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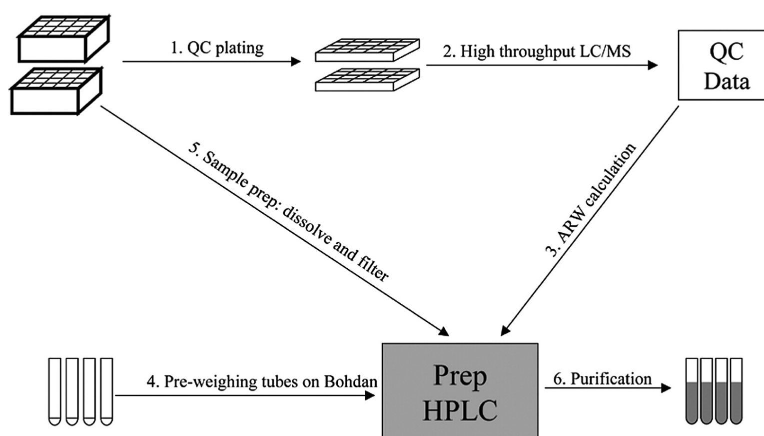

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High-Throughput Purification of Combinatorial Libraries I: A High-Throughput Purification System Using an Accelerated Retention Window Approach

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We have developed a high-throughput purification system to purify combinatorial libraries at a 50−100-mg scale with a throughput of 250 samples/instrument/day. We applied an accelerated retention window method to shorten the purification time and targeted one fraction per injection to simplify data tracking, lower QC workload, and simplify the postpurification processing. First, we determined the accurate retention time and peak height for all compounds using an eight-channel parallel LC/UV/MS system, and calculated the specific preparative HPLC conditions for individual compounds. The preparative HPLC conditions include the compound-specific gradient segment for individual compounds with a fixed gradient slope and the compound-specific UV or ELSD threshold for triggering a fraction collection device. A unique solvent composition or solvent strength was programmed for each compound in the preparative HPLC in order to elute all compounds at the same target time. Considering the possible deviation of the predicted retention time, a 1-min window around the target time was set to collect peaks above a threshold based on UV or ELSD detection. Dual column preparative instruments were used to maximize throughput. We have purified more than 500 000 druglike compounds using this system in the past 3 years. We report various components of this high-throughput purification system and some of our purification results.

Introduction

One of the driving forces to apply combinatorial chemistry[1] in drug discovery is to accelerate lead discovery and preclinical research in order to find the next drug. It is important that these combinatorial library compounds are as pure as possible when performing lead discovery screening. At this stage, any impurities in samples may lead to false positive results. Even with the rapid advances in solid phase[2] and solution phase[3] synthesis methods and intensive reaction optimization,[4] excess reagents, starting materials, synthetic intermediates, and byproducts are often found along with the desired product. Furthermore, strong solvents for swelling the resin bead used for solid-phase synthesis or scavenging treatment in solution-phase reactions can often bring in additional impurities extracted from resins and plastic plates. The requirement for *the absolute purity*[5] of combinatorial library compounds demands the development of high-throughput purification method (HTP)[6] at a scale that matches combinatorial or parallel synthesis (10−100 mg). An HTP method for purifying combinatorial libraries must possess three qualities: high throughput, full automation, and low cost.

Throughput is the most important consideration in purifying combinatorial libraries because parallel synthesis often produces a large number of samples per library (often ranging from 500 to 10 000 in our laboratories). Fast gradient separation[7] and parallel purification[8] have been reported as the major approaches. We built our system on the basis of the first approach.

Purifying a large combinatorial library is highly repetitious. Robotics can work around the clock and provide the highest precision in repetitious operations. This dramatically reduces the chance for human error.

A lengthy purification, a scale-up in library production, a low purification recovery, and solvent all boost the cost of the purified products. Mass-directed purification[9] systems have advantages in compound-specific collection; however, the cost of using multiple mass spectrometers as detectors is high. In a high-throughput purification setting, a "general" reversed-phase HPLC method may be sufficient to purify at least a major portion of a library; however, using conditions targeting individual compounds will be more likely to accomplish a successful purification of the whole library.

Our system achieves low-cost purification using a UV- and ELSD-triggered fraction collection of the predesigned elution of target compound and individualized solvent gradient to reduce solvent consumption. We report here our fully automated HTP system and systems for efficient postpurification processing and data tracking. The system offers increased throughput and speed, decreased operation cost for the process, and individualized separation conditions for each compound.

Results and Discussion

1. Process and System Overview. The flow of HTP operation and our HTP system are described in Figures 1

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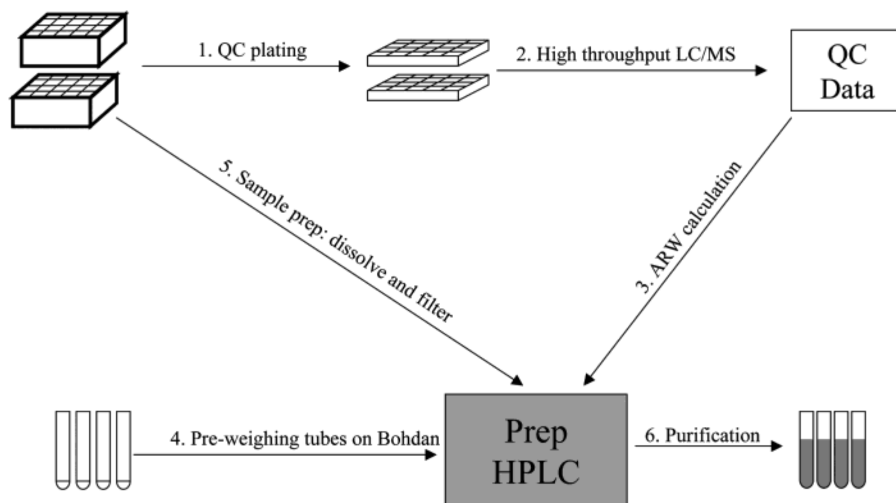

Figure 1. The first half of our HTP process: synthesis, QC plating, LC/UV/MS analysis, weighing, and HPLC purification.

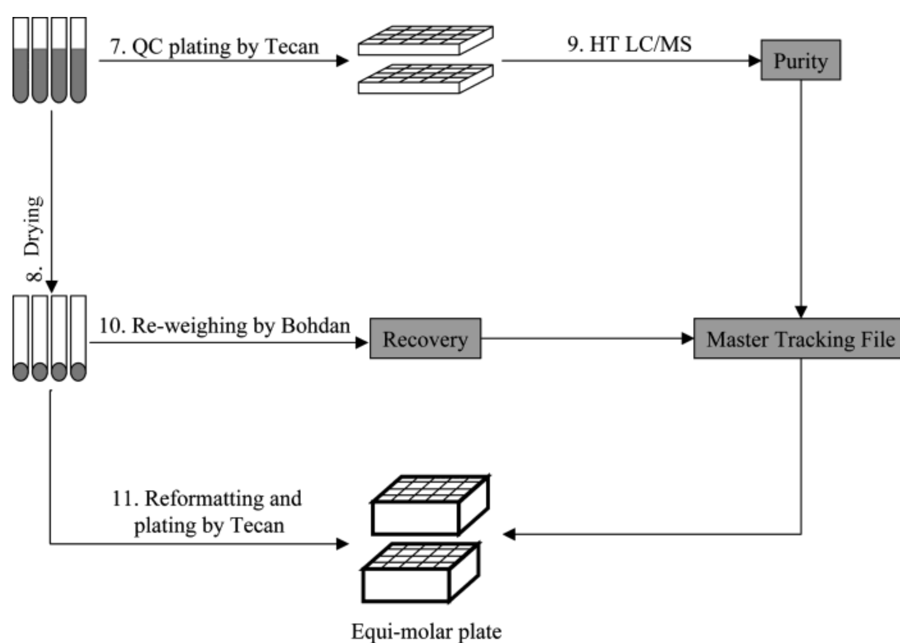

Figure 2. The second half of our HTP process: postpurification QC plating and LC/UV/MS analysis, drying, weighing, consolidation, reformatting, and plating.

and 2. The process started with the synthesis products in 96-well plates (0.1−0.2 mmol/well). We used Hydra 96-probe liquid handlers to make QC plates for all samples. We analyzed all samples with a MUX-LCT eight-channel parallel LC/MS instrument at a throughput of 2000 samples/day.[10] Because the purification yield of low purity compounds is always very low, only samples with purity higher than 10% were purified on Gilson dual column preparative HPLC systems. The retention time of each compound from analytical LC/MS was used to calculate the specific gradient segment (initial composition 1 to final composition) targeted to elute the compound at ~2.3 min. A 1-min window was set around 2.3 min for fraction collection. The analytical HPLC peak height was also used to calculate the threshold for collection trigger on the basis of UV or ELSD. All test tubes for fraction collection were preweighed automatically on Bohdan weighing stations. It took about 7 h to purify 96 samples from a 96-well plate.

As shown in Figure 2, QC plates were made from collected tubes using a Tecan liquid handling system. The postpurification QC was carried out again on another MUX-LCT eight-channel parallel LC/UV/MS system. The final identity and purity of each fraction was determined at a throughput of 2000 samples/day. Test tubes containing purified compounds were dried in a lyophilizer or a centrifugal vacuum evaporation system. The final tube weight was measured automatically on Bohdan weighing stations. On the basis of the final identity, purity, and weight measurements of the purified compounds, equimolar solutions of purified compounds were made in the final plates.

2. Accelerated Retention Window (ARW) Principle. In the analytical HPLC, the retention time of a compound on a column depends on the starting solvent strength (percent of strong solvent acetonitrile) during a gradient elution at a constant flow rate (Figure 3). For example, as shown in Figure 3, a compound is eluted at tg1 when a gradient elution

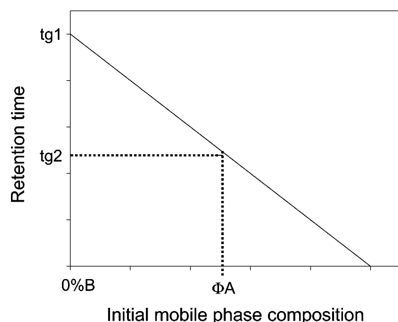

Figure 3. The relationship between retention time and the initial mobile phase composition.

starts from 0% ACN. The same compound is eluted at tg2 if the gradient starts from ΦA% ACN. From HPLC equations, the retention time of a compound in a preparative elution can be calculated on the basis of the compound's analytical retention time and the same conditions (gradient slope, linear velocity) and physical parameters (such as column dimension, dead volume, etc.) for both operations. It is also possible to calculate the starting solvent composition for individual compounds so that all compounds can elute at the same retention time. In other words, each individual compound is eluted using a unique starting solvent strength (%B) to elute every compound at the same predetermined retention time to facilitate the fraction collection for HTP. Abundant experimental results have proved this concept (to be published separately). Despite some variations in experimental retention time from the target time, they are all located within a predictable window. These variations are mostly structure-dependent and can be adjusted systematically after testing a small set of compounds. Since all compounds can be eluted into a predictable retention time range, a collection device can be set up to collect any HPLC peaks that surpass a certain threshold as defined by UV or ELSD detectors. The threshold can be determined by the peak height value in analytical HPLC analysis.

3. Prepurification LC/MS Analysis. The MUX-LCT eight-channel parallel LC/UV/MS system consists of an autosampler with eight injection probes, two pumps for generating a binary gradient, eight UV detectors, and an eight-way MUX with a TOF mass spectrometer. This two-pump arrangement keeps the system simple and cost-efficient; however, it does not provide pressure regulation for each LC channel. To ensure flow consistency across each channel, we selected identical tubing, joints, and columns. Columns are from the same manufacturer and the same batch. The tubing is the same length initially for each channel and is further adjusted by checking the flow at the end. With these precautions, the flow from this two-pump system could be split evenly among the eight channels. In addition, a standard mixture (A, theophylline (log P 0.05); B, 5-phenyl-1*H*-tetrazole (log P 2.41); C, reserpine (C log P 3.32); D, Fmoc-Asp(OtBu)-OH (log P 4.43)) is analyzed every 24 injections, and the retention times of these standards are closely monitored to ensure an even flow across the eight channels. The relative standard deviation (RSD%) in retention time variation among the eight channels over one month for standards A and B was <2%; for C and D, less than 1%. In addition to the identity and purity determinations,

Analytical data				Calculated prep HPLC conditior		
Sample	MW	Ret. Time (min)	Peak height	100*ΦA2	100*ΦB2	peak level
LIB005-1-A1	348.12	1.34	161407	9.49	43.28	15.33
LIB005-1-B1	358.14	1.56	169364	14.47	48.27	16.08
LIB005-1-C1	364.1	1.48	148540	12.48	46.27	14.11
LIB005-1-D1	364.1	1.48	155715	12.81	46.61	14.79
LIB005-1-E1	372.16	1.67	175596	17.8	51.59	16.68
LIB005-1-F1	372.16	1.65	172515	17.46	51.26	16.38
LIB005-1-G1	372.16	1.64	164138	17.46	51.26	15.59
LIB005-1-H1	376.14	1.5	136693	14.14	47.93	12.98
LIB005-1-A2	352.12	1.07	33184	0.52	34.31	3.15
LIB005-1-B2	368.1	1.21	11190	3.51	37.3	1.06
LIB005-1-C2	368.1	1.21	29101	3.51	37.3	2.76
LIB005-1-D2	376.16	1.4	98003	3.84	37.64	9.31
LIB005-1-E2	376.16	1.38	104106	8.83	42.62	9.89
LIB005-1-F2	376.16	1.38	70332	8.49	42.29	6.68
LIB005-1-G2	380.13	1.22	15394	8.83	42.62	1.46
LIB005-1-H2	352.12	1.16	163289	4.84	38.63	15.51

Figure 4. An example of ARW calculation for 16 compounds.

prepurification LC/MS analysis also provides the accurate retention time and peak height for each compound. The detailed LC/MS and sample rerun procedures are listed in the Experimental Section.

4. ARW Calculation. On columns of the same packing material and under the same chromatographic conditions (solvents, solvent strength, gradient, linear loading capacity), there is a correlation between a compound's retention times in analytical scale and in preparative scale. From basic chromatography equations, the preparative retention time can be calculated from the analytical retention time. Alternatively, we can calculate the starting solvent strength (B%) in order to elute all compounds at the same retention time. This is not true under overloading conditions. Most preparative chromatographic separations are under such conditions. Therefore, deviations from calculated values may occur.

Figure 4 shows the analytical LC/MS data and the calculated preparative HPLC parameters. For compound LIB005-1-A1, the elution condition is from 9.49%B (ΦA2) to 43.28% ACN (ΦB2). The peak level is the threshold for triggering the fraction collector during purification. It is calculated from the analytical peak height and is ~5% of the predicted preparative peak height.

5. Preparative HPLC Purification. A schematic outline of the instrument configuration is given in Figure 5. A dual column system illustrated in Figure 5 was used to increase throughput. Sample injection was done on one column while the other column was simultaneously regenerated from a prior sample injection. Regeneration consisted of using a 100% acetonitrile cleaning wash followed by bringing the column to starting conditions for the next sample. In this way, throughput was maximized. Sample injection and fraction collection were automated using two separate 215 liquid handlers. An alternative configuration was also used in which one liquid handler operated in both inject and collect modes. Samples to be injected were dissolved in an optimal ratio of DMSO, acetonitrile, and water to ensure complete dissolution of crude sample. The total sample volume was kept below 1000 μL. Samples were then drained through filter plates (Thomson Inc., Oceanside, CA). Fraction collection was triggered by UV_{214} or ELSD signal. HPLC purification and postpurification processing were facilitated by a computer program that tracks compounds and their associated fractions.

6. Postpurification Processing and LC/MS Analysis. After purification, a computer program assisted in determin-

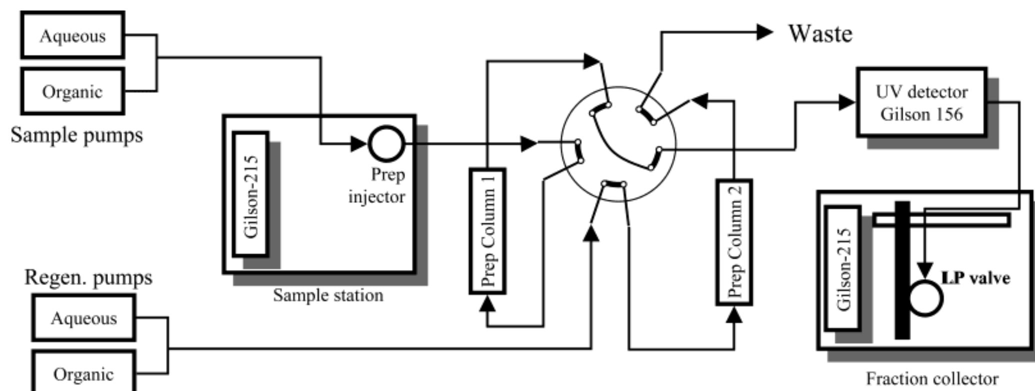

Figure 5. Diagram of preparative HPLC system.

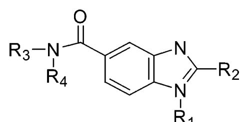

Figure 6. General structure of a 6336-member library purified by our HTP system.

ing the fraction containing the largest peak in the collection window when more than one fraction was collected. A manual process was sometimes used to search for the product when it was not the largest peak in the window. This process involved comparison of the preparative chromatogram to the analytical LC/MS chromatogram to determine the desired fraction. Desired fractions were consolidated and sampled using Bohdan/Tecan for LC/MS analysis. High-throughput LC/UV/MS analysis using an eight-channel LC/MS system (MUX-LCT) was carried out on desired fractions. Samples were assessed for their identity and purity as detailed in the Experimental Section.

7. Drying, Weighing, and Plating. Desired product fractions were dried by lyophilization or centrifugal vacuum evaporation in preweighed tubes. The dried sample tubes were weighed on a Bohdan weighing station to get the weight of purified compounds. On the basis of both the purity and weight, tubes containing compounds were consolidated and reformatted. Samples were dissolved to equimolar solutions. The final screening plates were prepared from these solutions.

8. Purification of a 6336-Member Library. A library (Lib 1, see Figure 6) containing 6336 compounds was synthesized at a 0.1 mmol scale using solid-phase parallel synthesis. QC plates were made from master plates and analyzed on an eight-channel LC/MS system (MUX-LCT). The average purity of the library was 65%. ARW calculations were carried out using the retention times generated in LC/MS analysis. The master plates were dissolved in DMSO and purified using the ARW method. Some purification chromatograms are shown in Figure 7. All compounds were expected to elute at 2.3 min, and the collection window was set between 1.8 and 2.9 min. Variations from the target eluting time can be seen in Figure 7. The collection starting time is marked as a down arrow and finishing time as an up arrow. We targeted to collect only one fraction for the postpurification LC/MS analysis. In most cases, only one fraction was collected. Occasionally, more than one fraction was collected in the window. As discussed before, a computer program automatically picked the correct fractions in most cases, whereas some correct fractions were manually picked. The postpurification purity distribution as compared with the prepurification data is shown in Figure 8. The purity of compounds was determined by a UV_{214} detector. This figure shows a significant improvement in compound purity.

During the course of the postpurification LC/MS analysis, purified fractions were dried. The weight of each compound

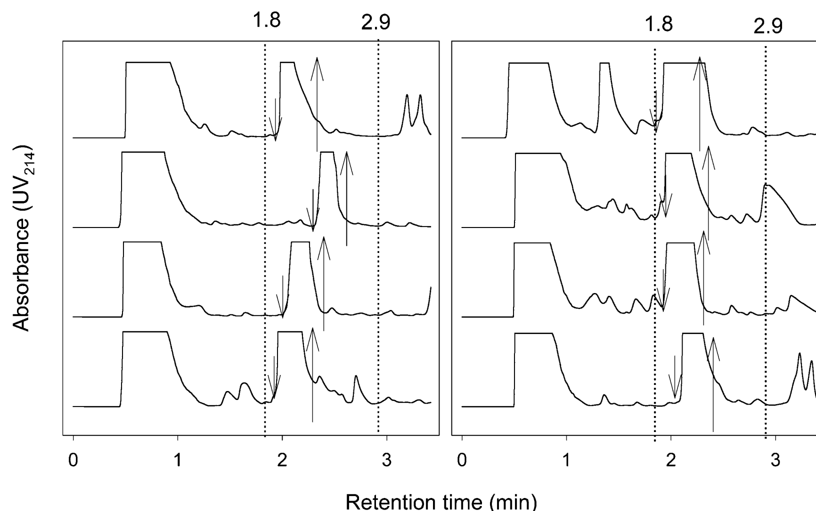

Figure 7. Purification chromatograms of eight representative compounds. Dotted lines show the collection window, and arrows show the start (down) and end (up) of the collection.

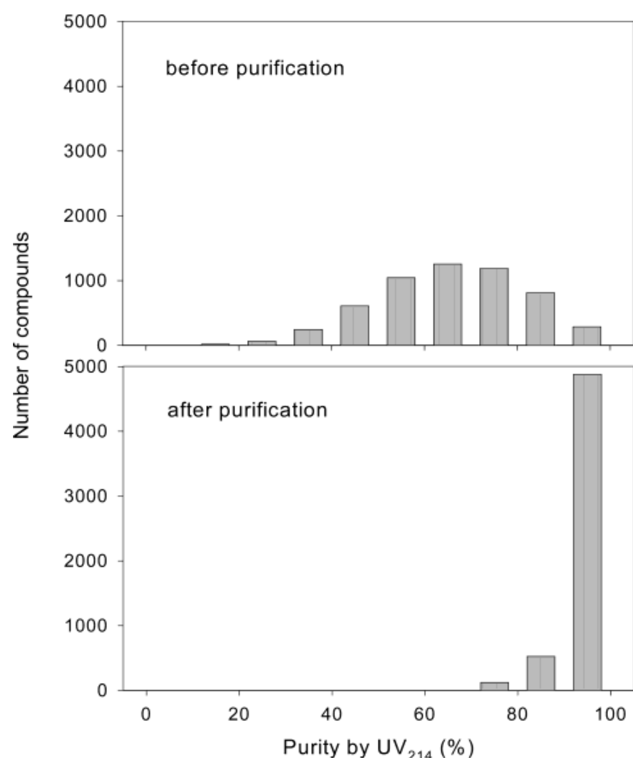

Figure 8. Purity distribution for the library before and after purification.

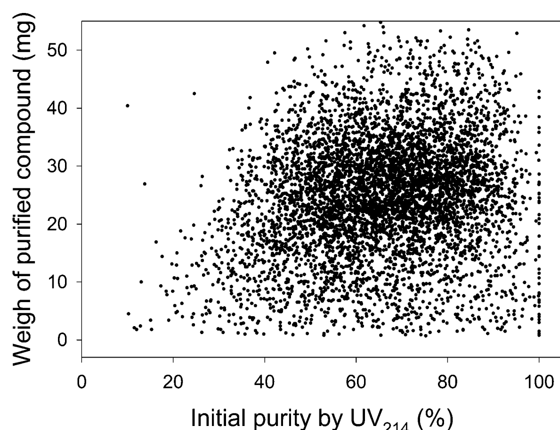

Figure 9. Correlation of the initial purity and the weight of purified compound for all compounds in this library.

was determined using Bohdan automated weighing stations. The weights of purified compounds and their relationship to the prepurification purity are shown in Figure 9. The weights of purified compounds mostly range between 15 and 35 mg. There is no clear correlation between the purified weights and the crude purity. Some compounds possessed high purity, yet were obtained with poor recovery. This poor recovery may be due to low synthesis yield or failed purification. Compounds with low purity often gave low purification recovery. Some compounds with intermediate purity yielded relatively large amount of purified compounds, presumably due to their high synthesis yield.

Experimental Section

1. Prepurification LC/MS Analysis. The LC/UV system consisted of a Gilson pump system (two 306 pumps, an 811C dynamic mixer, and an 805 manometric module), a Gilson 215 autosampler with an 889 injection module, and eight Gilson 115 UV detectors. (Gilson, Inc., Middleton, Wisconsin). The solvent delivered by the pump at 16 mL/min was equally split into eight LC columns with precolumn filters to carry out reversed-phase HPLC separation. Eight 4.6 × 50 mm RP-HPLC columns (particle size 5 μm) from Phenomenex (Torrance, CA) were used. Eight samples were simultaneously injected into eight columns, separated by the same gradient, and detected by individual UV detectors at 214 nm. Two mobile phases (mobile phase A, 99% water, 1% acetonitrile, 0.05% TFA; mobile phase B, 1% water, 99% acetonitrile, 0.05% TFA) were used. The gradient time was the following: 10−100% B in 3.0 min, 100% B for 0.5 min, and 10% B for 0.5 min. A flow of 50 μL/min from each column post UV detection was introduced into an eight-channel multiplexed electrospray ion source (MUX), while the remaining flow was directed to waste.

The mass spectrometer in the LC/MS system was a Micromass LCT orthogonal acceleration time-of-flight mass spectrometer (Micromass UK Limited, Manchester, England) equipped with an eight-channel MUX. The MUX interface consisted of eight electrospray probes and a sampling aperture positioned coaxial to the sampling cone. Each of the probes within the MUX source was indexed using an optical position sensor and selected using a programmable stepper motor controlled by the MassLynx software. The position of the sampling aperture in MUX was controlled by the stepper motor, which only allowed ions from one probe at a time to enter the sampling cone of the mass spectrometer. This arrangement made it possible to acquire discrete data files of electrospray ion current sampled from each channel. Therefore, an eight-channel MUX-LCT worked like eight individual ESI-MS systems. Acquisition times per spray were set to 0.1 s, with the interspray time of 0.05 s. This produced a data point for each spray every 1.2 s.

When the instrument was operated in positive mode, the following settings were used: capillary voltage, 3.5 kV; sample cone, 30 V; RF lens, 250 V; extraction cone, 5 V; RF DC offset 1, 4 V; RF DC offset 2, 7 V; aperture, 10 V; acceleration, 200 V; steering, 0 V; and ion energy, 34 V. Desolvation and source temperatures were set at 350 and 100 °C, respectively. The nitrogen desolvation and nebulizer gas flows were set at 900 and 300 L/h, respectively. For negative ion mode, the capillary voltage was 3.3 kV.

The raw data were processed using MassLynx 3.5 with OpenLynx software in five steps. (1) Each UV chromatogram from the corresponding analogue channel was aligned with the TIC chromatogram by adjusting to a premeasured delay time. (2) Each UV peak that passed a set of detection thresholds was integrated. (3) For each integrated peak, a mass spectrum was taken by averaging the two scans adjacent to the retention time. The target molecular ion within a 0.5 amu mass window was searched for in each spectrum. The peak was labeled as positive if the intensity of the molecular ion or an expected fragment ion was 80% of that of the base peak intensity. If the relative intensity was between 30 and 80%, it was labeled as tentative, and the spectrum was further inspected by the analyst to determine identity. (4) The relative area of the positive peak was then calculated as relative

purity. (5) These data were further reviewed. Blanks and standards are first reviewed to confirm that the instrument was working properly and there was no carryover between injections. Each sample well was examined for failures in sample injection, UV and MS signal abnormalities, and saturation. On the basis of this preliminary examination, a rerun sample list was generated based on the review.

For rerun, a Gilson liquid handler was programmed with Unipoint software to dilute and reformat failed samples. Solvent was allowed to evaporate at ambient temperature from the new plate, and a newly calculated volume of solvent was then added to each well in the plate, taking into consideration the cause of failure, such as over saturation, baseline drift, or low concentration. The new plate, reformatted and compressed, was analyzed with the MUX-LCT system using the new sample list. New data were then converted into the original format to replace the failed data. All data were then reviewed in detail, looking for coelution, peak integration problems, etc. Appropriate corrections were made to the report to revise product purity.

Fmoc-Asp(OtBu)-OH was purchased from Novabiochem. Theophylline, 5-phenyl-1*H*-tetrazole, and reserpine were purchased from Aldrich. All standards were weighted to the nearest 0.01 mg on an AT261 DeltaRange analytical balance (Mettler Toledo, Columbus, OH). A stock solution of 1.0 mg/mL of each standard was diluted using methanol to make a four-standard mixture for pre- and postpurification sample analysis.

2. ARW Calculation. To translate analytical HPLC conditions to the preparative scale, two factors are important for our operation. First, the analytical and semiprep columns should ideally come from the same manufacturer, and should contain the same lot of packing material. Second, after knowing the dimensions of both analytical and preparative columns, it is possible to calculate the flow rate for the preparative run so that the sample will ideally experience the same linear velocity as the analytical run. If the packing material and linear velocity are held constant between the two systems, they should have the same chromatographic environment. Consequently, samples should elute at the same time with the same mobile phase composition.

Unique solvent conditions are calculated in batches for each compound using in-house software and data from each analytical run. Channel-specific corrections of the sample's retention time are made on the basis of the retention times of standard compounds. A universal correction is made on the basis of the dead volume of the LC/MS. Knowing the gradient steepness, flow rate, and the dead volume of the preparative system, it is possible to calculate the necessary starting and ending mobile phase compositions to elute a compound at a target time.

As each sample is processed to determine the mobile phase compositions, a threshold for fraction collection is also calculated using that sample's analytical peak height and an empirically determined ratio between peak heights on the two systems.

Once generated, these calculations are exported to an Excel spreadsheet, and the sample names, locations, collection thresholds, and solvent conditions can easily be pasted into the Unipoint operation list.

3. Preparative Reversed-Phase HPLC Purification. Library compounds were purified using Gilson (Gilson Inc., Middleton, Wisconsin) liquid handlers and HPLC equipment. Unipoint Version 3.2 controlled instrument operation. Initial and final HPLC gradient conditions and the threshold setting for the fraction collector were set according to analytical LCMS data for each compound. Four pumps (three 306 piston pumps and one 305 piston pump) were used to control mobile phase flow through two 21.2 × 50-mm Phenomenex Hydro-RP columns (Phenomenex, Torrance, CA). Liquid streams were mixed in a Gilson 811C dynamic mixer, and pressure spikes were moderated using Gilson 806 manometric modules. Aqueous mobile phase consisted of HPLC grade water with 0.05% TFA; the organic mobile phase was HPLC grade acetonitrile with 0.05% TFA. The flow rate was 24.9 mL/min. A schematic outline of the instrument configuration is given in Figure 5.

Sample injection and fraction collection were automated using two separate 215 liquid handlers. Samples were dissolved in DMSO, and total sample volume was kept below 1000 mL. Samples were then drained through filter plates (Thomson Inc., Oceanside, CA). Fraction collection was triggered by a signal from a UV_{214} detector or a Sedex 55 evaporative light scattering detector (S.E.D.E.R.E., France). HPLC purification and postpurification processing were facilitated by software that tracks desired fractions. The software also coordinated the consolidation, postpurification analysis, drying, and final plating.

4. Solvent Evaporation. Solvent in the collected fractions was removed by either lyophilization (freeze-drying) or centrifugal vacuum evaporation. Lyophilization was achieved by freezing the sample solution at −80 °C in a Revco Ultima II freezer (Kendro Laboratory Products Inc., Ashville, NC) for 12−24 h and then dried under vacuum (below 200 mTorr) in a Virtis Ultra EL tray lyophilizer (Virtis Inc., Gardiner, NY) for 2−3 days until reaching a constant weight. Centrifugal vacuum-drying was achieved using a Discovery SpeedVac (Thermo Savant, Holbrock, NY). Solvent evaporation was performed in two stages. The first stage was the evaporation of acetonitrile, in which the samples were spun at a rate of ~900 rpm and the vacuum was ramped at a rate of 40 Torr/min to a final pressure of 6.5 mTorr. The temperature was held at 45 °C for a total of 4 h. The second evaporation stage was to remove water, where the samples were spun at a rate of ~900 rpm, and a vacuum of 2.0 mTorr was then applied with no vacuum ramp. Temperature was held at 65 °C for 10 hours.

5. Automatic Weighing of Purified Compounds. All fraction collection tubes (16 × 100 mm, fused-silica) were preweighed using a Bohdan BA-200 (Bohdan Automation Inc., Mundelein, IL). These tubes were held in a custom Gilson 207 test tube rack (Gilson Inc., Middleton, WI). After fraction collection, the prep chromatograms were processed by in-house software to pick the major product fraction. If necessary, preparative chromatograms were compared to the corresponding analytical chromatograms to pick correct product fractions. Product fractions from each run were then

placed into a master tracking file. The product tubes were then merged into a new set of 207 racks using the Bohdan BA-200. These tubes were then sampled, using an eight-probe Tecan Genesis system (Tecan AG, Hombrechtikon, Switzerland), into 200-μL, 96-well, polypropylene plates for LC/MS MUX analysis. The consolidated racks were then dried by the procedures detailed above. After drying, the tare weight for each tube was automatically transferred to the tracking file, and the dry tubes were reweighed using a Bohdan BA-200. The net weight of compound was calculated from the difference between the two weights.

6. Reformatting and Plating. Final plating of purified compounds was performed using a Tecan Genesis. The first step is uploading the weight and purity data for each compound into the tracking file. The tubes containing compounds that meet the required weight and purity criteria are then transferred to another Gilson 207 rack (the "S" rack) using the Bohdan BA-200. Samples in the "S" rack are then dissolved in the appropriate plating solvent (e.g., DMSO) and vortexed for at least 1 h using a digital vortexer (Thomson Instrument Company, Oceanside, California, USA). The dissolved samples are then transferred to the final plates using a Tecan Genesis system.

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References and Notes

(1) Fenniri, H. *Combinatorial Chemistry. A Practical Approach*; Oxford University Press: New York, 2000.

(2) Seneci, P. *Solid-Phase Synthesis and Combinatorial Technologies*; John Wiley & Sons, Inc.: New York, 2000.

(3) Baldino, C. M. *J. Comb. Chem.* **2000**, *2*, 89−103.

(4) Yan, B.; Czarnik, A. W. *Optimization of solid-phase combinatorial synthesis*; Marcel Dekker: New York, 2001.

(5) Yan, B.; Fang, L.; Irving, M.; Zhang, S.; Boldi, A.; Woolard, F.; Figliozzi, G. M.; Lease, T. G.; Johnson, C. R.; Collins, N. Qua *J. Comb. Chem.* **2003**, *5*, 547−559.

(6) Weller, H. N. *Mol. Diversity* **1999**, *4*, 47−52.

(7) Neue, U. D.; Carmody, J. L.; Cheng, Y. F.; Lu, Z.; Phoebe, C. H.; Wheat, T. E. *Adv. Chromatogr.* **2002**, *41*, 93−136.

(8) (a) Stump, H.; God, R. *Chem.-Ing.-Tech.* **2000**, *9*, 937−938. (b) Ripka, W. C.; Barker, G.; Krakover, J. *Drug Discovery Today* **2001**, *6*, 471−477.

(9) Kyranos, J. N.; Cai, H.; Wei, D.; Goetzinger, K. W. *Curr. Opin. Biotechnol*. **2001**, *12*, 105−111.

(10) Fang, L.; Demee, M.; Sierra, T.; Zhao, J.; Tokushige, D.; Yan, B. *Rapid Commun. Mass Spectrom*. **2000**, *16*, 1440−1447.

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