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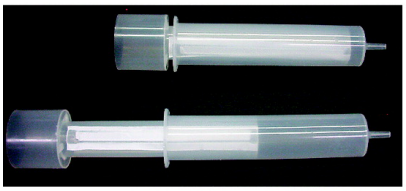

Further Development of a Robust Workup Process for Solution-Phase High-Throughput Library Synthesis To Address Environmental and Sample Tracking Issues

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During further improvement of a high-throughput, solution-phase synthesis system, new workup tools and apparatus for parallel liquid−liquid extraction and evaporation have been developed. A combination of in-house design and collaboration with external manufacturers has been used to address (1) environmental issues concerning solvent emissions and (2) sample tracking errors arising from manual intervention. A parallel liquid−liquid extraction unit, containing miniature high-speed magnetic stirrers for efficient mixing of organic and aqueous phases, has been developed for use on a multichannel liquid handler. Separation of the phases is achieved by dispensing them into a newly patented filter tube containing a vertical hydrophobic porous membrane, which allows only the organic phase to pass into collection vials positioned below. The vertical positioning of the membrane overcomes the hitherto dependence on the use of heavier-than-water, bottom-phase, organic solvents such as dichloromethane, which are restricted due to environmental concerns. Both small (6-mL) and large (60-mL) filter tubes were developed for parallel phase separation in library and template synthesis, respectively. In addition, an apparatus for parallel solvent evaporation was developed to (1) remove solvent from the above samples with highly efficient recovery and (2) avoid the movement of individual samples between their collection on a liquid handler and registration to prevent sample identification errors. The apparatus uses a diaphragm pump to achieve a dynamic circulating closed system with a heating block for the rack of 96 sample vials and an efficient condenser to trap the solvents. Solvent recovery is typically >98%, and convenient operation and monitoring has made the apparatus the first choice for removal of volatile solvents.

Introduction

High-throughput organic synthesis (HTOS) is now an established part of the medicinal chemists' armory to facilitate both the generation of new lead compounds in early drug discovery and focused libraries of compounds for lead optimization. Along with the growth of new strategies and chemical methods suitable for HTOS, considerable attention has been paid to the development of reliable and convenient apparatus to perform parallel synthesis.[1] The HTOS process consists of several sequential stages: library design, template synthesis, diversity reaction, workup, purification, evaporation, analysis, registration, and submission of the compounds for screening and storage. At Takeda, a modular process[2] for parallel solution-phase synthesis based on a number of commercially available instruments[3] and that is flexible enough to cope with a variety of chemistries (e.g. amidation, reductive amination, alkylation, Mitsunobu, Suzuki-Miyaura, Grignard, Buchwald reactions) has been constructed. Initially, both solid-phase and solution-phase synthesis was carried out, but after several years of experience, the limitations of solid-phase chemistry and the decline in the need for the combinatorial paradigm has led to the almost exclusive adoption of solution-phase chemistry for library synthesis. Figure 1 illustrates the modular HTOS process employed at Takeda, notably showing the workup stages between reaction and purification.

In solution-phase organic synthesis, workup is the process by which pure materials are isolated from reaction mixtures, which are often complex. The first step is usually liquid−liquid extraction, in which the reaction mixture is partitioned between aqueous and organic phases, and this is followed by phase separation, evaporation, and chromatography. Although these operations can be applied to most types of synthesis reactions and are easily undertaken by the chemist, workup is far from trivial to automate or carry out in parallel, and its diversity and complexity was the main reason that early attempts at HTOS focused on solid-phase synthesis. However, the clear limitations to chemistries that can be performed on the solid phase led us to focus on the development of tools to support the parallelization of solution chemistry.

Due to the recognized limitations of using only commercially available instruments, a program to systemize the whole HTOS process was undertaken through the introduction of several new procedures and in-house modifications. The key aims were to increase the capability and reliability,

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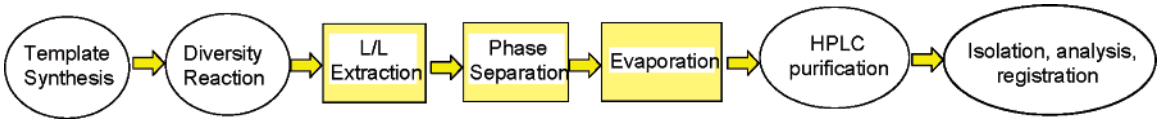


Figure 1. Modular HTOS process from synthesis to registration.

Table 1. Legal Restrictions for Handling Dichloromethane[4]

Japanese law	restriction limit
Air Pollution Control	<0.15 mg/m^3 (av for year)
Clean Water	<0.2 mg/L
Industrial Health and Safety	<340 mg/m^3 (100 ppm)

improve efficiency, and reduce errors. In particular, local restrictions[4] put on the use and disposal of chlorinated solvents (Table 1) encouraged us to develop new tools and apparatus for liquid−liquid extraction and solvent evaporation. Furthermore, better integration of apparatus through unification of formats and handling was identified as essential to reducing the need for manual intervention, a major cause of poor efficiency and error in the modular HTOS process. To maintain the open-access nature of the HTOS process, all new tools that were to be introduced had to be able to be easily used by chemists without specialist training.

The Tecan Genesis multichannel liquid handler has been used extensively for parallel workup purposes to purify products or simplify subsequent purification by HPLC chromatography. After liquid−liquid extraction and phase separation on the Genesis platform, it is generally necessary to evaporate the organic solvent and replace it with one, such as dimethyl sulfoxide (DMSO), that will dissolve a wide variety of samples without giving any precipitation during column charging and without interfering with the HPLC separation.

In this report, the following developments are described: (1) a parallel liquid−liquid extraction unit for use on the Tecan Genesis, (2) parallel phase separation using newly patented filter tubes that enable top-phase extraction with non-chlorinated solvents, and (3) a parallel solvent evaporation apparatus developed in collaboration with an external manufacturer that gives good solvent recovery and eliminates the need for manual reformatting of vials.

Results and Discussion

Top- and Bottom-Phase Liquid−Liquid Extraction Unit. It was decided to develop a unit for the Tecan Genesis multichannel liquid handler that would be capable of parallel liquid−liquid extraction with top-phase organic solvents. The Tecan Genesis has been widely used due its rapid and efficient eight-probe liquid-handling capacity and its intuitive operating software. In addition, the open and flexible deck of the Genesis permits easy customization, and its large deck size allows for processing batches of 48 or more samples. The liquid−liquid extraction unit was designed around two racks: a stirring rack to contain the reaction vessels and enable efficient mixing of the organic and aqueous phases and a filter rack for phase separation and sample collection. The stirring rack was custom-made[5] and accommodates 48 Myriad[6] solution-phase reaction vessels in a 6 × 8 grid. Each vessel is located immediately above a miniature high-speed motor fitted with two rare-earth magnets, which enable efficient mixing of the phases inside the reaction vessel by means of a PTFE-coated rare-earth magnetic stirrer bar.

In addition, a set of magnets are positioned so that they can be brought into close proximity with the flasks in a single movement to attract the stirrer bars away from the bottom of the reaction vessels, and thus allow the Genesis probe to access the bottom of the reaction vessel without fouling (see Figure 2). The filter rack can hold either 48 individual filter tubes or four microtiter plate format filters, under which glass collection tubes are located. The filters can be either bottom-phase,[7] or top-phase.[8] The design and development of the top-phase filter tubes is described below (Section 2).

Figure 3 illustrates the steps involved in the automated liquid−liquid extraction that was carried out after reaction in the Myriad vessels. The crude reaction mixtures are placed into the stirrer rack, and the required aqueous and organic solvents are added in parallel to the vessels using the multichannel dispenser. Dimethylformamide (1 mL) is commonly used as the reaction solvent, and liquid−liquid extraction generally involves the addition of ethyl acetate (3 mL), together with water or aqueous acid or base (2 mL), followed by vigorous stirring for a few minutes. The phases are allowed to separate by standing for awhile, and then the organic phase is transferred to a vial for evaporation. The Tecan Genesis does have the capability to sense the different electric conductivities of organic and aqueous phases, and this can be useful to detect the phase boundary and then discard most of the aqueous phase or collect most of the organic phase.

It was found, however, that the conductivity sensing could be difficult for some reaction mixtures, proceeding slowly and often causing the Tecan Genesis to pause for manual intervention. Thus, to achieve a robust and flexible process for the collection of the organic phase, it was found that the most reliable method was to set the robot to transfer all of the organic phase plus a fraction of the aqueous phase into filter tubes or plates containing hydrophobic membranes. From these filtering devices, only the organic fraction, either the top or bottom phase, passes through into numbered 8-mL glass vials positioned below.[9]

Top-Phase Separation Filter Tubes. Dichloromethane has often been used as the organic extraction solvent due to its good liquid−liquid extraction ability and high specific gravity, which results in the required organic extract's being recovered from the lower phase in high yield (>80%) with a single extraction; however, environmental issues and restrictions on the use of halogenated solvents encouraged us to develop a filter tube that would allow liquid extraction using top-phase organic solvents such as ethyl acetate and thereby enable us to avoid the use of dichloromethane. The size and format of the top-phase filter tubes were designed to match those of the commercially available bottom-phase

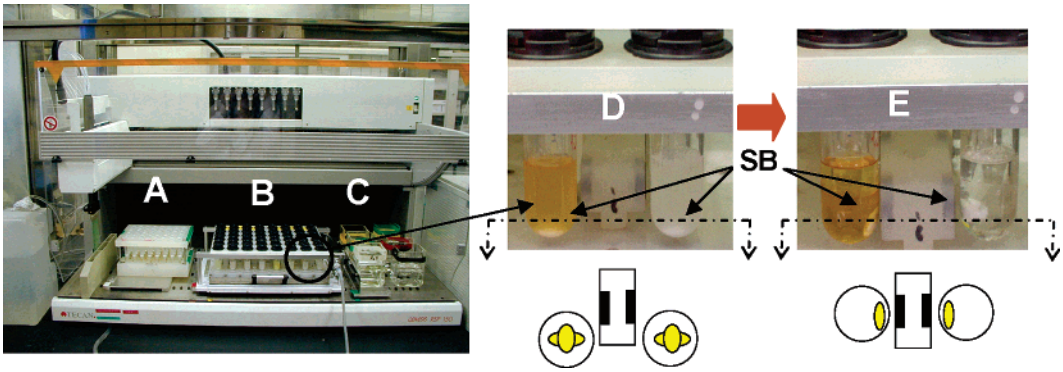


Figure 2. Parallel liquid−liquid extraction on the Tecan Genesis. A, filter tube rack; B, stirrer rack; C, solvents; D, rapid stirring gives efficient mixing of the phases; E, after standing, a magnet is slid into position next to the flasks, the phases separate, and the stirring bars (SB) are attracted away from the bottom.

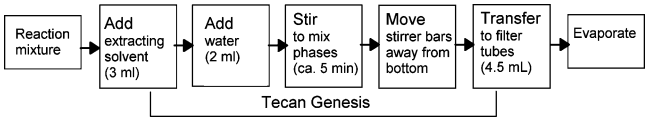


Figure 3. Typical procedure for liquid−liquid extraction.

filter tubes so that the same racks and software programs could be used. The bottom-phase filter tube has a hydrophobic PTFE membrane at its base that prevents passage of the aqueous phase. Thus, in the case of bottom-phase organic solvents, such as dichloromethane, the organic solvent first passes through the membrane, and the following aqueous phase is retained. Clearly, for top-phase organic solvents, the aqueous phase is located at the bottom of the tube, and no organic solvent can pass. To allow the top-phase organic solvents to be collected, a tube containing a hydrophobic PTFE membrane on the sides was designed so that the bottom aqueous phase would not hinder the passage of the organic phase through the membrane. The organic solvent passes through the membrane of the inner tube and then flows into the cavity between the inner and outer walls and is guided to the glass collection tube located below. The double-barreled tube design (Figure 4) was also useful to prevent the membrane from clogging, which could occur if the organic solvent quickly evaporated from the surface, and a precipitate formed.

The extraction efficiency of a single ethyl acetate aliquot was observed to be lower than for the same volume of dichloromethane, but it was generally greater than 60%, which is sufficient for the last step of a library synthesis. A second extraction could be performed to increase the yield when required, but the extra operations and time required mean such a strategy is unattractive for general use.

A larger size (60-mL) reservoir capacity top-phase filter tube has also been introduced for use in the parallel synthesis of template compounds, the common core structures that are combined with a diverse collection of reagents, for high-throughput library synthesis. Figure 5 shows a rack of 12 tubes being used for liquid−liquid extraction in a g-scale synthesis of templates.

Parallel Solvent Evaporation without Reformatting. A major part of the HTOS workup process involves solvent evaporation, typically after (1) liquid−liquid extraction, (2) purification by HPLC, and (3) sampling of the purified samples for quality control. Previously, all evaporation was carried out using vacuum centrifugation, which required samples to be transferred into robust 24-well aluminum racks. However, because the process from QC sampling through to submission is based on handling samples in a 96 (8×12) rack format, this requirement made it necessary to carry out sample reformatting steps. Not only was the manual reformatting a source of considerable inefficiency, it could result in the introduction of positional errors that would lead to incorrect compound registration. Thus, eliminating this source of error was made a priority, together with the need to obtain good solvent recovery to comply with strict environmental regulations.

A bar-coded vial-tracking system was introduced, and the time-consuming, error-prone, manual reformatting step was eliminated by developing an evaporation instrument, SolTrapper[10] (Figure 6), that could handle the 96-well-format rack of 8-mL, bar-coded vials. In contrast to most of the commercially available apparatus for parallel evaporation, SolTrapper does not use a vacuum, shaking, or centrifugation, but employs a diaphragm pump to achieve a dynamic circulating closed system in which solvent is blown off from the samples and collected in a highly efficient cold trap. The closed system enables very high recoveries of organic solvents to be achieved for compliance with the environmental regulations.

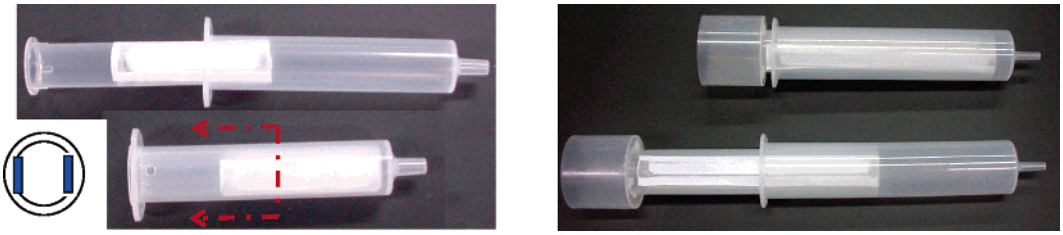


Figure 4. Double-barreled top-phase PreSep filter tubes. Left, 6 mL; right, 60 mL.

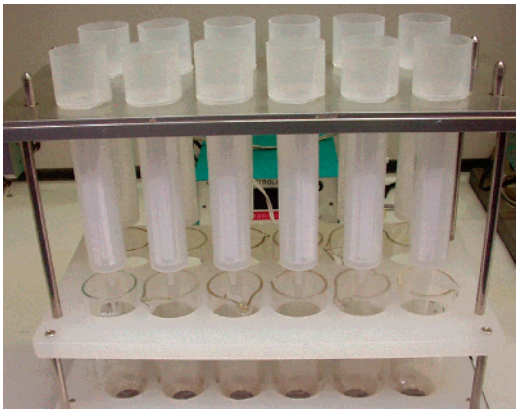


Figure 5. Parallel liquid−liquid extraction of intermediates and templates using 12 large, top-phase separation tubes.

Table 2. Time Required and Recoveries Obtained for Complete Evaporation of Typical Volatile Solvents Used in the HTOS Process Using SolTrapper and a Centrifugal Evaporator

	SolTrapper[b]		centrifugal evaporator[c]	
solvent[a]	*t* (h)	recovery[d] (%)	*t* (h)	recovery[d] (%)
acetonitrile	1	99	2	89
methanol	1	100	2	92
ethyl acetate	1	100	2	82

[a] 96 × 2 mL of solvent used in 8-mL glass tubes. [b] Aluminum rack at 60 °C, trap at −20 °C. [c] Genevac HT8, standard methods, aluminum racks, trap at −40 °C. [d] Calculated from the volume of solvent collected in the traps, with an estimated error of <2%.

Table 2 shows the recoveries of some common volatile solvents used in the HTOS process that were obtained using both SolTrapper and vacuum centrifugation. The data indicates that the solvents could be recovered much faster using SolTrapper, and improved recoveries could be obtained.[11] Furthermore, unlike the centrifugal vacuum evaporators, the samples are stationary, and evaporation can be stopped instantly, which allows for convenient, safer operation and the ability to visually inspect the condition of the samples at any time during evaporation. Hence, SolTrapper has become the instrument of choice for the removal of volatile solvents, and vacuum centrifugation is used only when it is necessary to remove solvents of high boiling point, such as DMF, DMSO, and *N*-methylpyrrolidone.

Samples can be evaporated in SolTrapper in the standard 96-well polypropylene rack without moving the individual vials, but due to the poor conductivity of polypropylene, samples containing water (generally obtained after HPLC purification) were found to require long evaporation times of 24 h or longer. To overcome this disadvantage, an apparatus was developed to transfer the vials from the polypropylene rack to an identical one made of aluminum, in a single operation without any handling of individual vials.[5] Exchange 96 (Figure 7) utilizes the holes in the bottom of the 96-well racks for pegs to be inserted to push the vials up into a temporary holding rack. A retaining plate is then slid across to prevent the vials from falling, the pegs are lowered, and the polypropylene rack is exchanged for an aluminum one of the same dimensions. Raising the pegs again, removing the retaining plate, and then lowering the pegs completes the transfer of the 96 vials into the aluminum rack without any possibility of introducing positional errors. After evaporation, the samples are redissolved in methanol, and QC sampling is performed, then evaporation is repeated, and the dry samples are transferred back to the polypropylene rack for weighing and submission for screening.

Synthesis of a Lead Generation Library of (*N*-Acylamino)benzamide Derivatives, from Template Synthesis to Library Synthesis. As shown in Figure 1, the HTOS synthesis process begins with parallel template synthesis, followed by incorporation of the diversity reagents. Parallel g-scale reaction[12] and workup employing the large-size top-phase separation tubes can be easily and safely performed in an efficient and environmentally benign process. A typical example of HTOS synthesis from four templates and 24 diversity reagents to give 96 library compounds is described here for the generation of biologically attractive (*N*-acylamino) benzamide derivatives (Scheme 1).[13a,b] First, commercially available methyl aminobenzoates (**1a**−**1d**) were acylated with benzoyl chloride or acetyl chloride to give the

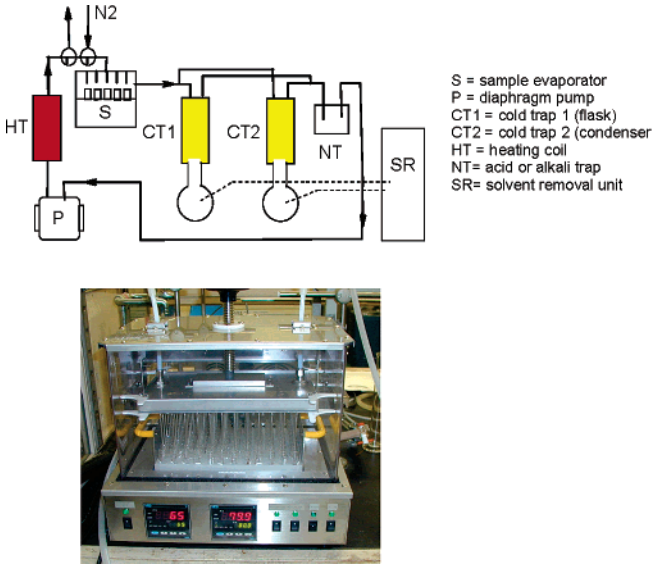


Figure 6. Diagram and photographs of the solvent evaporation apparatus SolTrapper.

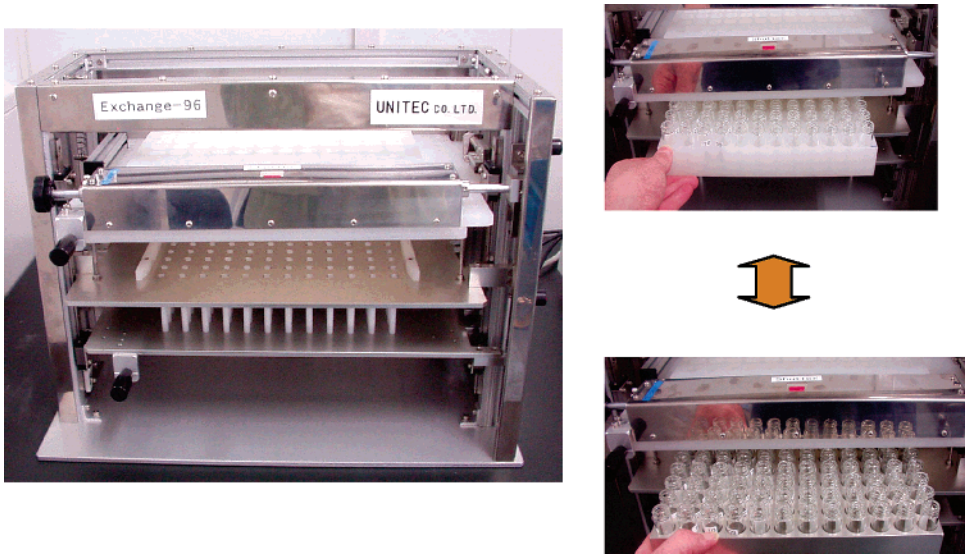
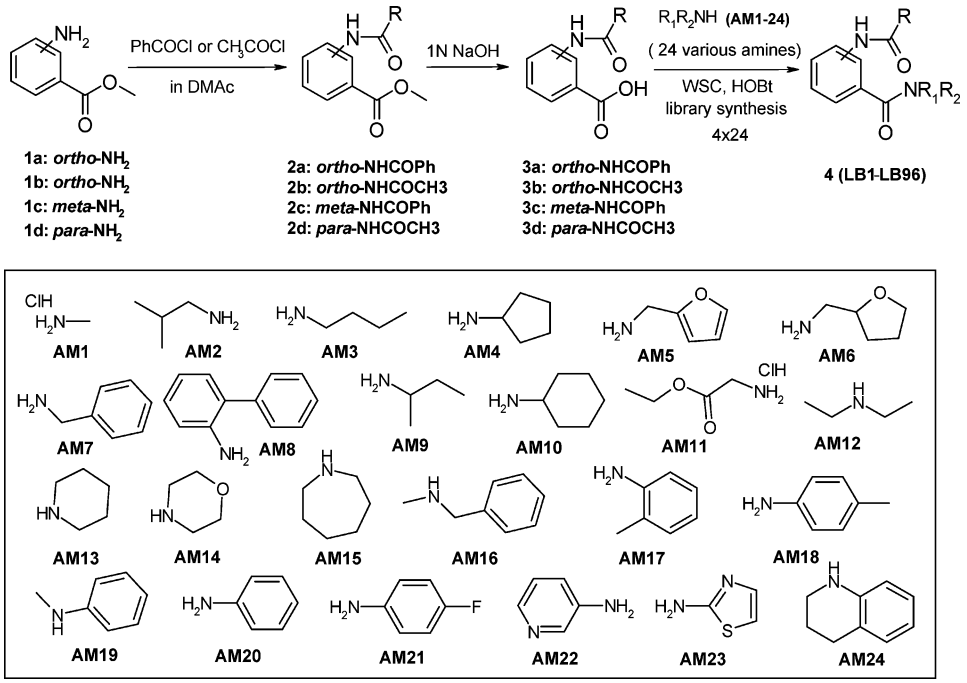


Figure 7. Exchange 96 apparatus for transfer of vials between 96-format polypropylene and aluminum racks.

Scheme 1. Synthesis of (*N*-Acylamino)benzamide Derivatives



intermediates (**2a**−**2d**), followed by hydrolysis with aqueous NaOH to give the template acids (**3a**−**3d**). Workup after the acylation step was achieved by adding ethyl acetate and 5% sodium bicarbonate and performing liquid−liquid extraction by high-speed stirring. Phase separation was performed in parallel using 60-mL PreSep tubes in the customized unit (see above, Figure 5). Subsequently, evaporation of the organic solvent was performed using a centrifugal evaporator,[14] and then 24 kinds of diverse amine reagents were selected to synthesize a library of (*N*-acylamino)benzamide compounds (**4**, LB1-LB96). Condensation of the templates with the selected amines was performed on the Myriad Core System. The workup of the library compounds was performed on a Tecan Genesis using the liquid−liquid extraction unit with 6-mL PreSep filter tubes, and SolTrapper was used for evaporation of the samples in an efficient, accurate, and environmentally clean process. After purification by preparative HPLC, the samples were submitted for screening.

Conclusion

New apparatuses and tools have been developed and incorporated into modular high-throughput synthesis protocols for both parallel template and library synthesis to give a robust process that has been used for over 2 years handling a variety of chemistries and samples in a safe, reliable, and efficient manner. The ability to perform parallel liquid−liquid extraction and phase separation with lighter-than-water organic solvents and also complete evaporation of multiple arrays of samples in an apparatus that enables extremely high solvent recovery has allowed us to greatly reduce the emission of harmful organic solvents and comply with regulations introduced for environmental protection. In addition, because the apparatus for parallel solvent evapora-

tion can accommodate the racks of 96 vials in their final format, it was possible to eliminate the movement of individual vials between different format racks, which prevents the accidental introduction of sample tracking errors and speeds the workup process.

Experimental Details

Starting materials were purchased from Wako Pure Chemical Industries, Ltd. ^{1}H NMR spectra were recorded on a Bruker-400 (400 MHz) with tetramethylsilane as internal standard. Combined liquid chromatography−mass spectrometry LC/MS (ESI-MS) analyses were carried out using a Waters Open-Lynx system. Flash chromatography was performed using Purif-Pack SI (60 μm, 200 mesh; manufactured by Fuji Silysia Chemical Ltd.) on a Purif-4 parallel HPLC system (Moritex, Japan) for template synthesis. Reaction workup was carried out using 60- or 6-mL PreSep tubes. Evaporation was carried out using a centrifugal evaporator (CE-1, Hitachi, Japan) for template synthesis or SolTrapper (Technosigma, Okayama, Japan) for library synthesis. Preparative HPLC was carried out using a Gilson system employing the following conditions: column, Shiseido Capcellpak UG120 5 μm × 120 Å 50 × 20 mm; mobile phase, CH_3CN/H_2O/TFA = 10:90:0.1 (0 min) → 10:90:0.1 (1 min) → 95:5:0.1 (4 min) → 95:5:0.1 (7.5 min) → 10:90:0.1 (8 min) → 10:90:0.1 (10 min); flow rate, 20 mL/min. For polar compounds: Nomura Develosil C30 5 μm × 120 Å 50 × 20 mm; mobile phase, CH_3CN/H_2O/TFA = 0:100:0.1 (0 min) → 0:100:0.1 (4 min) → 95:5:0.1 (6 min) → 95:5:0.1 (7.5 min) → 0:100:0.1 (8 min)→ 0:100:0.1 (10 min); flow rate, 20 mL/min; detection, UV 220 nm.

Template Synthesis

General Procedure for Acylation of Amine Compounds, Followed by Hydrolysis of Methyl Esters, To Give Acid Templates (3a−3d). To each stirred solution of methyl aminobenzoate (1.5 g, 10.0 mmol) in dimethylacetamide (10 mL) was added benzoyl chloride (4.2 g, 30 mmol) or acetyl chloride (2.4 g, 30 mmol). After the mixtures were stirred at 50 °C for 20 h, an aqueous solution of 5% sodium hydrogen bicarbonate (20 mL) was added to each of the reaction mixtures. Extraction was carried out by addition of EtOAc (30 mL), followed by vigorous stirring for 10 min. After the mixtures were allowed to stand for 5 min to allow the phases to separate, ∼90% of the aqueous phase was removed by suction, and the remainder was poured into a large top-phase separation tube. The organic extracts were collected and evaporated using a centrifugal evaporator, and the residues were chromatographed on silica gel to give the expected (*N*-acylamino)esters (**2a**−**2d**) in 35−75% yield, then the esters were hydrolyzed with 1 N NaOH (6−10 mL)/MeOH (6−10 mL) at 60 °C for 20 h. The residues were neutralized with 1 N HCl (6−10 mL), and the resulting precipitate was filtered and washed with water to give the acid templates (**3a**−**3d**) in 80−98% yield. The LC/MS and ^{1}H NMR data of the templates **3a**−**3d** shown below are identical to those reported in the literature.[15a,b]

2-(Benzoylamino)benzoic Acid (3a). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.21−7.28 (1H, m), 7.58−7.79 (4H, m), 7.95−8.01 (2H, m), 8.06−8.14 (1H, m), 8.73−8.78 (1H, m). LC/MS (ESI): *m*/*z* 242 (M + H).

2-(Acetylamino)benzoic Acid (3b). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.14 (3H, s), 7.14 (1H, t, J = 4.6 Hz), 7.58 (1H, t, J = 4.6 Hz), 7.98 (1H, d, J = 6.4 Hz), 8.46 (1H, t, J = 4.6 Hz). LC/MS (ESI): *m*/*z* 180 (M + H).

3-(Benzoylamino)benzoic Acid (3c). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.43−7.71 (5H, m), 7.97−8.08 (3H, m), 8.44 (1H, s), 10.44 (1H, brs). LC/MS (ESI): *m*/*z* 242 (M + H).

4-(Acetylamino)benzoic Acid (3d). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.09 (3H, s), 7.69 (2H, d, J = 8.8 Hz), 7.88 (2H, d, J = 8.8 Hz), 10.24 (1H, brs). LC/MS (ESI): *m*/*z* 180 (M + H).

Library Synthesis

Library synthesis was performed in parallel on the Myriad Core System. General procedure for amidation of acid templates was as follows. To stirred solutions of the acid templates (80 μmol in each vessel) in dimethylformamide (500 μL) were added the various amines (1-hydroxybenzotriazole, triethylamine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (96 μmol of each), and 4-(dimethylamino)pyridine (9.6 μmol of each)) in dimethylformamide (500 μL), and stirring was carried out overnight at 40 °C. Then ethyl acetate (3 mL) and water (2 mL) were added to the reaction mixtures, and vigorous stirring was performed for 5 min on the Tecan Genesis liquid−liquid extraction unit. After mixing was stopped and the phases had separated (∼5 min), the top 4 mL of the resulting mixtures were transferred to 6-mL PreSep tubes. The organic phase was collected in disposable glass tubes and then evaporated in SolTrapper to give the crude products. After chromatography on a preparative HPLC system, pure products were isolated by removal of the solvent (acetonitrile/water) using SolTrapper without any further necessity to reformat the vials. Average yield of the obtained products was 7.0 mg (32%), with average purity of 94%. Products were identified by LC/MS, and 20 of them were additionally confirmed by ^{1}H NMR analysis. The LC/MS data (*m*/*z*) of the compounds and ^{1}H NMR data of representative compounds are shown in Table 3.

2-(Benzoylamino)-*N*-(tetrahydrofuran-2-ylmethyl)benzamide (LB6). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.66−2.18 (4H, m), 3.32−3.49 (1H, m), 3.76−4.22 (4H, m), 6.69 (1H, brs), 7.12 (1H, brs), 7.29 (1H, brs), 7.48−7.65 (5H, m), 7.92−8.19 (2H, m), 8.87 (1H, brs). LC/MS (ESI): *m*/*z* 324 (M + H).

2-(Benzoylamino)-*N*-benzylbenzamide (LB7). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.66 (2H, d, J = 4.8 Hz), 6.52 (1H, brs), 7.09 (1H, t, J = 6.4 Hz), 7.28−7.42 (5H, m), 7.48−7.59 (5H, m), 8.07 (2H, d, J = 6.4 Hz), 8.83 (1H, d, J = 6.4 Hz). LC/MS (ESI): *m*/*z* 330 (M + H).

2-(Benzoylamino)-*N*-(4-fluorophenyl)benzamide (LB21). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.93 (1H, brs), 7.21−7.48 (8H, m), 7.61 (2H, d, J = 8.0 Hz), 7.90 (2H, d, J = 8.0 Hz), 8.95 (1H, brs). LC/MS (ESI): *m*/*z* 334 (M + H).

2-(Benzoylamino)-*N*-pyridin-3-ylbenzamide (LB22). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.91 (1H, brs), 7.35−7.46 (2H, m), 7.48−7.66 (4H, m), 8.01−8.05 (2H, d, J = 4.6 Hz),

Table 3. LC/MS Data (*m*/*z*) of the Library Compounds

Compound No.
amount
yield
M+H

∗ trace amount ; <0.1mg
∗ Low yield ; <80%

template	diversity AM1	AM2	AM3	AM4	AM5	AM6	AM7	AM8	AM9	AM10	AM11	AM12
3a	LB1	LB2	LB3	LB4	LB5	LB6	LB7	☆LB8	LB9	LB10	LB11	LB12
	1.2mg	3.2mg	3.8mg	2.1mg	0.4mg	8.2mg	3.9mg	∗ trace	1.0mg	4.3mg	5.5mg	1.7mg
	92%	99%	100%	100%	90%	97%	98%	∗ Low	94%	89%	91%	97%
	254	**296**	**296**	**308**	**320**	**324**	**330**	**392**	**296**	**322**	**326**	**296**
3b	LB25	LB26	LB27	LB28	LB29	LB30	LB31	LB32	LB33	LB34	LB35	LB36
	4.5mg	21.8mg	4.5mg	2.9mg	2.5mg	1.8mg	1.9mg	4.5mg	1.9mg	3.6mg	20.6mg	7.6mg
	∗ Low	97%	95%	93%	95%	98%	98%	∗ Low	81%	88%	95%	100%
	192	**234**	**234**	**246**	**258**	**262**	**268**	**330**	**234**	**260**	**264**	**234**
3c	LB49	LB50	LB51	LB52	LB53	LB54	LB55	LB56	LB57	LB58	LB59	LB60
	0.7mg	8.7mg	11.7mg	11.0mg	11.2mg	13.6mg	13.9mg	3.1mg	11.9mg	7.5mg	11.7mg	10.8mg
	∗ Low	99%	100%	100%	98%	100%	100%	∗ Low	100%	96%	85%	99%
	254	**296**	**296**	**308**	**320**	**324**	**330**	**392**	**296**	**322**	**326**	**296**
3d	LB73	LB74	LB75	LB76	LB77	LB78	LB79	LB80	LB81	LB82	LB83	LB84
	2.4mg	8.7mg	8.8mg	9.3mg	10.7mg	6.1mg	4.3mg	0.9mg	6.7mg	9.7mg	5.7mg	7.2mg
	∗ Low	97%	95%	91%	98%	93%	98%	∗ Low	98%	93%	93%	96%
	192	**234**	**234**	**246**	**258**	**262**	**268**	**330**	**234**	**260**	**264**	**234**

template	diversity AM13	AM14	AM15	AM16	AM17	AM18	AM19	AM20	AM21	AM22	AM23	AM24
3a	LB13	LB14	LB15	LB16	☆LB17	☆LB18	☆LB19	☆LB20	☆LB21	☆LB22	☆LB23	☆LB24
	5.2mg	6.5mg	1.8mg	6.5mg	0.2mg	0.3mg	0.2mg	0.3mg	2.3mg	3.3mg	0.6mg	∗ trace
	100%	87%	89%	95%	99%	93%	100%	99%	97%	99%	98%	∗ Low
	308	**310**	**322**	**344**	**330**	**330**	**330**	**316**	**334**	**317**	**323**	**356**
3b	LB37	LB38	LB39	LB40	LB41	LB42	LB43	LB44	LB45	LB46	LB47	LB48
	6.2mg	10.8mg	24.8mg	4.1mg	2.2mg	5.4mg	19.3mg	10.0mg	8.2mg	17.6mg	13.9mg	21.2mg
	99%	∗ Low	100%	85%	99%	99%	99%	94%	98%	96%	99%	∗ Low
	246	**248**	**260**	**282**	**268**	**268**	**268**	**254**	**272**	**255**	**261**	**294**
3c	LB61	LB62	LB63	LB64	LB65	LB66	LB67	LB68	LB69	LB70	LB71	LB72
	12.9mg	11.8mg	14.0mg	13.7mg	10.8mg	10.8mg	6.4mg	8.4mg	7.8mg	5.5mg	8.3mg	0.6mg
	100%	98%	99%	100%	99%	96%	98%	88%	96%	98%	∗ Low	∗ Low
	308	**310**	**322**	**344**	**330**	**330**	**330**	**316**	**334**	**317**	**323**	**356**
3d	LB85	LB86	LB87	LB88	LB89	LB90	LB91	LB92	LB93	LB94	LB95	LB96
	11.6mg	5.1mg	13.3mg	12.9mg	6.7mg	4.6mg	5.3mg	10.5mg	4.6mg	2.7mg	5.7mg	16.2mg
	98%	∗ Low	94%	87%	89%	91%	85%	98%	97%	98%	97%	∗ Low
	246	**248**	**260**	**282**	**268**	**268**	**268**	**254**	**272**	**255**	**261**	**294**

☆**LB8**, **LB17**−**LB24:** reaction was conducted using excess amine, either in pyridine or neat, and the reaction mixture was stirred at 80 °C for 20 h.

8.30 (1H, m), 8.49 (1H, m), 8.52 (1H, m), 8.81 (1H, brs), 8.95 (1H, brs). LC/MS (ESI): *m*/*z* 317 (M + H).

***N*-[2-(Piperidin-1-ylcarbonyl)phenyl]acetamide (LB37).** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45−1.79 (6H, m), 2.16 (3H, s), 3.42−3.80 (4H, m), 7.13 (1H, t, J = 4.6 Hz), 7.19 (1H, d, J = 4.0 Hz), 7.43 (1H, t, J = 4.6 Hz), 8.27 (1H, d, J = 4.0 Hz), 8.95 (1H, brs). LC/MS (ESI): *m*/*z* 246 (M + H).

***N*-[2-(Morpholin-4-ylcarbonyl)phenyl]acetamide (LB38).** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (3H, s), 3.62−3.87 (8H, m), 7.13 (1H, t, J = 4.6 Hz), 7.23 (1H, d, J = 4.0 Hz), 7.43 (1H, t, J = 4.6 Hz), 8.23 (1H, d, J = 4.0 Hz), 8.95 (1H, brs). LC/MS (ESI): *m*/*z* 248 (M + H).

2-(Acetylamino)-*N*-phenylbenzamide (LB44). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.45 (3H, s), 7.21−7.36 (5H, m), 7.61 (1H, t, J = 4.6 Hz), 7.90 (2H, d, J = 8.0 Hz), 8.30 (1H, t, J = 8.0 Hz). LC/MS (ESI): *m*/*z* 254 (M + H).

3-(Benzoylamino)-*N*-butylbenzamide (LB51). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (1H, t, J = 4.8 Hz), 1.31−1.47 (2H, m), 1.56−1.70 (2H, m), 3.42 (2H, t, J = 5.6 Hz), 6.28 (1H, brs), 7.41−7.65 (5H, m), 7.89 (3H, m), 8.15 (1H, s), 8.19 (1H, brs). LC/MS (ESI): *m*/*z* 296 (M + H).

3-(Benzoylamino)-*N*-cyclopentylbenzamide (LB52). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.52−2.15 (8H, m), 4.42 (1H, m), 6.18 (1H, brs), 7.41−7.67 (5H, m), 7.89 (3H, m), 8.05 (1H, s), 8.19 (1H, brs). LC/MS (ESI): *m*/*z* 308 (M + H).

3-(Benzoylamino)-*N*-(tetrahydrofuran-2-ylmethyl)benzamide (LB54). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.56−1.68 (1H, m), 1.88−2.13 (3H, m), 3.28−3.39 (1H, m), 3.72−4.12 (4H, m), 6.58 (1H, brs), 7.48−7.65 (5H, m), 7.82−8.09 (5H, m). LC/MS (ESI): *m*/*z* 324 (M + H).

3-(Benzoylamino)-*N*,*N*-diethylbenzamide (LB60). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.14 (3H, s), 1.24 (3H, s), 3.35 (2H, m), 3.56 (2H, m), 7.11 (1H, m), 7.35−7.69 (6H, m), 7.89 (2H, m), 8.21 (1H, brs). LC/MS (ESI): *m*/*z* 296 (M + H).

***N*-[3-(Morpholin-4-ylcarbonyl)phenyl]benzamide (LB61).** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.35−3.56 (4H, m), 3.58−

3.98 (4H, m), 7.16 (1H, m), 7.31−8.09 (8H, m), 8.34 (1H, brs). LC/MS (ESI): *m*/*z* 308 (M + H).

3-(Benzoylamino)-*N*-benzyl-*N*-methylbenzamide (LB64). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.96 (3H, d, J = 8.8 Hz), 4.55 (2H, s), 4.74 (2H, s), 7.18 (2H, m), 7.31−7.88 (12H, m), 8.14−8.24 (1H, brs). LC/MS (ESI): *m*/*z* 344 (M + H).

3-(Benzoylamino)-*N*-(4-methylphenyl)benzamide (LB66). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.46 (3H, s), 7.10 (1H, brs), 7.31−7.88 (13H, m), 8.25 (1H, brs). LC/MS (ESI): *m*/*z* 330 (M + H).

3-(Benzoylamino)-*N*-pyridin-3-ylbenzamide (LB70). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.87 (1H, brs), 7.06−7.46 (4H, m), 7.58−7.71 (2H, m), 7.86−7.96 (2H, m), 8.12 (1H, m), 8.30 (1H, m), 8.50 (1H, m), 8.82 (1H, brs), 8.98 (1H, brs). LC/MS (ESI): *m*/*z* 317 (M + H).

3-(Benzoylamino)-*N*-1,3-thiazol-2-ylbenzamide (LB71). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.01 (1H, d, J = 4.8 Hz), 7.48−7.60 (5H, m), 7.80−8.14 (5H, m), 8.26 (1H, s). LC/MS (ESI): *m*/*z* 323 (M + H).

4-Acetamido-*N*-(tetrahydrofuran-2-ylmethyl)benzamide (LB78). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.85−2.15 (4H, m), 2.21 (3H, s), 3.31−3.41 (1H, m), 3.74−4.15 (4H, m), 6.54 (1H, brs), 7.38 (1H, brs), 7.46−7.52 (2H, d, J = 4.2 Hz), 7.72−7.78 (2H, d, J = 4.2 Hz). LC/MS (ESI): *m*/*z* 262 (M + H).

4-(Acetylamino)-*N*-(*sec*-butyl)benzamide (LB81). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.97 (3H, t, J = 8.0 Hz), 1.23 (3H, d, J = 4.8 Hz), 1.45−1.68 (2H, m), 2.20 (3H, s), 4.11−4.18 (1H, m), 5.79 (1H, brs), 7.48 (2H, d, J = 6.4 Hz), 7.62 (2H, d, J = 6.4 Hz). LC/MS (ESI): *m*/*z* 234 (M + H).

4-Acetamido-*N*-(2-methylphenyl)benzamide (LB89). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.20 (3H, s), 2.83 (3H, s), 6.85 (1H, brs), 7.38−7.52 (4H, m), 7.86−7.98 (2H, m), 7.93−8.06 (1H, m), 8.25 (1H, brs), 8.86 (1H, brs). LC/MS (ESI): *m*/*z* 268 (M + H).

4-Acetamido-*N*-pyridin-3-ylbenzamide (LB94). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.89 (3H, s), 6.54 (1H, brs), 7.38−7.52 (5H, m), 7.64−7.84 (2H, m), 8.25 (1H, brs), 8.86 (1H, brs). LC/MS (ESI): *m*/*z* 255 (M + H).

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(3) Modular high-throughput apparatuses include Myriad Core System, Myriad Personal Synthesizer (Mettler Toledo, Japan), Genesis multichannel liquid handler (Tecan, Manndorf, Switzerland), centrifugal evaporator (Genevac, Ipswich, U.K.), preparative HPLC purification system (Gilson, Wisconsin, U.S.A.), LC/Mass instruments (Shimadzu, Kyoto, Japan and Waters, Massachusetts, U.S.A.), and Automated weigher (Mettler Toledo, U.S.A.).

(4) Restriction limits are included in the Japanese laws. See *Industrial Safety and Health law*, law 57, article 28, clause 3; *Air pollution control law*, law 32, article 18, clause 21; and *Clean Water law*, law 35, article 3, clause 1.

(5) The stirring rack to contain the reaction vessels and the filter rack to hold phase separation tubes and the Exchanger 96 to transfer the vials from the polypropylene rack to the aluminum rack were all custom-made by Unitech Ltd. (Unitech, Osaka, Japan).

(6) Hird, N.; MacLachlan, W. Robotic Workstations and Systems. In *Laboratory Automation in the Chemical Industries*; Cork, D. G., Sugawara, T., Eds.; Marcel Dekker Inc.: New York, 2002; pp 1−39.

(7) Filter tubes (Catalogue No. 6984-0610, 1.0 μm) sold by Whatman Inc., Clifton, NJ. http://www.whatman.com (accessed 09/06/05).

(8) The top-phase filter tube was developed in collaboration with Wako Pure Chemical Industries Ltd. (Osaka, Japan), who now sell the tube as PreSep. Cork, D. G.; Inoue, K. PCT Japanese Patent No. 15238, 2003.

(9) Traces of DMF or other organic compounds in the aqueous phase may cause the aqueous phase to also gradually seep through the PTFE membranes. The vials containing the organic phase should, therefore, be removed for further processing soon after filtration is observed to stop.

(10) The Soltrapper customized apparatus was developed by Technosigma, Okayama, Japan, using their patented technology. Torii, S.; Miki, K. PCT Japanese Patent Nos. 113927A, 2004; 004317, 2004; 131504A, 2005.

(11) Dichloromethane was evaporated rapidly using SolTrapper, and recovery was >90%, which was approximately twice the typical recovery obtained using vacuum centrifugation.

(12) A synthesizer with six positions for 100-mL vessels was used (Metz; Radleys Discovery Technologies, Saffron Walden, U.K.).

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