Solution-Phase Synthesis of Novel Linear Oxyamine Combinatorial Libraries with Antibacterial Activity

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Use of solution phase combinatorial library synthesis led to the discovery of several oxyamine-containing antibacterial compounds. Solution-phase simultaneous addition of meta-substituted benzyl bromides and "fix-last" combinatorial strategies were used to prepare libraries. Additional structure−activity relationship studies were conducted by reductive cleavage of the oxyamine moiety and led to loss of antibacterial activity. Several single compounds were designed and synthesized on the basis of library screening results and were shown to have antibacterial activity.

Introduction

Strategies to enhance drug discovery by devising, synthesizing, and screening combinatorial libraries in a high-throughput process are undergoing a very active phase of development.[1,2] Initial combinatorial libraries were prepared by solid-phase techniques.[1,2] Although solution-phase synthesis of combinatorial libraries is a relatively unexplored area, we believe such techniques provide some advantages over solid-phase synthesis.[3−5] One of our recent approaches in this area is to synthesize combinatorial libraries in solution-phase by adding groups of functionalities in a simultaneous manner.[5] As we desired to search for novel pharmacophores, we elected to base our combinatorial libraries on novel structures rather than known pharmacophore structures typically targeted in most combinatorial chemistries.[6−8] In addition, we desired to rapidly search novel structure space with a variety of scaffolds selected to provide various shapes (footprints). We sought to design and synthesize libraries containing molecules possessing either conformational constraints or flexibilities before the active conformer is known. If needed, structure−activity relationship studies could subsequently be performed on the basis of the information obtained from an active library. In previous work, a series of libraries was generated from unsymmetrical polyazaphane scaffolds.[5] In the present work, we selected the linear scaffold (Figure 1, **1**) for combination with meta-substituted benzyl bromides in order to investigate a totally different structure space. This scaffold has an oxyamine group and a primary alkylamine as combinatorial sites. In this paper, we describe the design and synthesis of the linear scaffold followed by the synthesis of several linear oxyamine combinatorial libraries utilizing solution-phase synthesis techniques.[5b]

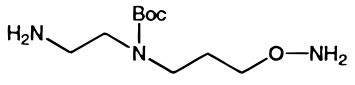

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Figure 1.

Results and Discussion

Prior to library synthesis, the alkylation reaction of the oxyamine group was investigated by two model studies (Scheme 1). Treatment of *O*-methylhydroxylamine with excess benzyl bromide at room temperature followed by consumption of unreacted halide with 3-mercaptopropane sulfonate and liquid−liquid extraction provided the corresponding monoalkylated hydroxylamine product (**2a**) in 67% yield. Compound **2a** was isolated as the hydrochloride salt due to the volatility of the free amine. Additional coupling of the monoalkylated product with benzyl bromide did not proceed at an appreciable rate at room temperature, as evidenced by the absence of higher molecular weight peaks in the mass spectrum of the crude reaction mixture. However, refluxing at 80 °C for 12 h exclusively gave the corresponding dialkylated product (**2b**, 53%) with no evidence of quaternary amine formation. This result demonstrated that the degree of

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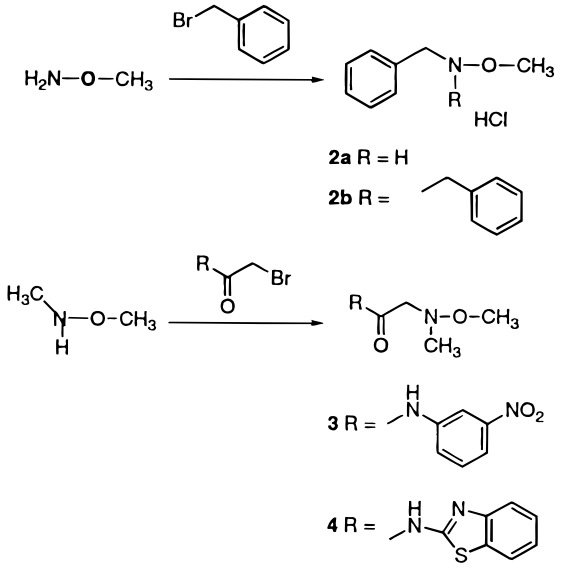
Scheme 1



alkylation could be controlled by the reaction conditions. For comparison, an attempt was made to prepare **2a** by Schiff base formation with benzaldehyde followed by reduction. However, removal of excess benzaldehyde from the reaction mixture was not as efficient as the sulfonate-mediated purification described above, thus precluding the use of Schiff base chemistry in our library synthesis. We also investigated the alkylation reaction with more reactive alkylating agents (α-bromoacetamides) using a commercially available secondary oxyamine derivative (*N*,*O*-dimethylhydroxylamine) as the substrate and employing the same quenching and purification procedure described for the preparation of **2a** and **2b** above. The expected tertiary oxyamines (Scheme 1, **3** and **4**) were obtained in 76% and 51% yields, respectively. No corresponding quaternary salts were isolated in these cases nor indicated by mass spectral analysis of the crude reaction mixtures. Having completed the model studies, we then sought to prepare oxyamine-containing combinatorial libraries based on the linear scaffold, compound **1**.

The synthesis of scaffold **1** is shown in Scheme 2. 3-Amino-1-propanol was monobenzylated by treatment with benzaldehyde in the presence of trimethyl orthoformate followed by reduction with sodium borohydride to afford **5** in excellent yield (93%).[9] Coupling of **5** and *N*-(2-bromoethyl)phthalimide in the presence of potassium carbonate and catalytic potassium iodide gave **6** in 56% yield. The benzyl group on the secondary amine of **6** was deprotected by hydrogenation and reprotected with di-*tert*-butyl dicarbonate (**7** and **8**; 68% and 95% yields, respectively). The oxyamino functionality was introduced onto **8** to give **9** by Mitsunobu reaction. Deprotection of **9** by treatment with hydrazine afforded scaffold **1** in 98% yield.

A "fix-last" combinatorial method[5,10] was devised to minimize the chemical reactions required to prepare the initial libraries. The *tert*-butyl carbamate group on the scaffold serves as a protecting group of an independent combinatorial site which could be functionalized last in the process of library synthesis. Libraries were generated by solution phase simultaneous additions of meta-substituted benzyl bromides to scaffold **1** (Scheme 3) and purified by the solution phase purification procedure described for the model studies above. Successful preparation of the libraries was confirmed by electrospray ionization mass spectrometry (ES/MS), which indicated the presence of the desired products. Subsequently, five tertiary amine libraries (Scheme 3, **10**−**14**) derived from scaffold **1** were generated via the solution-phase techniques described above.

Scheme 2

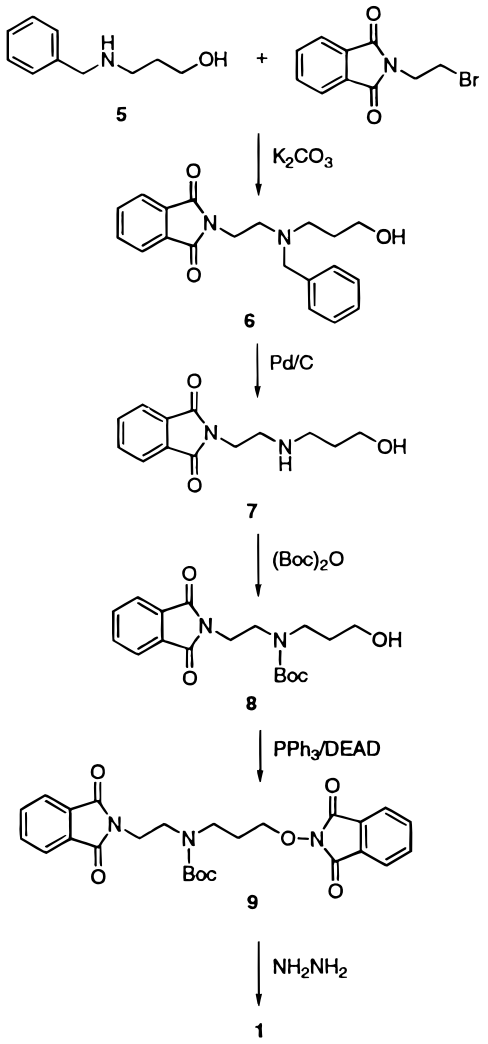


Table 1. Antibacterial Activity Screening Results of the First Round Libraries against One Gram-Positive Strain (*S. pyogenes* ATCC#14289) and One Gram-Negative Strain (*E. coli* imp-)

	minimum inhibition concentration, μM	
compd no.	*S. pyogenes*	*E. coli*
1	>100	>100
10	>100	>100
11	5−10	5−25
12	>100	>100
13	>100	>100
14	>100	>100

Antibacterial activity screening results against one Gram-positive strain (*Streptococcus pyogenes*) and one Gram-negative strain (*Escherichia coli*, imp-) of the first round libraries (libraries **10**-**14**) as well as scaffolds

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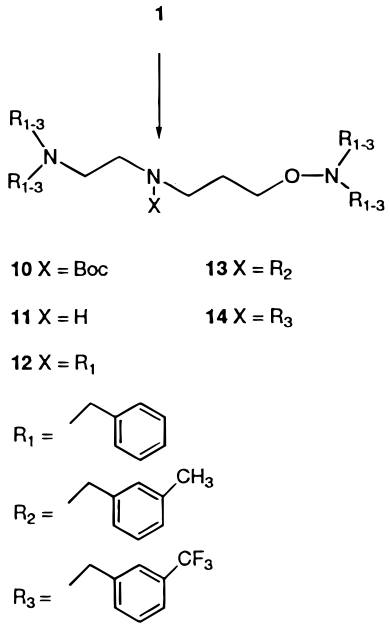
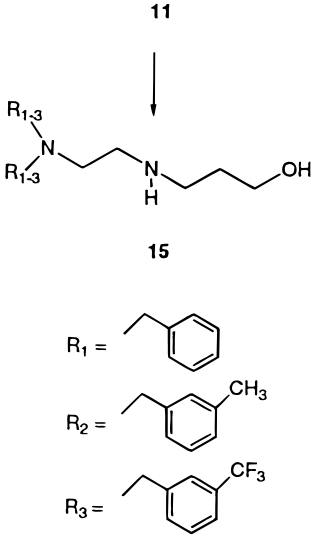
Scheme 3



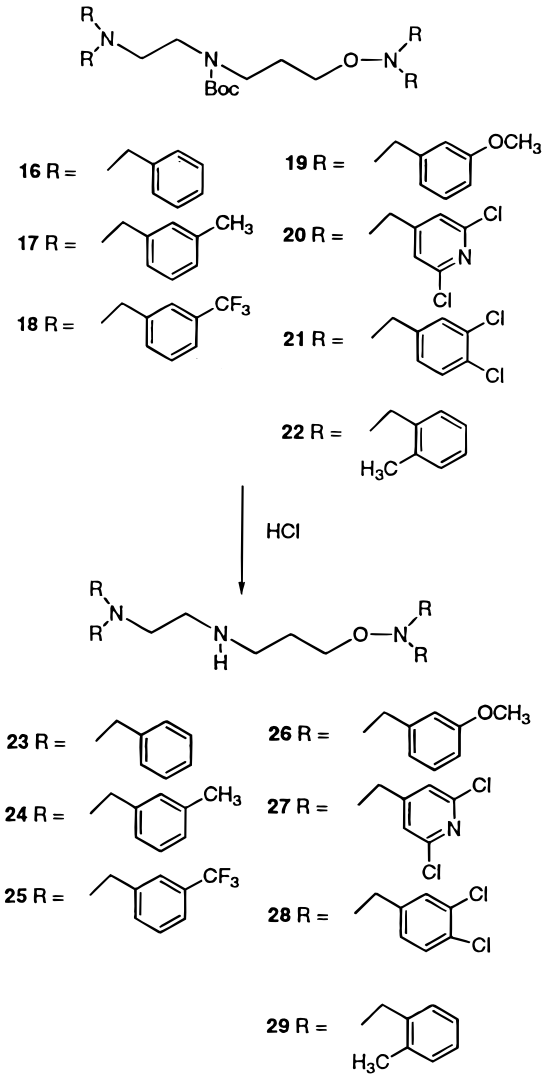
Scheme 4



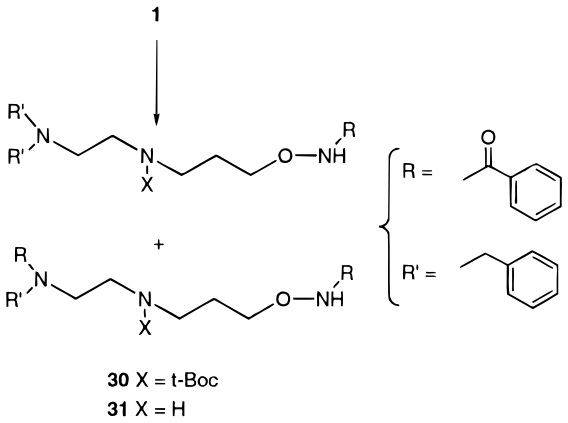
1 are listed in Table 1. The antibacterial activities were measured in solution by minimum inhibition concentration (MIC) of bacterial growth. One sublibrary prepared from scaffold **1** (library **11**) exhibited potent antibacterial activity. Instead of performing deconvolution of library **11**, the initial structure−activity relationship (SAR) studies were performed on the library mixture or by the synthesis of several single compounds (Scheme 4, **15**; Scheme 5, **16**−**22, 23**−**29**; Scheme 6, **30, 31**).

The oxyamine group of scaffold **1** allowed the SAR studies to be performed on the library mixture to investigate the importance of the benzyl moiety for antibacterial activity. Accordingly, the oxyamine bond of scaffold **1** was reductively cleaved by refluxing the library mixture (library **11**) with 1 M BH_3/THF to generate a new library (library **15**). The antibacterial activities of these two libraries are compared in Table 2. Library **15** was five to 10-fold less active against tier 1 bacterial strains. Therefore, through library SAR studies, we believe the benzyl functionality on the oxyamino group is essential to maintain antibacterial activity.

Scheme 5



Scheme 6



Nine single compounds, including three from the active sublibrary (library **11**) and six SAR compounds, were subsequently synthesized (Schemes 5 and 6). These SAR studies sought to optimize the stereoelectronic effects of the benzyl moiety. For this purpose, the remainder of the molecule was held constant as the active components in the active library (library **11**). Among these six SAR compounds, two of them were synthesized in the combinatorial fashion by utilizing the differing reactivities of

Table 2. Antibacterial Activity Screening Results for Various Libraries against Gram-Positive and Gram-Negative Strains

	minimum inhibition concentration, μM							
compd no.	*S. pyrgenes* ATCC#14289	*E. coli* imp-	*S. pyogenes* ATCC#49399	*S. aureus* ATCC#25923	*E. faecalis* ATCC#29212	*E. coli* ATCC#11775	*K. pneumoniae* ATCC#13883	*C. albicans* ATCC#10231
11	5−10	5−25						
15	25−50	~50						
23	2.5−5	5−10	3−6	6−12	2−12	12−25	6−12	>100
24	2.5−5	2.5−5	12−25	3−6	6−12	6−12	>100	>100
25	25−50	25−50						
26	1−5	5−25						>100
27	>40	10−20	12−25	6−12	>100	>100	>100	>100
28	>50	>50						
29	>50	>50						
30	>100	>100						
31	>20	>20						

the oxyamine moiety toward sp^2 and sp^3 carbons[11] in order to incorporate the acyl moiety of the benzyl group into the active compound, **23** (Scheme 6, **31**). The structures of these two compounds were determined by both the ES/MS and MS/MS techniques. Four of the nine compounds (**23**, **24**, **26**, **27**) exhibited potent antimicrobial activities in the low micromolar range as listed in Table 2. The active compounds were also screened against other wild-type bacterial strains, such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (Table 2). The specificity of these compounds was measured by the minimum inhibition concentration of the growth of the yeast cell *Candida albicans*.

Compounds **23**, **24,** and **26** showed equivalent or better activities compared with the library mixture, **11**. Compound **27** showed specificity for *S. aureus*. Two of the three single compounds in the original active pool, benzyl derivative **23** and *m*-methyl benzyl derivative **24,** showed interesting activities. The third compound, *m*-trifluoromethyl benzyl derivative **25**, however, was approximately 5-fold less active. Other different substituents on the benzyl moiety such as 3,4-dichloro derivative **28** and the *o*-methyl benzyl-containing compound **29** did not show inhibition against either Gram-positive or Gram-negative strains up to 50 μM. More importantly, among the active compounds, different specificity against different bacterial strains was observed, depending on the nature of the substituents on the benzyl moiety.

Conclusion

We have used solution-phase combinatorial library synthesis techniques to discover a novel class of antibacterial compounds. The use of an oxyamino-containing scaffold allowed control of product distribution obtained by reaction with both acylating and alkylating agents. Moreover, library SAR study was conducted by reductive cleavage of the oxyamine bond of the scaffold. Several single compounds and SAR compounds were synthesized on the basis of the initial antibacterial screening results of the library mixtures and were shown to have interesting antibacterial activities.

Experimental Section

General. All commercial reagents and solvents were used as received from their respective suppliers with the following exception. The specific benzyl bromide (*m*-methoxy benzyl bromide) used to synthesize compound **29** was prepared by the literature procedure.[12] Flash column chromatography[13] was performed using silica gel 60 (Merck Art 9385). Mass spectra were acquired using an electrospray ionization source in both positive and negative modes. MS/MS data were obtained by isolating ions of interest in a 1.5 amu window using resonance ejection. The bacterial and yeast antigrowth assays were performed in 96-well plate format in 150 μL volume in the presence of library or relevant antibiotic or antifungal controls.[14] Growth was monitored at 24 h for antibacterial assays and at 48 h for antifungal assays by measuring absorbance at 595 nm. The *S. pyogenes* strain was ATCC#14289 and was grown in 1× Todd-Hewitt broth. The *E. coli* imp- strain was a kind gift from Spencer Bensen and was grown in $^1/_2\times$ LB.[15] The *C. albicans* was ATCC#10231 and was grown in YM media.

***N*-Benzyl-*O*-methylhydroxylamine Hydrochloride (2a).** *O*-Methylhydroxylamine (0.25 g, 3 mmol) was dissolved in THF (20 mL), and benzyl bromide (1.4 mL, 12 mmol) and diisopropylethylamine (3.2 mL, 18 mmol) were added sequentially. The mixture was stirred at room temperature for about 12 h. 3-Mercapto-1-propanesulfonic acid sodium salt (2.2 g, 12.3 mmol) and potassium carbonate (3.5 g, 25 mmol) were then added, and the mixture was stirred for 2 h and concentrated in vacuo. The residue was partitioned between H_2O (30 mL) and Et_2O (2 × 30 mL). The organic layer was washed with saturated NaCl (20 mL) and added to 5 mL of 12 M HCl. After stirring for 1 h, the mixture was then diluted with H_2O (30 mL) and poured into a separatory funnel to get layer separation. The aqueous layer was then collected and concentrated in vacuo to give 346 mg (66.5% yield) of the title compound: TLC (R_f = 0.5; 20% EtOAc/hexanes); ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H), 4.40 (s, 2H), 7.50 (m, 5H), 12.10 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 137.8, 130.4, 128.7, 128.3, 60.6, 51.6.

***N,N*-Dibenzyl-*O*-methylhydroxylamine (2b).** *O*-Methylhydroxylamine (0.25 g, 3 mmol) was dissolved in THF (20 mL), and benzyl bromide (1.4 mL, 12 mmol) and diisopropylethylamine (3.2 mL, 18 mmol) were added sequentially. The mixture was stirred at 80 °C for 12 h. 3-Mercapto-1-propanesulfonic acid sodium salt (2.2 g, 12.3 mmol) and potassium carbonate (3.5 g, 25 mmol) were then added, and the mixture was stirred for 2 h and concentrated in vacuo. The residue was partitioned between H_2O (30 mL) and Et_2O (2 × 30 mL). The organic layer was separated, washed with brine (1 × 10 mL), and dried over Na_2SO_4. Removal of the solvent in vacuo provided **3b** as a yellow oil (0.36 g, 53% yield): TLC (R_f = 0.5; 20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 10H), 3.85 (s, 4H), 3.18 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.8, 129.5, 128.1,

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127.2, 62.3, 61.0; HRMS (FAB) *m/z* 227.1310 $(M + H)^+$ ($C_{15}H_{17}NO$ requires 227.1395).

2-(Methoxymethylamino)-*N*-(3-nitrophenyl)acetamide (3). 2-Bromo-*N*-(3-nitrophenyl)acetamide (1.17 g, 4.48 mmol) was dissolved in a 3/2 mixture of THF/DMF (10 mL) at ambient temperature, and *N*,*O*-dimethylhydroxylamine hydrochloride (0.22 g, 2.24 mmol) and diisopropylethylamine (0.75 mL, 8.96 mmol) were added sequentially. After stirring at ambient temperature for 12 h, a freshly prepared solution of 2-mercapto-1-ethanesulfonic acid sodium salt (1.52 g, 9.25 mmol) and potassium carbonate (1.23 g, 8.89 mmol) in water (10 mL) was added. After stirring of the biphasic reaction mixture for 30 min, EtOAc (50 mL) was added and the mixture was agitated. The organic layer was separated, washed with water (2 × 10 mL) and brine (1 × 10 mL), and dried over $MgSO_4$. Removal of the solvent in vacuo provided **4** as a solid (0.54 g, 76%): R_f = 0.5 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 8.90 (br, 1H), 8.0 (m, 2H), 7.51 (m, 1H), 3.63 (s, 3H), 3.50 (s, 2H), 2.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.8, 148.7, 138.7, 130.0, 125.2, 118.9, 114.2, 64.2, 59.8, 44.2; HRMS (FAB) *m/z* 240.0989 $(M + H)^+$ ($C_{10}H_{13}N_3O_4$ requires 240.0984).

***N*-Benzothiazol-2-yl-2-(methoxymethylamino)acetamide (4)**. 2-Bromo-*N*-(2-benzothiazolyl)acetamide (2.84 g, 10.5 mmol) was dissolved in a 3/2 mixture of THF/DMF (20 mL) at ambient temperature, and *N*,*O*-dimethylhydroxylamine hydrochloride (509 mg, 5.21 mmol) and diisopropylethylamine (2.0 mL, 12 mmol) were added sequentially. After stirring at ambient temperature for 12 h, a freshly prepared solution of 2-mercapto-1-ethanesulfonic acid sodium salt (1.71 g, 10.4 mmol) and potassium carbonate (1.44 g, 10.4 mmol) in water (10 mL) was added. After stirring of the biphasic reaction mixture for 30 min, EtOAc (50 mL) was added and the mixture was agitated. The organic layer was separated, washed with water (2 × 10 mL) and brine (1 × 10 mL), and dried over $MgSO_4$. Removal of the solvent in vacuo provided **4** as a solid (0.66 g, 51%): R_f = 0.2 (25% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 10.11 (br s, 1H), 7.82 (m, 2H), 7.41 (m, 2H), 3.62 (s, 3H), 3.60 (s, 2H), 2.73 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 167.7, 157.0, 148.4, 132.3, 126.2, 124.0, 121.4, 121.0, 63.3, 59.8, 44.2.

3-Benzylaminopropan-1-ol (5). 3-Amino-1-propanol (7.2 mL, 94.2 mmol) was added dropwise to a solution of benzaldehyde (10 g, 94.2 mmol) and trimethyl orthoformate (15.5 mL, 141 mmol) in MeOH (300 mL) at room temperature. The reaction was stirred at room temperature for 5 h and then was cooled in an ice bath. Sodium borohydride (3.6 g, 94.2 mmol) was added in two portions, and when gas evolution stopped, the solvent was then evaporated. The resulting residue was partitioned between EtOAc (300 mL) and H_2O (300 mL). The aqueous layer was extracted with more EtOAc (2 × 75 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and evaporated to give the title compound (14.4 g, 93%) as pale yellow, oily residue: TLC (R_f = 0.14; 70% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.27 (m, 5H), 3.80 (s, 2H), 3.60 (t, 2H, *J* = 5.6), 2.72 (t, 2H, *J* = 6.2), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 139.2, 128.5, 127.7, 127.2, 62.6, 53.7, 48.2, 31.2.

2-{2-[Benzyl(3-hydroxypropyl)amino]ethyl}isoindole-1,3-dione (6) *N*-(2-Bromoethyl)phthalimide (9.4 g, 37 mmol), potassium carbonate (1.5 g, 11 mmol), and potassium iodide (91.3 mg, 0.55 mmol) were added sequentially to a solution of **5** (6.0 g, 36.8 mmol) in DMF (300 mL). The resulting mixture was maintained at 65 °C for 10 h. The reaction mixture was evaporated in vacuo and partitioned between EtOAc (300 mL) and H_2O (300 mL). The aqueous layer was extracted with more EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to give a residue. Purification of the residue by flash column chromatography (gradient elution, 10% → 40% EtOAc/hexanes) provided **6** (7.0 g, 56%): TLC (R_f = 0.68; 100% EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.71 (m, 4H), 7.15 (m, 5H), 3.80 (t, 2H, *J* = 6.1), 3.63 (t, 2H, *J* = 5.5), 3.60 (s, 2H), 2.72 (q, 4H, *J* = 6.3), 1.70 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.4, 138.3, 133.9, 129.2, 128.3, 127.2, 123.2, 62.1, 58.3, 52.5, 51.8, 35.8, 28.7; HRMS (FAB) *m/z* 339.1693 $(M + H)^+$ ($C_{20}H_{22}N_2O_3$ requires 339.1709).

2-[2-(3-Hydroxypropylamino)ethyl]isoindole-1,3-dione (7). A mixture of **6** (4.6 g, 11.9 mmol), palladium on activated carbon (10%, 1.0 g), and MeOH/HOAc (100 mL, v/v, 95:5) was transferred to a 250 mL Parr hydrogenation flask. The flask was purged and filled with H_2 three times and then left under H_2 atmosphere at 55 psi with shaking for 4 h. The reaction mixture was filtered through a bed of Celite and the solvent was evaporated in vacuo to give a yellowish oil. The oil residue was purified by flash column chromatography using $MeOH/CH_2Cl_2$ as the eluent to afford 2.5 g (68%) of the title compound as a white solid: mp 94−95 °C; TLC (R_f = 0.38; 50% EtOAc/MeOH); ^{1}H NMR (DMSO-d_6) δ 7.87 (m, 4H), 6.20 (br, 2H), 3.72 (t, 2H, *J* = 6.3), 3.44 (t, 2H, *J* = 6.4), 2.84 (t, 2H, *J* = 6.3), 2.66 (t, 2H, *J* = 6.8), 1.83 (s, 3H), 1.57 (t, 2H, *J* = 6.6); ^{13}C NMR (DMSO-d_6) δ 172.8, 168.0, 134.3, 131.8, 122.9, 59.3, 46.6, 45.8, 36.8, 31.9, 21.9; HRMS (FAB) *m/z* 249.1247 $(M + H)^+$ ($C_{13}H_{16}N_2O_3$ requires 249.1289).

[2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)ethyl](3-hydroxypropyl)carbamic Acid *tert*-Butyl Ester (8). Di-*tert*-butyl dicarbonate (3.6 g, 16.3 mmol) was added to a solution of **7** (2.5 g, 8.1 mmol) and triethylamine (5.6 mL, 40.5 mmol) in CH_2Cl_2 (40 mL). The reaction was stirred at room temperature for 4 h. The reaction mixture was washed with H_2O (3 × 20 mL) and brine (1 × 10 mL) and dried over Na_2SO_4. Removal of the solvent in vacuo provided a residue which was purified by flash column chromatography using EtOAc/hexanes as the eluent to afford 2.4 g (81%) of the title compound as a colorless oil: TLC (R_f = 0.72; 95% EtOAc/MeOH); ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 2H), 7.73 (m, 2H), 3.84 (t, 2H, *J* = 6.0), 3.65 (br, 1H), 3.42 (m, 6H), 1.19 (m, 2H), 1.29 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 167.9, 156.5, 134.0, 131.9, 123.2, 80.4, 58.2, 42.4, 35.6, 30.2, 27.9; HRMS (FAB) *m/z* 371.1598 $(M + Na)^+$ ($C_{18}H_{24}N_2O_5$ requires 371.1583).

[2-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)ethyl][3-(1,3-dioxo-1,3-dihydroisoindol-2-yloxy)propyl]carbamic Acid *tert*-Butyl Ester (9). *N*-Hydroxyphthalimide (0.60 g, 3.7 mmol)) and triphenylphosphine (0.94 g, 3.6 mmol) were added to a solution of **8** (1.2 g, 3.3 mmol) in anhydrous THF (30 mL). The reaction mixture was cooled in an ice bath and diethyl azodicarboxylate (0.6 mL, 4.0 mmol) was added dropwise. The reaction was warmed to room temperature and maintained at that temperature for 12 h. The reaction mixture was concentrated in vacuo and Et_2O (100 mL) was added. The title compound was precipitated and filtered to afford 1.2 g (75%) as a white solid: mp 171−173 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (m, 4H), 4.22 (t, 2H, *J* = 6.2), 3.89 (t, 2H, *J* = 5.9), 3.63 (t, 2H, *J* = 4.9), 3.48 (t, 2H, *J* = 5.9), 2.02 (m, 2H), 1.20 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 168.1, 136.7, 155.6, 123.5, 134.0, 132.3, 129.0, 123.5, 123.3, 79.8, 76.1, 45.6, 43.8, 36.2, 28.1; HRMS (FAB) *m/z* 494.1912 $(M + H)^+$ ($C_{26}H_{27}N_3O_7$ requires 494.1927). Anal. Calcd for $C_{26}H_{27}N_3O_7$: C, 63.28; H, 5.51; N, 8.51. Found: C, 63.08; H, 5.52; N, 8.63.

(2-Aminoethyl)(3-aminooxypropyl)carbamic Acid *tert*-Butyl Ester (1). Anhydrous hydrazine (0.16 mL, 5 mmol) was added to a solution of **9** (0.5 g, 1.0 mmol) in absolute EtOH (15 mL). The mixture was stirred at 45 °C for 2 h. The reaction mixture was concentrated in vacuo to give a semicrystalline slurry. The slurry was taken into CH_2Cl_2 (25 mL) and the precipitate of phthalalhydrazide was filtered off and rinsed with CH_2Cl_2 (2 × 2 mL). The filtrate was concentrated in vacuo to give a pale yellow oily residue (0.23 g, 98%): TLC (R_f = 0.18; 100% MeOH); ^{1}H NMR ($CDCl_3$) δ 3.61 (t, 2H, *J* = 6.3), 3.20 (m, 4H), 2.76 (t, 2H, *J* = 6.7), 1.76 (t, 2H, *J* = 7.4), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 154.7, 79.6, 74.2, 55.3, 52.9, 43.5, 28.4, 25.8; HRMS (FAB) *m/z* 234.1818 $(M + H)^+$ ($C_{10}H_{23}N_3O_3$ requires 234.1818). Anal. Calcd for $C_{12}H_{25}N_3O_3 \cdot 1.15H_2O \cdot 0.5CH_3OH$: C, 48.97; H, 9.76; N, 14.90. Found: C, 48.94; H, 9.37; N, 15.26.

Preparation of Library 10. A solution of benzyl bromide (692 μL, 4.8 equiv), α-bromo-*m*-xylene (785 μL, 4.8 equiv), and α-bromo-α′,α′,α′-trifluoro-*m*-xylene (888 μL, 4.8 equiv) in THF (40 mL) was added dropwise to a stirred mixture of **1** (0.28 g, 1.21 mmol) in THF (10 mL) at room temperature. *N*,*N*-Diisopropylethylamine (1.7 mL, 9.68 mmol) was added and the resulting reaction mixture was refluxed for 12 h. The reaction mixture was then poured into a mixture of 3-mercapto-1-propanesulfonic acid sodium salt (2.85 g, 16 mmol) and

potassium carbonate (4.5 g, 32 mmol) in MeOH/H_2O (100 mL, v/v, 50:50). The mixture was stirred at room temperature for 2 h and concentrated in vacuo. The resultant residue was partitioned between Et_2O (40 mL) and H_2O (40 mL), and the aqueous layer was extracted with more Et_2O (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give the title library (0.94 g, 99% mass recovery). The resultant pale yellow oily residue was identified by ES(ESI) *m*/*z* 594, 608, 623, 636, 650, 662, 676, 690, 704, 730, 744, 758, 798, 812, 866 $(M + H)^+$.

Preparation of Library 11. A solution of HCl in EtOH (4 M, 26 mL) was added to library **10** (1.21 mmol) at room temperature. The resulting solution was stirred at room temperature for 12 h. The volatiles were evaporated in vacuo, and the residue was dissolved in H_2O (30 mL) and basicified with solid NaOH at 0 °C. The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to give the title library as a pale yellow, oily residue (0.81 g, 1.34 mmol, quantitative mass recovery). The resultant pale yellow, oily residue was identified by ES(ESI) *m*/*z* 494, 508, 522, 536, 550, 562, 576, 590, 604, 630, 644, 658, 698, 712, 766 $(M + H)^+$.

General Procedure for the Preparation of Libraries 12−14. A mixture of library **11**, a specific benzyl bromide (2 equiv), and *N*,*N*-diisopropylethylamine (2 equiv) in 5 mL of THF was stirred at room temperature for 12 h. The reaction mixture was then poured into a mixture of 3-mercapto-1-propanesulfonic acid sodium salt (0.05 g, 0.27 mmol) and potassium carbonate (0.1 g, 0.6 mmol) in MeOH/H_2O (10 mL, v/v, 50:50). The mixture was stirred at room temperature for 2 h and concentrated in vacuo. The resultant residue was partitioned between Et_2O (10 mL) and H_2O (10 mL), and the aqueous layer was extracted with more Et_2O (2 × 5 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give the desired library.

Library 12: pale yellow oil, yield 54 mg (quantitative yield); ES(ESI) *m*/*z* 584, 598, 612, 627, 640, 652, 666, 680, 694, 720, 734, 748, 788, 802, 856 $(M + H)^+$.

Library 13: pale yellow oil, yield 43 mg (91%); ES(ESI) *m*/*z* 598, 612, 627, 641, 655, 666, 680, 694, 708, 734, 748, 762, 802, 816, 870 $(M + H)^+$.

Library 14: pale yellow oil, yield 51 mg (76%); ES(ESI) *m*/*z* 652, 666, 680, 694, 710, 720, 734, 748, 762, 788, 802, 816, 856, 870, 924 $(M + H)^+$.

Preparation of Library 15. A solution of BH_3 in THF (1 M, 6 mL) was added to library **11** (0.04 mmol) in THF (10 mL) at room temperature. The resulting solution was refluxed for 12 h and then cooled to room temperature and to it was added a solution of HCl in H_2O (6 M, 50 mL). The volatiles were evaporated in vacuo and the aqueous solution was basicified with solid NaOH at 0 °C. The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to give a pale yellow, oily residue. Purification of the residue by flash column chromatography (gradient elution, 5% → 20% MeOH/CH_2Cl_2, then 20% MeOH/CH_2Cl_2, 1% H_2O) provided **15** (5 mg, 0.014 mmol, 35% mass recovery). The resultant colorless oily residue was identified by ES(ESI) [*m*/*z* 299, 313, 327, 367, 381, 435 $(M + H)^+$].

General Procedure for the Preparation of Compounds 16−22. A mixture of compound **1**, a specific benzyl bromide (6 equiv), and *N*,*N*-diisopropylethylamine (12 equiv) in THF (50 mL) was refluxed for 12 h. The reaction mixture was then poured into a mixture of 3-mercapto-1-propanesulfonic acid sodium salt (1 equiv to the specific benzyl bromide) and potassium carbonate (2 equiv to 3-mercapto-1-propanesulfonic acid sodium salt) in MeOH/H_2O (100 mL, v/v, 50:50). The mixture was stirred at room temperature for 2 h and concentrated in vacuo. The resultant residue was partitioned between Et_2O (100 mL) and H_2O (50 mL), and the aqueous layer was extracted with more Et_2O (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give an oily residue. The oily residue was either used directly for the next reaction or purified by flash column chromatography.

(2-Dibenzylaminoethyl)[3-(*N*,*N*-dibenzylaminooxy)propyl]carbamic acid *tert*-butyl ester (16): flash column chromatography (gradient elution, 0% → 20% EtOAc/hexanes); pale yellow oil, yield 1.06 g (50%); TLC (R_f = 0.5; 20% EtOAc/MeOH); ^{13}C NMR ($CDCl_3$) δ 155.2, 139.6, 137.8, 129.6, 129.2, 128.7, 127.6, 127.2, 126.8, 78.9, 70.7, 62.5, 58.6, 51.9, 45.3, 44.5, 28.4, 27.4; HRMS (FAB) *m*/*z* 594.3712 $(M + H)^+$ ($C_{38}H_{47}N_3O_3$ requires 594.3696).

{2-[Bis-(3-methylbenzyl)amino]ethyl}{3-[*N*,*N*-bis-(3-methylbenzyl)aminooxy]propyl}carbamic Acid *tert*-ButylEster (17): flash column chromatography (gradient elution, 0% → 10% EtOAc/hexanes); pale yellow oil, yield 1.25 g (60%); TLC (R_f = 0.7; 30% EtOAc/MeOH); ^{13}C NMR ($CDCl_3$) δ 155.2, 139.5, 137.7, 137.5, 130.3, 129.4, 127.9, 127.5, 126.6, 125.8, 78.9, 70.7, 62.5, 58.6, 52.0, 45.3, 44.5, 28.3, 27.0, 21.3; HRMS (FAB) *m*/*z* 650.4350 $(M + H)^+$ ($C_{42}H_{55}N_3O_3$ requires 650.4322).

{2-[Bis(3-trifluoromethylbenzyl)amino]ethyl}{3-[*N*,*N*-bis(3-trifluoromethylbenzyl)aminooxy]propyl}carbamic acid *tert*-butyl ester (18): pale yellow oil, yield 0.2 g (81%); TLC (R_f = 0.5; 30% EtOAc/MeOH); HRMS (FAB) *m*/*z* 998.2205 $(M + Cs)^+$ ($C_{42}H_{43}N_3O_3F_{12}$ requires 998.2167).

{2-[Bis(3-methoxybenzyl)amino]ethyl}{3-[*N*,*N*-bis(3-methoxybenzyl)aminooxy]propyl}carbamic acid *tert*-butyl ester (19): flash column chromatography (gradient elution, 0% → 30% EtOAc/hexanes); pale yellow oil, yield 0.59 g (67.2%); TLC (R_f = 0.5; 30% EtOAc/hexanes); ^{13}C NMR ($CDCl_3$) δ 159.6, 159.5, 155.3, 141.3, 139.4, 129.0, 121.9, 120.9, 115.1, 114.1, 112.6, 112.3, 79.0, 70.7, 62.4, 58.5, 55.1, 52.0, 51.9, 45.2, 44.6, 28.3; HRMS (FAB) *m*/*z* 714.4145 $(M + H)^+$ ($C_{42}H_{55}N_3O_7$ requires 714.4118).

{2-[Bis(2,6-dichloropyridine-4-methyl)amino]ethyl}{3-[*N*,*N*-bis(2,6-dichloropyridine-4-methyl)aminooxy]propyl}carbamic acid *tert*-butyl ester (20): flash column chromatography (gradient elution, 0% → 30% EtOAc/hexanes); red oil, yield 0.15 g (83.5%); TLC (R_f = 0.3; 30% EtOAc/hexanes); ^{13}C NMR ($CDCl_3$) δ 154.1, 151.4, 150.8, 123.4, 122.6, 80.2, 71.4, 60.8, 56.6, 52.4, 44.2, 42.6, 28.5, 27.4; HRMS (FAB) *m*/*z* 1006.2214 $(M + Cs)^+$ ($C_{34}H_{35}N_7O_3Cl_8$ requires 1006.2271).

{2-[Bis(3,4-dichlorobenzyl)amino]ethyl}{3-[*N*,*N*-bis-(3,4-dichlorobenzyl)aminooxy]propyl}carbamic acid *tert*-butyl ester (21): colorless oil, yield 0.13 g (74%); TLC (R_f = 0.5; 30% EtOAc/hexanes); HRMS (FAB) *m*/*z* 1001.9561 $(M + Cs)^+$ ($C_{38}H_{39}N_3O_3Cl_8$ requires 1001.9503).

{2-[Bis-(2-methylbenzyl)amino]ethyl}{3-[*N*,*N*-bis(2-methylbenzyl)aminooxy]propyl}carbamic acid *tert*-butyl ester (22): flash column chromatography (gradient elution, 0% → 20% EtOAc/hexanes); red oil, yield 0.16 g (62%); TLC (R_f = 0.7; 30% EtOAc/hexanes); ^{13}C NMR ($CDCl_3$) δ 155.1, 137.6, 137.2, 135.7, 131.2, 131.1, 130.2, 130.0, 129.8, 127.7, 127.4, 126.9, 125.4, 78.9, 70.7, 61.1, 57.3, 52.4, 44.4, 28.3, 27.5, 19.1; HRMS (FAB) *m*/*z* 650.4303 $(M + H)^+$ ($C_{42}H_{55}N_3O_3$ requires 650.4322).

General Procedure for the Preparation of Compounds 23−29 A solution of HCl in EtOH (4 M, 50 mL) was added to a specific compound (3 mmol) at room temperature. The resulting solution was stirred at room temperature for 12 h. The volatiles were evaporated in vacuo and the residue was dissolved in H_2O (30 mL) and basicified with solid NaOH at 0 °C. The aqueous layer was extracted with EtOAc (2 × 40 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to give the title compound as an oily residue. The oily residue was either used directly or purified by flash column chromatography for the antimicrobial screening.

***N*,*N*-Dibenzyl-*O*-[3-(2-dibenzylaminoethylamino)propyl]hydroxylamine (23)**: flash column chromatography (gradient elution, 0% → 5% MeOH/CH_2Cl_2); pale yellow oil, yield 0.62 g (92%); TLC (R_f = 0.3; 5% MeOH/CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.12 (m, 16H), 3.79 (s, 4H), 3.51 (s, 4H), 3.31 (t, 2H, J = 6.2), 2.53 (s, 4H), 2.15 (t, 2H, J = 7.2), 1.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 139.6, 137.9, 129.6, 128.8, 128.2, 128.1, 127.2, 126.9, 71.4, 62.6, 58.7, 53.5, 47.4, 46.8, 29.2; HRMS (FAB) *m*/*z* 494.3178 $(M + H)^+$ ($C_{33}H_{39}N_3O$ requires 494.3171). Anal. Calcd for $C_{33}H_{39}N_3O \cdot 1/4H_2O$: C, 79.56; H, 7.99; N, 8.43. Found: C, 79.80; H, 7.89; N, 8.51.

***O*-(3-{2-[Bis(3-methylbenzyl)amino]ethylamino}propyl)-*N*,*N*-bis(3-methylbenzyl)hydroxylamine (24)**: flash column chromatography (gradient elution, 0% → 5% MeOH/CH_2Cl_2); pale yellow oil, yield 0.78 g (84.3%); TLC (R_f = 0.4; 5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 20H), 3.82 (s, 4H), 3.54 (s, 4H), 3.28 (t, 2H, J = 6.3), 2.52 (s, 4H), 2.33 (s, 12H), 2.18 (t, 2H, J = 7.3), 1.70 (br, 1H), 1.39 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 139.2, 137.7, 137.5, 130.3, 129.6, 128.1, 127.9, 127.7, 127.6, 126.6, 125.9, 70.9, 62.6, 58.8, 52.6, 46.8, 46.5, 28.3, 21.3; HRMS (FAB) m/z 550.3812 $(M + H)^+$ ($C_{37}H_{47}N_3O$ requires 550.3797). Anal. Calcd for $C_{33}H_{39}N_3O \cdot {}^1/_2H_2O$: C, 79.53; H, 8.66; N, 7.52. Found: C, 79.45; H, 8.56; N, 7.41.

***O*-(3-{2-[Bis(3-trifluoromethylbenzyl)amino]ethylamino}propyl)-*N*,*N*-bis(3-trifluoromethylbenzyl)hydroxylamine (25)**: pale yellow oil, yield 0.16 g (94%); ^{1}H NMR ($CDCl_3$) δ 7.46 (m, 16H), 3.87 (s, 4H), 3.60 (s, 4H), 3.24 (t, 2H, J = 6.2), 2.65 (m, 2), 2.55 (s, 4H), 2.16 (t, 2H, J = 7.2); HRMS (FAB) m/z 766.2639 $(M + H)^+$ ($C_{37}H_{35}N_3OF_{12}$ requires 766.2667).

***O*-(3-{2-[Bis(3-methoxybenzyl)amino]ethylamino}propyl)-*N*,*N*-bis(3-methoxybenzyl)hydroxylamine (26)**: flash column chromatography (gradient elution, 0% → 10% MeOH/CH_2Cl_2); pale yellow oil, yield 0.41 g (81.6%); TLC (R_f = 0.5; 10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 4H), 6.89−6.76 (m, 12H), 3.79 (s, 4H), 3.76 (s, 12H), 3.52 (s, 4H), 3.37 (t, 2H, J = 6.3), 2.54 (s, 4H), 2.20 (t, 2H, J = 7.3), 1.70 (br, 1H), 1.39 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.5, 159.3, 141.2, 139.3, 129.0, 128.9, 121.8, 120.9, 115.0, 114.3, 112.5, 112.1, 71.3, 62.3, 58.6, 55.0, 53.5, 47.3, 46.8, 29.1; HRMS (FAB) m/z 614.3576 $(M + H)^+$ ($C_{37}H_{47}N_3O_5$ requires 614.3594). Anal. Calcd for $C_{37}H_{47}N_3O_5 \cdot 1/4HCl$: C, 71.34; H, 7.65; N, 6.75. Found: C, 71.29; H, 7.64; N, 6.56.

***O*-(3-{2-[Bis(2,6-dichloropyridine-4-methyl)amino]ethylamino}propyl)-*N*,*N*-bis(2,6-dichloropyridine-4-methyl)hydroxylamine (27)**: flash column chromatography (gradient elution, 0% → 5% MeOH/CH_2Cl_2); brown yellow oil, yield 0.78 g (63.8%); TLC (R_f = 0.3; 5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.29 (s, 8H), 3.82 (s, 4H), 3.60 (s, 4H), 3.28 (t, 2H, J = 5.8), 2.67 (m, 4H), 2.27 (t, 2H, J = 7.3), 1.70 (br, 1H), 1.54 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 153.7, 151.4, 151.0, 150.8, 123.4, 122.5, 71.4, 60.8, 57.3, 46.6, 46.3, 27.9. Anal. Calcd for $C_{29}H_{27}N_7OCl_8 \cdot {}^3/_4H_2O \cdot {}^1/_2HCl \cdot {}^1/_2CH_3OH$: C, 43.98; H, 3.75; N, 11.77. Found: C, 44.05; H, 3.34; N, 11.81.

***O*-(3-{2-[Bis(3,4-dichlorobenzyl)amino]ethylamino}propyl)-*N*,*N*-bis(3,4-dichlorobenzyl)hydroxylamine (28)**: flash column chromatography (gradient elution, 0% → 5% MeOH/CH_2Cl_2); colorless oil, yield 0.09 g (88.5%); TLC (R_f = 0.3; 5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.36−7.15 (m, 12H), 3.75 (s, 4H), 3.49 (s, 4H), 3.30 (t, 2H, J = 6.1), 2.52 (m, 4H), 2.20 (t, 2H, J = 7.3), 1.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 139.6, 137.6, 132.5, 132.2, 131.5, 131.1, 130.5, 130.3, 130.2, 128.9, 127.9, 127.7, 71.6, 61.4, 57.7, 53.6, 47.3, 47.0, 29.1; HRMS (FAB) m/z 768.0056 $(M + H)^+$ ($C_{33}H_{31}N_3OCl_8$ requires 768.0022).

***O*-(3-{2-[Bis(2-methylbenzyl)amino]ethylamino}propyl)-*N*,*N*-bis(2-methylbenzyl)hydroxylamine (29)**: flash column chromatography (gradient elution, 0% → 5% MeOH/CH_2Cl_2); pale yellow oil, yield 0.13 g (74.5%); TLC (R_f = 0.5; 10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.25−7.11 (m, 16H), 3.82 (s, 4H), 3.51 (s, 4H), 3.05 (t, 2H, J = 6.3), 2.50 (s, 4H), 2.32(s, 6H), 2.23 (s, 6H), 2.03 (t, 2H, J = 7.3), 1.70 (br, 1H), 1.19 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 137.6, 137.2, 137.1, 135.7, 131.1, 130.1, 130.0, 127.6, 127.4, 126.7, 126.9, 125.5, 71.1, 61.1, 57.5, 53.8, 47.3, 46.6, 28.9, 19.1; HRMS (FAB) m/z 550.3785 $(M + H)^+$ ($C_{37}H_{47}N_3O$ requires 550.3797). Anal. Calcd for $C_{37}H_{47}N_3O \cdot 1/4H_2O \cdot 1HCl$: C, 75.23; H, 8.28; N, 7.11. Found: C, 75.10; H, 8.11; N, 7.06.

Preparation of Library 30. Benzyl bromide (0.52 mL, 3 equiv) was added dropwise to a stirred solution of **1** (0.33 g, 1.44 mmol) in THF (30 mL) at room temperature. *N*,*N*-Diisopropylethylamine (2.5 mL, 14.4 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. Benzoyl chloride (0.67 mL, 4 equiv) was added to the reaction under argon. The resulting mixture was stirred at room temperature for 12 h and was then poured into a solution of 3-mercapto-1-propanesulfonic acid sodium salt (2.0 g, 11.1 mmol) and potassium carbonate (3.1 g, 22.2 mmol) in MeOH/H_2O (100 mL, v/v, 50:50). The mixture was stirred at room temperature for 2 h and concentrated in vacuo. The resultant residue was partitioned between Et_2O (40 mL) and H_2O (40 mL), and the aqueous layer was extracted with more Et_2O (2 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give the title library (0.68 g, 90% mass recovery). The resultant yellow oily residue was analyzed using ESI(ES) in both negative and positive modes yielding peaks of 516 and 530 amu. The structures of the two compounds were verified using MS/MS in the positive mode. Both the 516 and 530 amu peaks produced a fragment ion at 136.2 amu which corresponds to the mass of $ONHCOC_6H_5^+$.

Preparation of Library 31. A solution of HCl in EtOH (4 M, 26 mL) was added to library **30** (1.3 mmol) at room temperature. The resulting solution was stirred at room temperature for 12 h. The volatiles were evaporated in vacuo to give the title library as a brown yellow oily residue (quantitative mass recovery). The oily residue was analyzed using ESI(ES) m/z 417, 431 $(M - H)^-$.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **2b**, **28**; ^{1}H NMR spectra of **2a**, **4**, **6**, **7**, **8**, **18**, **21**, **25**; ^{13}C NMR spectra of **3**, **5**, **16**, **17**, **19**, **20**, **22**; high-resolution mass spectra of **18**, **20**, **21**, **25**; ESI spectra of libraries **10**, **11**, **12**, **13**, **14**, **15**, **30**, **31,** and MS/MS spectra of **30** (38 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any masthead page for ordering information.

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