liu, and Richard L. Simoneaux

Medicinal Chemistry Department, Janssen Research Foundation, Welsh & McKean Roads, Spring House, Pennsylvania 19477

Received September 13, 2000

A new inexpensive and practical apparatus for solid-phase chemistry and parallel synthesis is described. This new apparatus fills an important void in the availability of portable tools for the synthesis of libraries of compounds in multi-milligram amounts. Individual reaction tube capacities range in size from 4 mL to 500 mL of operating liquid volume. Reaction blocks of 36 tubes × 4 mL or 24 tubes × 150 mL allow flexibility of operation. Insert tubes with frit ends function as filter sticks for resin wash and for maintenance of inert atmosphere. An electronic controller device connects to the reaction tubes for programmable entry of pulses of inert gas for resin mixing or vacuum for resin wash. The utility of this apparatus is illustrated by the synthesis of libraries based on 4-methaneamine imidazoles.

Introduction

The practice of solid-phase and combinatorial chemistry is having an enormous impact on the pharmaceutical industry. It promises to significantly enhance the speed and efficiency of discovery of new chemical entities by allowing for the synthesis of hundreds of compounds in parallel fashion.[1] Individual chemists need not only be content with creating finely crafted molecules one at a time, but can accelerate the speed of synthesis as well. There are several elements that contribute to improvement of the synthesis speed. Development of efficient synthesis chemistries and reaction optimizations are always critical.[2] Equally important, however, are the practical aspects of the methods by which reactions are conducted in parallel leading to larger number of products. A number of key developments have contributed to process improvements in the speed of synthesis. Early developments included the Geysen Multi-Pin approach, Houghten's tea-bag technology, and the Parke-Davis Diversomer apparatus.[3] More recently, newer parallel synthesis instrumentation from a number of vendors has been introduced.[4]

Rationale and Background

When we began our work in solid-phase chemistry several years ago, we, much like others, had to wrestle with a myriad of issues relating more to the application than the science of solid-phase synthesis. Our work was undertaken within a traditional medicinal chemistry department, and we were not expected to synthesize extremely large libraries. We desired to improve total productivity while retaining a medicinal chemistry focus. Our productivity goal was the synthesis of 100 or more single pure final compounds in amounts of 7 mg and above per chemist per month. Trials of existing library synthesis technology were not extremely satisfying. Flexibility is critical when it comes to the practice of everyday organic synthesis, yet this was what we found lacking from commercially developed "high speed" synthesis equipment. While a majority of vendors advertised the capability of organic synthesis at high speed, their design often originated from peptide synthesis equipment, where chemistry is well developed. Adapting such equipment to organic synthesis was time-consuming and frustrating. We required an apparatus to synthesize moderate size libraries, where the scale and type of chemistry could be varied without the need to re-engineer the hardware for each new situation.

We have developed an inexpensive and practical manual synthesis apparatus for solid-phase chemistry based on the principles summarized in Table 1. A void clearly existed in the availability of portable solid-phase parallel synthesis tools that satisfied all these principles. Inverse filtration in round-bottom reaction tubes was the operating principle employed for resin wash. The advantage of such a design principle is enormous in that most standard organic reaction conditions are readily adaptable. Although several commercially available systems employ this method, the integration of compact reaction blocks, an array of reaction tube and block sizes along with a small portable electronic device to aid in resin wash and mixing made our apparatus highly useful and practical for everyday synthesis needs. In addition, we avoided robotics and thus achieved significant user freedom and flexibility.

Results and Discussion

The basic unit in our system is a two-piece reaction vessel comprised of a threaded glass reaction tube (A) and a glass insert ending in a frit (B, Figure 1). These pieces attached to each other via Schott bottle caps (C) fitted with a Teflon lined septa. A port (D) was designed in the collar section of the glass insert for the addition of liquids. This port was threaded to accept septa caps for those situations requiring very strict inert gas operations and syringe piercing for reagent addition. The frit end rested inside the suspension

* To whom correspondence should be addressed. E-mail: suaaj@msn.com.

10.1021/cc0000794 CCC: $20.00
Published on Web 02/16/2001

Table 1. Design Principles

principles on chemistry	principles on apparatus development
• 100 or more target-based single pure compounds per month per chemist	• Capability to handle a variety of reaction numbers and scales in parallel
• Dry weight of final compound should be 7 mg or higher	• Low cost and simple to maintain/replace
• All final compounds must be >90% pure by HPLC (214 nM) and characterized by mass spectroscopy	• Apparatus placed inside a normal chemistry fume hood
• NMR characterization must be conducted on all new and representative library structures	• Portable, lightweight, and flexible design
• Same overall design to allow chemistry development, validation, and library synthesis	• Apparatus to allow heating with reflux and cooling (−78 °C to 150 °C)
	• Ability to access and monitor individual reactions
	• Reactions requiring inert atmosphere readily feasible
	• Agitation by gas purges, shaking, and ultrasonic

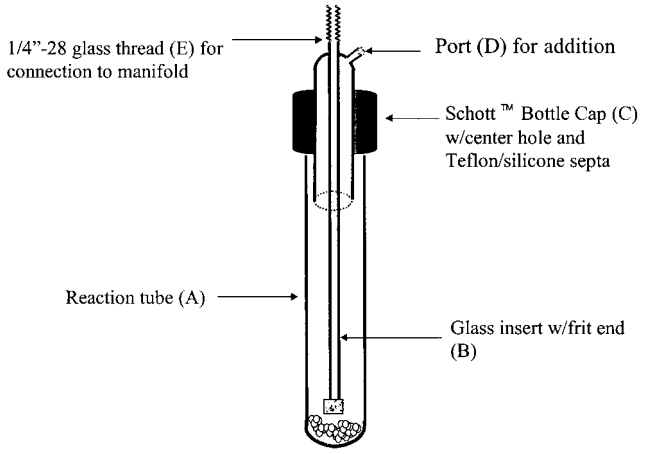


Figure 1. Design of the basic reaction vessel.

of reaction contents. For assembly, the bottle cap with septa w/center hole (C) was first screwed on to the reaction tube (A) followed by placement of the one-piece glass insert (B) through the center hole to complete assembly. The top end of the inserts carried a $^1/_4$ in.-28 tubing thread (E) for attachment to liquid/gas lines.

The manufactured reaction tube assembly is shown in Figure 2. A two-way Teflon coupler is shown attached to the $^1/_4$ in.-28 thread (E in Figure 1). The reaction tube had the dimension of 16 × 100 mm with a #18 bottle thread and was capable of accommodating reactions involving 50−200 mg of resin. We extended this design toward the manufacture of vessels that could accommodate larger amounts of resin to prepare building block libraries as well as starting materials on resin. Building block libraries typically required 500 mg to 2 g of resin per vessel. The vessels manufactured for this purpose are shown in Figure 3. The 50 mL and the 100 mL vessels were each comprised of a #25 bottle thread and 32 mm (OD) reaction tube with heights of 100 mm and 150 mm, respectively. A crimp was designed near the top of the reaction tubes, which served to prevent any unanticipated bump in the reaction content. Larger vessels of #32 bottle thread had capacities of 200 and 500 mL each and were self-standing. All vessels were readily immersible in hot or cold baths. The insert tubes were interchangeable for any given size reaction vessel. Each vessel could be closed with readily available bottle caps and thus served as convenient storage of dry resin-bound starting materials. The largest vessel was capable of holding reactions with 50 g of resin and was excellent for the initial loading of starting materials.

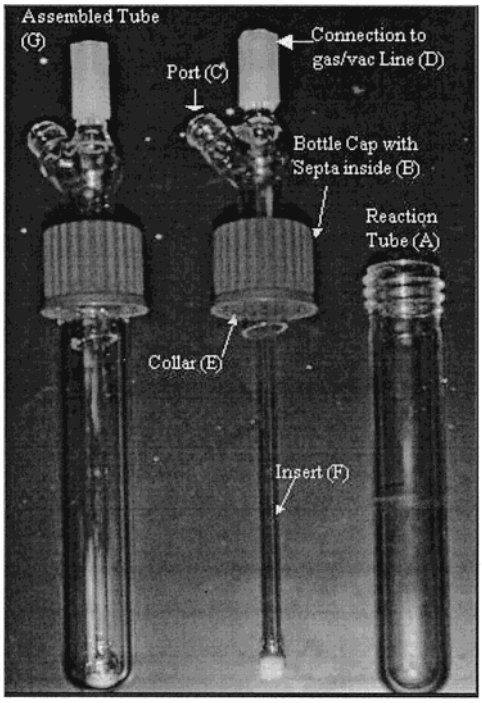


Figure 2. Manufactured reaction tube and assembly.

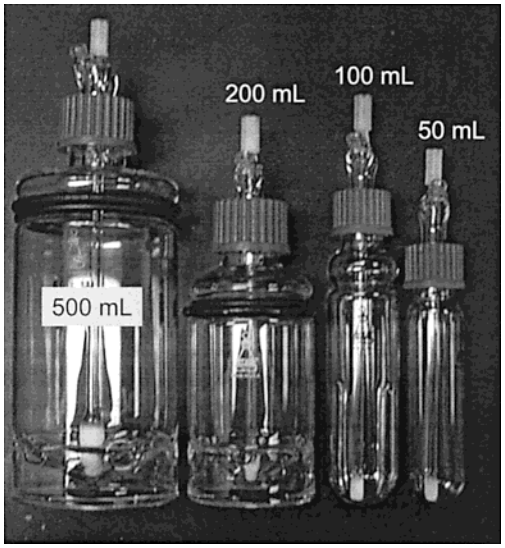


Figure 3. Larger size reaction vessels for reactions of 0.5−50 g of resin.

The synthesis of individual compounds employing these reaction vessels comprised the simple step of weighing in the desired amount of resin and any solid reagents into the open reaction tubes. The bottle cap and septa with center

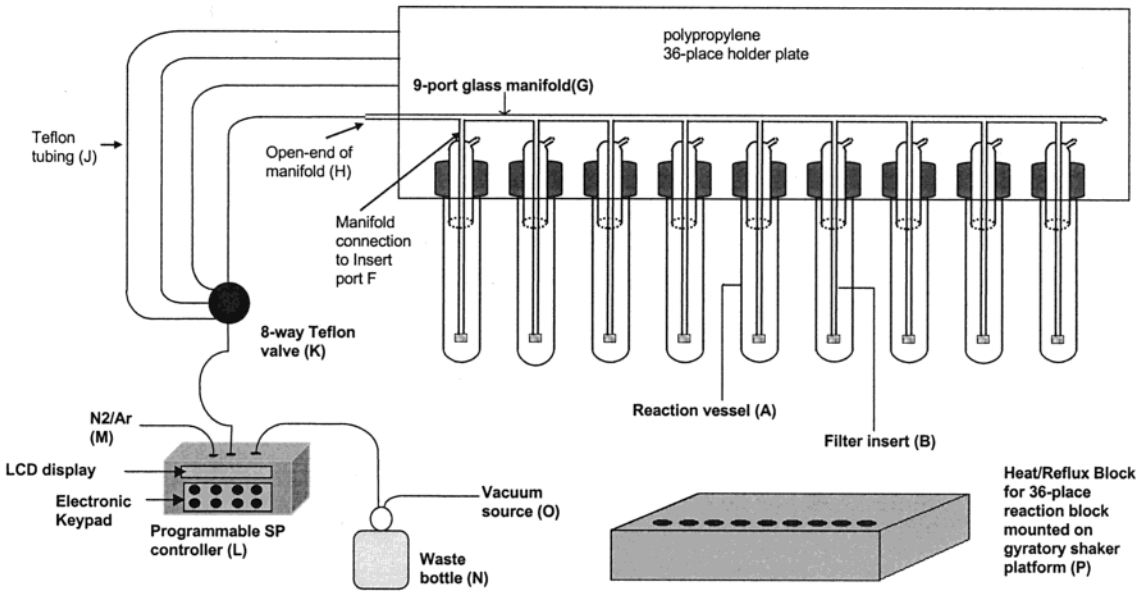


Figure 4. Design of 36-place reaction block and apparatus.

hole was then screwed on to the reaction tube followed by placing the insert through the center hole completing the assembly. Nitrogen gas purges were introduced via the connecting port (e.g., D in Figure 2), and liquid reagents and reaction solvent were introduced via pipet or syringes through reagent port C. The reaction tube was then placed in either heat block or cold bath depending on reaction need. After the reaction was complete, vacuum was applied through port D to drain the liquid contents of the reaction followed by addition of wash solvents through port C. The washing of resin was quite efficient through the repeated steps of solvent addition followed by nitrogen purges for agitation and application of vacuum for draining. At any point during the reaction, if desired, the reaction tube could be unscrewed from the insert assembly and either the resin or the supernatant liquid could be analyzed to monitor the progress of the reaction.

In order that the parallel synthesis of libraries could be carried out, the design of blocks accommodating 36 tubes in four rows of nine tubes each was considered (Figure 4). Glass manifolds (G) capable of being connected to the top ports in individual reaction tubes were designed. The open end (H) of this manifold could be connected to a multiport valve (K) with Teflon tubing (J). Four such liquid/gas lines were thus connected together to the multiport valve, which itself connected to a programmable synthesis controller (L). The main function of the controller was to allow entry of an inert gas into every reaction vessel and apply vacuum as needed for resin wash. Inside this controller was a three-way Teflon solenoid valve with connections to inert gas lines (M) and vacuum source (O) via a waste bottle (N). A simple program controlled the switching between the gas/vacuum lines at user defined time intervals. Agitation or draining of reaction vessels was effected via a small touch pad on the controller. A heat/reflux block (P) was designed such that the entire block could be simultaneously heated or refluxed.

AT-MAR Glass Co. of Kennett Square, PA, conducted all glass manufacture.[5] The finished 36-place reaction block is shown in Figure 5. The dimensions of the polypropylene holder plate for 36 reaction tubes is 12.5 in. × 5 in. The manufacture involved placement of the Schott bottle caps inside individual holes drilled on the plate to fit these caps snugly. All 36 tubes were then screwed onto the bottle caps exposed at the bottom part of holder plate. The insert pieces were then placed snugly through the top openings of holder plate. Efficient simultaneous gas purges and draining required that the manifold design be modified such that there are either five or four ports in each manifold. These manifolds were attached to the top end of insert threads with the aid of Teflon couplers. The assembled reaction block sat well in a compact lab bench with the aid of four detachable legs placed at the bottom corners of the holder plate. Two lips were designed at the ends of the plate such that the entire block could be lifted via these handles. The entire block could also rest well on these handles when suspended inside a standard ultrasonic bath or placed in a heat or cold bath.

The basic set up for library synthesis employing the 36-reaction block is shown in Figure 6. The block (A) is shown resting inside a custom heat/reflux block (B), which in turn is mounted on an Innova 2000 gyratory shaker (New Brunswick Scientific, New Brunswick, NJ). It was therefore possible to simultaneously effect heating, shaking, and agitation by gas purges for the duration of reactions. The SPS controller (C, Figure 6) containing the solenoid valve was compact, built with a LCD display and touch pad for easy access to programs. A single board computer inside this unit helped operate the solenoid valve. Several simple programs were pre-stored during manufacture with required user inputs for pulse duration, interval, number, and duration of pulse cycles, timing, and duration of vacuum application. J-KEM Scientific Inc. of St. Louis, MO, manufactured both the heat/reflux blocks and custom synthesis controller.[6] Most consumable items such as Teflon tubes, multiport valves, connectors, waste bottles, gripper fittings, and other tube connection supplies, while widely available, were purchased from Omnifit Limited.[7] As is displayed, the synthesis unit,

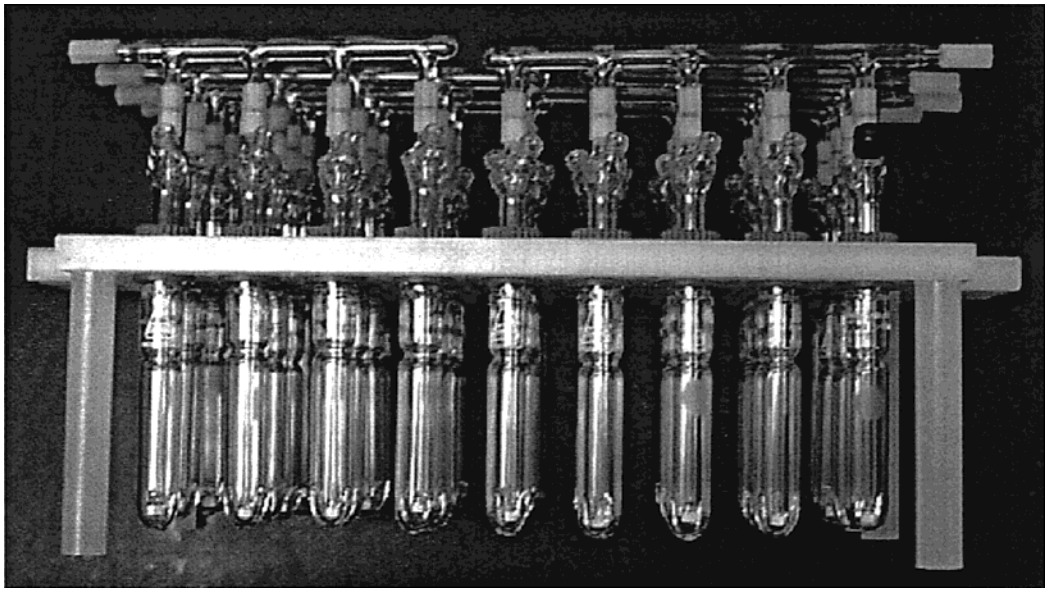


Figure 5. Manufactured, fully assembled 36-place reaction block.

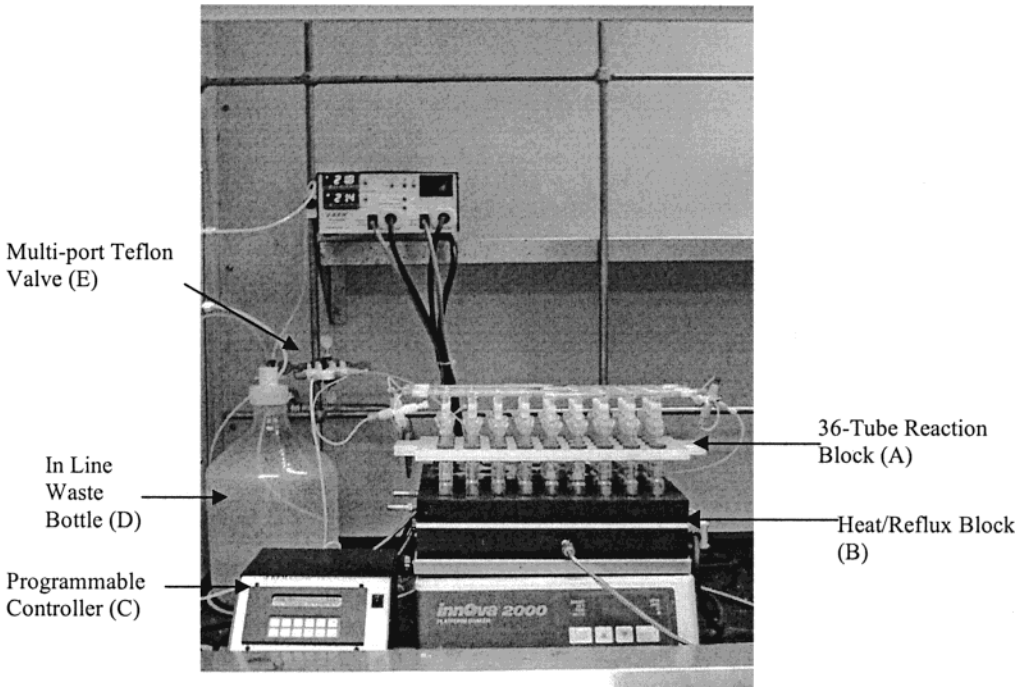


Figure 6. Apparatus setup for library synthesis.

waste bottle, and controller fit handily in a standard fume hood. Removal of the entire block from this space simply required disconnection of the liquid/gas lines from the multiport valve. When required, the entire block could be readily placed in a hot or cold bath while all reaction contents were maintained under an inert atmosphere with gas purges through the frit inserts. The bottom portion of the reaction tubes rested in liquid of the desired temperature, while the top part of the block containing manifolds, gas/vacuum connections, and reagent addition ports remained outside and available for any needed operations. Purges of inert gas also served to agitate the reaction contents without requiring placement on a shaker. The synthesis controller was custom programmed such that infinitesimal purges (e.g., 50−100 μs at desired intervals) could be applied in situations involving easily evaporated solvents such as methylene chloride or tetrahydrofuran.

We also designed mini-blocks of 20 or 16 reaction tubes for the preparation of smaller libraries (A, Figure 7). Blocks accommodating 24 vessels (B) of 100 mL capacity each were manufactured to facilitate the synthesis of larger scale libraries or building blocks. These blocks were constructed from thicker polypropylene plates and larger overall dimension. Metal stands with adjustable tilts were manufactured for both the 36-place and larger 24-place blocks such that workup and access to individual reaction tubes could be facilitated (C).

The ability to accommodate a variety of reaction sizes and numbers while retaining compactness, portability, and flexibility is clearly the strength of our design. Most operations

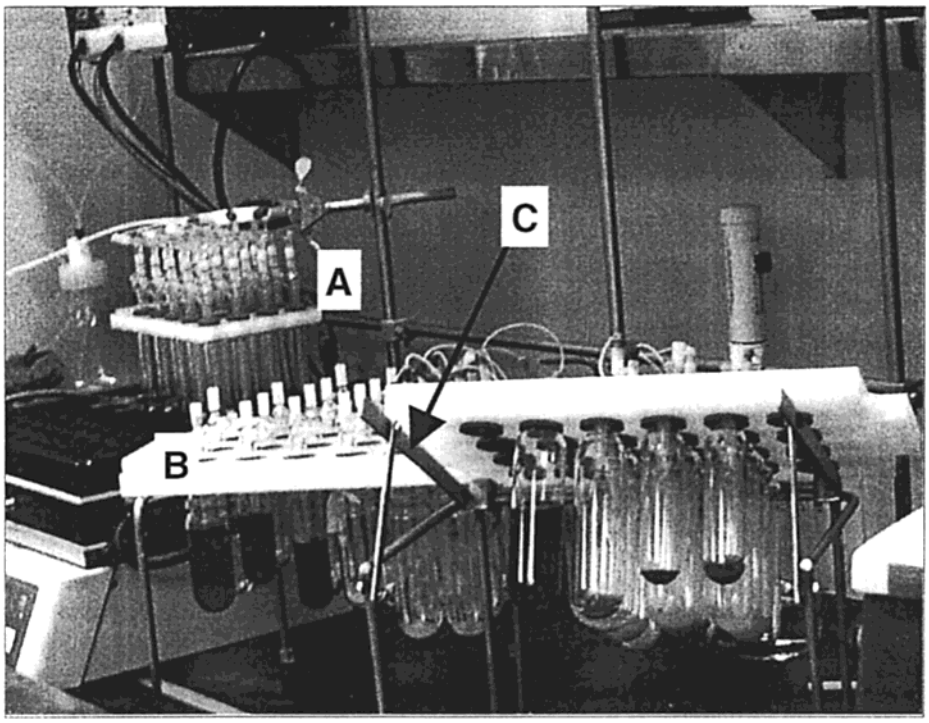


Figure 7. Larger size 24-place block and a mini-block of 20 vessels: (A) smaller 20-tube reaction block; (B) 24-tube reaction block for larger scale assemblies; (C) metal stands with adjustable tilt.

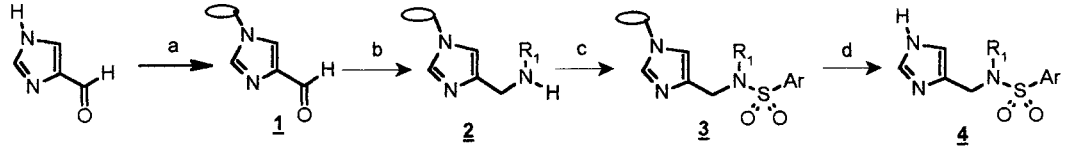
such as reagent and resin handling and solvent addition were handled manually. Cleavage was conducted after drying the resin directly in the reaction tubes. A manual transfer was required at the end of an incubation period with the cleavage solvent. We preferred to transfer the reaction contents into disposable frit-bottom columns resting on collection tubes in simple test tube racks. While this operation may be tedious for the synthesis of individual libraries of over 100−200 compounds, it usually posed no problem for a bench chemist to complete the cleavage of 72 tubes in about an hour. Thus, the synthesis of small to medium size libraries for which this apparatus was designed was very efficient. Remarkably, we also conveniently performed solution-phase protocols in this apparatus with optional resin capture for workup or library synthesis. We are currently investigating automated cleavage protocols and automated solvent addition for resin wash for larger libraries.

Synthesis Illustration

Examples of libraries synthesized in this apparatus are described below. These libraries were synthesized as part of a medicinal chemistry lead exploration and analogue synthesis program.[8]

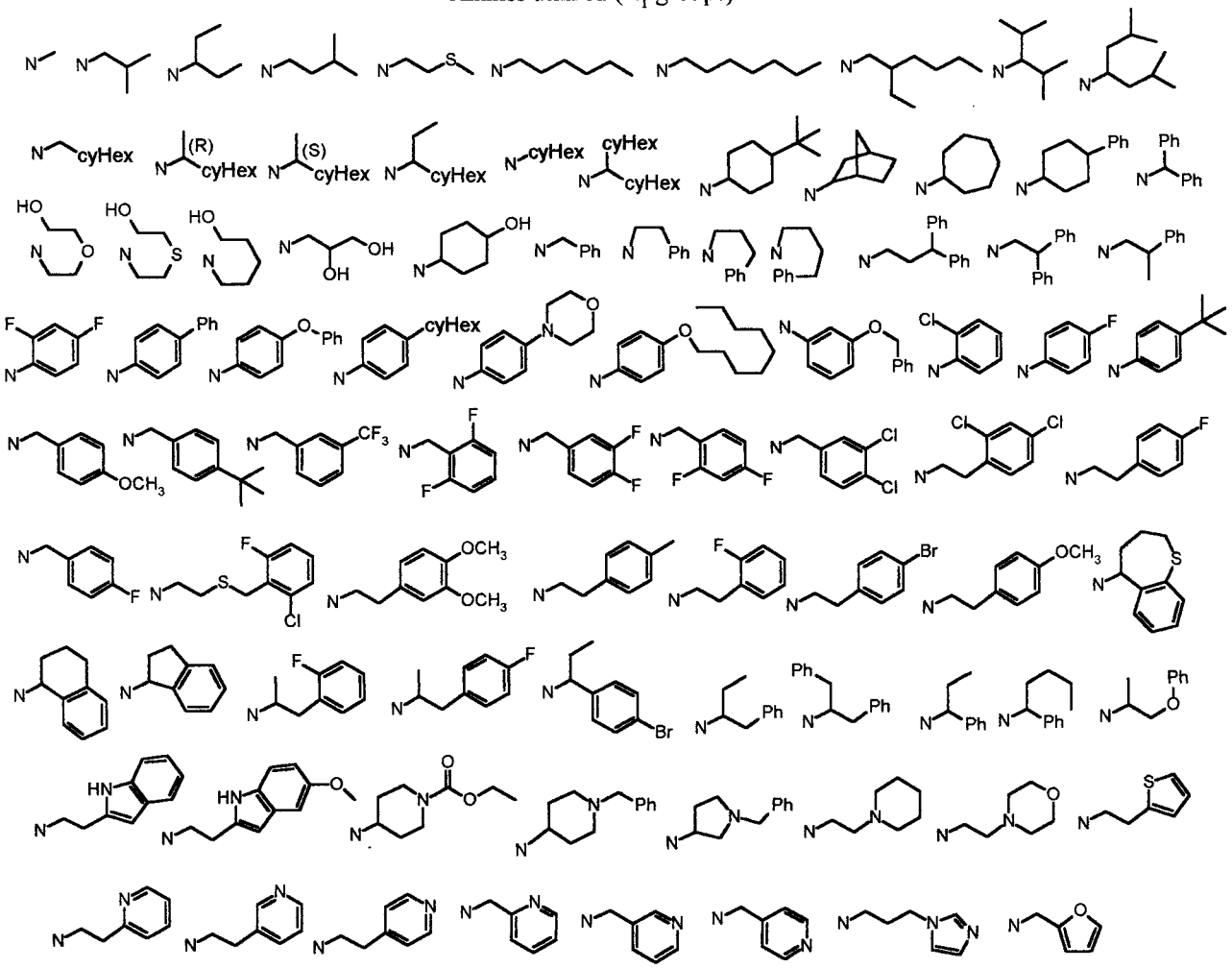
Reaction of 4-formyl imidazole with 2-chlorotrityl chloride PS resin[9] in the presence of triethylamine (Scheme 1) gave the immobilized aldehyde **1**. The extent of loading was assessed from aldehyde recovered after cleavage of measured amounts of dried resin and corresponded closely to loading of the trityl linker (0.9 to 1.3 mmol/g). The large 500 mL reaction vessel was employed to conveniently carry out this overnight reaction. Reductive amination[10] of resin bound aldehyde **1** with primary amines in the presence of 1% acetic acid went smoothly to give secondary amine products **2** (Scheme 1). Typically resin prepared in step one was weighed as either dry material for smaller numbers of reaction or as a slurry for larger sets of reaction. We used the 24-reaction block for the synthesis of a set of imidazole methylamine intermediates **2** at a scale of 4 g each. Suspending the entire block inside a standard ultrasonic bath affected agitation. A large variety of amines (Scheme 1) were utilized for this reaction with excellent results. Small amounts of dimeric products (0−5%; not shown) resulting from condensation of **2** with matrix-bound aldehyde **1** were observed; however, these products did not affect any subsequent chemistry. The amines were suspended in a suitable slurry solvent and transferred to tubes in 36-reaction blocks employing simple liquid transfer pipets. The blocks were then plumbed into the synthesis controller as described previously, and the swelling solvent drained. Reaction of amines **2** with commercially available sulfonyl chlorides gave sulfonamide products **3**. The reaction solvent and reagents were introduced via port C (Figure 2). All reactions were maintained and additionally agitated by minute intermittent purges of nitrogen gas. After completion, all reactions were subjected to manual washing utilizing suitable mix and drain time cycles on the synthesis controller. Cleavage from resin was conducted by addition of 10% trifluoroacetic acid in CH_2Cl_2 via the addition ports. Analytical RP-HPLC revealed that most compounds (**4**) were greater than 90% pure based on UV absorbance at 214 nM. Other compounds were purified by prep-reverse-phase HPLC. All library synthesis involved ca. 0.1 mmol of starting resin bound 4-formyl imidazole. The synthesis of a library of 72 compounds is completed in 2−3 days. The isolated yields for final compounds not requiring HPLC purification were in the range of 15−25 mg. Adequate amounts (usually > 7 mg) were obtained for compounds requiring chromatography. All compounds were characterized by HPLC and mass spectra.

Scheme 1

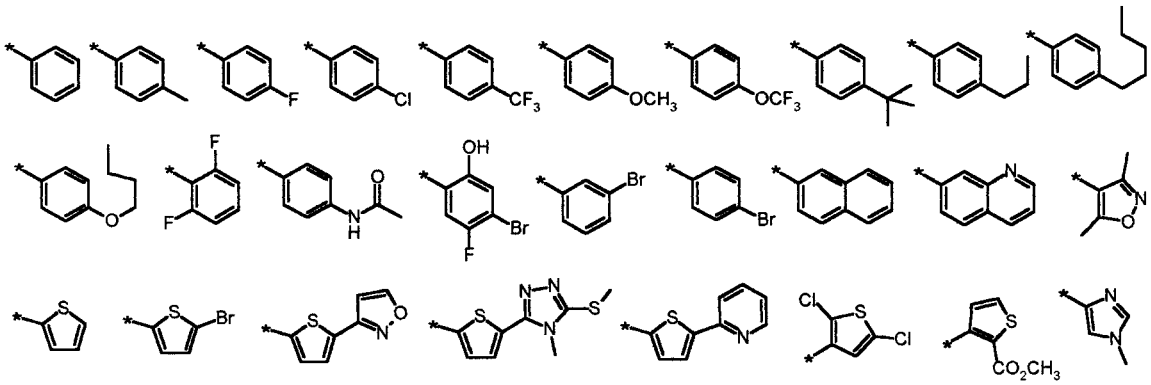


Reagents and conditions: (a) 2-Cl Trityl resin, DMF:DCE:Et_3N; (b) R_1NH_2, 1%HOAc-DCE, $NaBH(OAc)_3$; (c) $ArSO_2Cl$, iPr_2EtN, CH_2Cl_2; (d) $CH_3OSO_2CF_3$, CH_2Cl_2, RT, 2h

Amines utilized (R_1 groups)



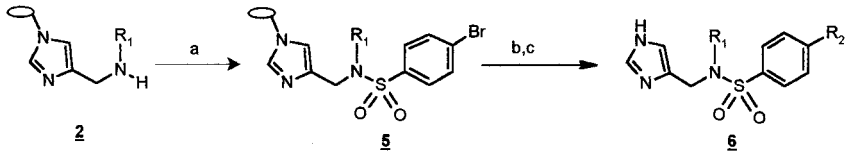
Sulfonamide groups utilized (Ar)



A selected number of compounds were also characterized by NMR techniques.

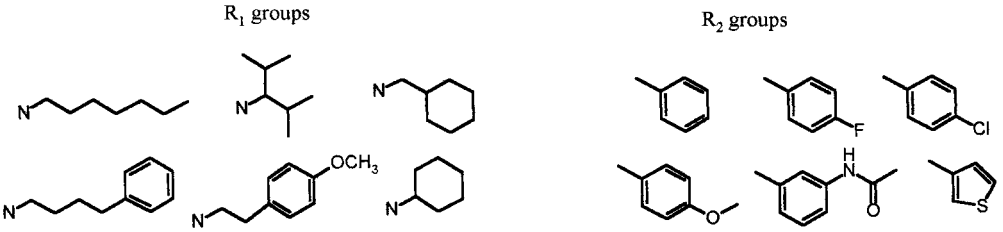
Reactions involving palladium catalysis could be conducted in this apparatus very efficiently. A small 36-compound library synthesized by employing the Suzuki biaryl coupling reaction[11] is exemplified in Scheme 2. Both inert atmosphere maintenance during reaction, heat/reflux, and aqueous workup was conducted without difficulty. More

Scheme 2

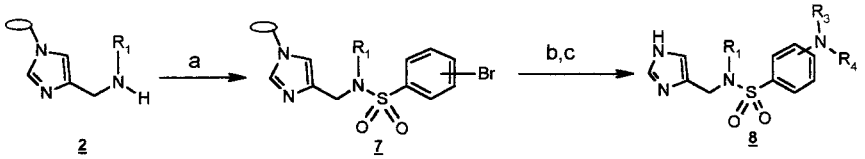


Reagents and conditions: (a) 4-Bromo benzene sulfonyl chloride, iPr_2EtN, CH_2Cl_2; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2M Na_2CO_3, dioxane 80°C; (c) $CH_3OSO_2CF_3$, CH_2Cl_2, RT, 2h

Library Fragments (Structure **6**)

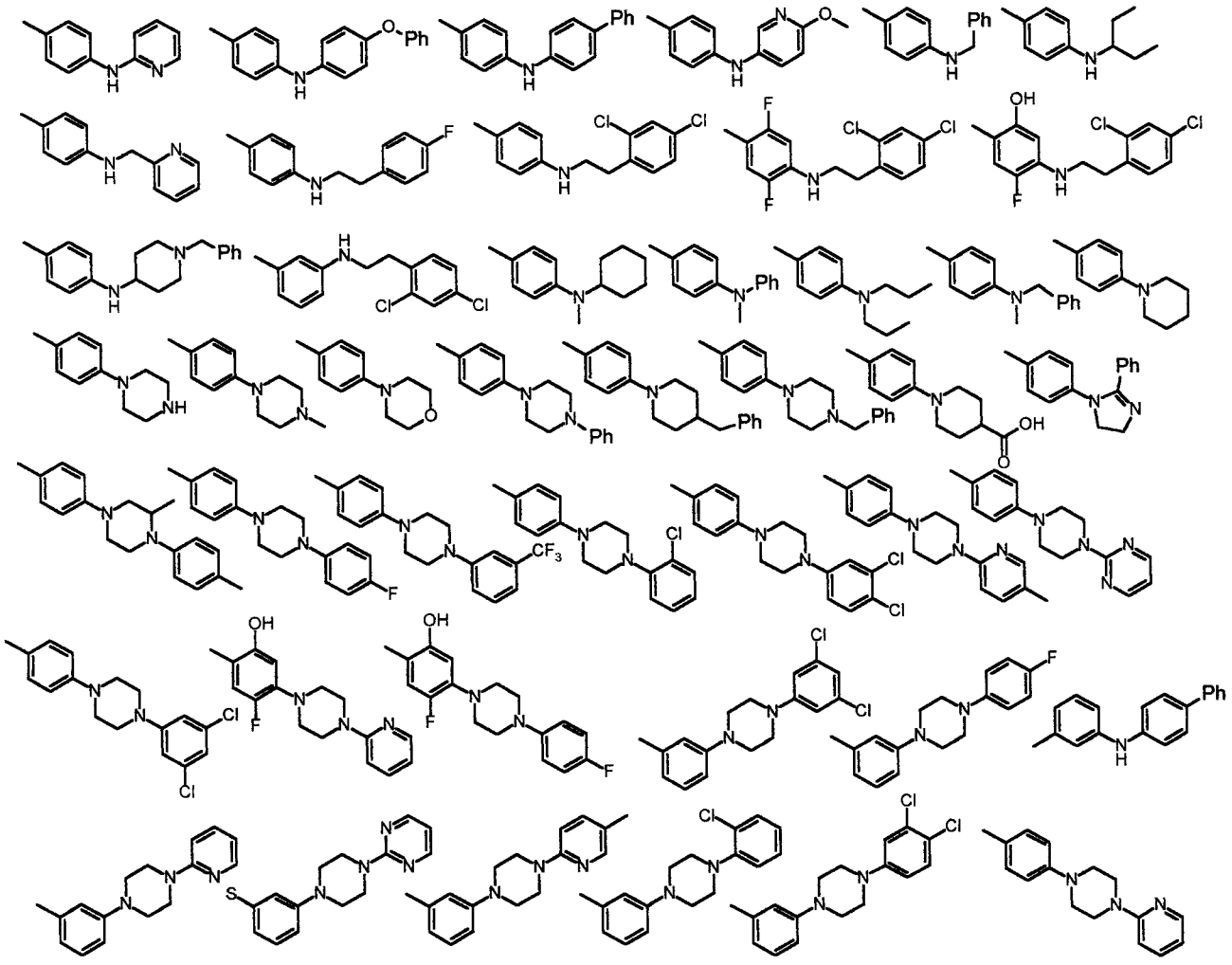


Scheme 3. Pd-Catalyzed Aryl Amination Reactions



Reagents and conditions: (a) 3 or 4-Bromo benzene sulfonyl chloride, iPr_2EtN, CH_2Cl_2; (b) Pd_2dba_3, rac-BINAP, R_3R_4NH, 80°C; (c) $CH_3OSO_2CF_3$, CH_2Cl_2, RT, 2h

List of Aryl Amine products synthesized (structure **8**, Ph-N-R_3R_4 group only shown)



than half of the compounds from this library were greater than 90% pure after resin cleavage. Excellent isolated yields of biaryl sulfonamides **6** (>60%) were obtained. The arylamine fragments synthesized toward additional sulfonamide modifications (structure **8**) are shown in Scheme 3. These palladium-catalyzed reactions were also conducted in parallel fashion employing single or multiple 36-place blocks according to procedures described in the literature.[12] Although the yields and purities of crude products were somewhat poorer than products from the Suzuki library, all compounds were isolated in >2−5 mg amounts from 100 mg of starting resin after purification on Gilson prep-HPLC.

In summary, we have developed an efficient solid-phase synthesis apparatus for the preparation of small to medium size libraries. A wide variety of reaction conditions and scales could be efficiently carried out on this apparatus system. No robotic or other expensive automation was necessary, making this apparatus highly practical and useful for everyday medicinal chemistry applications.

Acknowledgment. We are greatly indebted to Victor Pesce and Steve Pesce, AT-MAR Glass Co., and Bob Elliott, J-KEM Scientific, for their unfailing commitment and excellent collaboration. Adrian Argento is acknowledged for introducing us to Omnifit products. We also thank Dr. Allen B. Reitz for his helpful comments on this manuscript and William Leister and Kim Strauss for assistance with HPLC purification.

Supporting Information Available. HPLC traces, mass spectroscopy, purity, and yield information for representative compounds; ^{1}H NMR spectra for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Ellingboe, J. W. Solid-phase synthesis in lead optimization and drug discovery. *Curr. Opin. Drug Discovery Dev.* **1999**, *2* (4), 350−357. (b) Nuss, J. M.; Renhowe, P. A. Advances in solid-supported organic synthesis methods, 1998 to 1999. *Curr. Opin. Drug Discovery Dev.* **1999**, *2* (6), 631−650. (c) Edwards, P. J.; Gardner, M.; Klute, W.; Smith, G. F.; Terrett, N. K. Applications of combinatorial chemistry to drug design and development. *Curr. Opin. Drug Discovery Dev.* **1999**, *2* (4), 321−331. (d) Myers, P. L. Will combinatorial chemistry deliver real medicines? *Curr. Opin. Biotechnol.* **1997**, *8* (6), 701−707.

(2) (a) Franzen, R. G. Recent Advances in the Preparation of Heterocycles on Solid Support: A Review of the Literature. *J. Comb. Chem.* **2000**, *2* (3), 195−214. (b) Dolle, R. E. Comprehensive Survey of Combinatorial Library Synthesis: 1999. *J. Comb. Chem.* **2000**, *2* (5), 383−431.

(3) (a) Geysen, H. M.; Meloen, R. H.; Barteling, S. J. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3998−4002. (b) Houghten, R. A. General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen−antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5131−5135. (c) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M. R.; Pavia, M. R. "Diversomers": An approach to nonpeptide, nonoligomeric chemical diversity. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909−6913.

(4) Although literature citations or reviews on development of newer parallel synthesis apparatus are hard to find, the patent literature chronicles a very good history and is a source of much valuable information. Recent literature references on a few systems include (a) Krchňák, V. Semi-automated high throughput combinatorial solid-phase organic synthesis. *Biotechnol. Bioeng.* **1999**, *61* (3), 135−141. (b) Dapremont, L.; Valerio, R. M.; Bray, A. M.; Stewart, K. M.; Mason, T. J.; Wang, A. W.; Maeji, N. J. Multiple syntheses using the Multipin method. *Physiol. Chem. Phys. Med. NMR* **1995**, *27*, 339−343. (c) DeWitt, S. H.; Schroeder, M. C.; Stankovic, C. J.; Strode, J. E.; Czarnik, A. W. DIVERSOMER technology: solid phase synthesis, automation, and integration for the generation of chemical diversity. *Drug Dev. Res.* **1994**, *33* (2), 116−24.

(5) AT-MAR Glass Co., 611 W. State Rd., Kennett Square, PA 19348. Tel: (610) 444-5903.

(6) J-KEM Scientific Inc., 6970 Olive Blvd., St. Louis, MO 63130. Tel: (314) 863-5536.

(7) Omnifit products. Omnifit (U.K.), 2 College Park, Coldhams Lane, Cambridge CB1 3HD, U.K. Tel: +44(0) 1223 416642.

(8) Saha, A. K.; Liu, L.; Simoneaux, R. L.; Kukla, M. J.; Marichal, P.; Odds, F. C. *Bioorg. Med. Chem. Lett.* **2000**, *10* (19), 2175−2178.

(9) Calbiochem-Novabiochem Corp. Product # 01-64-0103.

(10) Gordon, D. W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47.

(11) Frenette, R.; Friesen, R. W. *Tetrahedron Lett.* **1994**, *35*, 9177.

(12) Wagaw, S.; Buchwald, S. L. *J. Org. Chem.* **1996**, *61*, 7240.

CC0000794