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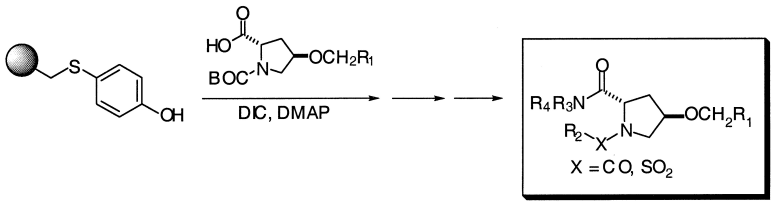

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Solid-Phase Library Synthesis of Alkoxyprolines

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The library synthesis of alkoxyprolines was achieved using an acid-stable, nucleophile-cleavable solid support. A hydroxythiophenol linker derived from Merrifield resin was esterified with the corresponding ethers of BOC-hydroxyproline. Removal of the BOC protecting group with trifluoroacetic acid followed by acylation gave solid-supported hydroxyproline derivatives. Cleavage from the solid support with excess primary amines or excess secondary amines followed by purification of the crude products from the excess amine by supported liquid−liquid extraction gave the alkoxyproline library in high purity.

Introduction

Recently, methods for constructing heterocyclic small molecules on solid support have attracted considerable attention.[1] In the context of an ongoing combinatorial chemistry development program, we were attracted to alkoxyprolines for their utility as scaffolds for protease inhibitors (**1** and **2** of Figure 1)[2] and receptor antagonists (**3** of Figure 1).[3] Compound **1** has an IC_{50} of 0.9 nM for thrombin. Compound **2** has an IC_{50} of 125 nM for Calpain I, and compound **3** is a fibrinogen receptor antagonist with an IC_{50} of 0.55 μM for inhibiting platelet aggregation. We have developed a novel and general library synthesis based on the 4-hydroxyproline scaffold intended for screening against a broad range of biological targets.

Results and Discussion

Identification of a Practical Synthetic Route. Several synthetic pathways were explored for the library synthesis of hydroxyproline derivatives. After amine addition to bromomethyl Wang resin (**4**),[4] Fmoc-Hyp-OH (**5**) was coupled to solid-supported amine with DIC (500 mol %) and HOBt (250 mol %) in NMP (Scheme 1). PyBrop (200 mol %), and *N*,*N*-diisopropylethylamine (400 mol %) in methylene chloride also gave the desired product. Mitsunobu reaction using DEAD/PPh_3 with phenols were initially performed in fritted polypropylene syringe cartridges with modest success. However, the Mitsunobu reaction completely failed to functionalize the secondary hydroxyl group when carried out in 96-well plates. We believe that heat generated from the high reaction concentration and poor mixing contributed to the failure of this reaction. In some cases, intramolecular cyclization or elimination occurred during the Mitsunobu reaction. TFA cleavage of the benzylic ether of the linker was also observed, a known side product from acylated amines derived from [(4-aminomethylphenoxy)methyl]polystyrene.[5] After the Mitsunobu reaction, the Fmoc group was removed, the amine acylated, and product **8** cleaved off solid support.

An alternative route involved the formation of the ester of Fmoc-Hyp-OH with bromomethyl Wang resin followed by Mitsunobu reaction, Fmoc deprotection, cleavage of the ether from the resin, and introduction of the final diversity element in solution as the last step (Scheme 2). While the Mitsunobu step appeared to give cleaner product than the corresponding amide, the coupling of primary and secondary

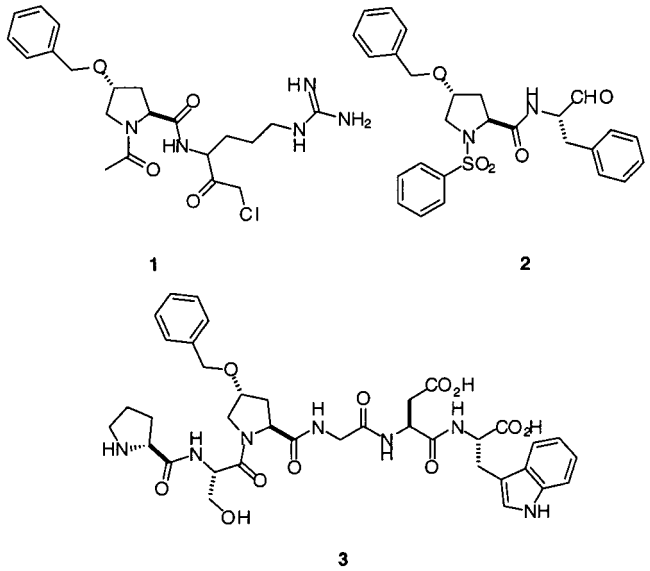
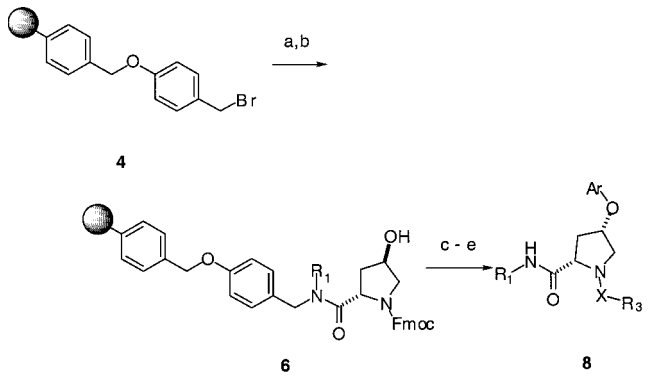


Figure 1. Biologically active alkoxyprolines.

Scheme 1[a]



[a] Reagents and conditions: (a) R_1-NH_2, CH_2Cl_2; (b) DIC, HOBT, NMP, Fmoc-Hyp-OH (**5**); (c) DEAD, Ph_3P, ArOH (**7**); (d) 30% piperidine/DMF; (e) acylation then cleavage with TFA.

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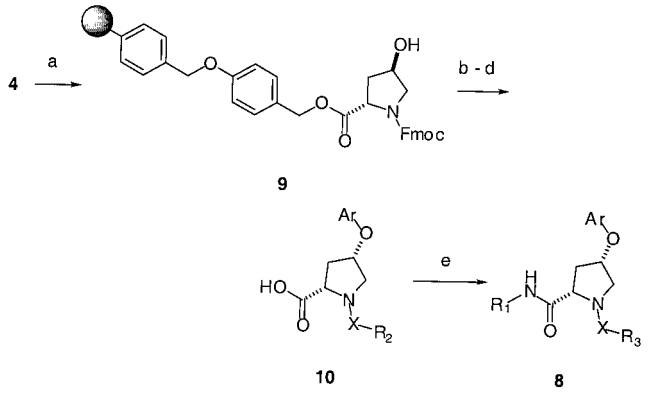
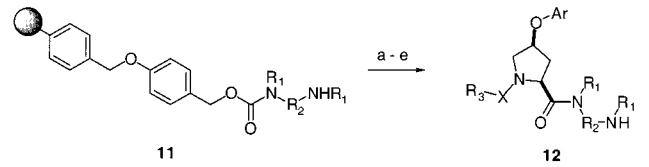
Scheme 2[a]



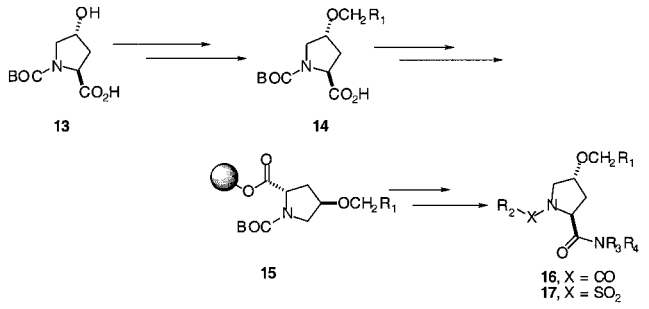
[a] Reagents and conditions: (a) **5**, CsI, DIEA, DMF; (b) DEAD, Ph_3P, ArOH (**7**); (c) 30% piperidine/DMF; (d) acylation then cleavage with TFA; (e) CDI, R_3R_4NH.

Scheme 3[a]



[a] Reagents and conditions: (a) DIC, HOBT, NMP, Fmoc-Hyp-OH (**5**); (b) DEAD, Ph_3P, ArOH (7); (c) 30% piperidine/DMF; (d) acylation; (e) cleavage with TFA.

Scheme 4

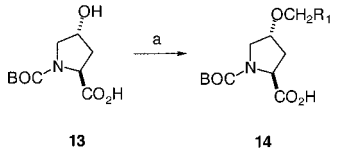


amines to the carboxylic acid was not satisfactory for library synthesis. We believe that TFA may have facilitated decomposition of CDI or the formation of the corresponding trifluoroacetamide. Coupling of Fmoc-Hyp-OH (**5**) to diamines on nitrophenol carbonate linker[6] **11**, Mitsunobu reaction, Fmoc deprotection, acylation, and cleavage off solid support gave low yields of the desired product **12** (Scheme 3).

Solution-phase synthesis of scaffolds containing the phenolic moiety was then considered as a strategy for library generation. Attempts to introduce the phenols onto the hydroxyproline scaffold via the Mitsunobu reaction were complicated by low yields, and the need for chromatographic purification made this route impractical for library generation. We then chose to alkylate the alcohol moiety of the hydroxyproline, a process that had literature precedent.[2a] Our revised approach is outlined in Scheme 4.

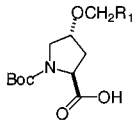
Scaffold Synthesis. A practical multigram synthesis of alkoxyproline derivatives (**14**) was accomplished by alkylation of BOC-hydroxyproline (**13**) with alkyl halides (Scheme 5), employing KOH and DMSO as base and solvent. This process was executed under anhydrous conditions and was driven to completion using 400 mol % alkylating reagent. Use of H_3PO_4 in the workup avoided the precipitation of solids that complicated product isolation when $KHSO_4$ was employed as the reagent for acidification. Purification of the crude BOC-4-alkoxyproline was accomplished via the cyclohexylamine salt in order to remove an unknown byproduct. No chromatography was required in the syntheses of the six carboxylic acids (Table 1).

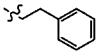
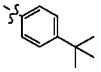
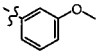
Scheme 5[a]



[a] Reagents and conditions: (a) KOH, RCH_2Br, DMSO.

Table 1. Representative BOC-Protected Alkoxyprolines Prepared in Solution

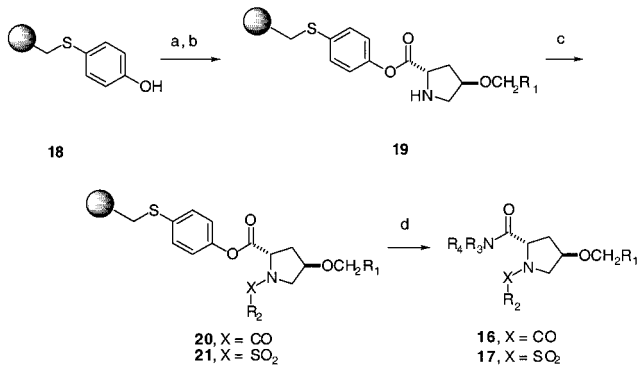


Entry	Compound	R_1	Yield[a] (%)
1	**14a**		58
2	**14b**		68
3	**14c**		95
4	**14d**		56
5	**14e**		57
6	**14f**		93

[a] Isolated yield.

Final Library Synthetic Route. BOC-protected alkoxyproline scaffolds **14** were coupled to the Marshall linker[7] **18** with DIC and catalytic DMAP. Complete coupling to the solid support was confirmed by a negative $FeCl_3$/pyridine test for phenols (Scheme 6).[7c,8] After scaffolds **14** were coupled to the solid-support, the *tert*-butoxycarbonyl protecting group was removed with trifluoroacetic acid in methylene chloride with anisole present as a cation scavenger. After the resins were washed, the proline nitrogen was acylated with carboxylic acids, acid chlorides, or sulfonyl chlorides. Acylation of amine **19** with carboxylic acids was successfully accomplished using DIC/HOBT in DMF. Acylation of amine **19** with acid chlorides or sulfonyl chlorides in the presence of *i*-Pr_2NEt in CH_2Cl_2 gave esters **20** or **21**, respectively. Treatment of esters **20** and **21** with various primary and secondary amines in 1,4-dioxane gave the alkoxyproline products **16** and **17**, respectively. A reaction time of >24 h was generally required for cleavage of products from the solid support. Cleavage with various secondary amines was

Scheme 6[a]



[a] Reagents and conditions: (a) DIC, DMAP **14**; (b) TFA/CH_2Cl_2/anisole (50:48:2); (c) R_2CO_2H, DIC, HOBT, DMF or R_2COCl, *i*-PrNEt, CH_2Cl_2 or R_2SO_2Cl, *i*-Pr_2NEt, CH_2Cl_2; (d) R_3R_4NH, 1,4-dioxane.

much slower (36−48 h) than with primary amines (24 h). Pyridine was also found to be a suitable solvent for cleavage, but 1,4-dioxane offered the advantage of solvent removal via lyophilization. Excess amine was removed by the previously described method of supported liquid−liquid extraction (SLE).[9] When 4:1 CH_2Cl_2/THF was used as the extraction solvent and 2 N HCl as the priming buffer on the Hydromatrix column, excess amine was efficiently removed. Representative compounds prepared by these methods are shown in Table 2.

In addition to the expected product, we occasionally observed several minor impurities in the final product wells (Figure 2). Although the $FeCl_3$/pyridine test was negative for all scaffolds coupled to solid support, some free thiophenol may be present because the limit of detection is 10% or less for the free phenol.[10] As a result, amide (compound **22**) was present in some cases. Carboxylic acid (compound **23**) and unacylated alkoxyproline (compound **24**) were seldom observed. If resin **19** was exposed to moisture over prolonged periods of time, carboxylic acid **23** and amine **24** were significant impurities. Amine **25** was observed as a contaminant if the SLE step was not efficient. Both compounds **22** and **25** generally ionize better than the library product by MS and complicated QC analysis.[11,12]

Conclusion

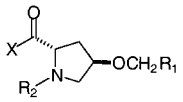
We developed a reliable large-scale solution-phase procedure for alkylation of BOC-hydroxyproline. Coupling onto the Marshall linker followed by BOC deprotection, acylation of the proline nitrogen, and cleavage off solid support gave fully functionalized alkoxyproline derivatives in high yield. A final SLE purification gave the products in high purity. These methods have been successfully applied to the synthesis of several spatially separated small-molecule libraries composed of over 17 000 compounds.[11,12] Each library consisted of about 5000 compounds. These libraries are currently being screened in a series of in vitro biological assays.

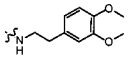
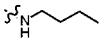
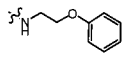
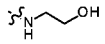
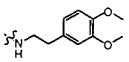
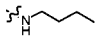
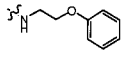
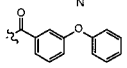
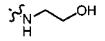
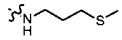
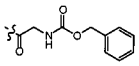
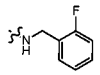
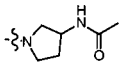
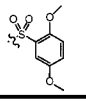
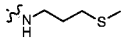
Experimental Section

1. General. All reactions were performed in standard glassware or suitable materials for parallel library synthesis. ^1H NMR and ^{13}C NMR spectra were measured on a JEOL 270 and 67.5 MHz spectrometer at 296 K, respectively. EI mass spectra were recorded on a Sciex 150 EX instrument equipped with an HP 1100 HPLC. Elemental analyses were done by Robertson Microlit Laboratories (Madison, NJ). Flash column chromatographies were done on silica gel 230−400 mesh (flash) from EM Science; thin-layer chromatographies (TLC) were all performed on glass plates coated with silica gel 60 F_{254} from Merck. Reversed-phase HPLC was performed on a HP1100 system (Hewlett-Packard, Palo Alto, CA) equipped with a vacuum degasser, binary pump, autosample, column compartment, a diode array detector, and a C18 column (3.0 mm × 100 mm, 5 μm, 100 Å) from Phenomenex (Phenomenex, Torrance, CA) at 40 °C with a flow rate of 1.0 mL/min. Two mobile phases (mobile phase A, 99% water, 1% acetonitrile, 0.05% TFA; mobile phase B, 1% water, 99% acetonitrile, 0.05% TFA) were employed to run a gradient condition from 0% B to 100% B in 6.0 min, 100% B for 2.0 min, and reequilibrated at 0% B for 2 min. An injection volume of 10 μL was used. All reagents and solvents were purchased reagent grade and were used without further purification. BOC-Hyp-OH (**13**) was obtained from Novabiochem.

2. General Procedure for the Preparation of BOC-Protected Alkoxyproline Scaffolds (14f). In a 2 L Morton three-neck round-bottomed flask equipped with a mechanical stirrer, finely powdered 85% KOH (93.5 g, 1.67 mol) was dissolved in HPLC-grade DMSO (400 mL) under an atmosphere of nitrogen. After the mixture was stirred at ambient temperature for 15 min, the apparatus was cooled to 0 °C with an ice−water bath. After the mixture was stirred for 10 min, Boc-Hyp-OH (48.6 g, 0.210 mol) was added in one portion. DMSO (ca. 10 mL) was used to rinse residual BOC-Hyp-OH off the neck of the flask. After being stirred for 5 min, the completely homogeneous solution was treated with one portion of 3-methoxybenzyl bromide (187 g, 0.928 mol). DMSO (10 mL) was used to rinse residual 3-methoxybenzyl bromide off the neck of the flask. After being stirred at 0 °C for 15 min, the reaction mixture was warmed to ambient temperature for 4 h. The reaction was monitored by the following procedure. The reaction mixture (0.5 mL) was aliquoted to a 4 mL glass vial and diluted with water (1 mL). The aliquot was acidified with 1 M aqueous $KHSO_4$ (0.5 mL) to pH 2−3 (pH paper). Diethyl ether (2 mL) was added to the acidic mixture. The organic phase was analyzed by TLC (9:1 CH_2Cl_2/MeOH) using UV light and ninhydrin spray detection. After the reaction was judged complete by TLC after 4 h, the reaction mixture was poured into water (1.2 L). The Morton flask was rinsed with an additional portion of water (400 mL), and the aqueous wash was transferred into the reaction mixture. After being stirred at ambient temperature for 5 min, the suspension was washed with diethyl ether (2 × 1.2 L). The aqueous phase was acidified with 87% concentrated H_3PO_4 (150 mL) to pH 2−3 (pH paper). This solution was then extracted with diethyl ether (2 × 1.2 L). The combined ether layers were washed with water (2 × 650 mL) and saturated aqueous NaCl (2 × 700 mL). The organic layer was dried over anhydrous $MgSO_4$ for 30 min, filtered, and concentrated in vacuo. The crude material was then triturated with hexane twice and concentrated in vacuo overnight.

Table 2. Representative Alkoxyprolines Prepared via Solid-Phase Library Syntheses



Compound	R_1	R_2	X	Yield[a] (%)	Purity[b] (%)
16a				>99	92
16b				86	90
16c				96	88
16d				>99	92
16e				63	77
16f				66	93
16g				71	98
16h				87	86
16i				66	93
16j				62	98
17a				57	82
17b				82	61

[a] Crude yield. [b] Area under the curve by UV at 214 nm.

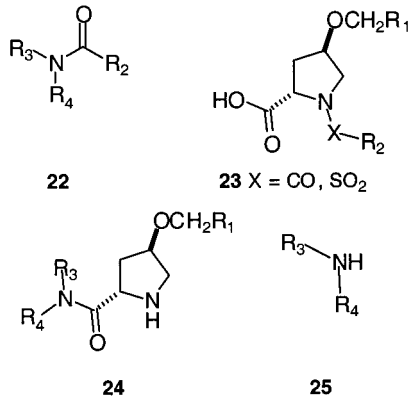


Figure 2. Observed side products.

If minor impurities are observed by HPLC at 214 nm, a solution of crude product in diethyl ether (1 L) was treated with cyclohexylamine (18 mL, 0.210 mol). Upon precipitation, the mixture was cooled with an ice bath, stirred for 20 min, and filtered through a Buchner funnel under vacuum. The complex was washed with diethyl ether (2 × 250 mL) and hexane (2 × 250 mL) and air-dried overnight.

A solution of the complex in water (500 mL) and diethyl ether (350 mL) was stirred at ambient temperature until all solid material dissolved. The solution was then acidified with 87% concentrated H_3PO_4 (40 mL) to pH 2−3. After separation of the organic layer from the aqueous layer, the aqueous layer was extracted with diethyl ether (350 mL). The combined organic layers were washed with 0.5 M $KHSO_4$ (350 mL), water (250 mL), and saturated aqueous NaCl (2 × 300 mL). The combined organic layers were dried over anhydrous $MgSO_4$ for 15 min and filtered. Concentration in vacuo provided 72.0 g (98%) of scaffold **14f** as a yellow oil.

14a: white solid; $[\alpha]_D^{25}$ −42.6° (*c* 1.06, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.93 (br s, 1 H), 7.34−7.29 (m, 5 H), 4.55−4.44 (m, 2 H), 4.37 (t, J = 7.9 Hz, 1 H), 4.18−4.13 (m, 1 H), 3.74−3.48 (m, 2 H), 2.45−2.31 (m, 2 H), 2.16−2.07 (m, 1 H), 1.43 (d, J = 15.1 Hz, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.72, 178.70, 175.3, 156.2, 153.8, 137.6, 128.5, 127.9, 127.64, 127.59, 81.6, 80.7, 76.3, 75.9, 71.3, 71.1, 57.9, 51.9, 51.3, 36.6, 34.5, 28.3, 28.2; MS (ESI) *m*/*z* 322 [$(M + H)^+$].

14b: tan solid; $[\alpha]_D^{25}$ −22.9° (*c* 1.04, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.78 (br s, 1 H), 7.30−7.24 (m, 2 H),

7.19−7.14 (m, 3 H), 4.46−4.30 (m, 1 H), 4.07−3.98 (m, 1 H), 3.64−3.32 (m, 4 H), 2.66 (t, J = 7.4 Hz, 2 H), 2.40−1.81 (m, 2 H), 1.44 (d, J = 14.6 Hz, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.71, 175.37, 156.22, 156.21, 153.84, 141.58, 128.39, 128.33, 125.82, 81.52, 81.49, 80.63, 76.64, 68.34, 68.20, 57.87, 51.89, 51.31, 36.60, 34.53, 32.13, 31.21, 31.16, 28.32, 28.20; MS (ESI) m/z 350 [(M + H)$^+$].

14c: yellow oil; $[\alpha]_D^{25}$ −56.6° (c 1.03, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.37 (br s, 1 H), 4.40−4.25 (m, 1 H), 4.07−3.95 (m, 1 H), 3.60−3.30 (m, 4 H), 2.39−2.00 (m, 3 H), 1.59−1.22 (m, 6 H), 1.40 (d, J = 14.6 Hz, 9 H), 0.86 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.5, 178.4, 178.3, 155.8, 153.9, 81.2, 81.1, 80.6, 76.3, 69.0, 57.8, 57.7, 51.8, 51.3, 36.5, 34.8, 31.7, 31.5, 28.3, 28.1, 22.6, 19.2, 13.7; MS (ESI) m/z 288 [(M + H)$^+$].

14d: pale-yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.44−4.27 (m, 1 H), 4.01−3.98 (m, 1 H), 3.62−3.42 (m, 3 H), 3.24−3.12 (m, 2 H), 2.39−2.02 (m, 3 H), 1.71−1.55 (m, 5 H), 1.42 (d, J = 16.3 Hz, 9 H), 1.28−1.05 (m, 2 H), 0.93−0.81 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.63, 178.60, 153.88, 81.64, 81.62, 80.57, 75.11, 75.04, 65.82, 57.97, 57.90, 51.86, 51.34, 38.00, 36.60, 34.49, 34.44, 29.94, 28.31, 28.20, 26.53, 25.76; MS (ESI) m/z 328 [(M + H)$^+$].

14e: yellow solid; $[\alpha]_D^{25}$ −47.2° (c 1.01, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.30−7.14 (m, 5 H), 4.46−4.30 (m, 1 H), 4.06−3.96 (m, 1 H), 3.68−3.30 (m, 4 H), 2.65 (t, J = 7.4 Hz, 2 H), 2.40−2.18 (m, 1 H), 1.91−1.81 (m, 2 H), 1.44 (d, J = 15.6 Hz, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.7, 175.0, 156.4, 153.8, 141.6, 132.7, 128.4, 128.4, 128.3, 125.8, 81.6, 80.6, 68.4, 68.2, 57.9, 51.9, 51.3, 36.6, 34.4, 32.2, 31.22, 31.17, 28.3, 28.2; MS (ESI) m/z 277 [(M − 101 + H)$^+$].

14f: dark-yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.67 (br s, 1 H), 7.24 (t, J = 7.2 Hz, 1 H), 6.85 (t, J = 13.9, 7.7 Hz, 3 H), 4.48−4.36 (m, 3 H), 4.16−4.14 (m, 1 H), 3.79 (s, 3 H), 3.71 (d, J = 12.4 Hz, 1 H), 3.60−3.43 (m, 2 H), 2.49−2.06 (m, 2 H), 1.42 (d, J = 14.8 Hz, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 178.5, 175.4, 159.7, 156.1, 153.8, 139.2, 129.5, 119.8, 119.8, 113.3, 113.3, 113.0, 81.5, 80.7, 76.28, 75.9, 71.1, 70.9, 57.9, 55.2, 51.9, 51.3, 36.6, 34.6, 28.3, 28.2; MS (ESI) m/z 352 [(M + H)$^+$].

3. General Procedure for the Preparation of Library Compounds. 3.1. Carboxylic Acid Coupling and BOC Deprotection (19). DIC (0.82 mL, 5.3 mmol) was added to a CH_2Cl_2 solution (6.0 mL) of carboxylic acid **14** (5.27 mmol), and the solution was allowed to sit for 15 min with occasional swirling. The mixture was then added to the Marshall resin (1.5 g, 1.8 mmol). The vial was rinsed with CH_2Cl_2 (5.0 mL), and this solution was added to the resin. DMAP (0.214 g, 1.76 mmol) was then added to the slurry, and the mixture was shaken for at least 18 h. The resin was then washed using the following solvents: CH_2Cl_2 then THF (4×); CH_2Cl_2 (4×), MeOH (3×). The resin was then dried on high vacuum overnight (ca. 16 h). The resin loading was determined by mass analysis as a percentage of the theoretical value. The resin was also qualitatively analyzed by IR and by the $FeCl_3$/pyr test. Resins (ca. 100 mg/well) were then transferred into a 96-well plate and washed with CH_2Cl_2 (2×). A solution (1 mL/well) of TFA/CH_2Cl_2/anisole (50:48:2) was added to each well. The plate was shaken for 1 h. After the bottom of the plates were freezed in dry ice, the plates were unclamped and TFA was allowed to drain. The resins were then washed using the following solvents: CH_2Cl_2 (3×), 20% Et_3N/CH_2Cl_2 (3×), MeOH (3×), CH_2Cl_2 (3×).

3.2. Acylation with Acid Chlorides or Sulfonyl Chlorides (20 and 21). Two different classes of reagents, sulfonyl chlorides and acid chlorides, were used to acylate the 4-aminoalkyl substituent. Each acylator (0.35 mmol) was dissolved in 0.70 M i-Pr_2NEt in CH_2Cl_2 (1.0 mL). A solution of each acylator (1.0 mL/well) was added to the appropriate well. The plate was shaken overnight (ca. 16 h). The resins were washed using the following solvents: CH_2Cl_2 then MeOH (4×), CH_2Cl_2 (4×).

3.3. Acylation with Carboxylic Acids (20). Each carboxylic acid (0.36 mmol) was dissolved in 0.36 M HOBT in DMF (1.0 mL) and treated with DIC (58 μL, 0.37 mmol). After the solutions were allowed to sit for 15 min with occasional swirling, a solution of each acylator (1 mL/well) was added to resin **19**. The plate was shaken overnight (ca. 16 h). The resins were washed using the following solvents: DMF (4×); MeOH (3×); CH_2Cl_2 (4×).

3.4. Product Cleavage (16 and 17). After being washed with dioxane (2×), the acylated resin **20** or sulfonylated resin **21** was treated with the appropriate amine (0.32 mmol, 0.80 mL of a 0.40 M solution in 1,4-dioxane). The plates were shaken at ambient temperature for 48 h. After the bottom of the plates were frozen, the crude products were collected in a deep-well plate. The resins were washed with 1,4-dioxane (0.35 mL, 2×). After freezing the dioxane solutions in −80 °C freezer for 1 h, the products were lyophilized for at least 4 h.

4. General Procedure for the Purification of Library Compounds. Removal of the excess amine starting material was accomplished by SLE using Varian Chem Elut material packed into a Polyfiltronics plate (10 μm PP/P). The Chem Elut (∼2.0 g) was treated with 2.0 N HCl (0.6 mL/well) followed by addition of the crude product in 4:1 CH_2Cl_2/THF (1 mL). After the product solution was allowed to elute for 15 min into a deep-well plate, the source plate was washed with 4:1 CH_2Cl_2/THF (0.35 mL) and each wash was transferred immediately to the SLE plate. Each 4:1 CH_2Cl_2/THF wash was allowed to drain for 15 min. The products were then concentrated in vacuo. Representative compounds below were purified by either reversed-phase preparative HPLC, normal-phase preparative HPLC, or normal-phase flash chromatography.

16a: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.36−7.27 (m, 5 H), 7.08 (t, J = 5.5 Hz, 1 H), 6.72 (dd, J = 19.5, 8.7 Hz, 3 H), 4.61 (t, J = 4.2 Hz, 1H), 4.52 (dd, J = 27.9, 11.9 Hz, 2 H), 4.34 (m, J = 5.7 Hz, 1 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.60 (dd, J = 10.6, 5.9 Hz, 1 H), 3.50 (dd, J = 10.6, 4.7 Hz, 1 H), 3.46−3.38 (m, 2 H), 2.70 (t, J = 7.4 Hz, 2 H), 2.65−2.56 (m, 2 H), 2.26−2.17 (m, 1 H), 2.01−1.93 (m, 1 H), 1.80−1.58 (m, 4 H), 1.49−1.31 (m, 1 H), 1.22−1.15 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 176.4, 171.0, 148.9, 147.5, 137.7, 131.3, 128.5, 127.9, 127.6, 120.6, 111.9, 111.2, 71.6, 58.3, 55.8, 52.0, 42.6, 40.8, 35.2, 32.6,

29.0, 28.4, 25.7, 25.5; MS (ESI) *m/z* 495 [(M + H)$^+$]. Anal. Calcd for $C_{29}H_{38}N_2O_5$·TFA: C, 61.17; H, 6.46; N, 4.60. Found: C, 61.55; H, 6.55; N, 4.59.

16b: colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.29−7.23 (m, 2 H), 7.19−7.13 (m, 3 H), 6.94 (br t), 4.62 (dd, J = 8.4, 4.7 Hz, 1 H), 4.21 (ddd, J = 10.4, 5.4, 5.4 Hz, 1 H), 4.02 (s, 2 H), 3.57 (dd, J = 10.9, 5.4 Hz, 1 H), 3.46−3.33 (m, 7 H), 3.29−3.10 (m, 1 H), 2.64 (t, J = 7.4 Hz, 2 H), 2.59−2.51 (m, 1 H), 2.24 (br s, 1 H), 1.96−1.80 (m, 3 H), 1.50−1.40 (m, 2 H), 1.37−1.23 (m, 2 H), 0.88 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.4, 169.5, 141.5, 128.4, 128.3, 125.8, 71.6, 68.5, 59.1, 58.7, 51.2, 39.3, 32.5, 32.2, 31.4, 31.2, 20.0, 13.7; MS (ESI) *m/z* 377 [(M + H)$^+$]. Anal. Calcd for $C_{21}H_{32}N_2O_4$·0.5TFA: C, 60.97; H, 7.51; N, 6.47. Found: C, 60.42; H, 7.25; N, 6.50.

16c: colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.47−7.33 (m, 5 H), 7.26−7.17 (m, 2 H), 6.94−6.84 (m, 3 H), 4.84 (t, J = 7.9 Hz, 1 H), 4.66 (br s, 1 H), 4.03 (t, J = 4.7 Hz, 3 H), 3.56 (d, J = 3.5 Hz, 2 H), 3.31 (ddd, J = 9.2, 6.4, 6.4 Hz, 1 H), 3.18 (ddd, J = 9.2, 6.4, 6.4 Hz, 1 H), 2.53 (ddd, J = 12.9, 7.7, 4.9 Hz, 1 H), 2.23−2.15 (m, 1 H), 1.47−1.37 (m, 2 H), 1.31−1.17 (m, 2 H), 0.83 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 171.8, 171.3, 158.4, 135.4, 130.5, 129.4, 128.3, 127.3, 121.0, 114.5, 68.6, 66.3, 58.7, 55.2, 39.2, 33.3, 31.7, 19.2, 13.8; MS (ESI) *m/z* 411 [(M + H)$^+$]. Anal. Calcd for $C_{24}H_{30}N_2O_4$·0.5TFA: C, 64.24; H, 6.53; N, 6.00. Found: C, 64.58; H, 6.57; N, 6.06.

16d: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.41 (d, J = 8.2 Hz, 2 H), 7.18 (d, J = 7.9 Hz, 2 H), 4.74 (t, J = 7.9 Hz, 1 H), 3.99 (br s, 1 H), 3.71−3.53 (m, 4 H), 3.50−3.41 (m, 1 H), 3.32−3.22 (m, 1 H), 3.14−3.09 (m, 3 H), 2.98 (dd, J = 8.9, 6.7 Hz, 1 H), 2.47−2.39 (m, 1 H), 2.36 (s, 3 H), 2.24−2.13 (m, 1 H), 1.70−1.53 (m, 5 H), 1.49−1.35 (m, 1 H), 1.23−1.05 (m, 3 H), 0.86−0.69 (m, 2 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 140.9, 132.6, 128.9, 127.5, 74.7, 61.6, 59.2, 55.5, 42.6, 37.9, 33.8, 30.0, 29.9, 26.5, 25.8, 21.4; MS (ESI) *m/z* 389 [(M + H)$^+$]. Anal. Calcd for $C_{22}H_{32}N_2O_4$·0.5TFA: C, 62.02; H, 7.30; N, 6.29. Found: C, 62.28; H, 7.22; N, 6.24.

16e: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.91 (d, J = 15.6 Hz, 1 H), 7.45 (d, J = 7.7 Hz, 1 H), 7.30 (br s, 7 H), 6.96−6.87 (m, 2 H), 6.71 (br s, 3 H), 4.81−4.73 (m, 1 H), 4.52 (q, J = 11.9 Hz, 2 H), 4.41−4.32 (m, 1 H), 4.09 (dd, J = 13.6, 6.7 Hz, 2 H), 3.83 (s, 2 H), 3.75 (s, 3 H), 3.62 (dd, J = 10.1, 5.2 Hz, 1 H), 3.48−3.41 (m, 2 H), 2.75−2.66 (m, 3 H), 2.03−1.93 (m, 3 H), 1.75 (br s, 1 H), 1.46 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.8, 167.0, 157.9, 148.8, 147.4, 139.3, 137.7, 131.5, 131.1, 129.8, 128.5 (3×), 127.8, 127.6 (3×), 123.6, 120.7, 120.8, 118.5, 111.9, 111.2, 71.7, 63.8, 58.6, 55.8, 55.7, 52.1, 40.9, 35.3, 32.7, 14.9; MS (ESI) *m/z* 559 [(M + H)$^+$]. Anal. Calcd for $C_{33}H_{38}N_2O_6$: C, 70.95; H, 6.86; N, 5.01. Found: C, 71.06; H, 6.87; N, 5.12.

16f: pale-yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.30−7.10 (m, 7 H), 6.96−6.81 (m, 2 H), 4.67 (dd, J = 8.4, 3.7 Hz, 1 H), 4.21 (ddd, J = 11.6, 5.9, 5.9 Hz, 1 H), 3.79 (s, 3 H), 3.62 (s, 2 H), 3.67−3.30 (m, 4 H), 3.23−3.09 (m, 2 H), 2.67−2.59 (m, 3 H), 1.95−1.80 (m, 4 H), 1.46−1.14 (m, 6 H), 0.86 (t, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 171.8, 170.6, 157.0, 141.6, 130.3, 128.4, 128.3, 125.8, 123.2, 120.8, 110.4, 68.6, 58.5, 55.4, 52.3, 39.2, 36.0, 32.7, 32.2, 31.5, 31.3, 20.0, 13.7; MS (ESI) *m/z* 453 [(M + H)$^+$]. Anal. Calcd for $C_{27}H_{36}N_2O_4$: C, 71.65; H, 8.02; N, 6.19. Found: C, 71.70; H, 8.14; N, 6.27.

16g: dark-yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.16 (s, 1 H, minor), 9.09 (s, 1 H, major), 8.61 (d, J = 1.7 Hz, 1 H, major), 8.50 (d, J = 1.5 Hz, 1 H, major), 8.21 (d, J = 1.7 Hz, 1 H, minor), 8.12 (d, 1 H, minor), 7.27−7.14 (m, 3 H), 6.97−6.71 (m, 2 H), 6.59 (br s, 1 H), 5.09 (t, J = 7.2 Hz, 1 H, minor), 4.82 (t, J = 7.2 Hz, 1 H, major), 4.19−3.26 (m, 7 H), 2.50−2.41 (m, 1 H), 2.36−2.28 (m, 1 H), 2.20−2.10 (m, 1 H), 1.96 (br s, 1 H), 1.55−1.39 (m, 1 H), 1.35−1.17 (m, 3 H), 0.92 (m, 3 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.8, 165.5, 158.4, 148.1, 146.6, 146.2, 145.8, 142.2, 141.4, 129.4, 121.0, 114.5, 77.6, 75.1, 68.9, 66.4, 59.9, 54.9, 39.1, 38.9, 38.0, 33.1, 31.7, 31.5, 22.6, 19.2, 14.1, 13.7; MS (ESI) *m/z* 413 [(M + H)$^+$]. Anal. Calcd for $C_{22}H_{28}N_4O_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.18; H, 6.83; N, 13.38.

16h: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.34−7.20 (m, 4 H), 7.13−6.97 (m, 5 H), 4.71 (dd, J = 7.9, 7.7 Hz, 1 H), 4.09 (q, J = 6.9 Hz, 1 H), 3.99 (br s, 1 H), 3.71−3.20 (m, 6 H), 3.11 (dd, J = 8.2, 6.9 Hz, 1 H, 2.98 (dd, J = 8.7, 6.9 Hz, 1 H), 2.80 (br s, 1 H), 2.46−2.36 (m, 1 H), 2.22−2.15 (m, 1 H), 2.01 (s, 1 H), 1.62 (br s, 5 H), 1.45−1.40 (m, 1 H), 1.23−1.04 (m, 6 H), 0.85−0.73 (m, 4 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 171.8, 170.7, 157.4, 156.4, 137.3, 129.9, 129.8, 123.8, 122.0, 120.6, 119.2, 117.5, 74.7, 61.6, 59.2, 55.3, 42.6, 37.9, 33.8, 31.5, 30.0, 29.9, 26.5, 25.7, 22.6, 14.1; MS (ESI) *m/z* 467 [(M + H)$^+$]. Anal. Calcd for $C_{27}H_{34}N_2O_5$: C, 69.50; H, 7.35; N, 6.00. Found: C, 69.58; H, 7.30; N, 5.89.

16i: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.48 (dd, J = 4.7, 1.8 Hz, 1 H), 7.42 (dd, J = 7.4, 1.8 Hz, 1 H), 7.32 (d, J = 8.2 Hz, 2 H), 7.15 (d, J = 8.2 Hz, 2 H), 7.02 (dd, J = 7.7, 4.9 Hz, 1 H), 4.87 (dd, J = 8.2, 6.2 Hz, 1 H), 4.36 (q, J = 11.4 Hz, 2 H), 4.22−4.16 (m, 1 H), 3.55 (dd, J = 11.6, 4.5 Hz, 1 H), 3.46−3.29 (m, 3 H), 2.69−2.59 (m, 1 H), 2.57 (s, 3 H), 2.53 (t, J = 7.2 Hz, 2 H), 2.32−2.21 (m, 2 H), 2.06 (s, 3 H), 1.87−1.77 (m, 1 H), 1.76 (br s, 1 H), 1.28 (s, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.1, 168.4, 155.9, 150.9, 150.0, 134.4, 133.9, 130.6, 127.4, 125.3, 118.9, 76.7, 70.9, 58.5, 53.5, 38.5, 34.5, 33.5, 31.5, 31.3, 28.7, 15.4, 13.0; MS (ESI) *m/z* 516 [(M + H)$^+$]. Anal. Calcd for $C_{27}H_{37}N_3O_3S_2$: C, 62.88; H, 7.23; N, 8.15. Found: C, 63.04; H, 7.19; N, 8.04.

16j: pale-yellow oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.32−6.80 (m, 13 H), 5.67 (br t, 1 H), 5.07 (s, 2 H), 4.63−4.30 (m, 6 H), 3.89 (d, J = 4.4 Hz, 1 H), 3.78 (s, 3 H), 3.60 (dd, J = 10.6, 5.2 Hz, 1 H), 3.50−3.42 (m, 1 H), 2.54−2.45 (m, 1 H), 2.15−2.06 (m, 1 H), 1.87 (br s, 1 H), 1.19 (t, J = 6.9 Hz, 1 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.4, 168.2, 159.8, 156.2, 139.1, 136.3, 129.8, 129.7, 129.6, 129.2, 129.0, 128.5, 128.1, 128.0, 125.0, 124.8, 124.3, 124.2, 119.7, 115.4, 115.1, 113.4, 113.1, 71.4, 66.9, 59.0, 55.2, 51.3, 43.4, 37.6, 37.5, 33.4, 30.3, 15.1; MS (ESI) *m/z* 550 [(M + H)$^+$]. Anal. Calcd for $C_{30}H_{32}FN_3O_6S_2$: C, 65.56; H, 5.87; F, 3.46; N, 7.65. Found: C, 65.31; H, 6.04; F, 3.61; N, 7.45.

17a: white solid; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.40−7.15 (m, 3 H), 6.88−6.46 (m, 5 H), 5.10 (br s 1 H), 4.72−4.60 (m, 1 H), 4.52 (br s, 1 H), 4.34−4.16 (m, 2 H), 4.00−3.37 (m, 20 H), 2.3−1.90 (m, 8 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 171.4, 171.03, 170.97, 170.65, 159.7, 152.8, 149.1, 139.0, 130.2, 130.0, 129.5, 129.4, 121.8, 121.5, 119.7, 119.7, 119.4, 113.25, 113.18, 113.10, 113.0, 112.9, 110.5, 110.4, 110.3, 70.8, 70.4, 58.1, 57.6, 56.3, 56.1, 55.2, 52.7, 52.2, 51.8, 50.4, 50.3, 47.8, 44.9, 44.4, 44.2, 37.2, 37.0, 32.3, 29.9, 22.9; MS (ESI) *m*/*z* 562 [$(M + H)^+$]. Anal. Calcd for $C_{27}H_{35}N_3O_8S\cdot 0.5TFA$: C, 54.37; H, 5.74; N, 6.80. Found: C, 55.77; H, 6.92; N, 6.87.

17b: colorless oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.43 (d, J = 3.0 Hz, 1 H), 7.30 (d, J = 8.2 Hz, 2 H), 7.02 (d, J = 8.2 Hz, 2 H), 6.99 (d, J = 3.2 Hz, 1 H), 6.95 (d, J = 2.9 Hz, 1 H), 6.77 (d, J = 9.2 Hz, 1 H), 4.81 (dd, J = 7.4, 6.7 Hz, 1 H), 4.17 (q, J = 24.7, 11.4 Hz, 2 H), 4.09−4.02 (m, 1 H), 3.80 (s, 3 H), 3.78 (s, 3 H), 3.56−3.27 (m, 4 H), 2.55 (t, J = 7.2, 6.2 Hz, 2 H), 2.30−2.25 (m, 2 H), 2.10 (s, 3 H), 1.90−1.79 (m, 2 H), 1.29 (s, 9 H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 173.3, 152.9, 151.2, 150.9, 134.1, 127.5, 125.3, 121.1, 116.8, 114.0, 76.3, 71.0, 61.4, 56.5, 56.0, 53.2, 39.0, 36.2, 34.5, 31.4, 31.3, 28.1, 15.5; MS (ESI) *m*/*z* 565 [$(M + H)^+$]. Anal. Calcd for $C_{28}H_{40}N_2O_6S_2\cdot 0.5TFA$: C, 56.04; H, 6.52; N, 4.51. Found: C, 56.36; H, 7.03; N, 4.36.

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References and Notes

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(8) The test was performed by dissolving about 50 mg of resin in pyridine (0.5 mL) and adding a 0.5 M chloroform solution of $FeCl_3$ (0.5 mL). The resin was washed with CH_2Cl_2 and examined. Any color change to a dark color indicates free thiophenol. With free thiophenol resin, the beads will turn dark green-black. The test will indicate no free phenol if no color change from the original resin color is observed. This is not a quantitative test but a qualitative measure of resin loading.

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(10) The limit of detection for free phenol was determined by adding known quantities of unacylated Marshall resin to a functionalized sample. Additional amounts of unacylated Marshall resin were added until a postive test for phenol was obtained by the ferric chloride test.

(11) Library analysis was performed by flow injection MS for identity of 12.5% of library wells. For each library, six reference samples were analyzed by LC−MS for qualitative purity (area under the curve by UV at 214 nm and evaporative light scattering detection) and subjected to a weight percent purity analysis. The weight percent purity analysis is a method for quantifying the amount of product in a production library well. Several compounds were purified to homogeneity. Standard detection curves for HPLC−UV were made for these purified and characterized standards to represent the diversity of the chemistry within the library. Compounds of the same structure found in the production library were sampled from the expected positions. The absolute mass of the product as determined by HPLC divided by mass recovery for a given well gave the weight percent purity.

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