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Article

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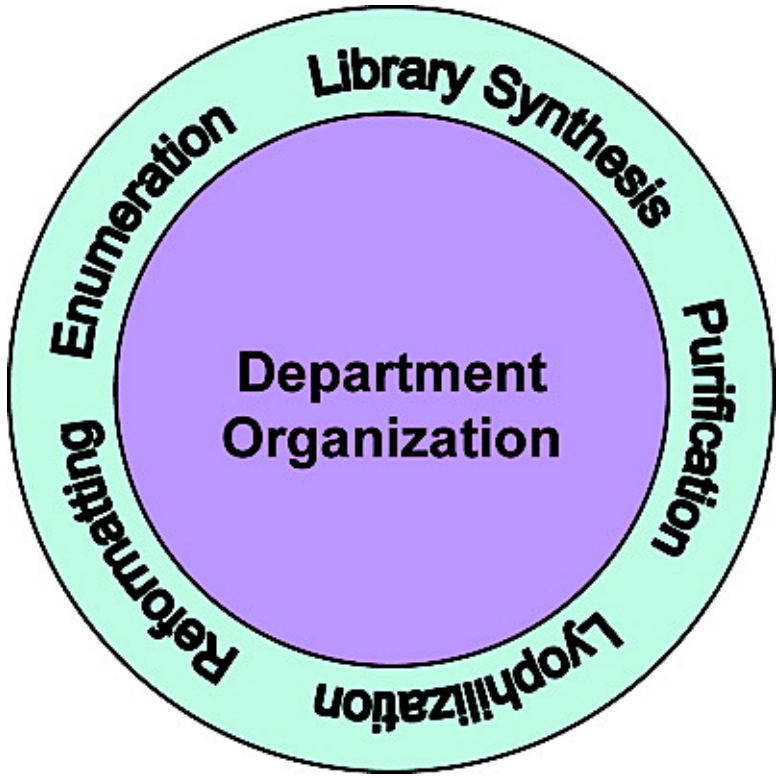

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Maximizing Efficiency in the Production of Compound Libraries

Marcus Koppitz*

Bayer Schering Pharma AG, Medicinal Chemistry, 13342 Berlin, Germany

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Efficiency is one of the most important criteria in departments responsible for the production of compounds in a library format. Consequently, this was a key factor in the initial design of our automated medicinal chemistry department, established some years ago. Nonetheless, we were able to improve and optimize our workflows and processes constantly. Here, we outline our current setup, from design to submission of libraries, and discuss which procedures and techniques appear to be useful for us and which ones turned out to be less effective. The aim of the manuscript is not to present individualized and tailor-made solutions in our laboratory but rather to describe approaches (often executed with commercial equipment) which might be of relevance for a broader readership working in this field.

Introduction

Compound synthesis in a parallel array format is a proven strategy not only for the enrichment of corporate compound collections or the establishment of more specific target-family oriented libraries but also for the support of medicinal chemistry programs in the hit-to-lead or lead-optimization phase.[1–4] Meanwhile, commercial or contract-based libraries are readily available from external vendors. Therefore, almost all activities in our automated medicinal chemistry department are focused on intellectual property sensitive project support, whereas external vendors are routinely considered for a general enrichment of the compound collection.

While efficiency in general is a key factor for any type of compound set produced in a parallel manner, the support of medicinal chemistry programs poses additional challenges to the process, like flexibility with respect to scope of applicable chemistry, compound amounts, and library sizes, as well as achievable turnaround times. Indeed, for efficient project support, cycle times for the delivery of novel libraries need to be competitive to those for individual compounds, as has been recently outlined by colleagues from BMS.[5] The following article describes our overall workflow for library production in different sections and presents our approaches and strategies to guarantee effective project support.

Experimental Details

For enumeration, the automated data processing software Pipeline Pilot from Accelrys was applied. For library synthesis, the Chemspeed Accelerator platform was used with the VLT100 upgrade for synthesis in glass reactors and the SLT100 upgrade for block synthesis. Six milliliter 48-well polypropylene microtiter plates were purchased from Abgene. Six milliter 48-well Teflon blocks with the dimensions of 128 × 86 × 75 mm, as well as the corresponding aluminum plate holders and the microwave turntable, were in-house developments. The appropriate 6 mL disposable glass tubes were from Schott (Duran, 12 × 75 mm). The multimode microwave oven was a MarsX from CEM. Parallel microstirrers were obtained from H+P Labortechnik AG. The HPLC platform has already been described in detail[6] and consists of 4-channel MUX systems for analysis and purification from Waters and a Hamilton robotics station for fraction pooling. For analysis, 3 × 50 mm Waters XBridge C-18, 5 μM particle size columns were used; for purification, we used 30 × 100 mm Waters XBridge C-18, 5 μM particle size columns. Our standard eluents on both systems are ACN and 0.1% TFA in water. All percentages refer to ACN. The standard analytical gradient employed is 1−99% in 5 min, followed by a hold of 1 min at 99%, then return to 1% within 0.25 min, and re-equilibrate at 1% for 1.25 min, all at a flow rate of 2 mL/min. For purification, we employ 6 min runs at a flow rate of 60 mL/min. The gradient pattern is hold *x*% for 0.2 min, then go to *y*% within 3.9 min, increase to 99% within 0.2 min, hold at 99% for 1.2 min, return to *x*% within 0.1 min, and re-equilibrate for 0.4 min. We employ six optimized flat gradients with UV/MS triggering calculated from the retention time/polarity of the target compounds and a generic 10−99% gradient for compounds with no UV or no MS signal. Time window/gradient combinations are 0 min $< t_R \leq$ 2 min/1−25%, 2 min $< t_R \leq$ 2.8 min/10−40%, 2.8 min $< t_R \leq$ 3.5 min/25−55%, 3.5 min $< t_R \leq$ 4.3 min/40−70%, 4.3 min $< t_R \leq$ 4.6 min/50−80%, and 4.6 min $< t_R \leq$ 6.5 min/65−99%.

Parallel filtration of compounds was achieved with an acrylic vacuum manifold from Porvair Sciences, 7.5 mL 25 μm polypropylene filter plates from Porvair Sciences, and 1200 μL 8-channel Pipet-Lite pipettes from Rainin. Removal of HPLC solvent was achieved in Christ lyophilizers type Epsilon 2−16 D with cooled storage plates (−60 °C) and Ilmvac Chemvac 23D-101 vacuum pumps. Reformatting and weighing of compounds was achieved with a custom-made Accelab reformatting robot.

Results and Discussion

Department Organization and Project Teams. Our automated medicinal chemistry unit is fully integrated within

* E-mail: Marcus.Koppitz@bayerhealthcare.com.

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Medicinal Chemistry and takes care of all library work required at the Berlin site. On the basis of our experience, parallel synthesis of libraries of more than 20 analogues is more efficiently executed in specialized units, having all necessary equipment and expertise available than in a decentralized manner. This of course does not exclude parallel synthesis in classical laboratories; however, as soon as a larger set of analogues is required, the synthesis is performed in the automated medicinal chemistry department.

There are various models in the pharmaceutical community dealing with the integration of parallel synthesis groups within medicinal chemistry.[7] This ranges from pure service groups that execute limited and well-defined chemistries with scaffolds that they receive from colleagues to more-or-less independent groups that cover the whole process including library design, chemistry development, and scaffold synthesis. Possible downsides to the latter approach are that only a limited number of projects can benefit from the libraries, the production cycle for libraries may be slowed down, and full integration into project work might be difficult. The first approach appears to be more efficient; however, significant restrictions with respect to target structures or types of chemistry are often inevitable. Moreover, "transfer times" from the library idea to the actual "order" of the library can be quite significant, slowing down the overall process.

We have established a mixed model trying to combine the best of both approaches: Early stage (hit-to-lead) projects are discussed and presented on a regular basis in indication-specific teams, for instance, for antiangiogenesis targets. These teams are responsible for the whole portfolio of the respective indication and are composed of scientists of all disciplines. Automated medicinal chemistry representatives are in each of those teams in which all new target structures and chemistry plans are discussed. This allows early participation in the ongoing chemistry and allows them to give input about the feasibility of library approaches. The scope of applied chemistry is thereby also expanded beyond standard libraries, for example, amide and Suzuki libraries. Through this, either service support for other projects is provided or libraries for their own projects are produced.

Enumeration and General Data Management. The commercial automated data processing software Pipeline Pilot from Accelrys is used for enumeration of libraries, as well as other necessary procedures such as automated uploading of report text files into our library database. Other tools that are used include an ISIS database that contains all data for our libraries and a proprietary software tool that allows handling of multiple structure data files. However, these solutions may be too specialized to be of general interest.

Building Block Sets. Immediate availability of building blocks is of utmost importance for fast library response times. Clearly, not all possibly required reagents can be stored in an in-house repository. To solve this problem, we established a reagent collection in Berlin of approximately 8000 reagents, covering many different building block classes and individual chemicals as well.

A key element for efficiency is the establishment of "standard sets" for the most common building blocks like amines, carboxylic acids, and boronic acids. Those sets contain around 300 members each, are always in stock, and are always available in preweighed, defined amounts in containers that we use on our synthesizers. This concept allows for very fast response times for standard library work, while flexibility is maintained with respect to number, scale, and structures of analogues to be synthesized. Key criteria for building blocks to be incorporated were SAR value, druglikeness/leadlikeness, diversity, and constant availability. At this stage, exclusivity is not a major factor because this quality is often a prerequisite for the core structure or can be considered in follow-up libraries. Moreover, those sets can quickly be supplemented with additional building blocks that are not included in the standard set.

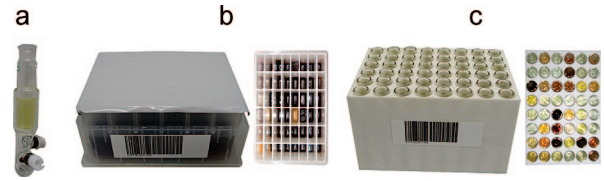


Figure 1. Typical reactors used on the Chemspeed Accelerator: (a) Chemspeed solution-phase reactor, (b) commercial 48-well polypropylene 6 mL MTP, and (c) custom-made 48-well Teflon block with commercial disposable 6 mL glass tubes.

Library Synthesis. The underlying concept for the synthesis platform was to be as broad as possible with respect to type and scale of chemistry that can be performed on the equipment. Routinely, compounds are synthesized on a scale between 0.1 and 0.3 mmol. Sophisticated automation that allows execution of complex chemistries, posing little restrictions on the selection of the library synthesis route, may be susceptible to breakdowns however. Therefore, our philosophy usually is to use equipment that is as simple as possible and as complex as necessary. A very versatile system that is frequently used is the Chemspeed Accelerator, because it combines both possibilities in one system. The system is known for the ability to perform delicate chemistries under inert conditions in proprietary glass reactors. However, because the system can handle a 4-needle head and has a relatively large pipetting space, it can also be used as a simple but very effective liquid handler for commercial or in-house reaction blocks. A preferred size of blocks is the standard microtiter plate format. The 48-well dimension is selected because synthesis on our preferred scale of 0.1−0.3 mmol is easily possible. Advantages of the block approach are a relatively compact footprint for the reactors and a higher throughput because the synthesizer is not occupied during the time needed for reaction (Figure 1).

The Accelerator surface can hold up to 15 standard MTP blocks, translating into a maximum of 720 individual compounds per run with 48-well blocks. Moreover, the double layer technology allows one to place up to 320 different building block stocks (8 mL), 30 scaffold stocks (60 mL), or 6 large volume reagents (1000 mL) on the synthesizer. We have preprogrammed many typical synthesis scenarios for bimolecular reactions, like 1 × 96, 7 × 96, 3 × 192, or 28 × 24, allowing instant production of most requests (Figure 2). The pipetting job itself is performed in less than 8 h.

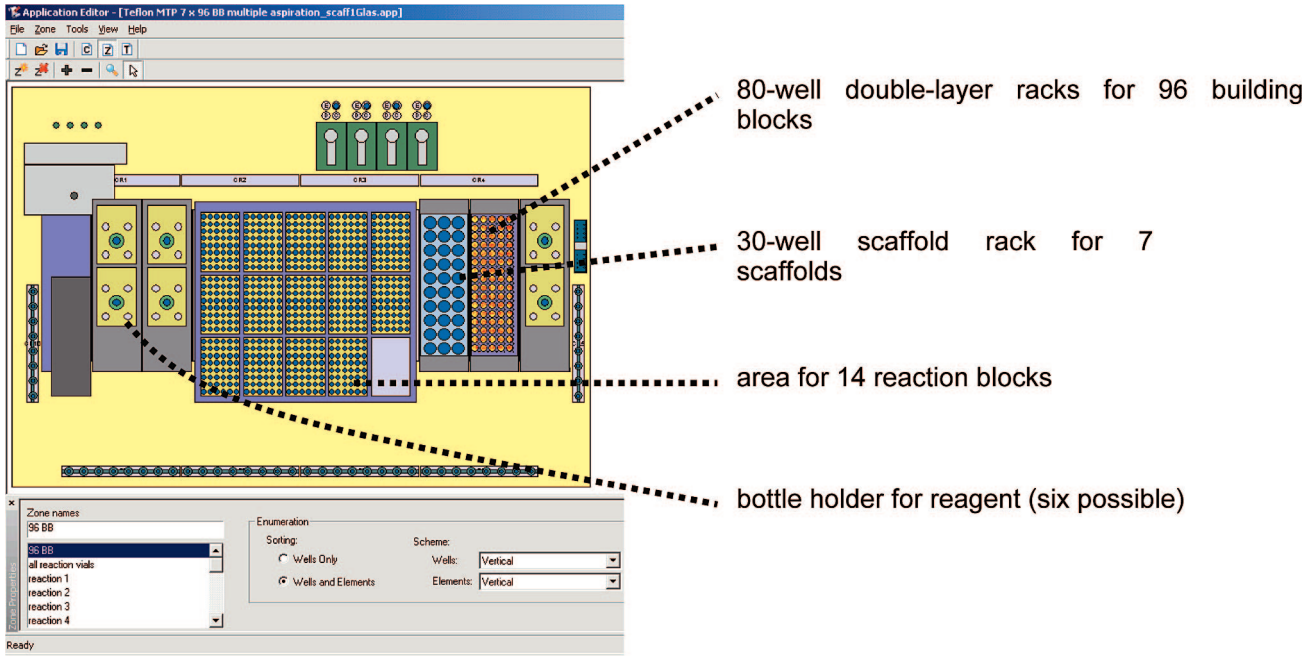


Figure 2. Program surface example for the 7 × 96 scenario (Chemspeed Autosuite Software).

Two different types of blocks are used. Commercial 6 mL 48-well polypropylene disposable plates are used for very simple chemistry from room temperature up to 70 °C. The 48-well Teflon blocks in MTP footprint that hold 6 mL disposable glass tubes are an in-house development. The advantage of those blocks is, that they can be used in standard multimode microwave systems to allow for true parallel microwave-assisted synthesis (Figure 3).

Those blocks are transformed into microwave-suitable pressure blocks that can withstand more than 15 bar, using an aluminum plate-based sealing device similar to a construction that has been recently described by Kappe et al.[8] Some developmental work was necessary to find the optimum design for the sealing mat because the commercial glass tubes used have tiny variations in their length, which makes it difficult to create tightly sealed reactors. Currently, Novasil SP 1078 mats with a thickness of around 3 mm, which perfectly even out the length differences, are used. In addition, the mat is dimpled at each glass tube to support the sealing process. The aluminum holders allow the use of the temperature and pressure sensor of our multimode microwave oven in one of the reactors making the block-microwave combination a very safe and reliable system (Figure 4). We have not encountered problems with inhomogeneous microwave fields leading to irreproducible or varying results, as reported by others.[9–12] Sequential monomode systems are also available for synthesis; however, for libraries we almost always use the multimode systems allowing the parallel synthesis of 96 compounds per microwave run on a 100 mg scale.

Insoluble building blocks are not easily used in laboratories, depending on the handling of solutions. Although an insoluble reagent often reacts in suspension, the addition of a defined amount of this material via stock solution is made impossible by sedimentation. Stirring during the liquid handling operation is an effective means to overcome this problem by generation of pseudohomogeneous solutions. We

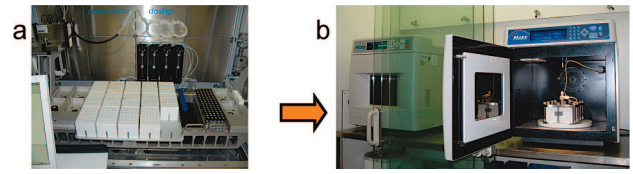


Figure 3. Typical workflow of microwave-assisted synthesis in Teflon blocks for the 7 × 96 scenario: (a) liquid handling operation on Accelerator and (b) parallel microwave synthesis of 96 compounds in multimode microwave oven.

have custom-built parallel microstirrers in various formats that allow one to handle suspensions of building blocks, scaffolds, or reagents on a synthesizer, when needles of a wider diameter (1−2 mm) are used for pipetting (Figure 5).

The choice of the reactor selected for synthesis depends on the required reaction conditions. Table 1 shows some library synthesis examples for the three different reactor types employed on the Accelerator platform. For the most delicate chemistries the proprietary Chemspeed glass reactors are used. Classical examples here are reactions that require deep temperatures (up to −70 °C) (entry 3), rigorous exclusion of water (entry 1), or an inert atmosphere. Surprisingly, it was not possible to form the amide bond of the sterically hindered carboxylic acid scaffold in the block systems (entry 2). Moreover, only thionyl chloride activation gave sufficient amounts of products with deactivated aromatic amines.

The Teflon blocks are the most popular systems for all kinds of metal-catalyzed cross-coupling reactions, for example, the Suzuki reaction, because these perform especially well in microwave-assisted synthesis. Another application includes all reactions that are performed or perform well at high temperatures, like many heterocycle condensation reactions (entries 5 and 6). Temperatures of up to 250 °C are possible with those blocks.

Synthesis in disposable plastic microtiter plates is performed for all robust reactions, which require no or only

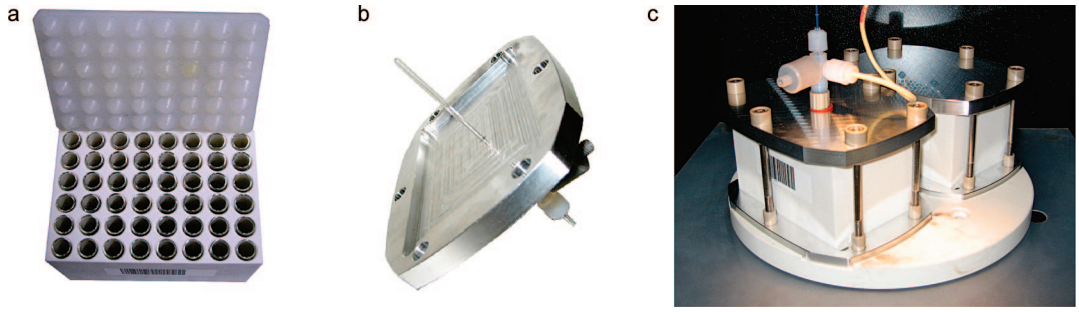


Figure 4. Teflon block design with (a) sealing mat and (b) aluminum lid with T/p sensor holding device and (c) the microwave turntable with two installed 48-well Teflon blocks.

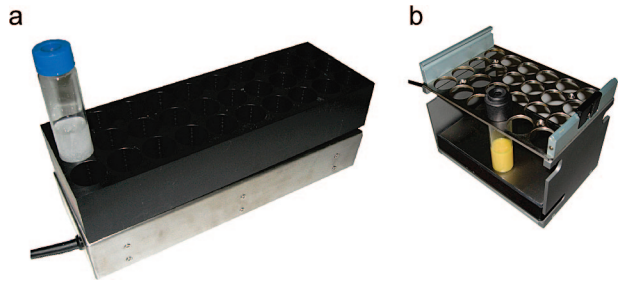


Figure 5. Magnetic stirrers for building block suspensions: (a) 30-fold stirrers for 30 mL vials and (b) 24-fold stirrer in MTP format for 12 mL vials.

moderate heat. The classical example here is the standard amide bond formation (entry 9).

Analysis and Purification. The platform we use in our department for analysis and purification has already been described in detail,[6] and here, only key aspects, as well as some improvements for fast analysis and purification, are addressed.

After synthesis, we try to do as little preparatory work as possible for samples submitted to HPLC. Precolumns are used on all analytical and preparative systems allowing us to purify compounds that are synthesized in an HPLC-compatible solvent and are devoid of solid material without further action. The advantage of the disposable plastic MTP blocks mentioned above is that in such cases a reformatting step is not even necessary because those plates can directly be loaded on our HPLC autosamplers. Incompatible solvents are evaporated and usually redissolved in up to 5 mL of DMSO. Samples containing solid are filtered. For filtration, commercial vacuum blocks and 48-well filter plates in combination with 2 mL 8-channel pipettes for manual liquid transfer are used (Figure 6). Difficult mixtures are filtered through commercial filter plates which are prefilled with various materials such as diatomaceous earth (sticky, slimy, fine solid material) or thiol resin (Pd-capture).

We always follow the procedure analysis of crudes−purification−reanalysis of products. The first analysis is used to select a tailor-made fast preparative gradient for purification (based on the retention time of the target compound), which is automatically performed by the FractionLynx software we use and to avoid purification of nonexisting products. In addition, for novel or delicate chemistries in situ reaction control by LC-MS analysis for selected library members is performed, which gives one the ability to modify the planned synthesis procedure (e.g., increase reaction times, temperatures, add catalysts), thereby reducing attrition rates significantly.

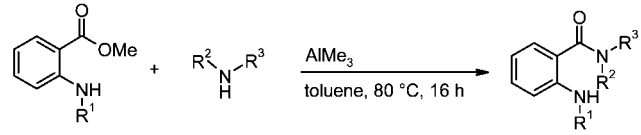
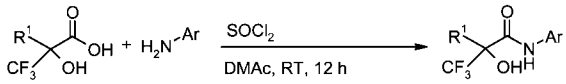
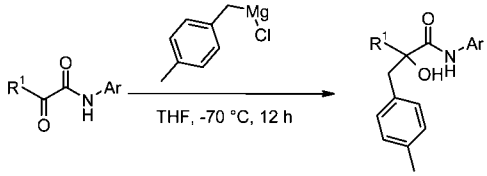
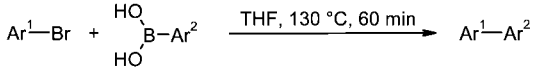
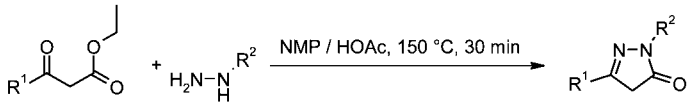
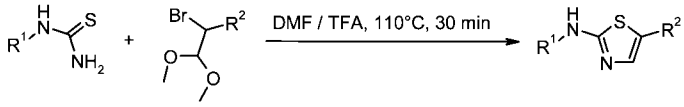
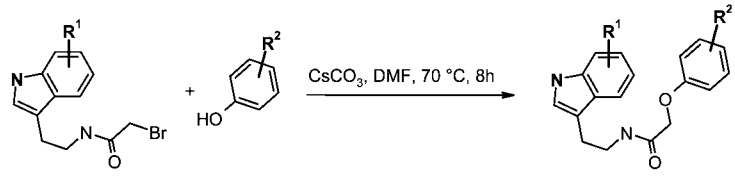
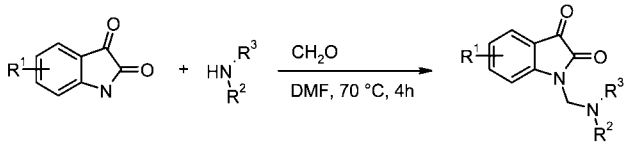
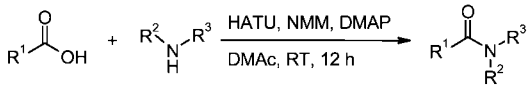
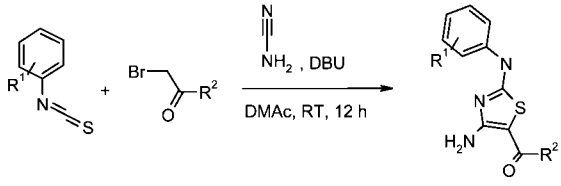
To improve resolution of preparative separations, we reinvestigated the narrow gradients and now established shallower 25−30% acetonitrile gradients for the six different preparative gradients that can be automatically assigned.

To improve throughput, the flow rates were increased on the preparative systems. As has been nicely demonstrated by colleagues from Sanofi-Aventis,[13] flow rate/run time combinations of even 100 mL/min over 5 min can give sufficient separation quality. Our own investigations revealed that doubling the flow rates from 25 to 50 mL/min and reducing the run times to 50% gave practically no loss of resolution, which is in line with the van Deemter curve available for the columns we use. The final design of the preparative runs was mainly guided by the goal to be able to separate 192 compounds (four 48-well plates) on the 4-channel preparative LC-MS MUX system under personal supervision and to start the subsequent pooling process of the product fractions as an overnight process. Now a 60 mL/min flow rate per preparative channel is used with 6.5 min run times, which add up to 7 min cycle times. Figure 7 shows an example of what separation quality can be achieved with the described procedure and displays UV chromatograms, total ion chromatograms TIC and extracted ion chromatograms EIC (total ion chromatogram is searched for target mass) for a library compound. Although many separation problems are simpler, the ability to extract products out of complex mixtures is of great value for project libraries, in which each member delivers helpful information.

High-Throughput Solvent Removal. The product fractions of the preparative separations are pooled into 40 mL Pyrex tubes, which are held by a rack for 48 tubes. Previously, we evaporated the products with vacuum centrifuges. Although this is a well-established process, we found that manual loading of the systems is quite labor-intensive because the tubes need to be transferred into suitable holders and the holder weight needs to be balanced. Sometimes, even rebalancing was necessary during runs. Other critical aspects were compound damage (heat, condensed TFA), nonstoichiometric amounts of residual TFA in the product, the danger of mixing up samples, and frequent system breakdowns.

We now use large lyophilizers with cooled storage plates that allow freeze-drying of acetonitrile/water mixtures of any composition.[14] The lyophilizers have a capacity for ten of the above racks, translating into 480 samples per run. It is

Table 1. Examples of Reactions and Selected Synthesis Equipment

Entry	Reaction	Synthesis System
1		Accelerator, glass reactors
2		Accelerator, glass reactors
3		Accelerator, glass reactors
4		Accelerator, Teflon block, Microwave
5		Accelerator, Teflon block, Microwave
6		Accelerator, Teflon block, Microwave
7		Accelerator, MTP, Microwave
8		Accelerator, MTP, Shaker
9		Accelerator, MTP, Shaker
10		Accelerator, MTP, Shaker

important that the systems are able to cool the storage plates to −60 °C to guarantee a frozen state for all samples. A key aspect for lyophilization was run-time because the nonoptimized process initially took several days. Parameters investigated were rack designs and the process parameters vacuum and storage plate temperature during freeze-drying runs. Lyophilization speed is a function of the temperature difference between the sample and the condenser of the freeze-dryer.[15] Because the maximum temperature of the sample in equilibrium is coupled to the applied vacuum via the vapor pressure curves, the lowest possible vacuum not leading to melting will produce fastest drying times. How-

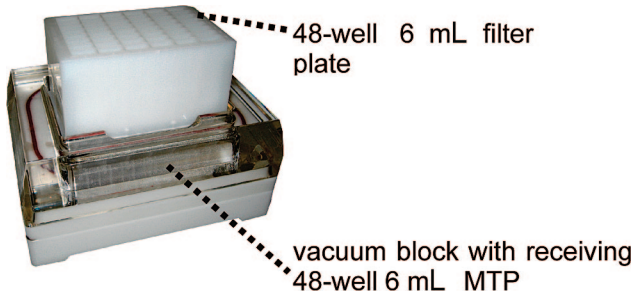


Figure 6. Filtration device for HPLC sample preparation.

ever, the composition of the LC-MS fractions after HPLC purification is not defined and can theoretically vary between 0 and 100% acetonitrile that makes an individual use of the "best" vacuum impossible. Therefore, a compromise needs to be found that reliably prevents melting of samples independent of its solvent composition in the first freeze-drying phase and still allows fast drying times. Later on, at decreased acetonitrile content, the cooling plate can also be used as a heater providing energy for sublimation, thereby speeding up the process.

The routine lyophilization process is as follows: The tubes are stored in a −70 °C freezer after pooling. This makes a freezing process in the lyophilizer obsolete and the freeze-drying can be started immediately after loading. The process is started in the freeze-dryer at −60 °C storage plate temperature and the maximum achievable vacuum of 0.03 mbar. After 5 h, the vacuum is reduced to 0.1 mbar within 1 h, without changing the storage plate temperature. Subsequently, temperature of the storage plates is slowly ramped up to 40 °C within 10 h. By separating theses two processes, it is possible to successfully reduce intermediate melting processes, which readily occur when those parameters are changed at the same time. The main drying phase now lasts 35 h with unchanged parameters. To remove residual water, the vacuum is reduced again to 0.03 mbar in the post drying phase. Using this program, lyophilization times can be reduced to 48 h, which is acceptable for us considering the added benefit we now have.

There is a dependence of lyophilization speed on rack design because the energy flow between sample tubes and storage plates is achieved via the holding racks. The most critical factor is the bottom of the rack. Plastic material turned out to be not suitable for lyophilization because of its insulating properties, and therefore, aluminum bottom plates with tiny dips adapting the round surface of the tube bottom are used now. Energy transfer via the tube mantel is also a factor. Although we measured a 5 h time advantage for racks with additional aluminum mantel, these racks significantly gain weight and lose their transparency, so we currently do not use them.

Sealing of sample tubes in lyophilizers is mandatory because the fluffy material generated otherwise leads to contamination of other samples and the whole interior of the lyophilizer. Various devices such as metal grids of different porosity, Teflon membranes, paper stoppers for individual tube sealing, and disposable filter mats were investigated. The best solution with respect to handling, lyophilization speed, and sealing quality turned out to be disposable cellulose tissues, which are put on top of the racks. Coverage with an aluminum plate with appropriate holes and fixation with plastic clips then forms a safe sealing system (Figure 8).

Reformatting of Compounds and Yield Determination. The final step in the process is the determination of isolated compound amounts after purification and the subsequent distribution of compound aliquots to various target vials and microtiter plates. We use a custom-made system from CyBio (former Accelab) that performs the required steps of weighing, dissolution, transfer, and evaporation in a fully automated manner and has a capacity for 384 compounds per run. Import format on the system are the 48-tube racks which contain the dry samples as lyophilized powder. Yields are determined by automated weighing of the pretared tubes. Transfer of compounds into destination vials is then performed by addition of DMSO, dissolution of compounds by shaking on a parallel shaker, and pipetting of the DMSO solutions into the required vial formats. In the final step, the vials and plates are automatically placed into a commercial vaccum centrifuge and evaporated.

Conclusion

Efficient production of compound libraries is only possible if all potential bottlenecks along the process are addressed equally well in an established workflow. Often, elimination of a bottleneck creates a new one downstream of the process, which requires subsequent optimization and adaptations. We divided the production process into several stages, and each stage was analyzed for possible improvements. For enumeration, the automated data processing software Pipeline Pilot turned out to be the tool of choice. For short library response cycle times, a large reagent repository and preweighed standard building block sets proved to be valuable. For synthesis, a flexible platform covering a broad range of chemistries was established. A central technology is the synthesis of libraries in Teflon blocks which can be used in

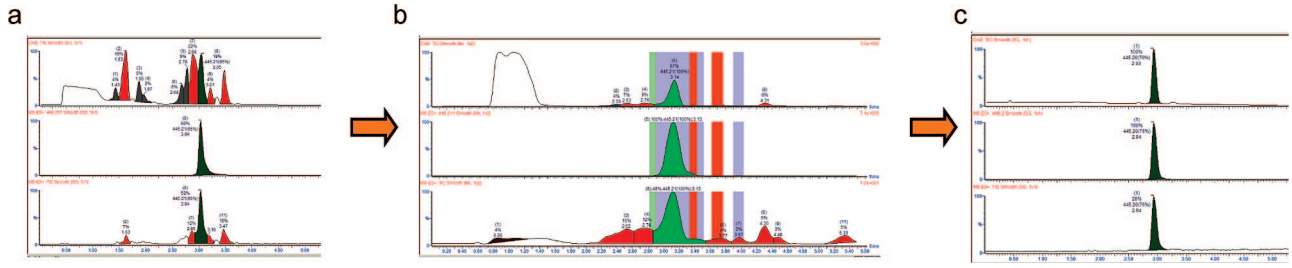


Figure 7. Example for routinely achieved separation quality using LC-MS purification with narrow gradients and postpurification fraction pooling. UV (upper), EIC (middle), and TIC (lower) traces of (a) crude product, (b) purification, and (c) purified product.

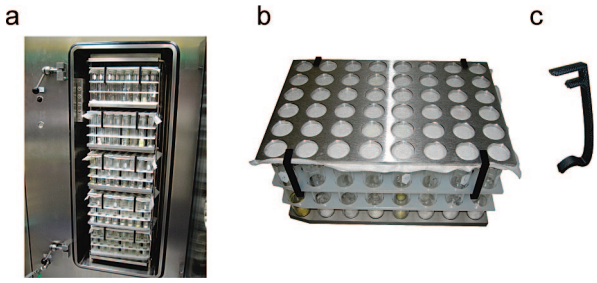


Figure 8. (a) Lyophilizer, (b) rack with aluminum bottom plate and sealing device, and (c) clip for fastening of sealing device.

monomode microwaves to allow true parallel microwave synthesis. LC/MS is the method of choice for analysis and purification of libraries. Key aspects for high-quality separations in HT mode are high flow-rates, automated assignment of narrow gradients, and a reliable and automated process for fraction pooling. HPLC solvents can be efficiently removed from purified products by lyophilization. Finally, an automated unit for yield determination by weighing and transfer of compounds into final destination vials concludes the process of library production. Using this platform, our current output per person and year is around 3500 submitted compounds.

The largest impact of libraries on medicinal chemistry projects is achieved if the turnaround time for libraries is similar to the time required for production of individual compounds, and all our efforts are directed to achieve that ultimate goal. However, efficiency also implies that all major systems in the production process, in particular, synthesizers, HPLC, or reformatting systems, are running under full capacity. This means that queuing cannot be avoided and that production time is not just the sum of the times required for the individual steps. For very urgent cases, a turnaround time of around 1 week is possible in our department by prioritization; however, average residence times for project libraries are around 2 −3 weeks, which is still sufficient for most project requirements.

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