Fragment-based domain shuffling approach for the synthesis of pyran-based macrocycles

Eamon Comer[1], Haibo Liu[1], Adrien Joliton, Alexandre Clabaut, Christopher Johnson, Lakshmi B. Akella, and Lisa A. Marcaurelle[2]

Chemical Biology Platform, Broad Institute, Cambridge, MA 02142

Edited by Stuart L. Schreiber, Broad Institute, Cambridge, MA, and approved January 28, 2011 (received for review November 16, 2010)

Complexity and the presence of stereogenic centers have been correlated with success as compounds transition from discovery through the clinic. Here we describe the synthesis of a library of pyran-containing macrocycles with a high degree of structural complexity and up to five stereogenic centers. A key feature of the design strategy was to use a modular synthetic route with three fragments that can be readily interchanged or "shuffled" to produce subtly different variants with distinct molecular shapes. A total of 352 macrocycles were synthesized ranging in size from 14- to 16-membered rings. In order to facilitate the generation of stereostructure-activity relationships, the complete matrix of stereoisomers was prepared for each macrocycle. Solid-phase assisted parallel solution-phase techniques were employed to allow for rapid analogue generation. An intramolecular nitrile-activated nucleophilic aromatic substitution reaction was used for the key macrocyclization step.

diversity | library | stereochemistry | high throughput

It has been demonstrated that compounds with increased complexity and one or more stereogenic centers have molecular attributes that contribute to their success throughout the drug discovery process, including increased solubility (1) and greater selectivity (2, 3). The vast majority of small-molecule screening collections however are heavily populated with sp^2-rich compounds (3, 4), perhaps due, in part, to the abundant methods for their synthesis (5). Although natural products have served to augment screening collections as a source of complexity, structural analogs of natural products are generally difficult to access. In order to facilitate downstream discovery, an up-front investment should be made to devise synthetic pathways that will yield compounds that are poised for optimization (6–8). As a step toward this goal, we have developed a modular synthesis of a small-molecule library with features aimed to increase the probability of success in the optimization phases of drug or probe discovery. To this end, our approach was to prepare a library of compounds consisting of three fragments that could be readily modified, interchanged, or shuffled (9, 10) to produce distinct structural variants (Fig. 1). Such an approach should not only yield useful structure-activity relationships of initial library members but allow rapid access to derivatives for follow up medicinal chemistry efforts.

In designing a small-molecule library with increased structural complexity accessible by a domain shuffling (DS) strategy, we were inspired by the numerous pyran-containing macrocycles found in nature, such as rapamycin (11), bryostatin (12), and spongistatin (13), among others (14–18), which contain a high ratio of sp^3-hybridized atoms and stereogenic centers. In light of their unique structural features (19), macrocycles in general have been the focus of a number of library synthesis efforts (9, 20–27), although only a few libraries centered on pyran-containing macrocycles (24–26). Initially, we chose to target 16-membered rings using an intramolecular nucleophilic aromatic substitution (S_NAr) reaction (20, 21) as the key macrocyclization step. The three fragments used in the preparation of the macrocyclic library represent three different structural types, namely, cyclic (A), linear (B), and aromatic (C) by design (Fig. 1). The cyclic pyran unit (**1** or **2**), which contains three stereogenic centers, originated from the chiral pool, whereas the linear component, a β-methoxy γ-amino acid (**3**), containing two stereogenic centers, was synthesized using an asymmetric aldol reaction (20). The aromatic component (3- or 5-cyano-2-fluorobenzoic acid, **4**-*o* or **4**-*p*) were both obtained from commercial sources. The pyran fragment (A) is a bis-nucleophile, whereas the linear (B) and aromatic (C) components each contain an electrophile suitable to react with (A), as well as functionality that would react with each other. Thus, pyran **1** could react with acids **3** and **4** to produce macro-

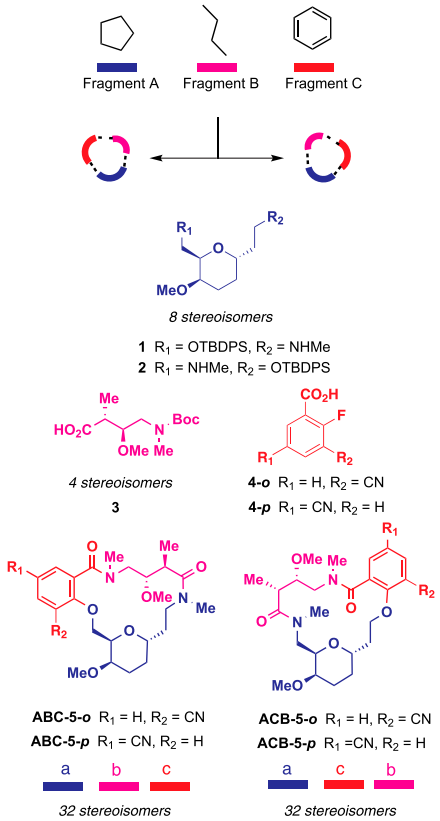


Fig. 1. Fragment-based DS strategy for the synthesis of pyran-based macrocycles.

Author contributions: E.C., H.L., and L.A.M. designed research; E.C., H.L., A.J., A.C., and C.J. performed research; L.B.A. performed computational work; E.C., H.L., C.J., L.B.A., and L.A.M. analyzed data; and E.C., H.L., L.B.A., and L.A.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]E.C. and H.L. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: lisa@broadinstitute.org.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015255108/-/DCSupplemental.

cycle **ABC-5**. Alternatively, pyran **2** can react with acids **3** and **4** to give the subtly different macrocycle **ACB-5**. In order to access valuable stereostructure-activity relationships (20, 28–30) directly from primary screening, we planned to synthesize all 32 stereoisomers for each macrocycle.

Results and Discussion

Synthesis of Pyran Fragments 1 and 2. The synthesis of pyran fragments **1** and **2** initiated with *C*-glycoside **6** (Scheme 1), which itself was derived from tri-*O*-acetyl D- and L-glycals according to methods previously developed in our laboratory (31). Methylation of **6** with Meerwein's reagent in the presence of Proton Sponge gave rise to the corresponding methyl ether in 69–94% yield, which in turn was hydrogenated under standard conditions (Pd/C, MeOH, H_2) to afford pyrans **7** in 94–100% yield. The resulting pyran ring systems served as a common precursor to fragments **1** and **2**. For the synthesis of pyrans **1**, the ester was reduced with lithium borohydride in the presence of a catalytic amount of lithium triethylborohydride (32). Oxidation of the resultant alcohol with Dess–Martin periodinane, followed by reductive amination with methylamine in the presence of sodium borohydride afforded the desired secondary amine **1** in 64–96% yield. This strategy was successful in generating all eight stereoisomers of pyran **1**.

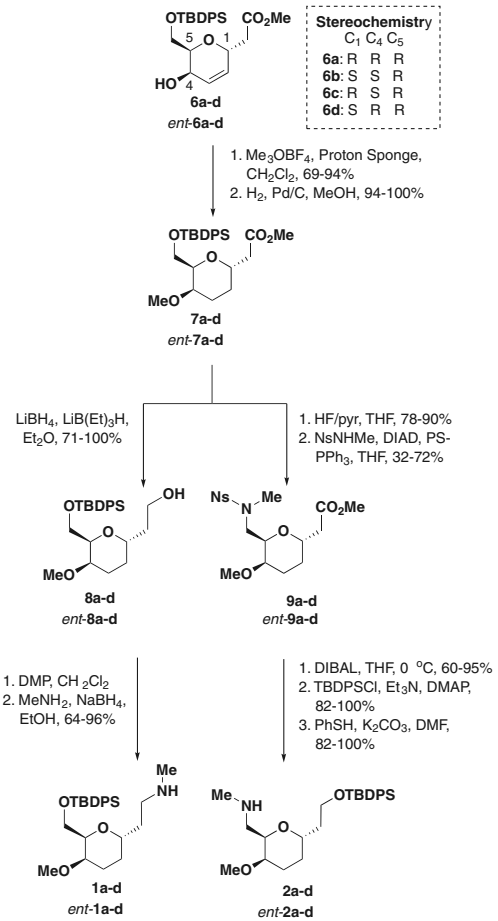
The synthesis of pyrans **2** began with the HF-pyridine-mediated removal of *tert*-butyldiphenylsilane (TBDPS) protecting group of **7**. The resulting primary alcohol was converted to the nosyl protected secondary amine **9** in 32–72% yield through a Mitsunobu reaction with *N*-methyl-2-nitrobenzenesulfonamide. Chemoselective ester reduction in the presence of nosyl group was achieved using diisobutylaluminum hydride at 0 °C (60–95% yield). Finally, TBDPS protection (82–100%) and nosyl deprotection (82–100%) led to the desired amine **2**. This reaction sequence was successfully applied to the synthesis of all eight stereoisomers of pyran **2**.

Synthesis of β-Hydroxy-γ-Amino Acids 3a-b. With the synthesis of pyrans **1** and **2** complete, we focused our attention to the preparation of the acyclic fragment, β-methoxy-γ-amino acid **3a-b**. The synthesis of **3** initiated from material prepared previously in our group, namely β-hydroxy-γ-amino acids **10**, accessible via asymmetric *syn*- and *anti*-aldol methodologies (20). As shown in Scheme 2, β-hydroxy-γ-amino acids **10** could be selectively methylated with NaH and MeI in THF at 0 °C to afford amino acids **3** in 77–98% yield.

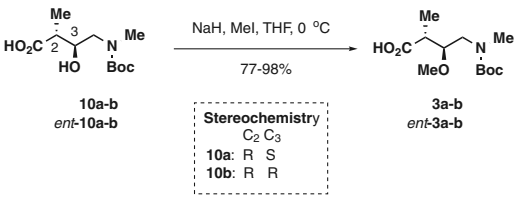
Parallel Solution-Phase Synthesis of Pyran-Containing Macrocycles. With all stereoisomers of pyrans **1** and **2** and γ-amino acids **3** in hand, we began to investigate methods for the union of these chiral fragments en route to macrocycles **ABC-5** and **ACB-5**. Although the efficiency of solid-phase synthesis for the preparation of large compound libraries has been well documented (33, 34), polymer-assisted parallel solution-phase synthesis offers several advantages (35, 36). Benefits include rapid analogue generation, reduced optimization time, and ease of reaction monitoring. We thus settled on a parallel solution-phase synthesis strategy utilizing silica-supported reagents and scavengers.

As shown in Scheme 3 we set out to produce a total of 128 products derived from monomers **1**–**3**, comprised of 32 stereoisomers and 2 regioisomers for each ring system. Acylation of pyran amines **1** and **2** with excess acid **3** in the presence of silica-bound dicyclohexylcarbodiimide (Si-DCC) and hydroxybenzotriazole (HOBt) proceeded smoothly to afford the corresponding amides. Residual acid was removed along with HOBt through the addition of a silica-bound tetraalkylammonium carbonate (Si-CO_3). Treatment with TFA in dichloromethane (DCM) efficiently removed both the *tert*-butoxycarbonyl (Boc) and TBDPS groups to provide the desired amino alcohols (**11** and **12**) along with a minor amount of the corresponding trifluoroacetyl esters. The latter underwent methanolysis in the presence of DCM:MeOH (3:1) and Si-CO_3 to afford the deprotected amino alcohols. Selective acylation of the amino alcohols with either the *ortho*- or *para*-regioisomer of benzoic acid **4** provided the corresponding amide S_NAr precursors. Again, the reaction products were treated with Si-CO_3 to remove excess benzoic acid and then with silica-bound carboxylic acid (Si-CO_2H) to remove any residual amine or potential products resulting from *O*-acylation (no bisacylation was observed).

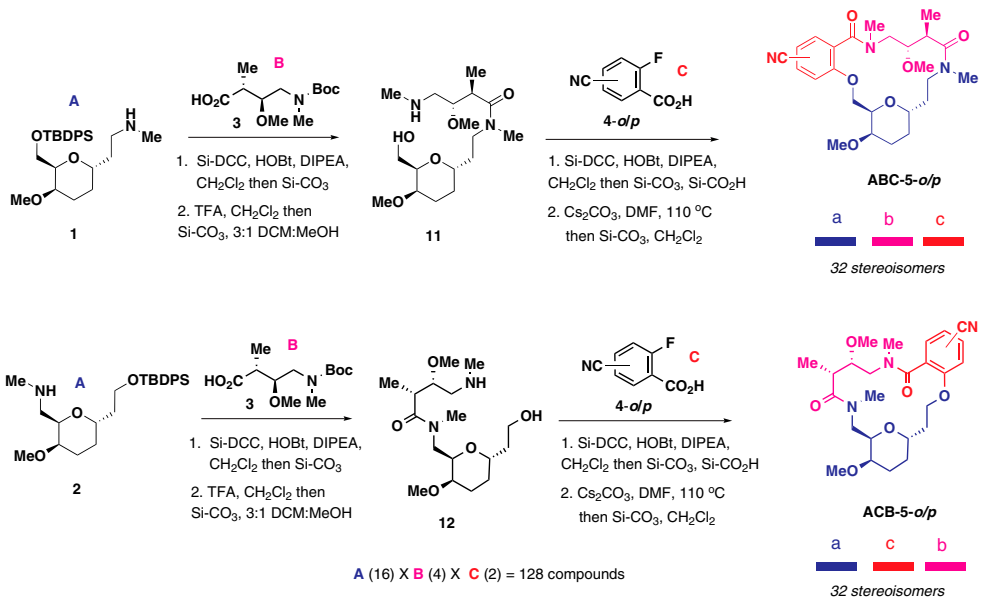
Traditionally intramolecular S_NAr reactions have been achieved using a nitro-activated aromatic component, however, given the toxicities associated with nitro-containing compounds (37), we instead chose to explore a nitrile-activated variant given the pharmaceutical relevance of aryl-nitriles (38). During initial feasibility studies, we found that nitrile-activated substrates underwent S_NAr cycloetherification with high efficiency. Aryl fluorides derived from **11** and **12** were treated with cesium carbonate and heated to 110 °C for 4 h to affect macrocyclization.



Scheme 1. Synthesis of pyran fragments **1** and **2** (only one stereoisomer shown).



Scheme 2. Preparation of chiral β-methoxy-γ-amino acid **3** (only one stereoisomer shown).



Scheme 3. Parallel solution-phase synthesis of pyran-containing macrocycles **ABC-5** and **ACB-5**.

For difficult macrocyclization reactions, aryl fluoride hydrolysis was occasionally observed. Fortunately, the corresponding phenol impurity could easily be removed upon treatment with Si-CO_3. Although intramolecular S_NAr reactions of nitrile-activated compounds have been reported (39), the work described here represents an example of a macrocyclization through this method.

Following completion of the four-step solution-phase synthesis the crude library products were subjected to mass-directed HPLC purification and analyzed by ultraperformance liquid chromatography-mass spectrometry (UPLC-MS). As shown in Table 1, the overall average purity of the library members ranged from 81–90% with the purity of macrocycles **ABC-5** (entries 1 and 2) being slightly greater than the corresponding **ACB-5** isomers (entries 3 and 4). The average isolated yield for the 128-membered library after HPLC purification was 22% over four steps with an overall pass rate of 95%.*

The modularity of the DS strategy allows for fragments to be easily interchanged thus facilitating rapid analogue synthesis including variations in ring size. To demonstrate this, commercially derived γ-amino acids **13** and **14**, along with β- and α-amino acids **15** and **16** (Fig. 2), were utilized for the preparation of 14- to 16-membered macrocycles **17**–**20** (Fig. 3).

As shown in Table 2, the efficiency of synthesis to access 16-membered macrocycles derived from γ-amino acids **13** and **14** was universally high in terms of purity (84–94%) with all compounds having purities >75% (entries 1–8). On average, the ABC macrocycles (**ABC-17** and **ABC-18**, entries 1, 2, 5, and 6) were isolated in higher yield than the corresponding ACB isomers (entries 3, 4, 7, and 8). In contrast, the shuffled 15-membered macrocycles **ACB-19** performed better in terms of overall purity and pass rate than their ABC counterparts (entries 9–12). Finally, the 14-membered compounds were isolated with similar purities (81–89%) for both the shuffled and nonshuffled systems (entries 13–16), although low yields were obtained for **ACB-20**-*p*(entry 16). The overall purity for the 224-membered sublibrary is satisfying at 87% given the complexity of the systems involved, especially considering that no attempts were made to tailor reaction conditions to each ring size. The overall average yield of 29% after HPLC purification provides ample material (approximately 6 mg) for future screening studies (the *SI Appendix* details full purity and yield analysis). Little differences were observed throughout the series between the *ortho*- and *para*-aromatic analogues, with the main determinants for success being the order of the fragments (ABC vs. ACB) and targeted ring size. Using this parallel synthesis strategy, typically 48-library members could be produced over the course of 1 wk by one chemist.

Physicochemical Properties and Shape Diversity of Library Members. We next examined various properties of the DS library as compared to two structurally distinct compound sets, namely natural products (AnalytiCon Discovery) and the National Institutes of Health Molecular Library Small Molecule Repository (MLSMR) (Table 3). The MLSMR serves to represent a typical small-molecule screening collection, because it is primarily comprised of commercial vendor libraries (>90%) (4). Not surprisingly, products resulting from the DS library more closely resemble natural products as compared to MLSMR compounds, having an increased number of stereogenic centers (>4) and greater structural complexity ($Fsp^3 = 0.59$). The degree of ring fusion is also higher with fewer aromatic ring systems than typical screening compounds. Whereas the oxygen count for the DS library is comparable to natural products, the nitrogen count is similar to that of MLSMR compounds. As an aside, it is interesting to note the differences in certain properties for natural products of different origins. Plant-derived natural products have a higher number of oxygen atoms, as well as stereogenic centers, as compared to microbial natural products. Lastly, all physico-

Table 1. Average purity and yield analysis for the formation of 16-membered macrocycles ABC-5 and ACB-5

Entry	Product	Ring size	Stereoisomers	% purity*	% pass rate†	% yield‡
1	**ABC-5**-*o*	16	32	90	100	29
2	**ABC-5**-*p*	16	32	90	100	16
3	**ACB-5**-*o*	16	32	86	94	26
4	**ACB-5**-*p*	16	32	81	84	18
5	Total		128	87	95	22

*Average purity was determined by UPLC monitoring at UV 210 nm.
†Pass rate is defined as the percentage of compounds with purity >75%.
‡Average isolated yield over four steps after HPLC purification (theoretical yield = 45 μmol).

*Library members with purity values <75% can be subjected to a second round of purification to access the full matrix of stereoisomers.

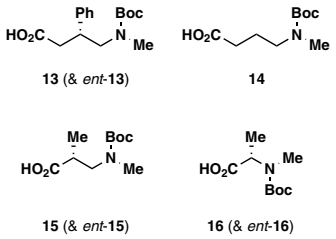


Fig. 2. Amino acids **13–16** (one enantiomer shown).

chemical properties affecting permeability and solubility (e.g., molecular weight, ALogP, rotatable bonds, topological polar surface area) for the DS library are within an acceptable range for a discovery library (40, 41).

Finally, in order to visualize the structural diversity obtained by the DS strategy, a molecular-shape-based diversity analysis derived from normalized principal moments of inertia ratios (42) was applied (Fig. 4). This simplistic representation categorizes molecular shape into three distinct topologies: rod-, disc-, and sphere-like character. It is gratifying to see the shape diversity brought about by the change in connectivity from ABC to ACB. Visual inspection shows the "nonshuffled" ABC scaffolds prefer the lower-right part of the triangle, whereas the shuffled ACB scaffolds occupy the upper-left part of the triangle. Varying ring size also contributed to the overall shape diversity.

Conclusions

We have developed a library of complex pyran-containing macrocycles with features that facilitate the initial discovery and subsequent optimization processes. A total of 352 macrocyles were prepared with increased structural complexity as compared to a typical screening collection. The library is modular and fragments can be readily shuffled or interchanged to afford analogues with distinct molecular shapes and ring sizes. The library anticipates a successful discovery phase by enabling rapid analogue synthesis both through the use of modular fragments and rapid solid-phase assisted parallel solution-phase techniques. The complete matrix of stereoisomers was prepared for each macrocycle. A single synthetic route afforded the collection of complex macrocycles with an average yield of 25% (over four steps) and an average purity of 87%.

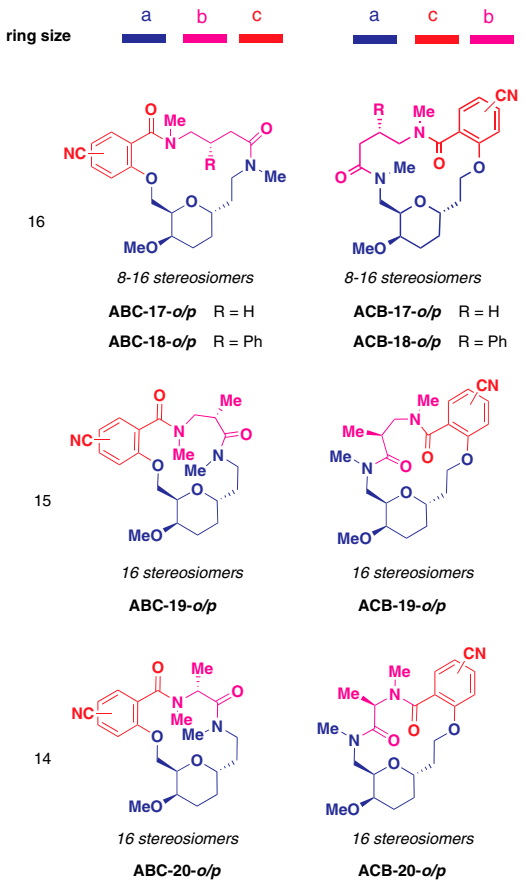


Fig. 3. Additional library members with varying ring size (only one stereoisomer shown).

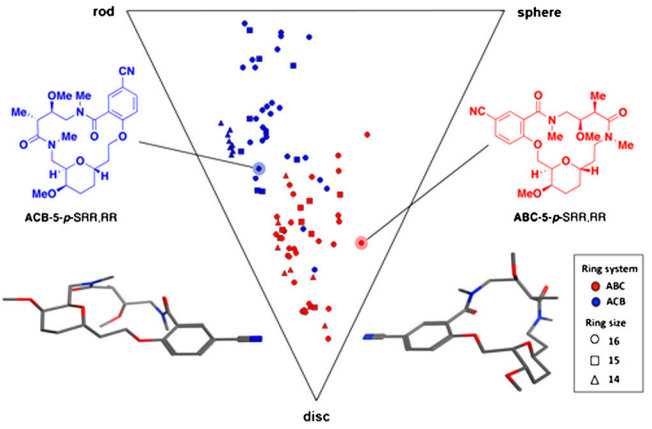


Fig. 4. Principal moments of inertia (PMI) analysis of the DS library products. PMI values (*x*, *y*, and *z*) were computed on the minimum energy conformer for each compound and normalized PMI ratios are plotted in the triangular scatter plot. The ABC (red) vs. ACB (blue) skeletons display significant shape diversity.

Materials and Methods

Details for the synthesis and characterization of all monomers are provided in the *SI Appendix*. SiliaBond® functionalized silica gels (Si-DCC, Si-CO_3, and Si-CO_2H) were purchased from SiliCycle. A Metler Toledo MiniBlock® and MiniBlock® XT were used for parallel synthesis. Compound purity and identity were determined by UPLC-MS (Waters). Purity was measured by UV absorbance at 210 nm. Identity was determined on a single quad mass spectrometer by positive electrospray ionization. Compound purification

Table 2. Average purity and yield analysis for macrocycles ABC-17-20 and ACB-17-20

Entry	Product	Ring size	Stereoisomers	% purity*	% pass rate†	% yield‡
1	**ABC-17-*o***	16	8	90	100	51
2	**ABC-17-*p***	16	8	84	100	47
3	**ACB-17-*o***	16	8	93	100	25
4	**ACB-17-*p***	16	8	94	100	35
5	**ABC-18-*o***	16	16	91	100	40
6	**ABC-18-*p***	16	16	86	100	37
7	**ACB-18-*o***	16	16	91	100	23
8	**ABC-18-*p***	16	16	92	100	28
9	**ABC-19-*o***	15	16	70	63	31
10	**ABC-19-*p***	15	16	72	63	24
11	**ACB-19-*o***	15	16	92	100	28
12	**ACB-19-*p***	15	16	90	100	30
13	**ABC-20-*o***	14	16	89	100	21
14	**ABC-20-*p***	14	16	88	100	17
15	**ACB-20-*o***	14	16	89	100	14
16	**ACB-20-*p***	14	16	81	94	6
17	Total		224	87	95	29

*Average purity was determined by UPLC monitoring at UV 210 nm.
†Pass rate is defined as the percentage of compounds with purity >75%.
‡Average isolated yield over four steps after HPLC purification (theoretical yield = 45 μmol).

Table 3. Mean values of different properties for DS library as compared to natural products and MLSMR

Property	DS library ($n = 352$)	NPs (plant/microbial)* ($n = 3,486/1,149$)	MLSMR† ($n = 337,890$)
Molecular weight	457	500/404	357
ALogP	1.4	1.6/2.2	2.9
TPSA	95	154/116	86
Rotatable bonds ‡	1.5	6.9/6.7	5.3
Nitrogen count	3.0	0.09/0.86	2.9
Oxygen count	5.4	9.7/6.5	3.0
Ring fusion degree	2.8	1.9/1.9	1.3
Aromatic to ring bonds	0.27	0.35/0.30	0.75
Stereogenic centers	4.3	8.0/4.1	0.4
Fsp^3	0.59	0.55/0.54	0.30

TPSA, topological polar surface area.
*Natural products (NPs) used for this analysis were obtained from the online database for AnalytiCon Discovery (www.ac-discovery.com).
†Compounds contained within the 2010 MLSMR were used for this analysis.
‡Rotatable bonds are defined as single bonds between heavy atoms that are not in a ring and not terminal.

was performed using a mass-directed approach on a Waters Autopurification System equipped with a ZQ mass spectrometer. Removal of organic solvents at intermediate stages of the synthesis was achieved using an EZ-2 Genevac centrifugal evaporator (or with the HT-24 Genevac workstation). HPLC-purified samples were concentrated using a VirTis 25L Genesis EL freeze dryer.

Amide Coupling. To a set of 16 test tubes equipped with stir bars was added Si-DCC (200 mg, 0.93 mmol/g), 1.0 mL solution of amine (20 mg, 0.045 mmol) in DCM, 1.0 mL solution of acid (0.077 mmol) in DCM. To the reaction mixture was then added 2.0 mL solution of HOBt and diisopropylethylamine (DIEA) in DCM, 0.038 mM and 0.135 mM, respectively. After 16 h, LC-MS showed complete coupling. Each reaction was scavenged with Si-CO_3 (400 mg, 0.63 mmol/g) for 30 min, filtered and dried on a Genevac for 3 h. The crude reaction products were taken on directly to the next step.

Boc/TBDPS Removal. Each reaction was treated with 0.2 mL TFA in 1.0 mL DCM for 3 h, then solvent was removed on a Genevac overnight. The samples were diluted with DCM (1.0 mL) and coevaporated for 30 min on a Genevac. The crude product was then treated with Si-CO_3 (400 mg, 063 mmol/g) in MeOH/DCM (4.0 mL, 1:3 vol/vol) to hydrolyze the TFA-ester. After filtration, solvents were removed on a Genevac for 1.5 h, followed by coevaporation with DCM (1.0 mL x2). Both Boc and TBDPS were removed cleanly based on LC-MS data. Crude products were carried on to the next step without purification.

Acylation. The crude amino alcohols, cyano-fluorobenzoic acid **4**-*o* or **4**-*p* (0.022 g, 0.135 mmol), Si-DCC (200 mg, 0.93 mmol/g), and DIEA (0.016 mL, 0.090 mmol) were combined in 2% dimethylformamide (DMF)/DCM (3.0 mL), and stirred at room temperature overnight. In cases where acylation is slow, additional Si-DCC (200 mg) and a solution of HOBt (5 mg) in DMF/DCM (1.0 mL) and DIEA base (6 μL) were added. After acylation was deemed complete, reactions were scavenged with Si-CO_3 (400 mg per reaction) and Si-CO_2H (200 mg per reaction) for 30 min and then filtered and evaporated on a Genevac for 4 h.

S_NAr Macrocyclization. All crude products from above were dissolved in DMF (4.0 mL) and heated at 110 °C with Cs_2CO_3 (approximately 200 mg per reaction) for 4 h. Reaction mixtures were filtered through Celite, washed with DCM, and solvents removed on a Genevac. Crude products were dissolved in DCM and treated with Si-CO_3 (400 mg) and Si-CO_2H (200 mg) for 30 min, and then filtered through Celite and concentrated on a Genevac. The crude products were purified by mass-directed preparative HPLC, dried via lyophilization, and analyzed by LC-MS.

ACKNOWLEDGMENTS. This work was funded in part by the National Institute of General Medical Sciences sponsored Center of Excellence in Chemical Methodology and Library Development (Broad Institute, P50 GM069721), as well as the National Institutes of Health (NIH) Genomics-Based Drug Discovery U54 Grants Discovery Pipeline RL1CA133834 (administratively linked to NIH Grants RL1HG004671, RL1GM084437, and UL1RR024924).

1. Lovering F, Bikker J, Humblet C (2009) Escape from flatland: Increasing saturation as an approach to improving clinical success. *J Med Chem* 52:6752–6756.
2. Clemons PA, et al. (2010) Small molecules of different origins have distinct distributions of structural complexity that correlate with protein-binding profiles. *Proc Natl Acad Sci USA* 107:18787–18792.
3. Feher M, Schmidt JM (2003) Property distributions: Differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci* 43:218–227.
4. Dandapani S, Marcaurelle LA (2010) Accessing new chemical space for "undruggable" targets. *Nat Chem Biol* 6:861–863.
5. Cooper TWJ, Campbell IB, Macdonald SJF (2010) Factors determining the selection of organic reactions by medicinal chemists and the use of these reactions in arrays (small focused libraries). *Angew Chem Int Ed* 49:8082–8091.
6. Kodadek T (2010) Rethinking screening. *Nat Chem Biol* 6:162–165.
7. Schreiber SL (2009) Molecular diversity by design. *Nature* 457:153–154.
8. Nielsen TE, Schreiber SL (2008) Towards the optimal screening collection: A synthesis strategy. *Angew Chem* 47:48–56.
9. Looper R, Pizzirani D, Schreiber S (2006) Macrocycloadditions leading to conformationally restricted small molecules. *Org Lett* 8:2063–2066.
10. Su S, et al. (2005) Convergent synthesis of a complex oxime library using chemical domain shuffling. *Org Lett* 7:2751–2754.
11. Schreiber SL (1991) Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* 251:283–287.
12. Hale KJ, Hummersone MG, Manaviazar S, Frigerio M (2002) The chemistry and biology of the bryostatin antitumour macrolides. *Nat Prod Rep* 19:413–453.
13. Pietruszka J (1998) Spongistatins, cynachyrolides or altohyrtins? Marine macrolides in cancer therapy. *Angew Chem Int Ed* 37:2629–2636.
14. Cereghetti DM, Carreira EM (2006) Amphotericin B: 50 years of chemistry and biochemistry. *Synthesis* 6:914–942.
15. Smith AB, Dong S, Brenneman JB, Fox RJ (2009) Total synthesis of (+)-sorangicin A. *J Am Chem Soc* 131:12109–12111.
16. Nicolaou KC, et al. (1996) Total synthesis of swinholide A. *J Am Chem Soc* 118:3059–3060.
17. Hoye TR, Danielson ME, May AE, Zhao H (2010) Total synthesis of callipeltoside A. *J Org Chem* 75:7052–7060.
18. Jackson KL, Henderson JA, Phillips AJ (2009) The Halichondrins: Chemistry and biology. *Chem Rev* 109:3044–3079.
19. Driggers EM, Hale SP, Lee J, Terrett NK (2008) The exploration of macrocycles for drug discovery—an underexploited structural class. *Nat Rev Drug Discovery* 7:608–624.
20. Marcaurelle LM, et al. (2010) An aldol-based build/couple/pair strategy for the synthesis of medium- and large-sized rings: Discovery of macrocyclic histone deacetylase inhibitors. *J Am Chem Soc* 132:16962–16976.
21. Goldberg M, Smith L, II, Tamayo N, Kiselyov AS (1999) Solid-supported synthesis of 14-membered macrocycles containing 4-hydroxyproline structural unit via SNAr methodology. *Tetrahedron* 55:13887–13898.
22. Peng LF, Stanton BZ, Maloof N, Wang X, Schreiber SL (2009) Syntheses of amino-alcohol derived macrocycles leading to the small-molecule binder to and inhibitor of Sonic Hedgehog. *Bioorg Med Chem Lett* 19:6319–6325.
23. Grimwood ME, Hansen HC (2009) Synthesis of macrocyclic scaffolds suitable for diversity-oriented synthesis of macrolides. *Tetrahedron* 65:8132–8138.
24. Beeler AB, et al. (2005) Synthesis of a library of complex macrodiolides employing cyclodimerization of hydroxy esters. *J Comb Chem* 7:673–681.
25. Grimwood ME, Hansen HC (2009) Synthesis of macrocyclic scaffolds suitable for diversity-oriented synthesis of macrolides. *Tetrahedron* 65:8132–8138.
26. Kim YK, et al. (2004) Relationship of stereochemical and skeletal diversity of small molecules to cellular measurement space. *J Am Chem Soc* 126:14740–14745.
27. Wessjohann LA, Ruijter E (2005) Strategies for total and diversity-oriented synthesis of natural product(-like) macrocycles. *Top Curr Chem* 243:137–184.
28. Zhang Q, Lu H, Richard C, Curran DP (2004) Fluorous mixture synthesis of stereoisomer libraries: Total synthesis of (+)-murisolin and fifteen diastereomers. *J Am Chem Soc* 126:36–37.
29. Dandapani S, Jeske M, Curran DP (2005) Synthesis of all 16 stereoisomers of pinesaw fly sex pheromones—tools and tactics for solving problems in fluorous mixture synthesis. *J Org Chem* 70:9447–9462.
30. Wrona IE, et al. (2009) Synthesis of a 35-member stereoisomer library of bistramide A: Evaluation of effects of actin state, cell cycle and tumor cell growth. *J Org Chem* 74:1897–1916.

31. Gerard B, et al. (2011) Large-scale synthesis of all stereoisomers of a 2,3-unsaturated *C*-glycoside scaffold. *J Org Chem*, in press.
32. Brown HC, Narasimhan S (1982) New powerful catalysts for the reduction of esters by lithium borohydride. *J Org Chem* 47:1604–1606.
33. Hudson D (1999) Matrix assisted synthetic transformations: A mosaic of diverse contributions. I. The pattern emerges. *J Comb Chem* 1:333–360.
34. Hudson D (1999) Matrix assisted synthetic transformations: A mosaic of diverse contributions. II. The pattern is completed. *J Comb Chem* 1:403–457.
35. Flynn DL, Devraj RV, Parlow J (1998) Recent advances in polymer-assisted solution-phase chemical library synthesis and purification. *Curr Opin Drug Discovery Dev* 1:41–50.
36. Ley SV, et al. (2000) Multi-step organic synthesis using solid-supported reagents and scavengers: A new paradigm in chemical library generation. *J Chem Soc Perkin Trans 1* 23:3815–4195.
37. Purohit V, Basu AK (2000) Mutagenicity of nitroaromatic compounds. *Chem Res Toxicol* 13:673–692.
38. Fleming FF, Yao L, Ravikumar PC, Funk L, Shook BC (2010) Nitrile containing pharmaceuticals: efficacious roles of the nitrile pharmacophore. *J Med Chem* 53:7902–7917.
39. Marivingt-Mounir C, Mettey Y, Vierfond JM (1998) A facile synthesis of cyanophenothiazines. *J Heterocycl Chem* 35:843–845.
40. Blake JF (2004) Integrating cheminformatic analysis in combinatorial chemistry. *Curr Opin Chem Biol* 8:407–411.
41. Shelat AA, Guy RK (2007) The interdependence between screening methods and screening libraries. *Curr Opin Chem Biol* 11:244–251.
42. Sauer WHB, Schwarz MK (2003) Molecular shape diversity of combinatorial libraries: A prerequisite for broad bioactivitiy. *J Chem Inf Comput Sci* 43:987–1003.