ACS Combinatorial Science

Research Article

pubs.acs.org/acscombsci

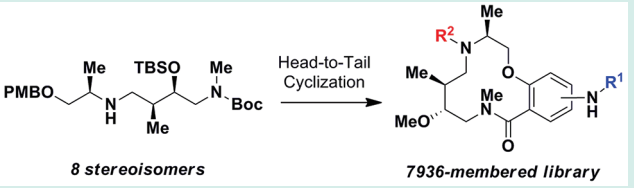
Build/Couple/Pair Strategy for the Synthesis of Stereochemically Diverse Macrolactams via Head-to-Tail Cyclization

Mark E. Fitzgerald, Carol A. Mulrooney, Jeremy R. Duvall, Jingqiang Wei, Byung-Chul Suh, Lakshmi B. Akella, Anita Vrcic, and Lisa A. Marcaurelle*,†

Chemical Biology Platform, Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, United States

Supporting Information

ABSTRACT: A build/couple/pair (B/C/P) strategy was employed to generate a library of 7936 stereochemically diverse 12-membered macrolactams. All 8 stereoisomers of a common linear amine precursor were elaborated to form the corresponding 8 stereoisomers of two regioisomeric macrocyclic scaffolds via head-to-tail cyclization. Subsequently, these 16 scaffolds were further diversified via capping of two amine functionalities on SynPhase Lanterns. Reagents used for solid-phase diversification were selected using a sparse matrix design strategy with the aim of maximizing coverage of chemical space while adhering to a preset range of physicochemical properties.



KEYWORDS: *diversity-oriented synthesis, macrocycle, stereochemistry, solid-phase synthesis*

INTRODUCTION

We have a long-standing interest in the design and synthesis of small-molecule libraries that combine the structural complexity of natural products and the efficiency of high-throughput synthesis.[1−4] Recent evidence has shown the importance of structural complexity as measured by sp^3 content and the presence of stereocenters for the successful transition from discovery to the clinic.[5] As such, macrocycles are an important class of organic molecules showing a variety of impressive biological activities.[6,7] There are numerous macrocycles currently in development or approved by the FDA for clinical use, including natural products (**1**, Figure 1)[8] and their derivatives (**2**),[9] as well as some designed and synthesized de novo (**3**).[10] The large occurrence of macrocycles in nature and their impressive biological activities may be derived from the fact that they often exhibit both preorganization as well as flexibility, both of which can aid binding to biological targets.[11,12] Based off of these observations, we have utilized macrocyclic compounds in the past as a source of diversity and structural complexity within our screening collection.[1−3] In this work, we present a continuation of this effort to build a small-molecule library that contains macrocyclic compounds but occupies a distinct area of chemical space.

Utilizing a three phase build/couple/pair (B/C/P) synthetic strategy,[13] we have previously reported a route to medium-sized rings and macrocycles (Figure 2) starting from 1,2-amino alcohol **4** and aldol-derived γ-amino acid **5**.[1,14] A key feature of the B/C/P strategy is the ability to generate the complete matrix of stereoisomers lending to the study of stereo/structure−activity relationships (SSAR) upon biological screening.[1,15] Thus all eight stereoisomers of amine **6** were prepared and utilized in downstream pairing reactions including nucleophilic aromatic substitution (S_NAr), Huisgen macrocyclization and ring-closing metathesis (RCM) to yield scaffolds **7**−**11**. In the current study, we have expanded this chemistry to include a pairing reaction, which cyclizes the linear template from "head-to-tail" (HtoT) leading to macrocycles **12** and **13**. This pairing strategy increases the number of stereogenic centers contained within the ring as compared to the previously prepared scaffolds, as well as decreases the number of rotatable bonds present in the final compounds. As evident by a molecular shape-based diversity analysis derived from

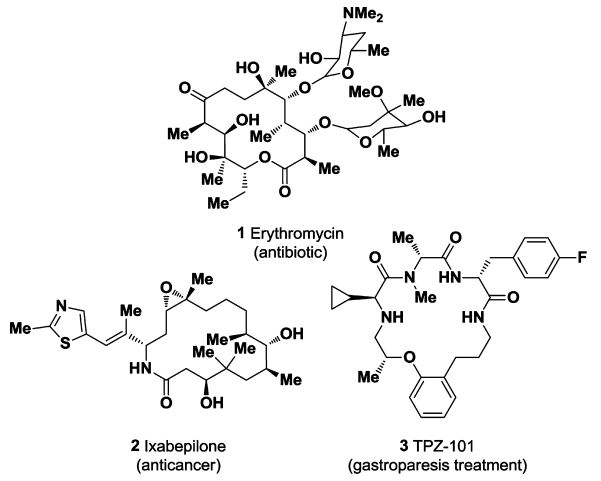


Figure 1. Select bioactive macrocycles in development or approved for clinical use.

Received: September 29, 2011
Revised: December 20, 2011
Published: January 17, 2012

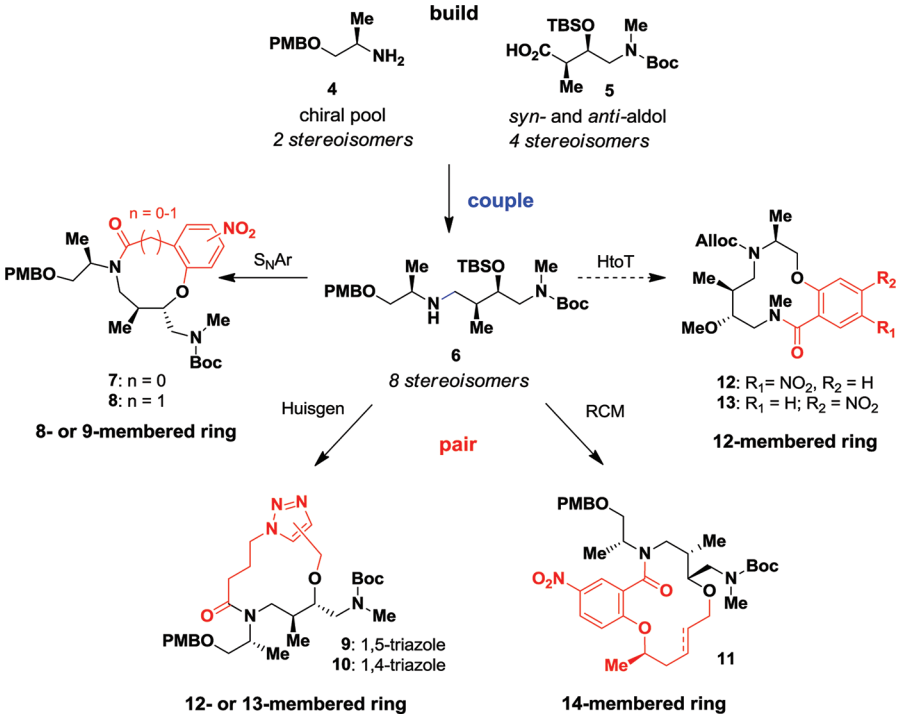


Figure 2. An aldol-based B/C/P strategy generating macrocycles and medium-sized rings via nucleophilic aromatic substitution (S_NAr), ring-closing metathesis (RCM), Huisgen cycloaddition and head-to-tail (HtoT) cyclization.

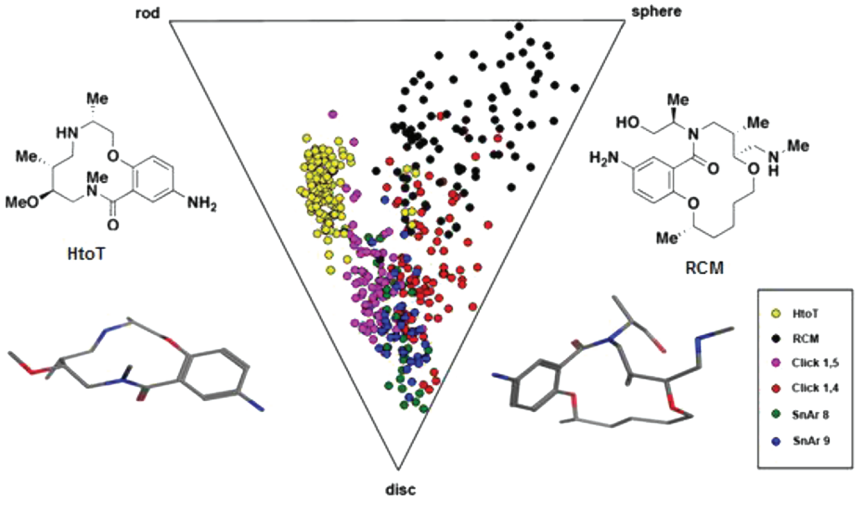


Figure 3. Principal moments of inertia (PMI) analysis of the HtoT macrolactam scaffolds as compared to previously prepared aldol-based scaffolds: RCM, Click (1,5 and 1,4) and SnAr (8 and 9).[1] PMI ratios are plotted in the triangular scatter plot. Minimum energy conformers ≤3 kcal/mol from the global minimum are plotted on the graph for all diastereomers.

normalized principal moments of inertia (PMI) ratios,[1,16] macrolactams **12** and **13** occupy a distinct region of 3-dimensional space as compared to scaffolds **7**–**11** (Figure 3). Surprisingly, the HtoT scaffolds are much more rod-like than the RCM-derived macrolactams.

RESULTS AND DISCUSSION

At the onset of this project, we envisioned two potential pairing strategies to effect HtoT macrocyclization: (1) an intramolecular S_NAr reaction or (2) macrolactamization (Figure 4). Because of the overall success of the intramolecular S_NAr reaction for the formation of the medium sized rings **7** and **8** in our initial study,[1] we first set our sights on the

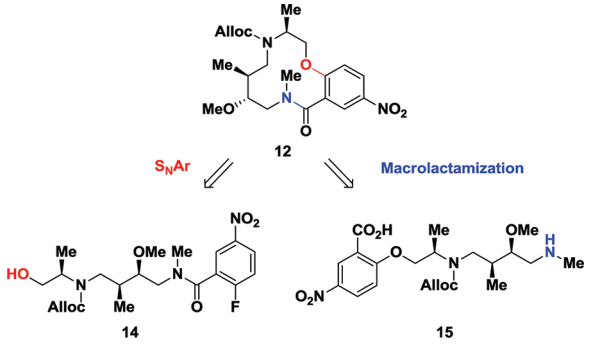


Figure 4. Potential HtoT macrocyclization strategies.

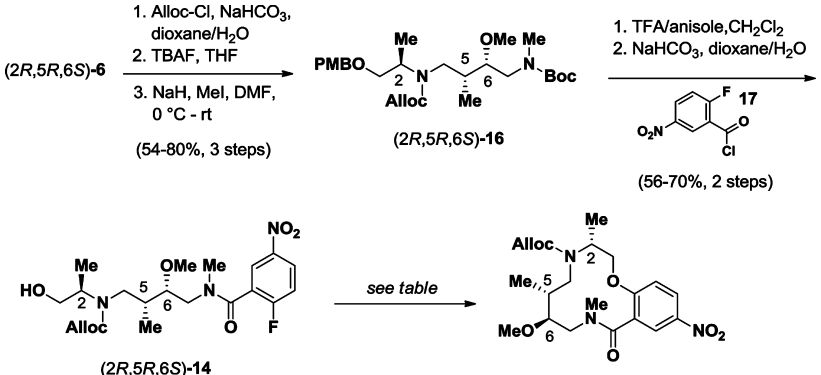
dx.doi.org/10.1021/co200161z | ACS Comb. Sci. 2012, 14, 89–96

application of this method to access macrolactam **12**. The synthesis of the S_NAr substrate begins with allyl carbamate formation accomplished by treatment of amine **6** with allyl chloroformate under Schotten−Baumann conditions (Scheme 1). This was followed by TBS removal with TBAF and methylation of the resultant secondary alcohol with methyl iodide. This synthetic sequence led to orthogonally protected diamine **16** in 54−80% yield. The choice of DMF as a solvent and the use of excess MeI was imperative to prevent intramolecular carbamate formation. The terminal PMB and Boc protecting groups were removed simultaneously upon treatment with TFA in the presence of anisole as a cation scavenger. Amidation of the resulting crude amino alcohol was then accomplished resulting in S_NAr substrate **14** in yields of 56−70% for the two steps.

The key S_NAr macrocyclization was first attempted utilizing conditions developed in our previous studies.[1,4] Treatment of **14** with NaH in THF at 0 °C led solely to the formation of oxazolidinone **18**, which could not be avoided even with careful exclusion of water and the use of KH. Heating alcohol **14** in the presence of excess CsF was also attempted, which showed no consumption of starting material by LC/MS at room temperature but ultimately led to decomposition after extended heating (>1 day). The use of DBU in DMF[17] was also unsuccessful even after extended heating. Finally, desired product **12** could be obtained using TBAF in THF/DMF (in the presence of 4 Å molecular sieves)[18] however the yields were generally low (25−45%) and highly variable. In light of the problems encountered promoting this transformation we decided to shift our efforts to a macrolactamization approach via intermediate **15** (Figure 4).

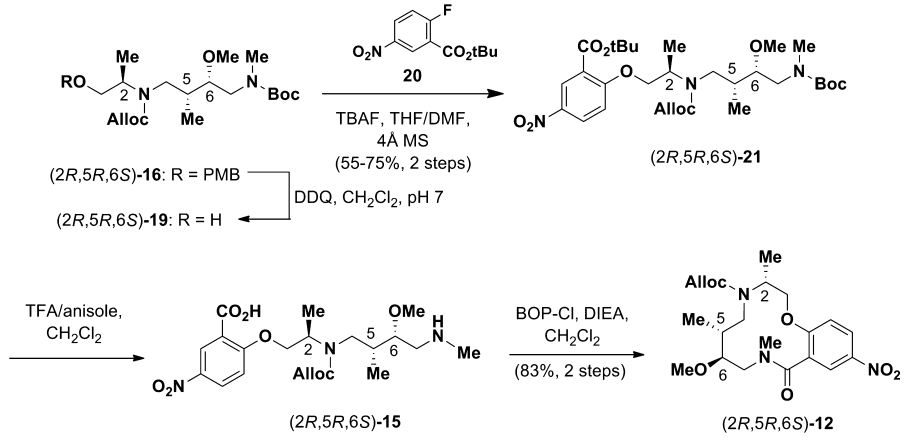
The synthesis of **15** (Scheme 2) commenced with PMB deprotection of intermediate **16** via treatment with DDQ in buffered CH_2Cl_2. The primary alcohol was subsequently subjected to an intermolecular S_NAr reaction with aryl fluoride **20**, in the presence of TBAF and 4 Å molecular sieves in THF/DMF resulting in ether **21** in good yield (55−75%). Bis-deprotection of the Boc and *t*-butyl ester groups provided crude amino acid **15** which was gratifyingly converted to macrocyle **12** in good yield (83%) upon treatment with BOP-Cl in the presence of DIEA.

Scheme 1. Synthesis of Scaffold 12 via S_NAr Macrocyclization



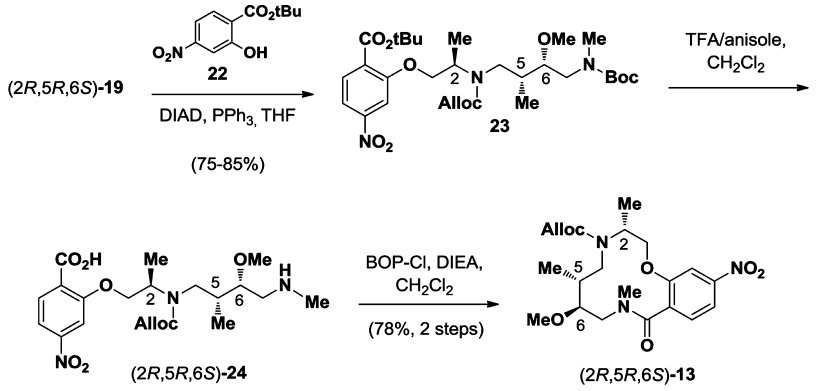
conditions	products
NaH, THF, 0 °C	**18**
CsF, DMF, 85 °C	**14** (decomp.)
DBU, DMF, 20-50 °C	recovered **14**
TBAF, THF/DMF, 4Å MS	**12** (25-45%)

Scheme 2. Synthesis of Scaffold 12 via Macrolactamization



dx.doi.org/10.1021/co200161z | *ACS Comb. Sci.* 2012, 14, 89−96

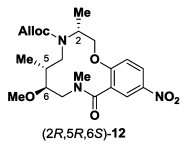
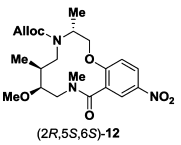
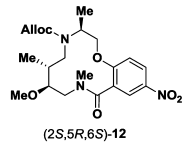
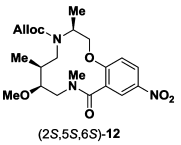
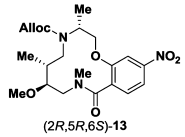
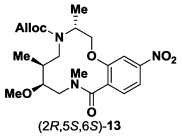
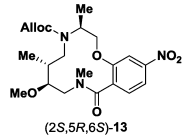
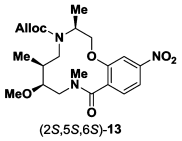
Scheme 3. Synthesis of Scaffold 13 via Macrolactamization



In order to access the *meta*-alkoxynitro macrocycle (**13**), we next decided to investigate a modified synthetic route in which the intermolecular S_NAr reaction was replaced with a Mitsunobu reaction[19] to install the nitrobenzoic acid. As shown in Scheme 3, primary alcohol **19** was treated with phenol **22** in the presence of DIAD and PPh_3 to give phenyl ether **23** in 75−85% yield. The Boc and *t*-butyl ester groups were removed with TFA and the resulting amino acid (**24**) cyclized in the presence of BOP-Cl to afford macrocycle **13** in 78% yield over two steps.

With established routes to both the *para*- and *meta*-alkoxynitro scaffolds, all eight isomers of macrocycles **12** and **13** were synthesized in 5-g quantities in preparation for solid-phase library production. The key macrocyclization reaction proceeded in high yield for all eight isomers of the linear amine scaffold (Table 1). The stereochemistry of precursors **15** and **24**, along with the regiochemistry of the aryl nitro substitutent, did not appear to influence the cyclization efficiency with combined yields for the double deprotection and lactamization ranging from 75 to 88%.

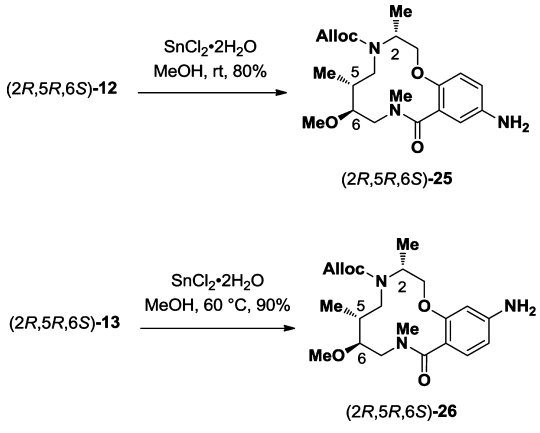
Table 1. Macrolactamization Yields for all Stereo- and Regioisomers

syn-aldol derived product	%yield[a]	*anti*-aldol derived product	%yield[a]
para-			
 (2R,5R,6S)-12	83%	 (2R,5S,6S)-12	83%
 (2S,5R,6S)-12	75%	 (2S,5S,6S)-12	87%
meta-			
 (2R,5R,6S)-13	78%	 (2R,5S,6S)-13	88%
 (2S,5R,6S)-13	79%	 (2S,5S,6S)-13	81%

[a]Isolated yields after silica gel chromatography.

To load the scaffolds onto solid-phase for library production, SynPhase Lanterns[20] functionalized with a monomethoxy Backbone Amide Linker (BAL)[21−23] were selected. Use of the BAL linker allows for loading via an amine (in this case an aniline) without sacrificing a diversity site. Immobilization would be achieved via reductive alkylation, while subsequent N-capping with sulfonyl chlorides, acid chlorides and isocyanates would afford the corresponding sulfonamides, amides and ureas upon cleavage with TFA. Thus, chemoselective reduction of the nitro functionality was achieved with $SnCl_2$ to afford anilines **25** and **26** (Scheme 4)

Scheme 4. Reduction of Aryl Nitro Group (Only One Stereoisomer Shown)

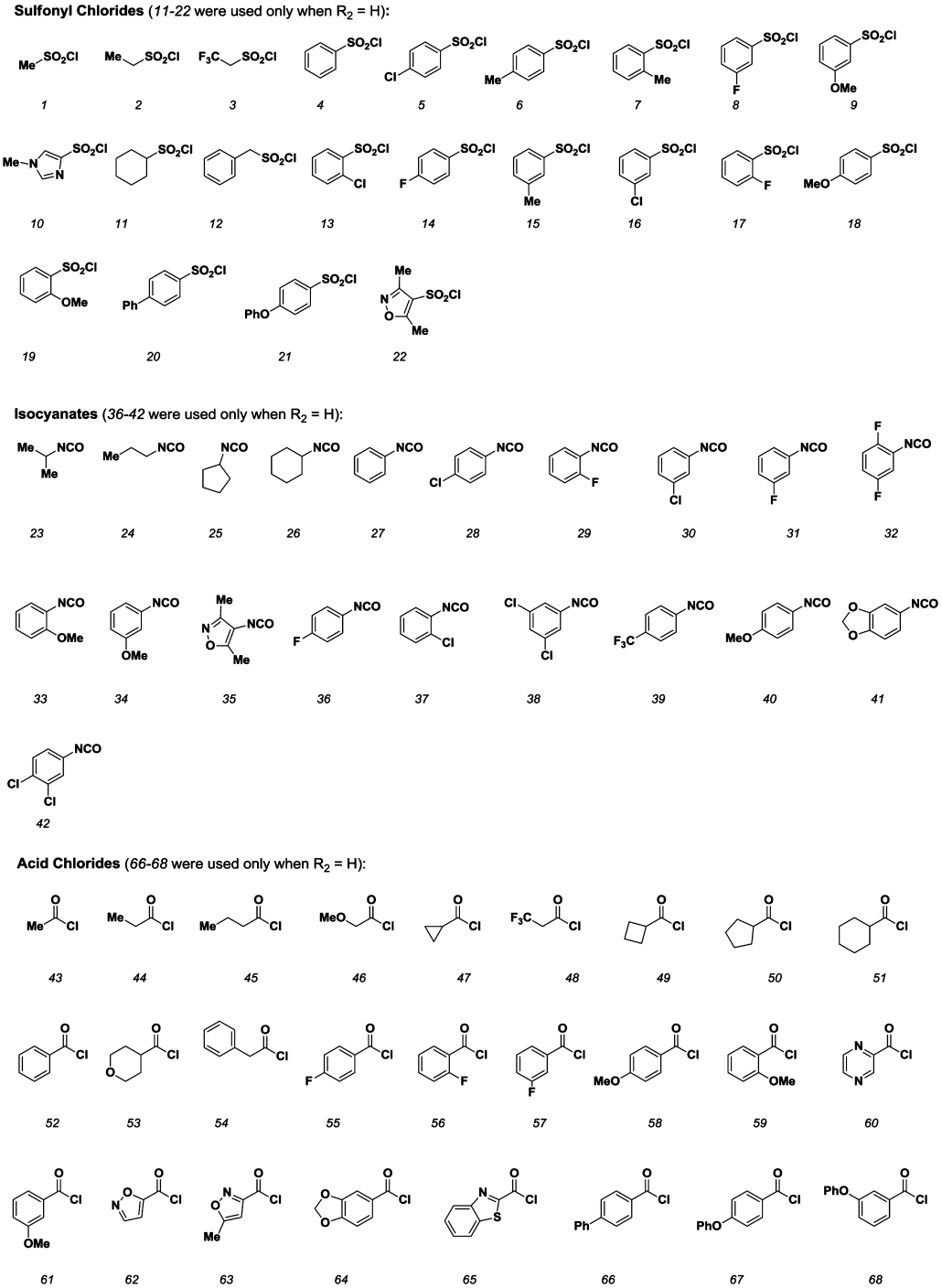


for loading onto solid-support. Of note, reduction of the *meta*-alkoxynitroaryl group proved more sluggish than the *para*-regioisomer requiring heating at 60 °C for full conversion. Nitro reduction proceeded smoothly for all isomers in yields ranging from 80 to 89%.

With ample quantities of all stereoisomers of anilines **25** and **26** in hand, a sparse matrix design strategy was implemented to select library members to be synthesized.[24] A virtual library was constructed (for each scaffold) incorporating all possible building block combinations at R_1 (aniline) and R_2 (secondary amine) using a master list of reagents (R_1 = sulfonyl chlorides, isocyanates, and acid chlorides; R_2 = sulfonyl chlorides,

dx.doi.org/10.1021/co200161z | ACS Comb. Sci. 2012, 14, 89−96

Chart 1. Building Blocks for Aniline Capping (R_1)

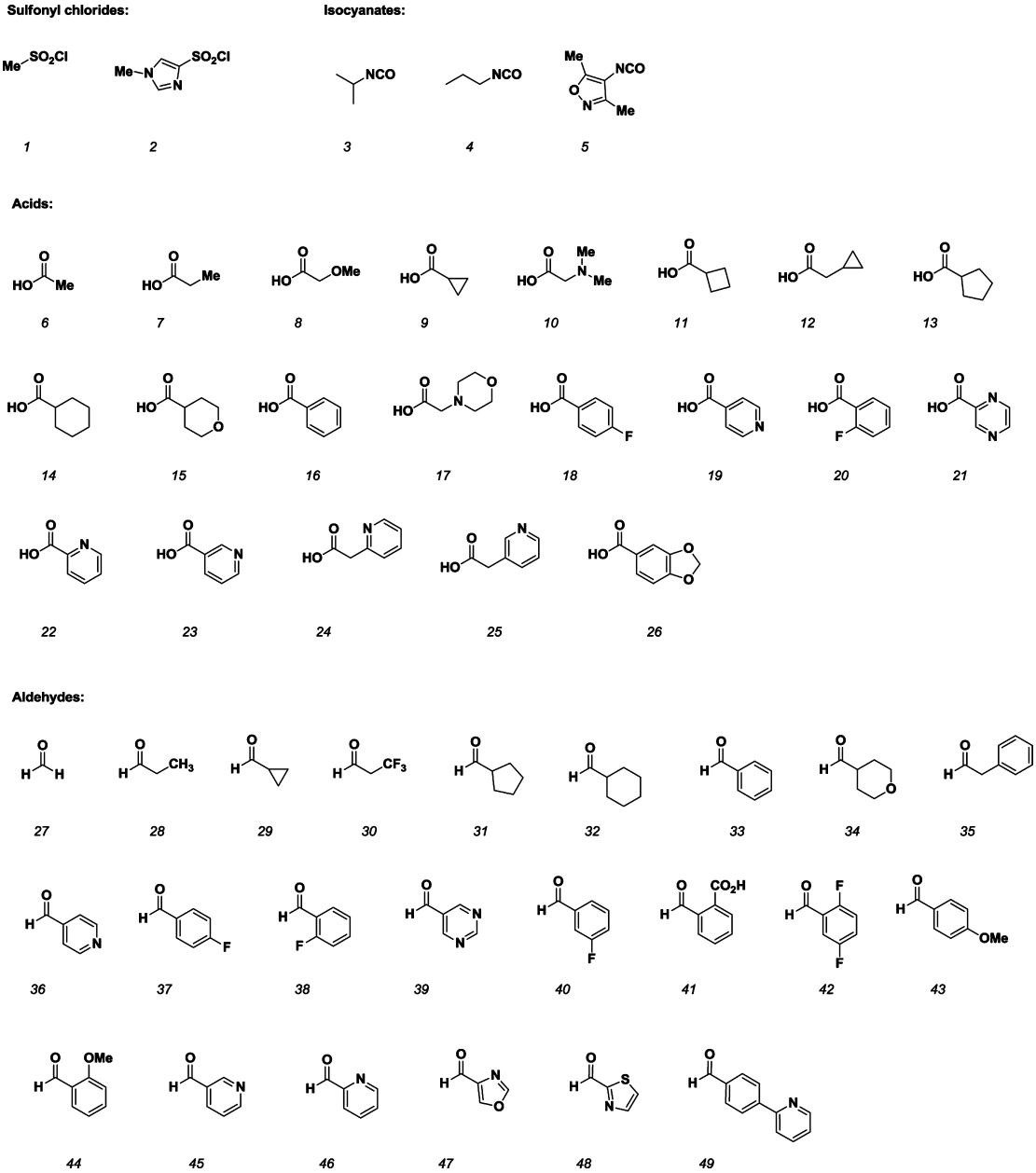


isocyanates, acids, and aldehydes).[25] This provided a virtual library of 7845 compounds per scaffold. Physicochemical property filters were then applied to eliminate building block combinations that led to products with undesired physicochemical properties.[26] The following property filters were applied to yield a total of 5363 compounds: MW ≤ 625, ALogP −1 to 5, H-bond acceptors and donors ≤ 10, rotatable bonds ≤ 10, and TPSA ≤ 140. To increase the percentage of "Lipinski compliant" products, a "75/25" rule was also implemented where 75% of all library members had MW < 500. A total of 496 compounds per scaffold were selected from the remaining set using chemical similarity principles, maximizing diversity but retaining near neighbors for built-in SAR. The reagents selected for library production include 22 sulfonyl chlorides, 19 isocyanates and 26 acid chlorides for aniline capping (Chart 1) along with 2 sulfonyl chlorides, 3 isocyanates, 21 acids, and 22 aldehydes for secondary amine capping (Chart 2). The same set of reagents was used for each stereo- and regioisomer, thereby maintaining the ability to generate SSAR for each building block combination. The property distribution for the selected products (7936 compounds total) is shown in Figure 5 and Table 2.

As shown in Scheme 5, scaffolds **25a−h** and **26a−h** were loaded onto the BAL functionalized SynPhase L-Series Lanterns

dx.doi.org/10.1021/co200161z | ACS Comb. Sci. 2012, 14, 89−96

Chart 2. Building Blocks for Amine Capping (R_2)



via treatment with an excess of $NaBH_3CN$ in 2% AcOH/DMF at 40 °C for 3 days. (Lanterns were equipped with radio frequency transponders to enable tracking and sorting of library members.) Scaffolds **25** and **26** contain two handles for the introduction of appendage diversity: the immobilized aniline and a protected secondary amine, both suitable for reaction with various electrophiles. The first diversity site, the aniline, was reacted with a total of 68 building blocks including isocyanates, sulfonyl chlorides and acid chlorides.[27,28] The second diversity site was then revealed via removal of the Alloc group upon treatment with $Pd(PPh_3)_4$ in the presence of excess 1,3-dimethyl barbituric acid. This secondary amine was capped with 49 building blocks including isocyanates, sulfonyl chlorides, acids, and aldehydes. This was followed by cleavage from the Lantern, which was achieved by treatment with a 1:1 solution of TFA:1,2-dichloroethane, to afford a total of 7936 products with an average yield of 8.5 μmol/Lantern. On average, yields were slightly higher for the *para*-aniline derived compounds than the *meta*-aniline compounds (9.1 μmol vs 8.0 μmol).[29]

All library products were analyzed by ultraperformance liquid chromatography, and compound purity was assessed by UV detection at 210 nm. An overview of library purity with respect to building blocks and stereochemistry is provided in Figure 6. The average purity of the library was 86% with 84% of the library being >75% pure. In general, all building blocks performed well during library production other than a few which are worth discussion. Reagents used for capping diversity site 1 which resulted in lower purities include ethyl sulfonyl chloride (R_1 = 2) and cyclopentyl isocyanate (R_1 = 25), both of which affected products predominantly for the *meta*-cores across most diversity site 2 building blocks. Isoxazole acid

dx.doi.org/10.1021/co200161z | *ACS Comb. Sci.* 2012, 14, 89−96

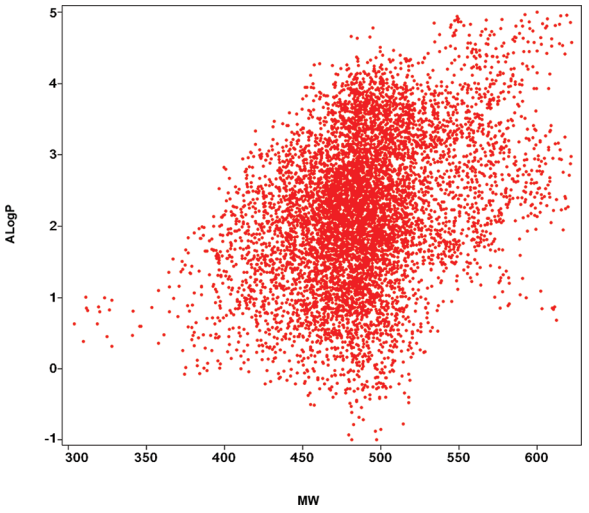


Figure 5. Molecular weight and ALogP distribution for HtoT library members.

Table 2. Property Analysis for the HtoT Library

property	HT scaffold[a] (n = 1)	HT library (n = 7936)
MW	321	489
ALogP	0.7	2.2
TPSA	77	97
rotatable bonds	1	4.7
HBA	5	5.8
HBD	2	1.4

[a]Property analysis of bare scaffolds, where R_1 and R_2 = H.

chloride (R_1 = *63*) led to low purity final compounds for most of the 16 cores and diversity site 2 building blocks. Overall, building blocks used to diversify the secondary amine faired better in terms of final product purity, with only one building block (oxazole-4-carbaldehyde, R_2 = *47*) showing reduced purities across R_1 building blocks.

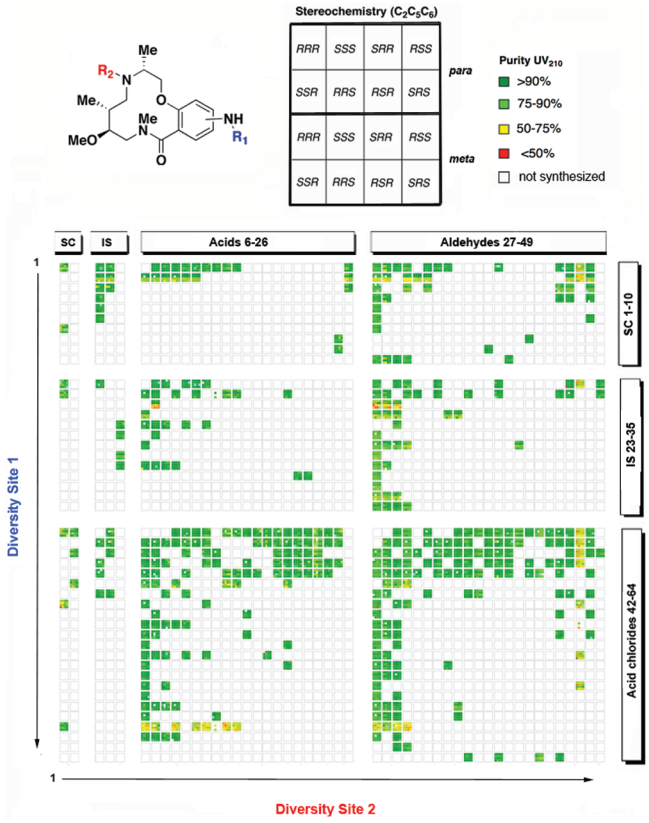
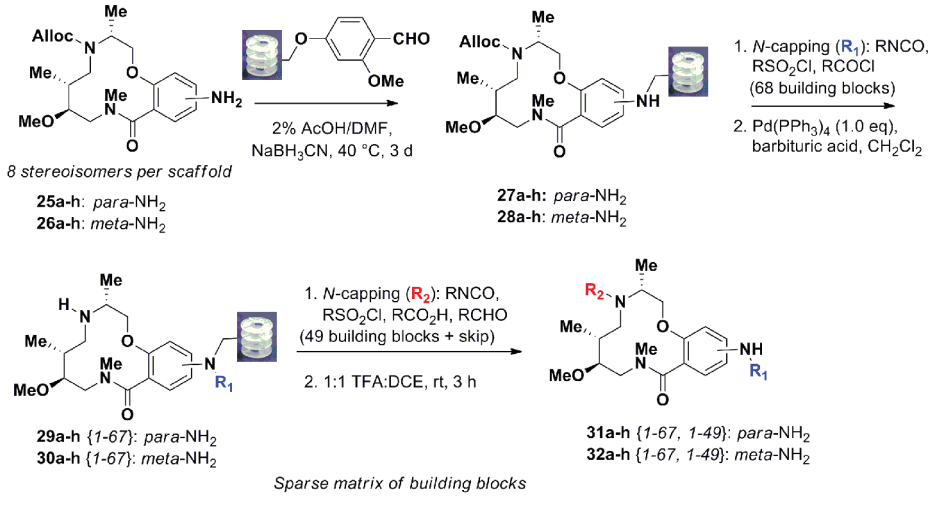


Figure 6. Purity analysis (UV 210 nm) for the HtoT Library. Library members are displayed as blocks of 16 isomers (8 stereoisomers X 2 regioisomers) (see legend) and reagents used for solid-phase diversification are shown on the x- and *y*-axes. See Charts 1 and 2 for detailed list of reagents. Products where R_2 = H are not shown. (SC = Sulfonyl chlorides, IS = Isocyanates).

CONCLUSIONS

A novel library of 7936 12-membered macrocycles was successfully prepared via a macrocyclization pairing reaction. Sixteen cores were synthesized from 8 isomers of linear amine scaffold **9** to establish "built-in" stereo/structure-activity relationships

Scheme 5. Solid-Phase Library Synthesis on SynPhase Monomethoxy BAL Lanterns



dx.doi.org/10.1021/co200161z | ACS Comb. Sci. 2012, 14, 89−96

(SSAR). Two regioisomers of aniline capping site were also included in our design to further increase skeletal diversity. This library was generated utilizing a traceless BAL linker to provide final compounds that contained less rotatable bonds and one less potential H-bond donor in an effort to provide more "drug-like" compounds for our screening collection. A sparse matrix of building blocks was employed to further enhance the appendage diversity of our library, while including built-in structure activity relationships and honing in on a desirable range of physicochemical properties.

■ ASSOCIATED CONTENT

Supporting Information

General information, library scaffold synthesis, solid-phase synthesis, QC analysis, and computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: lisa_marcaurelle@h3biomedicine.com.

Present Address

[†]H3 Biomedicine, 300 Technology Square, Cambridge, MA 02139

■ ACKNOWLEDGMENTS

This work was funded in part by the NIGMS-sponsored Center of Excellence in Chemical Methodology and Library Development (Broad Institute CMLD; P50 GM069721), as well as the NIH Genomics Based Drug Discovery U54 grants Discovery Pipeline RL1CA133834 (administratively linked to NIH grants RL1HG004671, RL1GM084437, and UL1DE019585).

■ REFERENCES

(1) Marcaurelle, L. A.; Comer, E.; Dandapani, S.; Duvall, J. R.; Gerard, B.; Kesavan, S.; Lee, M. D.; Liu, H.; Lowe, J. T.; Marie, J.-C.; Mulrooney, C. A.; Pandya, B. A.; Rowley, A.; Ryba, T. D.; Suh, B.-C.; Wei, J.; Young, D. W.; Akella, L. B.; Ross, N. T.; Zhang, Y.-L.; Fass, D. M.; Reis, S. A.; Zhao, W.-N.; Haggarty, S. J.; Palmer, M.; Foley, M. A. 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.* **2010**, *132*, 16962−16976.

(2) Comer, E.; Liu, H.; Joliton, A.; Claubat, A.; Johnson, C.; Akella, L. B.; Marcaurelle, L. A. Fragment-based domain shuffling approach for the synthesis of pyran-based macrocycles. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6751−6756.

(3) Kelly, A. R.; Wei, J.; Kesavan, S.; Marie, J. C.; Windmon, N.; Young, D. W.; Marcaurelle, L. A. Accessing skeletal diversity using catalyst control: formation of *n* and *n* + 1 macrocyclic triazole rings. *Org. Lett.* **2009**, *11*, 2257−2260.

(4) Gerard, B.; Duvall, J. R.; Lowe, J. T.; Murillo, T.; Wei, J.; Akella, L. B.; Marcaurelle, L. A. Synthesis of a stereochemically diverse library of medium-sized lactams and sultams via S_NAr cycloetherification. *ACS Comb. Sci.* **2011**, *13*, 365−374.

(5) Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52*, 6752−6756.

(6) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. The exploration of macrocycles for drug discovery: An underexploited structural class. *Nat. Rev. Drug Discovery* **2008**, *7*, 608−624.

(7) Drahl, C. Big hopes ride on big rings. *Chem. Eng. News* **2009**, *87*, 54−57.

(8) Pal, S. A journey across the sequential development of macrolides and ketolides related to erythromycin. *Tetrahedron* **2006**, *62*, 3171−3200.

(9) Lee, F.; Borzilleri, R.; Fairchild, C.; Kamath, A.; Smykla, R.; Kramer, R.; Vite, G. Preclinical discovery of ixabepilone, a highly active antineoplastic agent. *Cancer Chemother. Pharmacol.* **2008**, *63*, 157−166.

(10) Marsault, E.; Peterson, M. L. Macrocycles are great cycles: applications, opportunities, and challenges of synthetic macrocycles in drug discovery. *J. Med. Chem.* **2011**, *54*, 1961−2004.

(11) Tyndall, J. D. A.; Fairlie, D. P. Macrocycles mimic the extended peptide conformation recognized by aspartic, serine, cysteine and metallo proteases. *Curr. Med. Chem.* **2001**, *8*, 893−907.

(12) Wessjohann, L. A.; Ruijter, E.; Garcia-Rivera, D.; Brandt, W. What can a chemist learn from nature's macrocycles?—a brief, conceptual view. *Mol. Diversity* **2005**, *9*, 171−186.

(13) (a) Nielsen, T. E.; Schreiber, S. L. Towards the optimal screening collection: a synthesis strategy. *Angew. Chem., Int. Ed.* **2008**, *47*, 48−56. (b) Schreiber, S. L. Organic chemistry: Molecular diversity by design. *Nature* **2009**, *457*, 153−154 and references sited therein.

(14) Pandya, B. A.; Dandapani, S.; Duvall, J. R.; Rowley, A.; Mulrooney, C. A.; Ryba, T; Dombrowski, M.; Harton, M.; Young, D. W.; Marcaurelle, L. A. Practical asymmetric synthesis of *β*-hydroxy *γ*-amino acids via complimentary aldol reactions. *Tetrahedron* **2011**, *67*, 6131−6137.

(15) (a) Zhang, Q.; Lu, H.; Richard, C.; Curran, D. P. Fluorous mixture synthesis of stereoisomer libraries: Total syntheses of (+)-murisolin and fifteen diastereoisomers. *J. Am. Chem. Soc.* **2004**, *126*, 36−37. (b) Dandapani, S.; Jeske, M.; Curran, D. Synthesis of all 16 stereoisomers of pinesaw fly sex pheromones—Tools and tactics for solving problems in fluorous mixture synthesis. *J. Org. Chem.* **2005**, *70*, 9447−9462.

(16) Sauer, W. H.; Schwarz, M. K. Molecular shape diversity of combinatorial libraries: a prerequisite for broad activity. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 987−1003.

(17) Cristau, P.; Vors, J.-P.; Zhu, J. Solid-phase synthesis of natural product-like macrocycles by a sequence of Ugi-4CR and S_NAr-based cycloetherification. *Tetrahedron Lett.* **2003**, *44*, 5575−5578.

(18) Temal-Lab, T.; Chastanet, J.; Zhu, J. A convergent approach to cyclopeptide alkaloids: Total synthesis of sanjoinine G1. *J. Am. Chem. Soc.* **2002**, *124*, 583−590.

(19) Swamy, K. C. K.; Kumar, N. N. B.; Balaraman, E.; Kumar, K. V. P. P. Mitsunobu and related reactions: advances and applications. *Chem. Rev.* **2009**, *109*, 2551−2651.

(20) www.mimotopes.com

(21) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. Backbone amide linker (BAL) strategy for solid-phase synthesis of C-terminal-modified and cyclic peptides. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452.

(22) Boas, U.; Brask, J.; Jensen, K. Backbone amide linker in solid-phase synthesis. *J. Chem. Rev.* **2009**, *109*, 2092−2118.

(23) Higher loading levels were obtained using Lanterns functionalized with the monomethoxy BAL as compared to the traditional (dimethoxy) BAL.

(24) Akella, L. B.; Marcaurelle, L. A. Application of a sparse matrix design strategy to the synthesis of DOS libraries. *ACS Comb. Sci.* **2011**, *13*, 365−374.

(25) The master list of reagents used for library enumeration is provided in reference 24. Some building blocks were removed due to synthetic feasibility. Sulfonyl chlorides *2*, *3*, and *11* were also added.

(26) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.

(27) While the use of aldehydes for reductive alkylation was investigated, cleavage yields for the resulting products were generally low.

(28) Please see Supporting Information for solid-phase experimental procedures and reaction conditions for N-capping.

(29) The theoretical loading level for the L-series BAL functionalized Lanterns is 15 *μ*mol/Lantern however the highest loading levels that could routinely be obtained for the HtoT scaffolds was ∼10 *μ*mol even with an excess of scaffold.