JOC*Note*

pubs.acs.org/joc

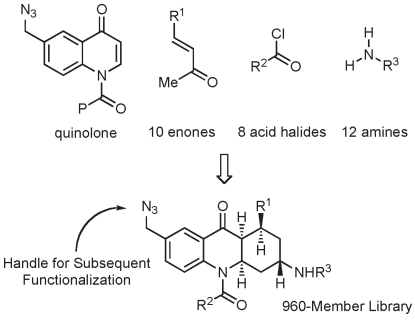
Synthesis of an Azide-Tagged Library of 2,3-Dihydro-4-quinolones

Hajoong Lee, Masato Suzuki, Jiayue Cui, and Sergey A. Kozmin*

Department of Chemistry, University of Chicago, 5735 S. Ellis Ave., Chicago, Illinois 60637

skozmin@uchicago.edu

Received December 1, 2009



We describe the assembly of a 960-member library of tricyclic 2,3-dihydro-4-quinolones using a combination of solution-phase high-throughput organic synthesis and parallel chromatographic purification. The library was produced with high efficiency and complete chemo- and diastereoselectivity by diversification of an azide-bearing quinolone via a sequence of [4 + 2] cycloadditions, N-acylations, and reductive aminations. The azide-functionalization of this library is designed to facilitate subsequent preparation of fluorescent or affinity probes, as well as small-molecule/surface conjugation.

Assembly of nitrogen-containing heterocyclic libraries has been a major focus of high-throughput organic synthesis due to the prevalence of such chemotypes among bioactive compounds.[1] Representative examples include 1,4-benzodiazepines,[2] 1,4-dihydropyridines,[3] dihydropyrimidines,[4] pyrrolidines,[5] and purines.[6] However, despite a number of innovative synthetic approaches to such small-molecule libraries reported in the past, the existing compound collections occupy only a small fraction of biogenic chemical space.[7] Thus, synthesis of new heterocyclic libraries to enable discovery of bioactive chemotypes continues to be of significant importance. We describe the assembly of a 960-member library of tricyclic dihydroquinolones, which was produced with high efficiency and excellent chemical purity by a combination of miniaturized solution-phase synthesis and high-throughput chromatographic purification. Azide-functionalization of this library was designed to facilitate subsequent cellular target identification and construction of small-molecule microarrays.

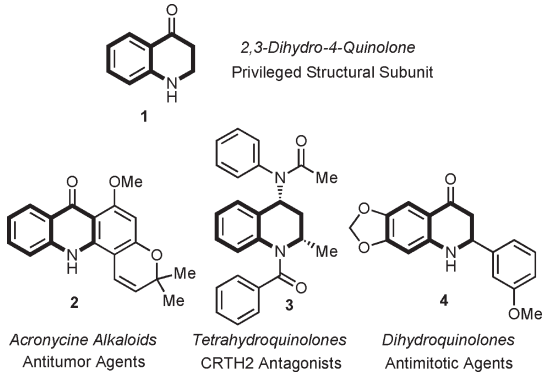


FIGURE 1. Structure of 3,4-dihydro-4-quinolone (**1**), which is found in several bioactive chemotypes, including antitumor alkaloids (**2**), CRTH2 antagonists (**3**), and antimitotic agents (**4**).

Bicyclic 2,3-dihydro-4-quinolone subunit (**1**, Figure 1) is found in a number of bioactive small molecules, which include the acronycine family of alkaloids with potent anticancer properties (**2**),[8] antagonists of CRTH2 receptor (**3**),[9] as well as a class of antimitotic agents (**4**).[10] Such diverse level of functionalization of 2,3-dihydro-4-quinolone moiety promoted us to examine this structural motif as an attractive platform for the assembly of the corresponding heterocyclic library, which was aimed at preserving favorable physicochemical properties of the resulting compounds in order to enable new lead discovery.

(1) (a) Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123–131. (b) Ellman, J. A. *Acc. Chem. Res.* **1996**, *29*, 132–143. (c) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600. (d) Balkenhohl, F.; von dem Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed.* **1996**, *35*, 2289–2337. (e) Lam, K. S.; Lebl, M.; Krchnak, V. *Chem. Rev.* **1997**, *97*, 411–448. (f) *Combinatorial Chemistry Synthesis and Application*; Wilson, S. R., Czarnik A. W., Eds.; John Wiley & Sons, Inc.: New York, 1997. (g) Gordon, E. M.; Kerwin, J. F., Jr. *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Wiley-Liss, Inc.: New York, 1998. (h) Schreiber, S. L. *Science* **2000**, *287*, 1964–1969. (i) Arya, P.; Chou, D. T. H.; Baek, M. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 339–346.

(2) (a) Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997–10998. (b) DeWitt, S. H.; Kiely, J. S.; Stankovic, C. J.; Schroeder, M. C.; Cody, D. M. R.; Pavia, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909–6913.

(3) Gordeev, M. F.; Patel, D. V.; Gordon, E. M. *J. Org. Chem.* **1996**, *61*, 924–928.

(4) Wipf, P.; Cunningham, A. *Tetrahedron Lett.* **1995**, *36*, 7819–7822.

(5) Murphy, M. M.; Schullek, J. R.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 7029–7030.

(6) Gray, N. S.; Wodicks, L.; Thunnissen, A. H.; Norman, T. C.; Kwon, S. J.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S. H.; Lockhart, D. J.; Schultz, P. G. *Science* **1998**, *281*, 533–538.

(7) Hert, J.; Irwin, J. J.; Laggner, C.; Keiser, M. J.; Shoichet, B. K. *Nat. Chem. Biol.* **2009**, *5*, 479–483.

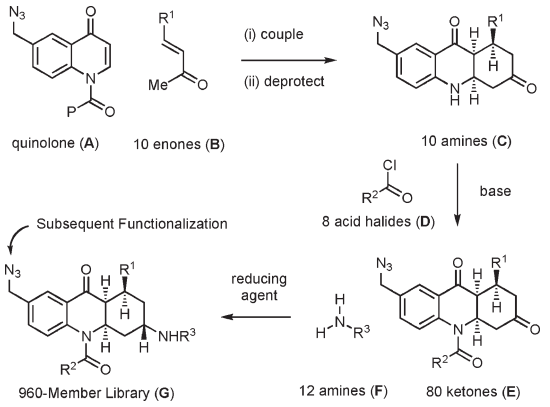
(8) (a) Huges, G. K.; Lahey, F. N.; Price, J. R.; Webb, L. J. *Nature* **1948**, *162*, 223–224. (b) Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Bodor, G. B. *J. Pharm. Sci.* **1966**, *55*, 758–768.

(9) Liu, J.; Wang, Y.; Sun, Y.; Marshall, D.; Miao, S.; Tonn, G.; Anders, P.; Tocker, J.; Tang, H. L.; Medina, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6840–6844.

(10) Zhang, S.-X.; Feng, J.; Kuo, S.-C.; Brossi, A.; Hamel, E.; Tropsha, A.; Lee, K. H. *J. Med. Chem.* **2000**, *43*, 167–176.

Published on Web 02/09/2010 DOI: 10.1021/jo9025447

SCHEME 1. Synthetic Strategy to Azide-Functionalized Library of Tricyclic 3,4-Dihydro-4-quinolones



We selected quinolone **A** (Scheme 1) as the starting point for introduction of molecular diversity. Indeed, the nitrogen atom can be readily functionalized by N-acylation. The vinylogous amide moiety can enable efficient fusion of additional carbocyclic rings using the [4 + 2] annulation developed by Beifuss and co-workers.[11] Furthermore, the aromatic ring can be readily equipped with an azidomethyl group, which would enable subsequent functionalization of each library member via alkyne-azide [3 + 2] cycloaddition.[12] The assembly process was designed to begin with a series of [4 + 2] cycloadditions of quinolone **A** with enones **B** to give the initial set of tricyclic amines **C**, which would be subsequently diversified via N-acylation using readily available acid chlorides **D** to give the resulting diketones **E**. Chemoselective and diastereoselective reductive amination of diketones **E** with amines **F** would be employed as the final diversity-generating step to deliver the target heterocyclic library **G**. High-throughput synthesis employing all possible combinations of 10 enones **B**, 8 acid chlorides **D**, and 12 amines **F** was expected to deliver 960 tricyclic dihydroquinolones.

Our initial objectives were 2-fold. First, we intended to validate the generality and efficiency of the synthetic route shown in Scheme 1. Second, we aimed to develop a simple protocol that would enable production of all final compounds in high chemical purity. To this end, azido quinolone **5** (Figure 2A), which was readily prepared from 4-nitrobenzyl alcohol, was first subjected to the [4 + 2] cycloaddition with *trans*-3-nonen-2-one using the protocol developed by Beifuss and co-workers (TESOTf, 2,6-lutidine, CH_2Cl_2, 20 °C).[11] Protodesilylation of the resulting TES enol ether with TFA, followed by subsequent Alloc deprotection ($Pd(PPh_3)_4$, morpholine) afforded tricyclic diketone **6**, which was next N-acylated with benzoyl chloride to give amide **7**. The final reductive amination step required differentiation of the two ketones in **7**, as well as a high level of diastereoselection. Initially, we examined the use of $NaBH(OAc)_3$, which promoted reductive amination with high chemoselectivity but with only moderate diastereoselectivity (dr 8:2−9:1), which was not acceptable for a final step of the library production. Examination of a number of other reducing agents revealed that this problem could be circumvented by using the bulkier reagent $NaBH(OEh)_3$ (Eh = 2-ethylhexanoate),[13] which produced the required amine as a single diastereomer detected by 500 MHz ^{1}H NMR. Using this protocol, we evaluated the reductive amination of diketone **7** with 12 commercially available primary amines (Figure 2B). To facilitate parallel chromatographic purification, all reactions were performed on 2−4 μmol scale to deliver the corresponding products (**8**−**19**) in good chemical yields (61−71%), high diastereomeric ratios (>95:5), and excellent chemical purities (Figure 2C,D).

The library production began with a selection of enones **B** to be employed for the first diversification step of the process (Figure 3). While a range of unsaturated carbonyl compounds successfully participated in the Beifuss [4 + 2] cycloaddition, only 3-substituted enones proved to be viable for the production of the final library as other substitution patterns were not tolerated at a later stage of the synthesis. We selected 10 readily available enones **B** (Figure 3B), which were employed to produce a set of corresponding diketones **C** following *in situ* protodesilylation of the TES enol ether and Alloc deprotection. For the second diversification step, we selected a set of seven acid chlorides **D** to be used for N-acylation of amines **C** (Figure 3C), as well as the parent Alloc-protected amine. Each reaction proceeded efficiently in the presence of Et_3N as a base to deliver the required amides **E**, setting the stage for the completion of the library synthesis. The final reductive amination was carried out using $NaBH(OEh)_3$ as a reducing agent to enable high diastereoselectivity of this transformation. Each of the 80 diketones **E** was treated with 12 amines **F** (Figure 2D) to produce tricyclic dihydroquinolones **G** on 4.5 μmol scale, followed by parallel preparative TLC purification. Initial analysis revealed that all 960 reactions proceeded successfully to deliver final library members in high chemical purity (see Supporting Information). The purity and efficiency was further quantified by 500 MHz ^{1}H NMR characterizations of 120 randomly selected library members. In addition, five diketones, five amides, and five amines were subjected to full analytical characterization (Figure 3E). While the efficiency of the reductive amination ranged from 42% to 75% after silica gel purification, this protocol provided sufficient amounts of material (1.0−1.5 mg of final products) for preparation of DMSO stock solutions to enable 200−500 subsequent high-throughput biological screens. While the selection of this reaction scale greatly facilitated high-throughput purification, standard LCMS purification methods could be readily employed for production of larger amount of material if desired.

Azide−alkyne [3 + 2] cycloaddition has emerged as a powerful, general, and bioorthogonal strategy for covalent molecular conjugation.[12] The azide functionalization of dihydroquinolone library was designed to explore attachment of affinity probes, fluorophores, and radiolabels to final library members. Alternatively, a similar approach could be used for surface immobilization of this small-molecule library.[14] Such functionalization would enable development of new high-throughput screens and facilitate cellular target identification of active compounds discovered

(11) Beifuss, U.; Ledderhose, S. *Synlett* **1995**, 938–940.
(12) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137.
(13) McGill, J. M.; LaBell, E. S.; Williams, M. *Tetrahedron Lett.* **1996**, *37*, 3977–3980.
(14) Uttamchandani, M.; Walsh, D. P.; Yao, S. Q.; Chang, Y. T. *Curr. Opin. Chem. Biol.* **2005**, *9*, 4–13.

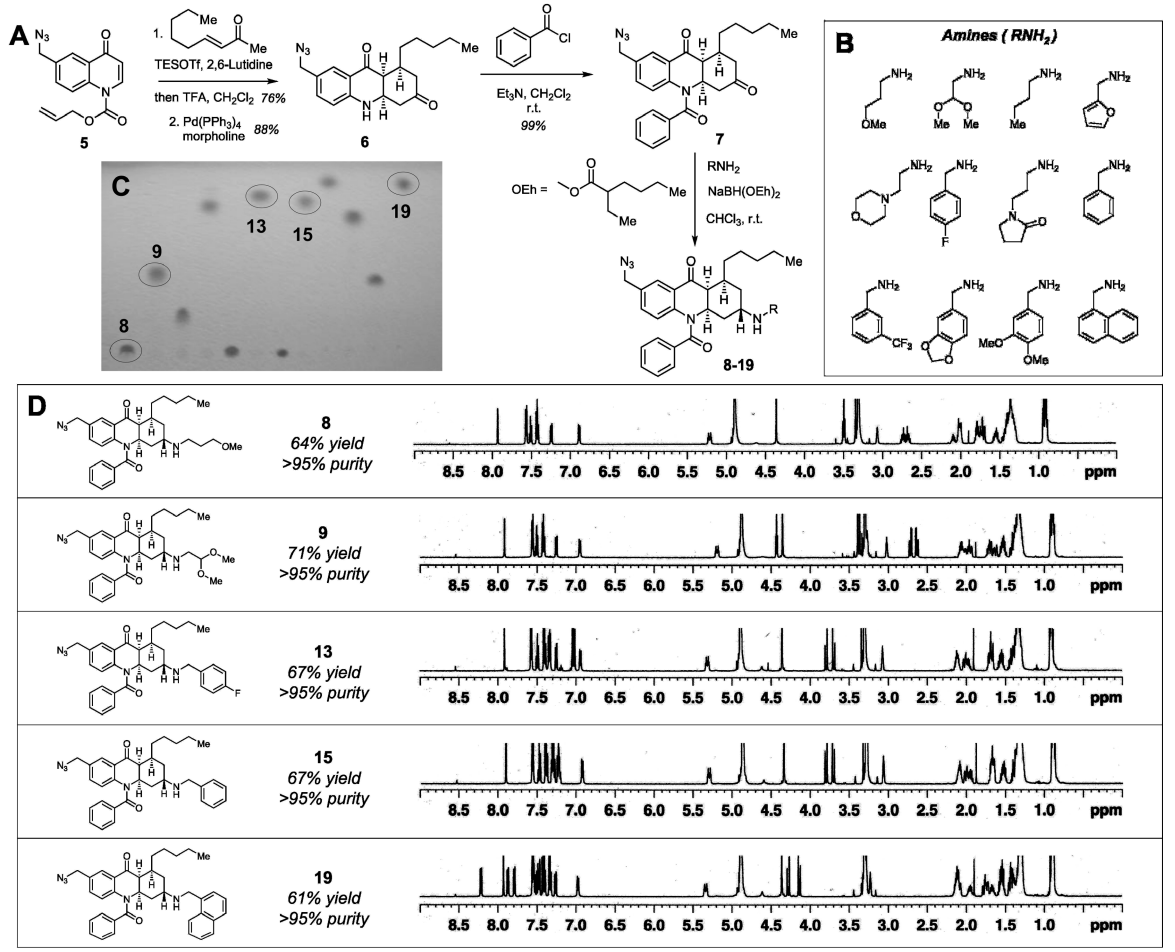


FIGURE 2. Validation studies. (A) General reaction sequence. (B) Structures of amines used for reductive amination. (C) Purity analysis of 12 purified products by TLC. (D) ^{1}H NMR spectra, structures and yields for five selected compounds. Yields were determined by 500 MHz ^{1}H NMR spectroscopy using an internal standard.

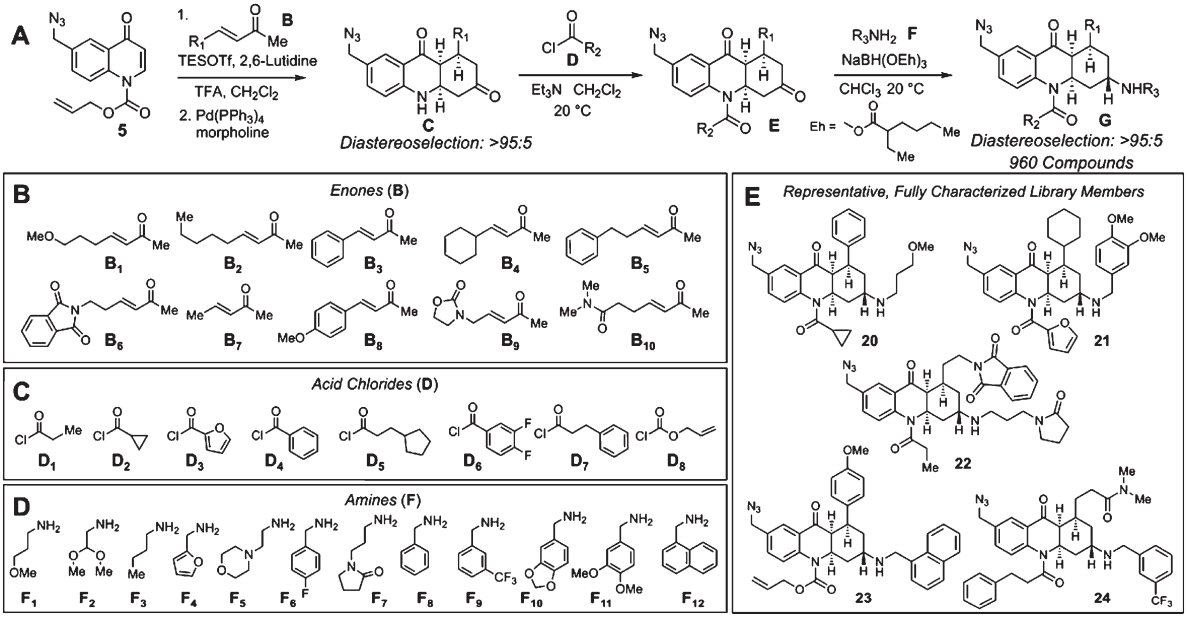
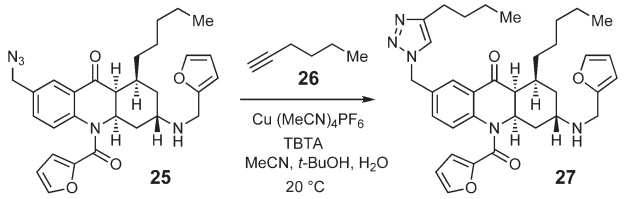


FIGURE 3. Assembly of azide-tagged small-molecule library. (A) Reaction sequence. (B) Structures of 10 enones employed for the first diversification step. (C) Structures of 8 acid chlorides employed for the second diversification step. (D) Structures of 12 amines employed for the final diversification step. (E) Representative, fully analytically characterized library members.

SCHEME 2. Cu-Catalyzed Azide−Alkyne Cycloaddition



in phenotypic assays. Treatment of a representative azide **25** with alkyne **26** in the presence of $Cu(MeCN)_4PF_6$ and TBTA afforded the expected [3 + 2] cycloadduct **27** in 62% yield (Scheme 2).[15] This experiment validated that the cycloaddition protocol is efficient and fully compatible with a range of functional groups present in dihydroquinolones and could be potentially employed for subsequent derivatization of the entire chemical library.

In closing, we have assembled a 960-member library of tricyclic 2,3-dihydro-4-quinolones using a combination of miniaturized solution-phase high-throughput organic synthesis and parallel chromatographic purification. Importantly, this approach enabled rapid validation of synthetic sequences and building blocks for library synthesis, which often represents the bottleneck in the process of generating new chemical libraries. Second, parallel chromatographic purification of each individual library member enabled rapid production of all final compounds in sufficient quantity to enable hundreds of cell-based or target-based high-throughput screens. Broad biological evaluation of this library, as well as the construction of small-molecule microarrays, is currently in progress. Results of these studies will be reported in the due course.

Experimental Section

General Protocol for Library Synthesis. The following procedure represents the synthesis of a representative set of 96 compounds (plate 4). To a solution of quinolone **5** (1.0 g, 3.52 mmol) in CH_2Cl_2 (30 mL) were added 2,6-lutidine (1.4 mL, 12.3 mmol), TESOTf (2 mL, 8.8 mmol), and a solution of enone $\mathbf{B_4}$ (1.1 g, 7.04 mmol) in CH_2Cl_2 (10 mL). The resulting solution was stirred for 2 h at room temperature and was treated with TFA (0.54 mL, 7.04 mmol). The reaction was quenched by addition of saturated aqueous solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried with anhydrous $MgSO_4$, concentrated *in vacuo*, and purified by flash chromatography on silica gel (ethyl acetate/hexane = 1:3) to give 1.1 g (70% yield) of the corresponding tricyclic ketone. This product (0.72 g, 1.83 mmol) was dissolved in THF (20 mL) and treated with $Pd(PPh_3)_4$ (10.5 mg, 0.09 mmol) and morpholine (0.2 mL, 3.66 mmol). The resulting solution was stirred for 30 min at 20 °C, concentrated *in vacuo*, and purified by flash chromatography on silica gel (ethyl acetate/hexane = 1:1) to give 580 mg (90% yield) of the corresponding tricyclic amine **C** (compound **50**, see Supporting Information). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.76 (m, 1H), 0.88 (m, 1H), 1.14 (m, 1H), 1.30 (m, 2H), 1.49 (m, 1H), 1.72 (m, 3H), 1.82 (m, 1H), 1.94 (m, 1H), 2.26 (m, 1H), 2.37 (m, 1H), 2.56 (m, 2H), 2.81 (t, 1H, J = 13.4 Hz), 3.44 (br s, 1H), 3.83 (m, 1H), 4.23 (br s, 2H), 4.83 (br s, 1H), 6.68 (d, 1H, J = 8.4 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.73 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.2, 26.4, 26.5, 314, 32.3, 39.0, 41.7, 43.66, 43.74, 46.0, 54.4, 54.9, 116.7, 118.4, 125.1, 127.3, 135.8, 147.9, 194.2, 208.9; MS (APCI) calculated for $C_{20}H_{24}N_4O_2$ 352.2, found 387.1 $[M + Cl]^-$.

A solution of this amine (350 mg, 0.98 mmol) in CH_2Cl_2 (14 mL) was next divided into 7 equal batches and treated with pyridine (57 μL, 0.7 mmol) and 7 acid halides ($\mathbf{D_1}$−$\mathbf{D_7}$) (0.42 mmol each). The resulting solutions were stirred for 3 h at room temperature, concentrated *in vacuo*, and purified by flash chromatography on silica gel (ethyl acetate/hexane = 1:2) to give seven corresponding amides **E** (51−63 mg, 85−99% yield). The parent *N*-alloc dihydroquinolone was used as the eighth amide. Each of the amides **E** was diluted in $CHCl_3$ to a final concentration of 0.2 M, divided into 12 equal batches (20 μL, 4 μmol per well), and arrayed into a polypropylene 96-well PCR plate. The plate was treated with 12 amines $\mathbf{F_1}$−$\mathbf{F_{12}}$ (0.74−1.15 μL) according to the plate map shown in Supporting Information. Each reaction mixture was treated with the 1.3 M solution of $NaBH(OEh)_3$ in CH_2Cl_2 (12.3 μL per well). Upon completion, the reaction mixtures were transferred onto preparative TLC plates as circular spots using a multichannel pipettor. The plates were developed using ether/hexanes = 2:1. The products were detected under UV light and removed from TLC plates as silica gel pellets, from which the final compounds were eluted with 0.8 mL of CH_3OH. Following analysis of purity of each compound **G** by TLC, the solvent was removed *in vacuo*. Twelve randomly selected compounds were dissolved in CD_3OD (0.5 mL) and analyzed by ^{1}H NMR. The amount of material in each sample was determined by integration using residual CH_3OH as a precalibrated internal standard. This protocol was used next to prepare all the remaining library members.

Representative Library Member 21. Obtained in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.73 (m, 1H), 0.86 (m, 1H), 1.13 (d, 1H, J = 12.7 Hz), 1.29 (m, 3H), 1.39 (m, 1H), 1.70 (m, 3H), 1.82 (m, 4H), 1.95 (m, 1H), 2.07 (m, 2H), 3.16 (s, 1H), 3.34 (s, 1H), 3.72 (m, 1H), 3.81 (m, 1H), 3.89 (s, 3H), 3.90 (s, 3H), 4.34 (m, 2H), 5.30 (m, 1H), 6.51 (m, 1H), 6.82 (d, 1H, J = 8.0 Hz), 6.89 (m, 2H), 7.11 (d, 1H, J = 8.4 Hz), 7.16 (d, 1H, J = 3.3 Hz), 7.33 (m, 2H), 7.94 (d, 1H, J = 2.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.4, 26.5, 26.8, 29.8, 30.9, 32.7, 38.7, 40.3, 49.7, 51.5, 52.1, 54.1, 56.0, 111.2, 111.6, 112.2, 118.3, 120.3, 124.3, 125.0, 126.6, 131.6, 133.3, 141.4, 144.8, 147.9, 148.2, 149.1, 160.0, 195.6; HRMS (ESI) calculated for $C_{34}H_{40}N_5O_5$ $[M + H]^+$ 598.3029, found 598.3029.

Acknowledgment. Partial support of this research was provided by NIH P50 GM086145. S.A.K thanks the Alfred P. Sloan Foundation, the Dreyfus Foundation, Amgen and GlaxoSmithKline for additional financial support.

Supporting Information Available: Additional experimental protocols, plate maps, images of TLC plates and copies of NMR spectra. This information is available free of charge via the Internet at http://pubs.acs.org.

(15) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853–2856.