Small-Molecule Library Synthesis on Silicon-Functionalized SynPhase Lanterns

Jeremy R. Duvall,[1] Anita Vrcic,[1] and Lisa A. Marcaurelle[1]

[1]Chemical Biology Platform, The Broad Institute of MIT and Harvard, Cambridge, Massachusetts

ABSTRACT

Silicon-functionalized SynPhase Lanterns are useful for the combinatorial synthesis of small-molecule libraries. Lanterns bearing an alkyl-tethered diisopropylarylsilane are first activated with triflic acid to afford the corresponding diisopropylsilyl triflate, which is then reacted with a library scaffold bearing a free alcohol. Once the scaffold has been loaded onto the solid phase, a variety of transformations can be run, including amine cappings, cross-coupling reactions, and amide bond formation. These reactions can yield a variety of products when run sequentially using split-pool synthesis strategies. Upon completion of the solid-phase transformations, the small molecules are released from the Lanterns using HF/pyridine. Using the techniques described here, libraries can be made ranging from a few compounds to >10,000 members in a highly efficient manner. *Curr. Protoc. Chem. Biol.* 2:135-151 © 2010 by John Wiley & Sons, Inc.

Keywords: solid-phase • combinatorial • synthesis • diversity • Lanterns • silicon

INTRODUCTION

Combinatorial chemistry is a powerful tool in the synthesis of small-molecule libraries for the development of biological probes and novel therapeutics (Crooks and Charles, 2000; Dolle et al., 2009). The use of combinatorial strategies, such as diversity-oriented synthesis (DOS; Nielsen and Schreiber, 2008), can afford highly diverse and structurally complex small molecules with great synthetic efficiency. Combinatorial libraries can be synthesized in either solution- or solid-phase formats, both of which have their merits. Solution-phase parallel synthesis has the benefit of being compatible with a broad spectrum of reactions and allows for easy reaction monitoring. Meanwhile, solid-phase synthesis has the advantage of employing simple "split-pool" techniques, allowing for rapid generation of large compound libraries without need for automation. Assuming a successful synthesis, products of solid-phase synthesis are generally of sufficient purity for biological testing, while libraries produced by solution-phase synthesis often require purification, typically by HPLC. This need for purification can be reduced or avoided through the judicious selection of solid-phase scavengers and reagents (Ley et al., 2000; Ley and Baxendale, 2002; Weinbrenner and Tzschucke, 2006).

Advances in solid-phase combinatorial synthesis have provided a variety of methods for compound immobilization, including different options for solid supports and a wide array of linkers (Scott, 2009). SynPhase Lanterns (*http://www.mimotopes.com*) are a practical alternative to conventional resins for library synthesis due to favorable reaction kinetics, easy handling, and simple washing procedures. Figure 1 shows a schematic of the composition of SynPhase Lanterns, as well as the different sizes offered by Mimotopes. SynPhase Lanterns are cylindrical in shape, containing a rigid polypropylene base coated with either a polystyrene (PS) or polyamide (PA) surface. The PS-Lanterns are most suitable for general organic synthesis in nonpolar solvents, while PA-Lanterns are useful for conducting reactions in hydrophilic or aqueous conditions. Many different linkers

Current Protocols in Chemical Biology 2: 135-151, July 2010
Published online July 2010 in Wiley Interscience (www.interscience.wiley.com).
DOI: 10.1002/9780470559277.ch100038

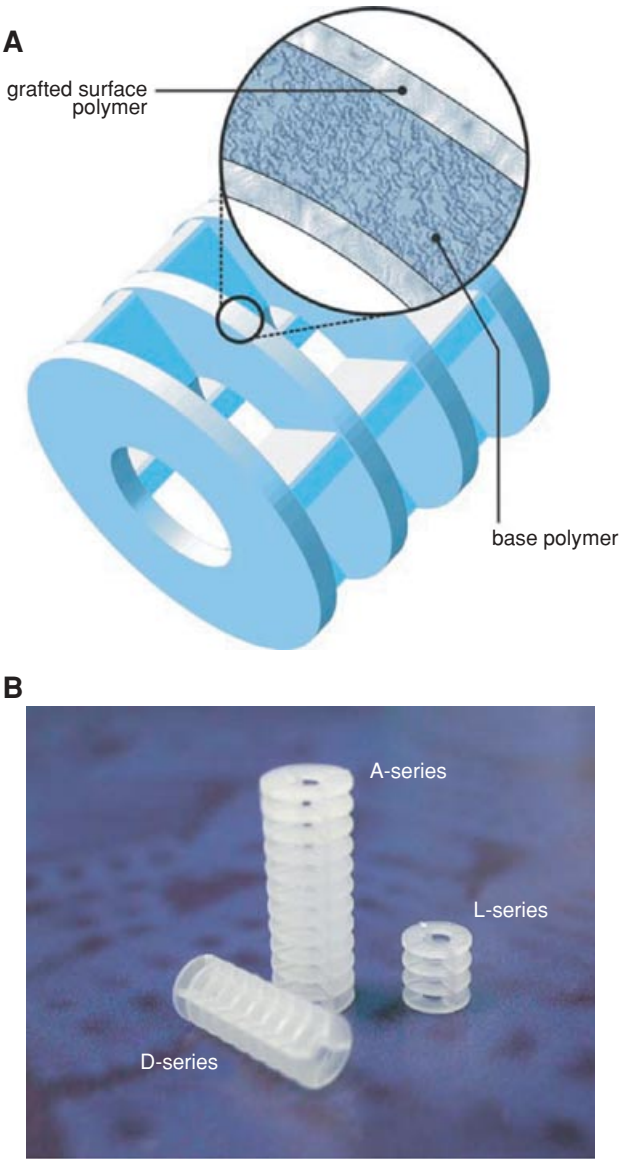

Figure 1 (**A**) Composition of SynPhase Lanterns: an unreactive base polymer and an outer polymer graft consisting of polystyrene or polyamide. (**B**) Lanterns are available in three sizes to accommodate different loading needs. A-series: 75 μmol/Lantern (5 mm × 5 mm). D-series: 35 μmol/Lantern (12.5 mm × 5 mm). L-series: 15 μmol/Lantern (17 mm × 6 mm). Figure reproduced with permission from Mimotopes.

are available, such as the Rink Amide (Verdié et al., 2008; Brucoli et al., 2009) and Backbone Amide (Zajdel et al., 2009) linkers. Herein, we focus on the L-series silicon-functionalized SynPhase PS-Lanterns (Ryba et al., 2009), due to the compatibility of this linker with a wide array of transformations and proven robustness for library synthesis (Tallarico et al., 2001; Taylor et al., 2004; Marcaurelle et al., 2009).

As depicted in Figure 2, the following protocol is divided into three sections: (1) activation of Lanterns and loading of the library scaffold, (2) solid-phase transformations of the immobilized scaffold via split-pool synthesis, and (3) cleavage of the small molecule from the Lantern. Activation of the Lantern is achieved via treatment with TfOH to form the intermediate diisopropyl silyl triflate. This reactive intermediate is then immediately treated with the library scaffold bearing an alcohol in the presence of excess

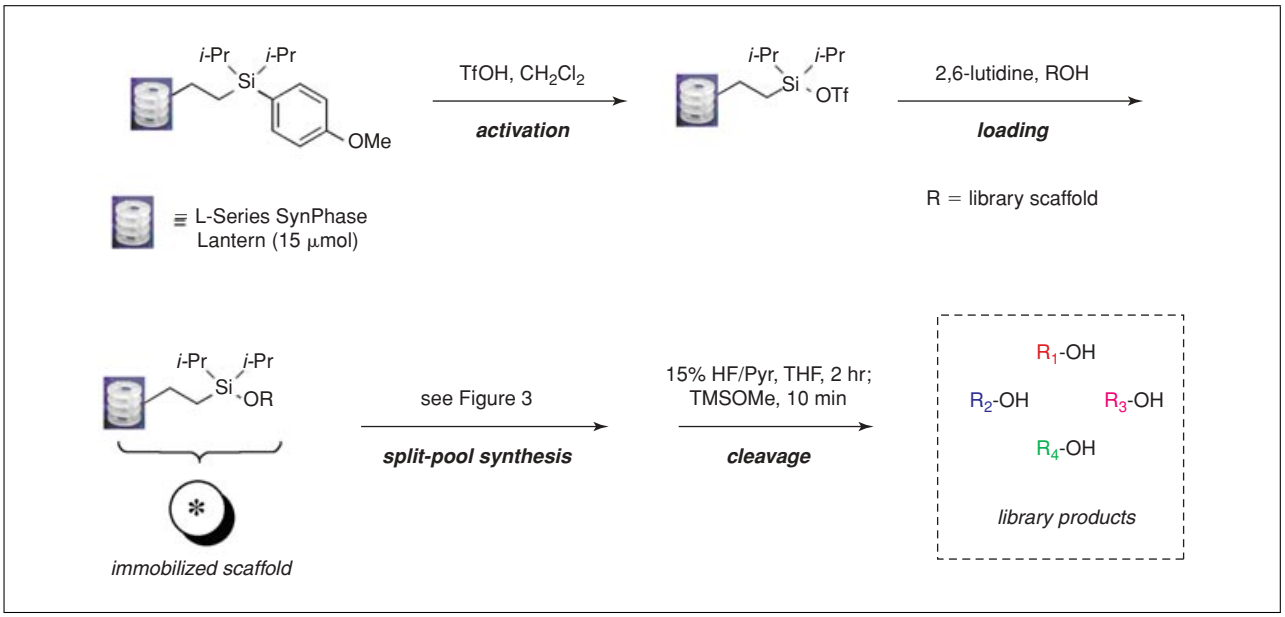

Figure 2 Use of silicon-functionalized Lanterns for library synthesis: activation, loading, split-pool synthesis, and cleavage.

2,6-lutidine to form a silyl ether. A variety of solid-phase transformations can then be carried out depending on the functional groups present on the library scaffold. Representative reactions compatible with the silyl ether linker are shown in Figure 3, and detailed procedures are provided in Basic Protocol 2. A number of nitrogen protecting groups are compatible with the silicon linker including Fmoc, Alloc, and Nosyl. Capping of the resulting amines can be achieved with sulfonyl chlorides, isocyanates, acids, and aldehydes to afford the corresponding sulfonamides, ureas, amides, and tertiary amines, respectively. An azide can also serve as a masked amine, as reduction can be performed with PBu_3 in aqueous THF. Alternatively, azides can be converted to triazoles via a Huisgen 1,3-dipolar cycloaddition with alkynes. Esters can be hydrolyzed under mild conditions (using KOTMS) to provide an acid for coupling with amines. Lastly, aryl halides can undergo cross-coupling reactions such as Suzuki and Sonogashira reactions, with boronic acid and alkynes, respectively. Cleavage of library products (Fig. 2) from the Lantern can be achieved via treatment with HF/pyridine in THF. Quenching of the reaction with TMSOMe provides volatile by-products TMSF and MeOH, which can be removed by evaporation.

STRATEGIC PLANNING

As mentioned above, solid-phase synthesis is a proven method for developing collections of small molecules, whether the objective is a small focused library for medicinal chemistry purposes or a large discovery library for initial screening efforts. Regardless of the type of library being synthesized, it is important to be mindful of the physicochemical properties (e.g., molecular weight, logP, polar surface area) of the library products at the outset of the synthesis (Blake, 2004). In order to prioritize library synthesis, cheminformatics methods such as principal component analysis (PCA; Feher and Schmidt, 2003), multi-fusion similarity (MFS) maps (Medina-Franco et al., 2007), ChemGPS (Oprea and Gottfries, 2001), and principal moments of intertia (PMI) plots (Sauer and Schwarz, 2003) can be utilized to analyze chemical diversity.

Having designed a set of small molecules for synthesis, another critical element is the tracking of compounds during the library production. There are several options for tagging Lanterns, depending on the library design. If the library size is large (>100 compounds),

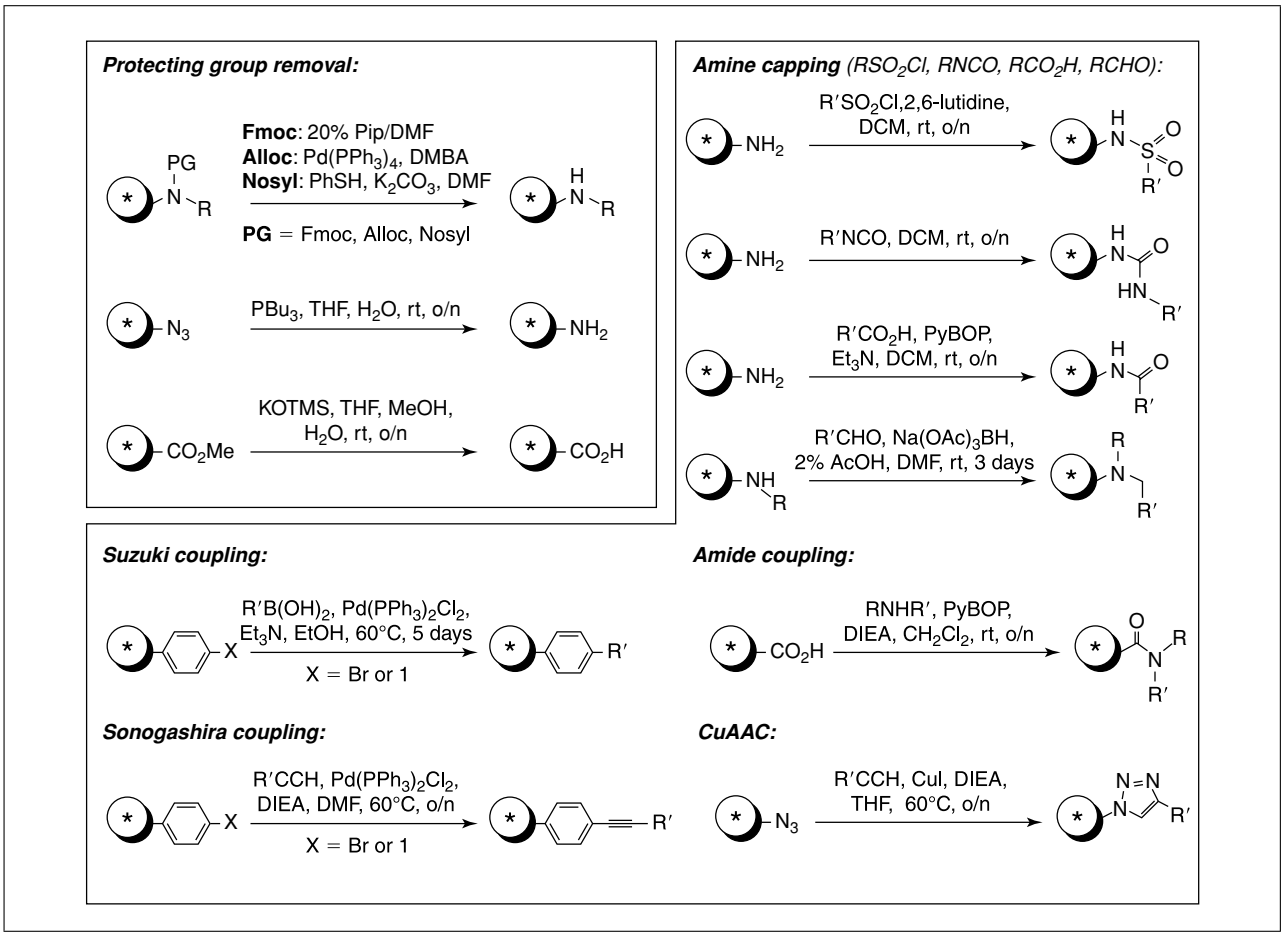

Figure 3 Representative solid-phase transformations useful for split-pool library synthesis on silicon-functionalized Lanterns.

radiofrequency (RF) stems can be used for their ease in sorting and identification of the immobilized small molecule. The RF Transtems (Fig. 4A) fit conveniently onto the Lanterns, and a Transort RF reader (Fig. 4C) can be used to sort the library at each synthesis step. RF-directed sorting requires the enumeration of the library and encoding of compound identities onto the Transtems. Several tools are available for library enumeration, including Pipeline Pilot, Chemaxon, Cambridgesoft, and Daylight. Enumeration and encoding for smaller libraries can be avoided by tracking members with colored spindles and cogs (Fig. 4B). Record keeping is essential when using this method of identifying compounds, especially for sorting Lanterns, to ensure that the intended compounds are synthesized.

To ensure that a library of high purity is synthesized, quality control (QC) Lanterns should be included at each synthesis step for LCMS (or NMR) analysis. Colored RF Transtems are commercially available for this purpose to allow easy identification from a reaction flask. For smaller libraries, the color-coded cogs and spindles can be used. If yield determination is required, then a full Lantern should be cleaved; otherwise Lanterns can be cut into quarters for cleavage and analysis to preserve material. When cleaving full Lanterns, the inclusion of multiple QC copies is recommended in case resubjection of the reaction is necessary. In general it is a good idea to determine yield at each step of the synthesis to ensure that premature cleavage from the Lantern is not occurring under the reaction conditions. The number of QC Lanterns per reaction flask will depend on the size of the library and the number of combinatorial steps. It is recommended to sample a variety of compounds per reaction, as the success of the reaction may depend on the building block used in the previous step.

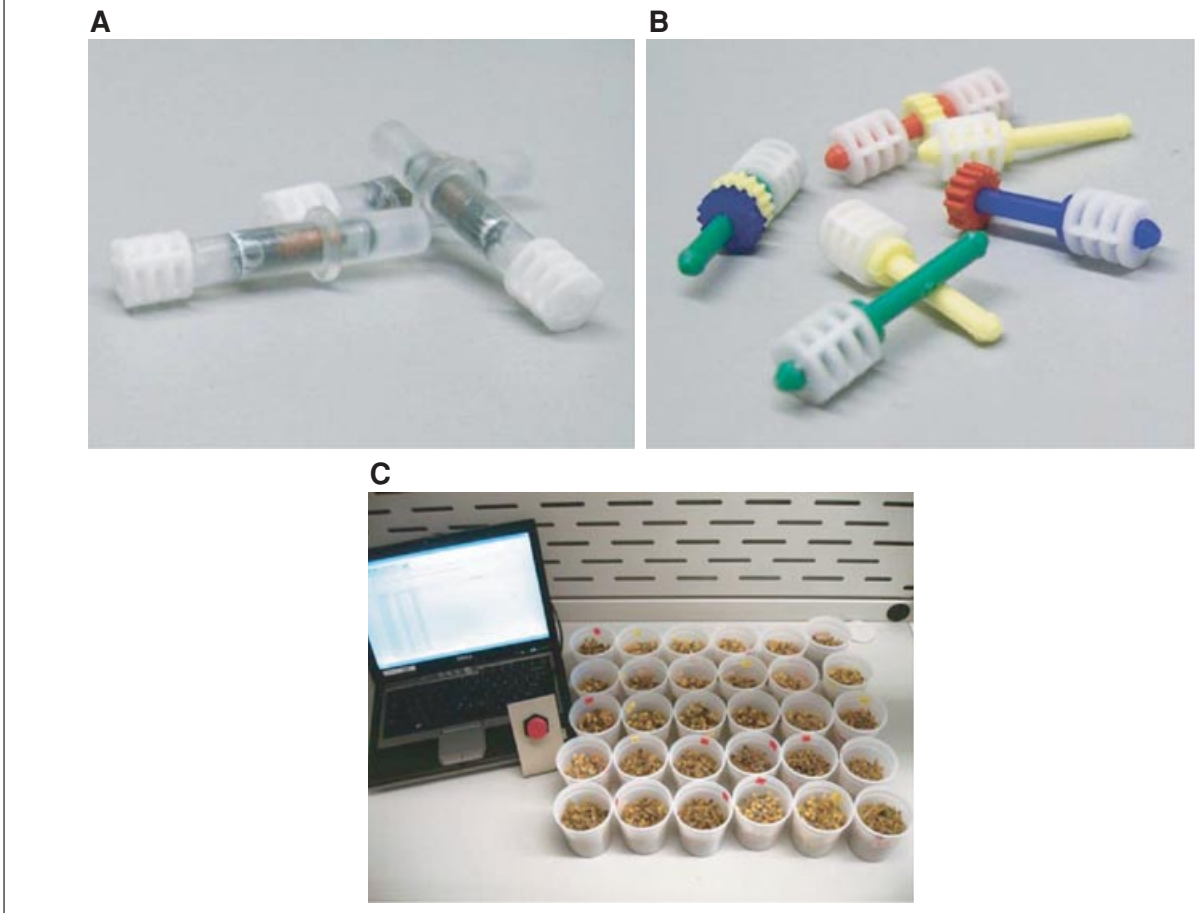

Figure 4 L-Series SynPhase Lanterns equipped with (**A**) radio frequency (RF) tags and (**B**) color-coded spindles and cogs. (**C**) Work station for RF-directed sorting.

LOADING OF A LIBRARY SCAFFOLD ONTO A SILICON-FUNCTIONALIZED LANTERN

BASIC PROTOCOL 1

Once a library has been designed and Lanterns have been suitably tagged and encoded (if necessary), production of the solid-phase library can begin. The following protocol describes the loading of a library scaffold bearing a free alcohol onto a silicon-functionalized L-series Lantern (~15 μmol/Lantern). After the loading has been completed and before subsequent reactions are run, QC analysis is carried out to determine the success of the loading. Generally, this is done via cleavage and recovery of the scaffold (see Basic Protocol 3), but Fmoc quantitation (Gude et al., 2002) can also be used, if applicable.

Materials

L-series alkyl tethered diisopropylarylsilane Lanterns (Mimotopes, cat. no. MIL10431000;*http://www.mimotopes.com*; also see Ryba et al., 2009)
Transtems (stems with enclosed RF transponder, Mimotopes, cat. no. MIT10260010)
Standard color tagging kit (colored cogs and spindles, Mimotopes, cat. no. MIT10430001)
3% trifluoromethanesulfonic acid solution in dichloromethane (TfOH in DCM; see recipe)
Dichloromethane (DCM, anhydrous for reactions; HPLC grade for washings)
Nitrogen source
2,6-lutidine (anhydrous; Aldrich, cat. no. 336106)

Library scaffold containing primary or secondary alcohol (coevaporated from benzene or toluene)
N,N-dimethylformamide (DMF, HPLC grade)
Tetrahydrofuran (THF, containing BHT as inhibitor)
Isopropanol

Oven-dried reaction vessel with screw top: e.g., ChemGlass, cat. no. CG-1880-42; (*http://www.chemglass.com/*) or Mimotopes, cat. no. MIA10140006 (*http://www.mimotopes.com*)
Incubator shaker (New Brunswick Scientific, model M1353-0004 or similar for large libraries)
Rubber septa (Sigma-Aldrich, cat. no. Z512222 or similar)
Washing apparatus (ceramic Buchner funnel and waste container)
Lyophilizer or high-vacuum manifold
SynPhase work station (cleavage tray, Lantern tray, stem ejector, SynPhase press and stem tray; Mimotopes, cat. no. MIA10910001)
Transort RF reader and software (Mimotopes, cat. no. MIT10520001)

CAUTION: TfOH is highly corrosive and personal contact can result in injury. Extreme caution should be exercised.

NOTE: Transtems can be attached to Lanterns using the SynPhase press (see SynPhase workstation) to enable RF sorting with the Transort reader and software.

Activate Lanterns with TfOH

1. To an oven-dried shaker vessel containing the Lanterns equipped with RF or color-coded tags (e.g., Transtems; see Fig. 4A), add a 3% TfOH solution in DCM under a flow of nitrogen. Add enough TfOH solution to cover all Lanterns. Cap vessel and shake at room temperature for 10 min.

   In general, for large-scale reactions (>100 Lanterns), the TfOH solution can be poured directly into the reaction vessel under a flow of nitrogen. For smaller reactions, a syringe can be used for transferring this reagent to a flask equipped with a rubber septum.

   The Lanterns should turn an orange or deep red color upon addition of TfOH.

Carry out loading

2. After 10 min, exchange the cap for a rubber septum and remove the TfOH solution with a syringe (or cannula) while maintaining an inert atmosphere (e.g., nitrogen) inside the flask.

3. Add anhydrous 2,6-lutidine (12.0 eq. with respect to Si). Purge with nitrogen and shake Lanterns for several minutes.

   The number of equivalents of reagent added are based on the equivalents of Si per Lantern. For L-series Lanterns, an average of 15 μmol/Lantern is observed.

   The Lanterns should turn white after several minutes. Add additional 2,6-lutidine as needed until Lanterns turn white.

4. Add the library scaffold (alcohol, 1.2 eq. with respect to Si) in anhydrous DCM via syringe or cannula and purge with nitrogen. Exchange rubber septum for a screw cap, and transfer reaction vessel to incubator shaker. Shake at room temperature overnight.

   The scaffold should be coevaporated with either benzene or toluene (benzene is recommended) prior to the loading to remove any residual water, and then placed on high vacuum overnight.

   DCM should be used conservatively during the loading to maintain as high a concentration of the scaffold as possible, while still covering all the Lanterns. A slight excess of solvent should be used to account for swelling of Lanterns.

For scaffolds with low reactivity or limited solubility in DCM, longer reaction times (up to 4 days) can be used to facilitate loading. In general, higher loading levels are achieved with primary alcohols than secondary alcohols.

Wash and dry Lanterns

5. After shaking overnight, remove the reaction mixture and set aside. Wash Lanterns with DCM for 5 min in the reaction vessel.

   The reaction mixture is kept until the analysis for the loading has been completed, to ensure that loading was successful. If the loading was unsuccessful, the scaffold can be salvaged from this mixture via acidic aqueous wash to remove excess 2,6-lutidine, and purified by silica gel purification.

   For washing Lanterns, the washing solvent and Lanterns are filtered through a ceramic Büchner funnel via gravity filtration, with the wash going into a waste container and the Lanterns being returned to the reaction flask. Alternatively, a drilled cap with filter holes (Mimotopes, cat. no. MIA10070012) can be used to drain the solvent from the reaction flask.

6. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM.

   THF containing BHT as an inhibitor should be used to avoid possible oxidation of amines.

7. Place the washed Lanterns on a lyophilizer or high vacuum overnight before proceeding to the next reaction.

Determine loading level

8. Follow cleavage protocol (see Basic Protocol 3) with three Lanterns for loading analysis.

   At least three Lanterns should be used to measure consistency of loading across all Lanterns. Expected loading amounts are calculated based on recovered amounts of compound and typically average ~15 μmol/Lantern. Where applicable, Fmoc quantitation (Gude et al., 2002) can also be used to measure success of loading. QC analysis ensures there was no decomposition during the loading step.

9. Sort the Lanterns using Transort RF reader and software for upcoming reactions, if necessary.

   If color-coded stems and cogs were used rather than RF tags, refer back to the master sheet to sort for the upcoming reactions.

SOLID-PHASE TRANSFORMATIONS OF SMALL-MOLECULE LIBRARIES ON LANTERNS

BASIC PROTOCOL 2

Once the loading of a library scaffold has been achieved, a split-pool combinatorial approach is applied for the introduction of appendage diversity. Below, a series of solid-phase transformations is presented that have been found to be compatible with silicon-functionalized Lanterns. The protocol includes conditions for the removal of a variety of protecting groups (e.g., Fmoc, Alloc, Nosyl), amine capping, amide coupling, azide-alkyne cycloaddition, and cross-coupling reactions. The reaction conditions provided are intended to be a starting point for use with a wide variety of building blocks (e.g., sulfonyl chloride, isocyanates, acids, etc.). Some optimization may be required. Once a solid-phase transformation has been carried out, LC-MS analysis can be utilized to determine the success of the reaction. If a reaction is deemed incomplete, the Lanterns can simply be re-subjected to the reaction conditions until the desired results are observed. Between synthesis steps, Lanterns can be sorted using either the color-coded spindles/cogs or RF tags (using the Transort reader). The next reaction can then be run, and the QC process repeated. This process is continued until the synthesis is complete.

The following set of procedures are not intended for sequential use, but rather implemented as desired to fit the design of the library. The success of each reaction may be dependent on the library scaffold used; therefore, some optimization may be required.

Materials

Lanterns, loaded (see Basic Protocol 1)
Tetrahydrofuran (THF), anhydrous, containing BHT inhibitor
Tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$; Aldrich, cat. no. 216666)
1,3-dimethylbarbituric acid (DMBA)
N,N-dimethylformamide (DMF, HPLC grade)
0.1 M sodium cyanide in a 1:1 THF/H_2O solution (prepare fresh)
Isopropanol
Dichloromethane (DCM, anhydrous for reactions; HPLC grade for washings)
20% (v/v) piperidine in DMF (store up to 1 month at 25°C)
Thiophenol
Potassium carbonate
Methanol (MeOH)
Potassium trimethylsilanolate (KOTMS; Aldrich, cat. no. 324868)
Tributylphosphine (PBu_3)
2,6-lutidine (anhydrous)
Sulfonyl chlorides
Isocyanates
Triethylamine (EtN_3, anhydrous)
Carboxylic acids
(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP; Aldrich, cat. no. 377848)
Sodium triacetoxyborohydride [$Na(OAc)_3BH$]
2% (v/v) acetic acid in DMF
Aldehydes
Ethanol (EtOH)
Boronic acids
Bis(triphenylphosphine)palladium(II) dichloride ($Pd(PPh_3)_2Cl_2$; Aldrich, cat. no. 412720)
Nitrogen source
Alkynes
N,N-diisopropylethylamine (DIEA)
Copper (I) iodide (CuI)
Amines

Oven-dried reaction vessel with screw top (e.g., ChemGlass, cat. no. CG-1880-42, Mimotopes, cat. no. MIA10140006)
Ceramic Büchner funnel
Incubator shaker (New Brunswick Scientific, model M1353-0004 or similar for large libraries)

Additional reagents and equipment for quality control analysis (Basic Protocol 3)

CAUTION: Some washes require the use of a solution of NaCN to remove residual metals (e.g., Pd, Cu). Exercise great caution when performing these washes as NaCN is highly toxic.

For Alloc removal

1a. To a reaction vessel containing Lanterns, add THF (0.8 ml/Lantern), followed by $Pd(PPh_3)_4$ (1 eq. with respect to Si), and 1,3-dimethylbarbituric acid (30 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

In general, 1,3-dimethylbarbituric acid is superior π-allyl scavenger as compared to other reagents (e.g., phenysilane, morpholine) for preventing N-allylation.

2a. Remove the reaction mixture and wash the Lanterns in the reaction vessel with DMF, followed by three washes (30 min each) with 0.1 M NaCN in 1:1 THF/H_2O, removing solvent after each wash by gravity filtration through a ceramic Büchner funnel.

3a. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete or *N*-allyation is observed, repeat starting at step 1a.

Fmoc removal

1b. To a reaction vessel containing Lanterns, add a solution of 20% piperidine in DMF (0.8 ml/Lantern). Seal the vessel and shake for 30 min.

2b. Remove the piperidine solution. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1b.

Nosyl removal

1c. To a reaction vessel containing Lanterns, add DMF (0.8 ml/Lantern) followed by thiophenol (20 eq. with respect to Si) and potassium carbonate (30 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2c. Remove the reaction mixture. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1c.

Ester hydrolysis

1d. To a reaction vessel containing Lanterns, add THF/MeOH/H_2O (5:2:2) (0.8 ml/Lantern) followed by KOTMS (10 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2d. Remove the reaction mixture. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1d.

Azide reduction

1e. To a reaction vessel containing Lanterns, add 9:1 THF/H_2O (0.8 ml/Lantern) followed by PBu_3 (10 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2e. Remove the reaction mixture, then add THF/H_2O (3:1). Seal the vessel and shake at room temperature for 6 hr.

3e. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1e.

Amine capping: sulfonyl chlorides

1f. To a reaction vessel containing Lanterns, add DCM (0.8 ml/Lantern) followed by 2,6-lutidine (25 eq. with respect to Si) and a selected sulfonyl chloride (20 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2f. Remove the reaction mixture, and wash Lanterns with DCM in Büchner funnel. Continue to Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1f.

Amine capping: isocyanates

1g. To a reaction vessel containing Lanterns, add DCM (0.8 ml/Lantern) followed by a selected isocyanate (15 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2g. Remove the reaction mixture and wash Lanterns with DCM in the reaction vessel. Continue to wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1g.

Amine capping: carboxylic acids

1h. To a reaction vessel containing Lanterns, add DCM (0.8 ml/Lantern), followed by Et_3N (30 eq. with respect to Si), a selected carboxylic acid (20 eq. with respect to Si), and PyBOP (20 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2h. Remove the reaction mixture and wash with DCM in the reaction vessel. Continue to wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1h.

Occasionally, using the above protocol with a primary amine, bis-capping can result in imide formation. Treatment with a 3:1 THF/pyrrolidine solution at room temperature overnight typically will provide the desired amide.

Amine capping: aldehydes

1i. To a reaction vessel containing Lanterns, add DMF containing 2% acetic acid (0.8 ml/Lantern) followed by a selected aldehyde (20 eq. with respect to Si). Seal the vessel and shake at room temperature for 1 hr.

2i. Remove cap and add $Na(OAc)_3BH$ (22 eq. with respect to Si). Seal the vessel and shake at room temperature for 3 days.

3i. Remove the reaction mixture and wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1i.

The amine capping with aldehydes is ideally suited for secondary amines (or the di-alkylation of primary amines), as it is difficult to selectively mono-alkylate primary amines using this protocol.

Cross coupling: boronic acids

1j. To a reaction vessel containing Lanterns add EtOH (0.8 ml/Lantern), followed by a selected boronic acid (20 eq. with respect to Si), Et_3N (40 eq. with respect to Si), and $Pd(PPh_3)_2Cl_2$ (1 eq. with respect to Si). Purge vessel with nitrogen, cap, and shake at 60°C for 5 days.

2j. Remove the reaction mixture and wash Lanterns with DMF several times in the reaction vessel. Next, wash Lanterns three times, each time for 30 min, with a solution of NaCN (0.1 M in 1:1 THF/H_2O solution).

3j. Continue to wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1j.

Cross coupling: alkynes

1k. To a reaction vessel containing Lanterns add DMF (0.8 ml/Lantern), followed by a selected alkyne (20 eq. with respect to Si), DIEA (30 eq. with respect to Si), CuI (30 eq. with respect to Si), and $Pd(PPh_3)_2Cl_2$ (2.0 eq. with respect to Si). Purge vessel with nitrogen, cap and shake at 60°C overnight.

2k. Remove the reaction mixture and wash Lanterns with DMF several times in the reaction vessel. Next, wash Lanterns three times with a solution of NaCN (0.1 M in 1:1 THF/H_2O).

3k. Remove the reaction mixture and wash Lanterns with DMF several times in the reaction vessel. Next, wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1k.

Cu-catalyzed azide/alkyne cycloaddition (CuAAC)

1l. To a reaction vessel containing Lanterns add THF (0.8 ml/Lantern) followed by a selected alkyne (30 eq. with respect to Si), CuI (10 eq. with respect to Si), and DIEA (30 eq. with respect to Si). Seal the vessel and shake at 60°C for 24 hr.

Although the example here is for solid-support azides, in theory, solid-supported alkynes could be used with a variety of azides to yield similar triazoles.

2l. Remove the reaction mixture and wash Lanterns with DMF several times in a Büchner funnel. Next, wash Lanterns three times, each time for 30 min, with a solution of NaCN (0.1 M in 1:1 THF/H_2O).

3l. Continue washing the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1l.

Amidation: PyBOP coupling

1m. To a reaction vessel containing Lanterns add a 3:1 DCM/DMF solution (0.8 ml/Lantern), followed by a selected amine (20 eq. with respect to Si), DIEA (10 eq. with respect to Si), and PyBOP (10 eq. with respect to Si). Seal the vessel and shake at room temperature overnight.

2m. Wash the Lanterns (in the reaction vessel) using the following solvents for 30 min each (~0.8 ml/Lantern): DMF (two washes), THF/H_2O (3:1), THF/isopropanol (3:1), THF, and DCM. Follow Basic Protocol 3 for QC analysis. If reaction is incomplete, repeat starting at step 1m.

CLEAVAGE OF FUNCTIONALIZED SCAFFOLD FROM SOLID SUPPORT

BASIC PROTOCOL 3

Once the desired solid-phase transformations have been performed, the product is removed from the Lantern via treatment with HF/pyridine. Cleavage may be carried out during the course of a library synthesis to determine yield after loading (see Basic Protocol 1) or to monitor the success of solid-phase reactions (see Basic Protocol 2). Here, a standardized cleavage protocol is presented for use with silicon-functionalized Lanterns, which can be carried out in a deep 96-well plate or microcentrifuge tube. When processing multiple samples at once, it is preferable to use a 96-well plate as this allows for

the use of a multichannel pipet. The following protocol describes cleavage of up to 96 Lanterns in a single 96-well plate using a multichannel pipet. If producing a large-scale library, the use of an automated liquid handler is preferred.

Materials

Lanterns, loaded, subjected to desired solid-phase transformations (see Basic Protocol 2)
Cleavage solution (see recipe)
Methoxytrimethylsilane (TMSOMe; 99%, Aldrich, cat. no. 253006-250g)
Methanol (MeOH)
Dichloroethane (DCE)
Dimethylsulfoxide (DMSO)

Labeled deep 96-well plate (Seahorse Biosciences, cat. no. XPO128; *http://www.seahorsebio.com/*)
Other 96-well plates to use as covers
Spreadsheet software
Multichannel pipettors and polypropylene reservoir
Labeled tube rack with preweighed 2-D barcoded glass mini-tubes (Tradewinds, 1.2-ml hi-recovery mini-tube, cat. no. 063227-0301, *http://www.twdtradewinds.com/*, or similar)
Centrifugal evaporator (e.g., Genevac HT12 or HT24, SP Industries)
Automated weighing station (e.g., FlexiWeigh, Mettler Toledo)
2-D barcode reader (e.g., VisionMatePlus, Thermo Fisher Scientific)

Additional reagents and equipment for liquid chromatography/mass spectrometry (LC-MS)

CAUTION: Use proper personal protection equipment when handling HF/pyridine (safety glasses, nitrile gloves, and lab coat). Labware that comes into contact with HF/pyridine should be quenched with methoxy- or ethoxytrimethylsilane. Prepare a solution of 25% methoxytrimethylsilane in THF and keep it nearby at all times.

NOTE: Remove any identifiers (Transtems or colored spindles) prior to cleavage. For removing Transtems, the use of a Stem Recycler Module (SynPhase stem recycler module for L- and D-series Lanterns; Mimotopes, cat. no. MIA10880001) is required.

Cleave Lanterns with HF/pyridine

1. Put one Lantern per well in a deep 96-well plate. Note the position of each Lantern in spreadsheet software.

   For testing at an intermediate stage of the synthesis, it is not necessary to cleave a whole Lantern if yield determination is not required. Lanterns can be cut into quarters using a razor blade. The amount of cleavage solution and TMSOMe should be reduced accordingly. For 1/4 Lantern, 200 μl of HF/pyridine solution is sufficient (see step 2).

   After extensive testing of different deep 96-well plates, Seahorse Biosciences cleavage plates were selected for use, as they released the least amount of detectable plasticizers.

2. Using multichannel pipet add 350 μl of cleavage solution to each well containing a Lantern.

   The Lantern should be fully submerged in cleavage solution.

   When using the multichannel pipet, the cleavage solution should be transferred from a polypropylene reservoir, due to the reactive nature of HF/pyridine and THF.

3. Cover cleavage plate with another 96-well plate and let sit for 3 hr at room temperature.

4. Quench reaction by adding two volumes (700 µl) of methoxytrimethylsilane.

   Addition of methoxytrimethylsilane should be slow, as the reaction is exothermic.

Transfer cleavage mixture to glass tubes

5. After 10 to 15 min, use a multichannel pipettor to mix the contents of the wells and transfer quenched solution from each well into its own preweighed 2-D barcoded glass tube (or other appropriate glass tube).

   Glass tubes should be used to avoid release of plasticizers into compound solution.

6. Add 200 µl of MeOH to each well containing Lanterns.

7. Using a multichannel pipettor, mix the contents of the wells to wash the Lanterns, and transfer the wash into its respective tube.

8. Dry contents of the tubes using centrifugal evaporator.

   To ensure proper drying conditions, program the evaporator to remove most of the THF first (~2 hr at 23 mbar; use REMP feature to prevent solvent bumping), then apply full vacuum for at least 12 hr to remove pyridine.

Determine yield and purity

9. Weigh tubes using automated weighing station.

10. Calculate yield for each compound by subtracting tare tube weight from filled tube weight. Determine loading level if required.

11. Dissolve product in 1:1 DCE/MeOH to ~10 mg/ml.

12. Remove a 5-µl aliquot and add it to 45 µl of DMSO. Mix well and analyze by LC-MS.

    Analysis can be done in LC-MS vial or from a 96- or 384-well microplate depending on the number of samples.

    At times the formation of a TMS adduct may be observed by LC-MS (M+72). This impurity can be reduced by resuspending the compound in MeOH, soaking for 4 hr at room temperature, and drying in a centrifugal evaporator.

REAGENTS AND SOLUTIONS

Use deionized water in all recipes and protocol steps.

Cleavage solution

To prepare 100 ml of cleavage solution, add 15 ml of 30% (v/v) hydrofluoric acid in pyridine (Aldrich, cat. no. 184225-100g) to 85 ml of anhydrous stabilized tetrahydrofuran (THF) containing BHT as an inhibitor (Aldrich, cat. no. 401757-1L). Use a polypropylene bottle to store the solution. The cleavage solution can be made in advance and stored in a freezer up to 3 months at −20°C for future use.

TfOH in DCM, 3%

Remove the Sure-Seal cap of a 100-ml Sure-Seal bottle of dichloromethane (Sigma-Aldrich; do not discard Sure-Seal cap) and pour 5 g of triflic acid (from a 5-g ampule; Aldrich, cat. no. 347817) directly into the dichloromethane bottle. Replace the Sure-Seal cap, along with the screw cap, and shake well. Use the solution soon after preparing.

COMMENTARY

Background Information

SynPhase PS-Lanterns produced by Mimotopes are solid supports containing a rigid polypropylene base coated with a polystyrene (PS) mobile surface. The unique "Lantern" shape allows for the attachment of snap-fitting tags for encoding purposes, and allows for the free flow of reactants and rapid drainage of washing solvents. PS-Lanterns can be functionalized with a variety of different linkers allowing for flexibility in loading strategies. For example, amines can be immobilized via reductive alkylation using Backbone Amide (BAL) Lanterns while carboxylic acids and phenols can be loaded through Hydroxymethylphenoxy (HMP) Lanterns. Meanwhile, primary and secondary alcohols can be loaded using a variety of linkers (Nam et al., 2003), including silicon-functionalized Lanterns (Ryba et al., 2009), now commercially available from Mimotopes. This latter technology was chosen as the focus of this article due to the compatibility and robustness of the linker for library synthesis applications (Tallarico et al., 2001; Taylor et al., 2004; Marcaurelle et al., 2009). Primary and secondary alcohols can also be used as a handle for affinity chromatography, small-molecule microarrays, and the attachment of biasing elements during subsequent follow-up in biological assays (Wong et al., 2003; Radner et al., 2006; Wang et al., 2008).

The mobile surface of the silicon-functionalized Lantern contains a *p*-methoxyphenyl (PMP)–protected diisopropylsilyl linker. Upon activation with triflic acid, the PMP group is removed and the subsequent silyl triflate is formed. Due to the highly reactive nature of silyl triflates, inert reaction conditions are required to prevent hydrolysis of the activated Lanterns, resulting in unsatisfactory loading. Under these inert conditions, in the presence of base, a scaffold containing a free alcohol can be easily immobilized onto the Lantern.

The silicon-functionalized linker can withstand a variety of reaction conditions, but does have certain limitations. Although the linker is stable to a wide range of basic conditions, including reductions and metal-mediated reactions, the use of strongly acidic conditions, as well as sources of fluoride, should be avoided, as this can result in premature cleavage of compound from the Lantern. In addition, while a library scaffold can be recovered if the loading is unsuccessful, the Lanterns cannot be reused or resubjected to the loading reaction. Thus, great care should be taken during the loading step.

Critical Parameters

The success of a library synthesis depends largely on the extent to which solid-phase feasibility studies are carried out prior to production. For example, loading levels may vary from one scaffold to another; thus, it is highly recommended that loading be tested on a minimum of 5 Lanterns prior to library synthesis. While the procedures provided in Basic Protocol 2 serve as a good starting point for solid-phase diversification, some optimization may be required, as the success of each reaction may be scaffold-dependent. It is recommended that a representative set of building blocks with varying reactivity (e.g., alkyl, aryl, electron-withdrawing, electron-donating, hindered) be tested under the suggested reaction conditions prior to library production. The cross-coupling reactions, in particular, may require the screening of multiple building blocks. Varying the recommended reaction conditions (e.g., temperature, solvent, Pd source) may also be necessary. In general, scouting reaction conditions in solution phase is not necessary, or even worthwhile, as the conditions often do not translate to the solid phase.

It is important to consider the sequence of steps to be employed in a library synthesis, particularly with respect to protecting groups. When the reaction sequence is not obvious, multiple options may be pursued in parallel during feasibility studies. In general, Fmoc is quite labile and should be removed early in the library synthesis, while Alloc is compatible with most solid-phase transformations with the exception of cross-coupling procedures. In order to prevent premature cleavage of products from the Lantern, protecting groups that require the use of harsh acid or base for removal should be avoided.

Other critical parameters for a successful library production include the use of reagents and scaffolds of high purity to ensure clean reactions, and, ultimately, clean products directly from the library production. In general, it is recommended that 3% to 5% of the library be sampled for QC to ensure good results overall. The synthesis of a pilot library (100 to 500 compounds) can also be beneficial prior to engaging in a large-scale (>1000 compounds) library production.

Troubleshooting

Poor loading

The easiest problem to diagnose when there is low loading of a library scaffold is poor solubility. If a scaffold has low solubility in DCM, the amount of solvent employed in the loading can be increased, and reaction time can be extended to up to 5 days to try improving loading levels. Sonication of the scaffold in DCM prior to addition to Lanterns may also be beneficial. If results are still suboptimal, then changes to the library scaffold may be required to improve solubility. For example, a simple change in protecting groups may facilitate dissolution in DCM. Heating the loading reaction generally does not lead to improved results, and cosolvents such as THF, DMF, or MeOH are incompatible with the loading reaction.

An unsuccessful loading reaction can also be due to moisture. Although difficult to detect, trace amounts of water in the triflic acid, 2,6-lutidine, or DCM can lead to poor loading results. The TfOH solution should always be prepared fresh prior to use, and the 2,6-lutidine obtained from an unopened Sure-Seal bottle (Sigma-Aldrich), or freshly distilled. The Lanterns should also be washed with anhydrous DCM and dried under high vacuum prior to use, and the library scaffold coevaporated from benzene or toluene. The use of an oven- or flame-dried reaction vessel is also recommended.

If the library scaffold is highly soluble and great care has been taken to exclude moisture from the loading reaction, and loading levels are still low (<10 μmol), the loading site may be inaccessible. In general, higher loading levels are obtained with primary alcohols than secondary alcohols; however, reasonable loading levels (>10 μmol) can be achieved for secondary alcohols if the loading site is not sterically hindered. Phenols can also be utilized as a handle for loading, but the aryl silyl ether linkage is slightly more sensitive to basic conditions. If modifications to the loading site are not feasible (or desirable), then the use of an alternate linker strategy may be needed.

Low conversion

Depending on the type of reaction or building block employed, low conversion may be observed that is not overcome by simply repeating the recommended reaction conditions. This typically can be remedied by employing harsher reaction conditions, assuming the required conditions are compatible with the silyl linker. For example, when capping an amine with a sterically hindered isocyanate, heating the reaction in toluene may be required to drive the reaction to completion. Meanwhile, sluggish reaction when capping an amine with a sulfonyl chloride can often be overcome by switching from 2,6-lutidine to pyridine. Reaction optimization will vary from substrate to substrate, and feasibility testing can prevent low conversion from happening during the actual library production.

Low purity

Reagent quality can have the greatest impact on the purity of final products. It is recommended that detailed and precise records be maintained during production of a library in case undesirable results are obtained for a particular building block. These records should also include reagent supplier information. In some cases, impurities may be traced back to a reagent. In other instances, a "combinatorial effect" may be observed where certain building-block combinations yield products of low purity. For the most part, this problem cannot be avoided even with thorough solid-phase feasibility studies.

A common impurity that may be observed upon HF cleavage is a TMS adduct (M+72), resulting from quenching of the reaction mixture with TMSOMe. Fortunately, this impurity can be reduced by treatment with MeOH at room temperature.

On rare occasions, unexpected rearrangements may occur under the HF/pyridine cleavage conditions. In such instances, the use of buffered HF/pyridine (e.g., 15% HF/pyridine in 1:1 THF/pyridine) may prevent or minimize undesired rearrangement. If the product yield is lower than expected using buffered conditions, then the cleavage can be repeated. For the cleavage of highly acid-sensitive products, the use of TBAF may be preferred. In this case, HPLC purification of the final products will be needed to remove excess TBAF.

For products containing tertiary amines, there is a risk of *N*-oxide formation if unstabilized THF is used during HF-cleavage or washing steps. This problem is easily detectible by LCMS (M+16). For this reason, THF containing BHT as an inhibitor of oxidation should be used for all washing and cleavage steps. The use of DCM/MeOH (or DCE/MeOH) is recommended for all volatile transfers post-cleavage, as the continued use of stabilized THF leads to an increase in BHT impurities.

Low yield

If a low yield of final product is obtained after treatment with HF/pyridine, a second round of cleavage should be carried out to ensure all compound has been removed from the Lantern. Assuming initial loading levels were satisfactory, if additional product is not recovered after a second cleavage event, this may indicate premature release of compound from the Lantern.

Anticipated Results

The loading step (Basic Protocol 1) typically yields ~15 μmol of core/Lantern for L-series Lanterns. During the subsequent steps, this number should remain constant and the final product obtained in similar amounts. The solid-phase manipulations are usually clean, but the conversions may not always be complete. Re-subjecting the Lanterns to the reaction conditions can be expected at some point during the library production. Driving the reaction to completion is always recommended unless byproducts begin to appear and counter the progress of increasing the conversion. Purity levels can vary based on the choice of reactions, building blocks, and number of steps. Feasibility studies and optimization of reactions should be carried out to ensure that a majority of the library is above the desired purity threshold. Typically, with the reactions described in Basic Protocol 2 and simple building blocks, >85% of the compounds can be expected to be >80% purity for a 4- to 6-step sequence.

Time Considerations

The amount of time needed to produce a library of small molecules using silicon-functionalized Lanterns depends on several factors and can range from a few weeks to several months. This timeframe does not include time required for solid-phase feasibility studies, which depends highly on the scaffold and associated chemistry. Very little time, relative to the whole, is spent on the library setup. The main factors in contributing to the length of time are size of the library, number of steps, and difficulty of the reaction sequence. The size of the library does not increase the reaction time, but does add more time during the sorting and cleavage steps, as well as LC-MS analysis. A very difficult reaction can result in poor conversion and the need for repeating a reaction step. Some reactions require longer reaction times (such as the Suzuki reaction), which may add to the overall production time line. For a medium- to large-sized library, typically 1 week should be set aside for each step in the library synthesis to account for reaction setup, reaction time (assuming overnight reaction), washing, drying, quality control, and sorting. For example, the solid-phase synthesis of 1000-membered library involving six synthesis steps, including loading, protecting group removal, amine capping, ester hydrolysis, amide coupling, and cleavage, would take approximately 6 weeks, assuming no resubjections are required.

Acknowledgments

This work was supported in part by CMLD funding from the NIH (Broad Institute CMLD; P50 GM069721). The authors would like to thank Dr. Lakshmi Akella for helpful reading of this Protocol.

Literature Cited

Blake, J.F. 2004. Integrating cheminformatic analysis in combinatorial chemistry. *Curr. Opin. Chem. Bio.* 8:407-411.

Brucoli, F., Howard, P.W., and Thurston, D.E. 2009. Efficient solid-phase synthesis of a library of distamycin analogs containing novel biaryl motifs on SynPhase Lanterns. *J. Comb. Chem.* 11:576-586.

Crooks, S.L. and Charles, L.J. 2000. Overview of combinatorial chemistry. *Curr. Protoc. Pharmacol.* 10:9.3.1-9.3.16.

Dolle, R.E., Le Bourdonnec, B., Goodman, A.J., Morales, G.A., Thomas, C.J., and Zhang, W. 2009. Comprehensive survey of chemical libraries for drug discovery and chemical biology: 2008. *J. Comb. Chem.* 11:739-790.

Feher, M. and Schmidt, J.M. 2003. Property distributions: Differences between drugs, natural products, and molecules from combinatorial chemistry. *J. Chem. Inf. Comput. Sci.* 43:218-227.

Gude, M., Ryf, J., and White, P.D. 2002. An accurate method for the quantitation of Fmoc-derivatized solid phase supports. *Lett. Pept. Sci.* 9:203-206.

Ley, S.V. and Baxendale, I.R. 2002. New tools and concepts for modern organic synthesis. *Nat. Rev. Drug Discov.* 1:573-586.

Ley, S.V., Baxendale, I.R., Bream, R.N., Jackson, P.S., Leach, A.G., Longbottom, D.A., Nesi, M., Scott, J.S., Storer, R.I., and Taylor, S.J. 2000. Multi-step organic synthesis using solid-supported reagents and scavengers: A new paradigm in chemical library generation. *J. Chem. Soc., Perkin Trans. 1* 3815-4195.

Marcaurelle, L.A., Johannes, C., Yohannes, D., Tillotson, B.P., and Mann, D. 2009. Diversity-oriented synthesis of a cytisine-inspired pyridone library leading to the discovery of novel

inhibitors of Bcl-2. *Bioorg. Med. Chem. Lett.* 19:2500-2503.

Medina-Franco, J.L., Maggiora, G.M., Giulianotti, M.A., Pinilla, C., and Houghten, R.A. 2007. A similarity-based data-fusion approach to the visual characterization and comparison of compound databases. *Chem. Biol. Drug Des.* 70:393-412.

Nam, N.-H., Sardari, S., and Parang, K. 2003. Reactions of solid-supported reagents and solid supports with alcohols and phenols through their hydroxyl functional group. *J. Comb. Chem.* 5:479-546.

Nielsen, T.E. and Schreiber, S.L. 2008. Towards the optimal screening collection: A synthesis strategy. *Angew. Chem. Int. Ed.* 47:48-56.

Oprea, T.I. and Gottfries, J. 2001. Chemography: The art of navigating in chemical space. *J. Comb. Chem.* 3:157-166.

Radner, J.E., McPherson, O.M., Mazitschek, R., Barnes-Seeman, D., Shen, J.P., Dhaliwal, J., Stevenson, K.E., Duffner, J.L., Park, S.B., Neuberg, D.S., Nghiem, P., Schreiber, S.L., and Koehler, A.N. 2006. A robust small-molecule microarray platform for screening cell lysates. *Chem. Biol.* 13:493-504.

Ryba, T.D., Depew, K.M., and Marcaurelle, L.A. 2009. Large scale preparation of silicon-functionalized SynPhase polystyrene Lanterns for solid-phase synthesis. *J. Comb. Chem.* 11:110-116.

Sauer, W.H.B. and Schwarz, M.K. 2003. Molecular shape diversity of combinatorial libraries: A prerequisite for broad bioactivity. *J. Chem. Inf. Comput. Sci.* 43:987-1003.

Scott, P.J.H. 2009. Linker strategies in solid-phase organic synthesis. Wiley, Chichester, U.K.

Tallarico, J.A., Depew, K.M., Pelish, H.E., Westwood, N.J., Lindsley, C.W., Shair, M.D., Schreiber, S.L., and Foley, M.A. 2001. An alkylsilyl-tethered, high-capacity solid support amenable to diversity-oriented synthesis for one-bead, one-stock solution chemical genetics. *J. Comb. Chem.* 3:312-318.

Taylor, S.J., Taylor, A.M., and Schreiber, S.L. 2004. Synthetic strategy toward skeletal diversity via solid-supported, otherwise unstable reactive intermediates. *Angew. Chem. Int. Ed.* 43:1681-1685.

Verdié, P., Subra, G., Averland-Petit, M.-C., Amblard, M., and Martinez, J. 2008. Solid-phase synthesis of 4-methylcarboxy-1,4-benzodiazepine-2,5-diones. *J. Comb. Chem.* 10:869-874.

Wang, X., Imber, B.S., and Schreiber, S.L. 2008. Small-molecule reagents for cellular pull-down experiments. *Bioconjugate Chem.* 19:585-587.

Weinbrenner, S. and Tzschucke, C.C. 2006. Purification principles in high-speed solution-phase synthesis. *In* Methods and Principles in Medicinal Chemistry, Vol. 26: Combinatorial Chemistry (W. Bannwarth, B. Hinzen, eds.) pp. 1-31. Wiley-VCH, Weinheim, Germany.

Wong, J.C., Hong, R., and Schreiber, S.L. 2003. Structural biasing elements for in-cell histone deacetylase paralog selectivity. *J. Am. Chem. Soc.* 125:5586-5587.

Zajdel, P., Subra, G., Verdie, P., Gabzdyl, E., Bojarski, A.J., Duszyńska, B., Martinez, J., and Pawłowski, M. 2009. Sulfonamides with the *N*-alkyl-*N′*-dialkylguanidine moiety as 5-HT_7 receptor ligands. *Bioorg. Med. Chem. Lett.* 19:4827-4831.

Internet Resources

http://www.mimotopes.com/knowledgeBase.asp?cid=26,34

An introduction to SynPhase Lanterns is provided on the Mimotopes Web site, as well as a variety of protocols for Lanterns with alternate linkers.