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X = Cl, Br

63,238 Ethambutol Analogs

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Combinatorial Lead Optimization of [1,2]-Diamines Based on Ethambutol as Potential Antituberculosis Preclinical Candidates

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Despite relatively modest potency, ethambutol (EMB, (*S*,*S*)-[*N*,*N*-di-2-amino-1-butanol]ethylenediamine) is a mainstay of contemporary chemotherapy for the treatment of tuberculosis. We have developed a solid-phase synthesis of 1,2-diamine analogues of EMB using a novel acylation−reduction sequence that is compatible with high-throughput 96-well format chemistry. Using this procedure, we have synthesized 63 238 diamine analogues in pools of 10 that are suitable for testing. MIC and a target-based reporter assay were used to direct deconvolution of 2796 individual compounds from these mixtures, and the 69 most potent molecules were resynthesized in milligram quantities for hit confirmation. Purification of these individual active diamine analogues allowed the identification of 26 compounds with activity equal to or greater than EMB. Amines which occurred most frequently in active compounds included many with large hydrophobic moieties, suggesting that optimization was perhaps selecting for the isoprenoid binding site of the arabinosyltransferase target of EMB. *N*-Geranyl-*N*′-(2-adamantyl)ethane-1,2-diamine (**109**), the most active of these diamines, displayed a 14−35-fold improvement in activity in vitro against *Mycobacterium tuberculosis*, as compared to EMB.

Tuberculosis (TB) is the cause of the largest number of human deaths attributable to a single etiologic agent; nearly 3 million people infected with the tubercle bacillus perish every year.[1] Current chemotherapy consists of two phases, an intensive two-month period of daily therapy with four drugs, isoniazid, rifampicin, pyrazinamide, and ethambutol (EMB), followed by a four-month continuation phase with two of these agents, isoniazid and rifampicin.[2] Despite most patients' clearing their sputum of live bacteria two months after they are placed on oral therapy, the full six-month course is required to prevent relapse with active disease after therapy is discontinued.[3−5] Decades of poor compliance with such a prolonged and complex regimen has had two unfortunate consequences: first, treatment of the disease is often unsuccessful, and tuberculosis continues to spread and cause immense mortality; and second, there is an expanding epidemic of drug resistance that threatens TB control programs worldwide.[6−8] One potential route to decreasing the length of treatment would be to improve the potency of the currently used anti-tuberculosis agents. Such a strategy would allow higher effective dosing of patients and could thereby improve the therapeutic effect of the agent by maintaining the drug concentration above the MIC for longer periods of time or by enhancing the ratio of the peak concentration to the MIC. Drugs which affect the cell wall of the bacteria are known to have concentration-dependent cidal effects in vitro that may be achievable in vivo with enhanced potency.

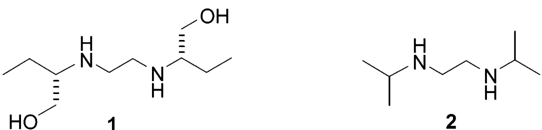

Figure 1. Structures of ethambutol (EMB, **1**) and *N*,*N*-diisopropylethylenediamine (**2**).

EMB (**1**) was developed by Lederle Laboratories in the 1950s from the simple lead molecule, *N*,*N*-diisopropylethylenediamine (**2**) (Figure 1).[9−12] EMB was a useful addition to tuberculosis chemotherapy, despite a relatively modest MIC of 10 μM, in part because of very low toxicity and relatively few side-effects.

Although a lead optimization program was conducted at the time that resulted in selection of EMB from about 2000 mostly symmetrical diamines, a lack of information about the precise molecular target of EMB and a lack of access to high-throughput synthetic schemes limited the potency that was achievable. Nonetheless, it was apparent that the size and nature of the alkyl groups on the ethylenediamine nitrogens were critical for determining activity. Small, α-branched alkyl groups were much more active than either longer aliphatic chains or chains branched at positions other than α.[13] *N*,*N*-Diisopropylethylenediamine (**2**) was the original lead molecule, but other amines that were as active

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included symmetrical diisobutyl- or di-*tert*-butylethylenediamine derivatives. β-Branched alkyl substituents and cycloalkyl and aryldiamines were all substantially less active than the parent molecule. N-Methylation of diisopropylethylenediamine, EMB or other related diamines to form tertiary amines retained but did not significantly enhance activity, presumably because of facile N-demethylation in vivo.[11] Removal or significant alteration of the basicity of either amino group resulted in a loss of potency, with the exception that the corresponding amides retained partial activity in some analogues.[11] Alteration of the linker region of the molecule was detrimental, and lengthening the ethylene unit by even a single methylene or introducing a variety of heteroatoms into the chain resulted in loss of activity.[11,13] Although some branching in the ethylene linker was permissible with lower activity, most branched alkyl, aryl, or cycloalkyl substitutions resulted in diminished activity.

Because of the absolute requirement for the 1,2-substituted ethylenediamine pharmacophore and the known propensity of similar diamines to form stable chelates with divalent metal ions such as copper, a series of derivatives were made that incorporated hydroxyl groups in positions that might be available to stabilize a metal complex.[11] EMB was then identified as a 1,2-amino alcohol that possessed four times the in vivo activity of the lead molecule when prepared chirally pure as the dextro isomer from (*S*)-2-amino-1-butanol. Strikingly, the opposite levo isomer has $<1/500$ the activity.[14] Although the additional potency from the introduction of a hydroxyl group that is β to the amine appears consistent with the chelation hypothesis, asymmetrical derivatives lacking that moiety on one of the amines were fully as active as EMB, suggesting that the hydroxyl groups may play a more complex role than simply contributing to chelate stability.

All of the early SAR that has been reported has focused on the results of in vivo tests of infected animals, and relatively little in vitro MIC information is available. This complicates the interpretation of these results, since effects on absorption, distribution and metabolism may make highly active molecules appear less active. Furthermore, the lack of an in vitro assay that would confirm that the newly synthesized diamines maintained the same mechanism of action on the bacterium also limited the ability to rationally extend the correlation between structural changes and effects on activity. Recently, a small number of closely related amino alcohol EMB analogues have been synthesized and screened against *Mycobacterium smegmatis,* but none were found to be more potent than EMB, suggesting that there was little room for improvement.[15] No attempt was made to equate these results to the earlier results obtained on *Mycobacterium tuberculosis* by Wilkinson and Shepard.[11]

Because of recent genetic and biochemical evidence suggesting that the enzyme target of EMB action is likely to be an integral membrane arabinosyltransferase for which crystallographically derived information is unlikely to become available to guide chemical optimization, we sought to develop and implement information-rich, high-throughput screening and synthesis of EMB analogues that would circumvent these problems.[16−18] We therefore revisited the relationship between diamine structure and activity in this series of molecules using modern methods of split-and-pool synthesis on a solid support to produce a large and diverse diamine library. We desired to move away from simple symmetrical amino alcohol-containing diamines, which have been extensively analoged, and explore asymmetric diamines. Such a library could then be screened directly against *M. tuberculosis* using modern high-throughput screening (HTS) techniques, such as bioluminescent reporter strains that produce light in response to inhibition of cell wall synthesis by EMB.[19] It was hoped that this would lead to the discovery of new compounds that have the potential to be more clinically effective as treatments for *M. tuberculosis* and other clinically important mycobacteria while maintaining potency against EMB-resistant strains.

Results and Discussion

Design of a Solid-Phase Approach to a Diamine Library. Solid-supported syntheses of 1,2 diamine libraries that relied upon the reduction of short peptides[20] have previously been reported. However, we desired to synthesize a library using amine building blocks that allowed for greater diversity of monomers than could be achieved with simple amino acids. Anchoring the diamines through nitrogen required that the synthetic strategy end with cleavage of a resin-bound tertiary amine to yield a secondary amine. Secondary amines are not ideal leaving groups requiring either the use of hyperlabile acid linkers or chemical activation by, for example, α-chloroethyl chloroformate (ACE).[21] Exploratory studies showed that ACE-promoted cleavage was not flexible enough for a large and diverse library because of side reactions of ACE with other functional groups in our amine monomer set (notably amino alcohols). We also evaluated a number of acid labile linkers, including Wang, HMB, Rink, and chlorotrityl. The Rink linker proved to be most flexible for the proposed chemistry because it allowed for good stability under the reaction conditions and was cleaved with a relatively low concentration of TFA (10% in dichloromethane).[22] In our initial studies, we attempted to use direct dibromoethane addition to immobilized amines to form the linker, but this route suffered from two major flaws: first, it resulted in the formation of a highly reactive nitrogen mustard intermediate that rapidly reacted with intrabead nucleophiles. Second, the resin-bound tertiary amine was frequently overalkylated and, consequently, was released from the solid support. To solve these problems, we developed a synthetic strategy that relied upon acylation of a resin-immobilized amine (N1) with an α-haloacyl halide. This was followed by addition of the second amine (N2) to form an intermediate aminoamide (Scheme 1 and Figure 2). This intermediate could then be fully reduced to the diamine while on the resin and subsequently cleaved.

In essence, the amine attached to the resin is protected from over-alkylation and the inadvertent release from the solid support by the intermediate amide. Use of an intermediate resin-bound α-haloamide allowed for the efficient addition of the second amine building block, which could be added in large excess.

Optimization and Analysis of Rink Resin Amine Loading and Acylation. Attachment of the first amine to the

Scheme 1. Solid-Phase Synthesis of EMB Analogs

Figure 2. General structure of the EMB analogue libraries.

support was done according to the Garigipati protocol: Rink acid resin (Novabiochem) was converted to the Rink−chloride resin upon treatment with triphenylphosphine and hexachloroethane in THF (Scheme 1).[22,23] This activated resin was then loaded by the addition of an amine (N1) in the presence of Hünig's base in dichloroethane. Acylation of the resultant resin-bound secondary amine was accomplished using either (i) the corresponding chloroacetyl chloride or α-alkylbromoacetylbromides in the presence of pyridine and THF, or (ii) via peptide coupling with α-chloro-α-alkylacetic acids in the presence of PyBrOP and EtN(*i*-Pr)$_2$ in dichloromethane.[24] The acylations were repeated to improve product yields.[24]

Resin loading was assessed initially by mass changes in dried resin as well as by cleavage of the amine and quantification of the resulting TFA salt. However, elemental analysis proved to be useful for assessing both loading and the efficiency of acylation and was predictive of ultimate success in producing diamine products in many cases. For example, Table 1 shows the analysis results from eight resins with different N1 substituents. These resins have 0.43 mmol/g capacity based on the starting Rink acid. Analysis for nitrogen content revealed that the content of N1 was within the range of 0.46−0.70 mmol/g, a value in good agreement with the predicted capacity of the Rink resin. In these same samples, the chlorine content ranged from 0.58 to 1.53 mmol/g. On the basis of MS data, resins **5**, **6**, **7**, and **10** yielded diamine products and had nitrogen/chlorine ratios that did not exceed 1:1.3. In contrast, **8**, **9**, **11**, and **12** did not yield the expected ethylenediamines, but the corresponding nitrogen/chlorine ratio was found to be within a range 1:1.5 to 2.9. Notably, all of the failed amines contained a second N-acylation site, which may have accounted for failure of the synthesis and the large amount of chlorine present. In fact, heterocycles, such as 3-amino-1,2,4-triazine and 3-aminopyrazole and also *N*,*N*-disubstituted diamines, for example, 1-(2-aminoethyl)pyrrolidine (but not pyrrolidinone) or 4-(2-aminoethyl)morpholine, never produced products when they were used as N1 in the reaction with Rink−Cl, probably as a result of base-promoted intramolecular transformations. In contrast, the same amines gave products in good yields when introduced as N2 into the second position.

Introduction of N2, Reduction and Cleavage. Incorporation of the second nitrogen moiety as a secondary amine into the molecule was achieved by reacting the α-halo-amide resin **3** with various amines in the presence of Hünig's base in DMF at 70−75 °C for 16−20 h in the presence of catalytic sodium iodide.[25] Reduction of the aminoethyleneamides **4** into corresponding diamines **5** (Scheme 1) was carried out using the soluble reducing reagent 65+ w% Red-Al at room temperature.[26] Cleavage of the products from the resin was achieved with a 10% solution of TFA in dichloromethane. The resulting TFA salts were analyzed by LC/MS for the presence of the expected diamine.

Synthesis of EMB on Solid Phase. To validate the synthetic procedure, we attempted to synthesize EMB and a number of analogues immobilized on the Rink resin, as described. This synthesis was carried out on a Quest synthesizer, starting from 0.2 g of Rink acid resin per tube.

Table 1. Results of Elemental Analysis for the Resins of General Formula

Resins	N, mmol/g	# N atoms	Cl, mmol/g	N : Cl ratio	Ethylenediamine products
6	0.65	1	0.85	1 : 1.3	products formed
7	0.58	1	0.58	1 : 1.0	products formed
8	0.59	1	0.63	1 : 1.1	products formed
9	0.92	2	1.30	1 : 2.8	no products
10	2.12	4	1.53	1 : 2.9	no products
11	0.70	1	0.91	1 : 1.3	products formed
12	1.30	2	0.98	1 : 1.5	no products
13	1.08	2	1.24	1 : 2.3	no products

6 **7** **8** **9** **10** **11** **12** **13**

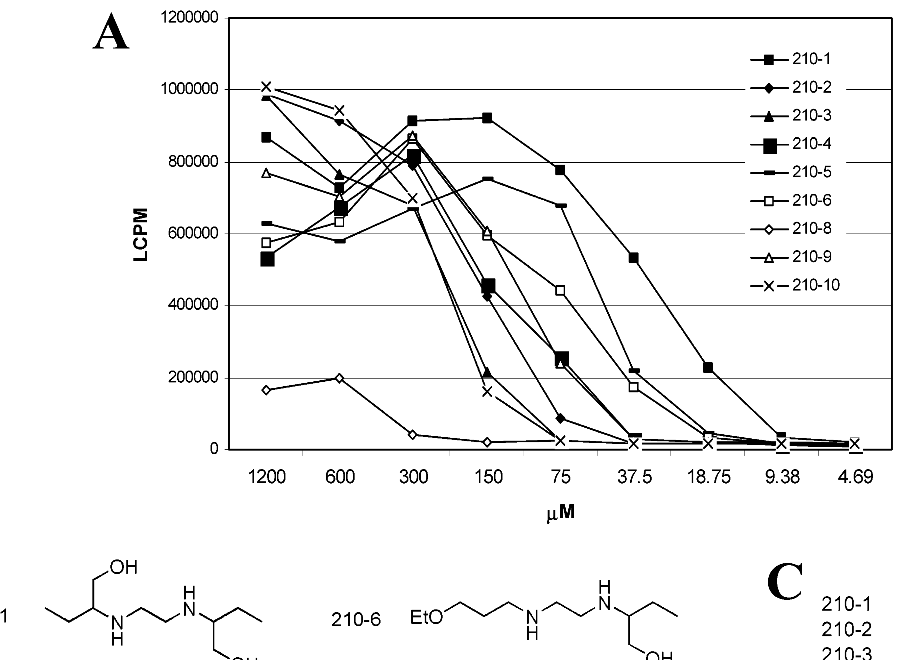
In this synthesis, N1 was varied by using a selection of 10 amino alcohols and cyclic amines, and N2 was always the amino alcohol corresponding to the racemic EMB parent monomer (±)-2-amino-1-butanol. Chloroacetyl chloride was used as the linker to produce the simple ethylenic series. The crude material from TFA cleavage was analyzed by NMR and MS. Of the 10 tubes, 9 contained a single product, and one failed to produce the predicted material. The nine products were shown to be >90% pure by LC/MS and were bioassayed directly (Figure 3). The data appeared consistent with previous studies showing that the symmetrical diamino-dialcohol EMB is optimal for activity in this set and that there are strict stereochemical requirements for activity: the racemate (compound 210-1) was only 25% as active as enantiomerically pure EMB. Further, introduction of (*R*)-2-amino-1-butanol into the N2 position (compound 210-10) reduces activity even further. The bioassay data also demonstrated that the library compounds could be tested directly as TFA salts in the whole cell bioluminescence assay (Figure 3A), and these values were shown to correlate with the experimentally determined MIC values in agreement with previous reports[27] (Figure 3C). To establish an accurate chemical yield, compound 210-1 (racemic EMB) was subsequently purified by HPLC using a semipreparative C-18 column and was isolated as the acetate salt in 48% chemical yield with respect to resin loading and >95% purity by NMR.

Synthesis of a Library of Ethylenic Diamines. Our initial attempts at synthesizing a diamine library involved focusing on the ethylenic series by utilizing chloroacetyl chloride acylation of a structurally diverse group of amines in both positions. A complete list of the amines used is available as Supporting Information, but an indication of the diversity sampled is shown in Table 2. Two hundred and eighty-eight amines were chosen for the synthesis, including a set of commercially available primary and secondary aliphatic, aromatic and cyclic amines as well as amino alcohols and amino acid esters. These were selected in part by comparison with other published collections of amines, representing a range of different sizes, charges and shapes.[28,29]

For library production, the first three steps of the synthetic scheme (resin activation, amine loading and acylation) were typically carried out using a Quest 210 synthesizer on scale of 0.2−0.5 g of resin per tube. Amines for the initial steps (position N1) were limited to the 177 primary amines, since secondary amines and aromatic amines loaded poorly and failed to give products. Additionally, amino acid esters were excluded from the N1 position, because they were found to cyclize, yielding 1,3-substituted piperazines under the reaction conditions. Following acylation of the resin, the α-haloacetamide-containing resins **2** were thoroughly washed, dried and then pooled into groups of ten. A small amount of each resin (~0.05 g) was archived prior to pooling to facilitate resynthesis and deconvolution of active molecules.

Addition of the N2 component, reduction and cleavage were carried out in 96-well filter plates using the Combiclamp system (Whatman Polyfiltronics), which was replaced over time with the more reliable FlexChem system filter plates and blocks (Robbins Scientific). A neutral buoyancy suspension of the pooled resins in dichloromethane/THF was evenly distributed into three reaction plates, resulting in ~15−20 mg of total resin per well. The 288 diverse amines (each 1 M in DMF) were arrayed in three 96-well plate templates and added, 1 amine per well, to each individual pool of 10 resins, resulting in an anticipated 960 diamines produced per plate or a total theoretical library size of 50 976 products. Reduction was carried out in the same plates, and cleavage and filtering into storage plates was followed by evaporation of the TFA/DCM cleavage solution prior to biological assay.

Quality assessment of the prepared library of ethylenediamines was performed by electrospray mass spectrometry using two randomly selected rows (20 samples, 1 hori-



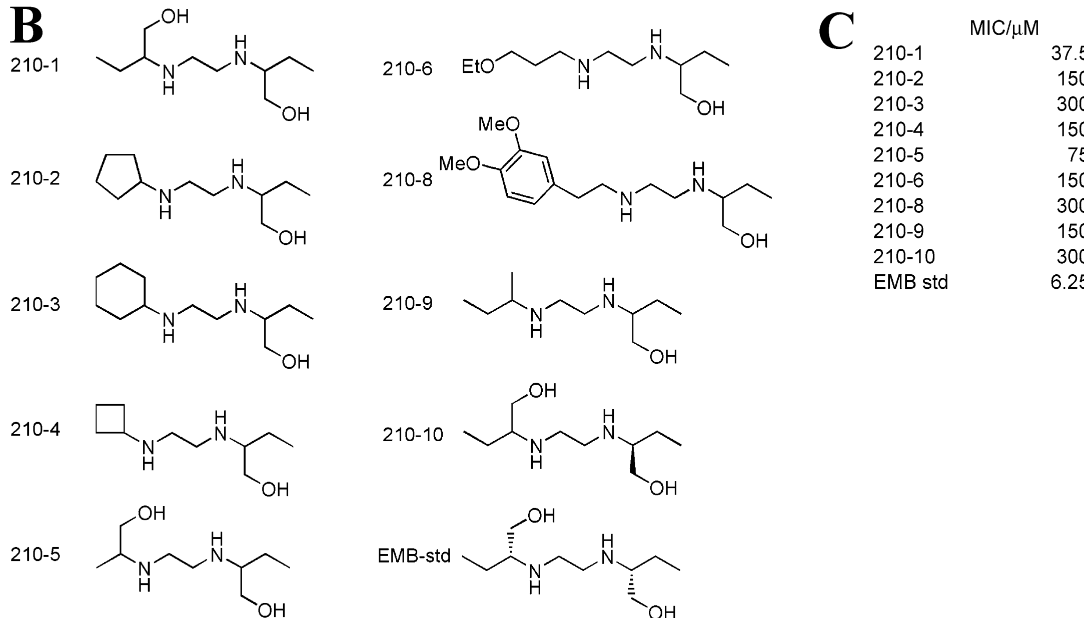


Figure 3. Biological assay data for EMB and selected analogues. (A) Luminescence assay data for 10 analogues of EMB, using a luciferase reporter strain of *M. tuberculosis* containing the Rv0341 promoter fused to firefly luciferase, assayed by serial dilution. Each well contains a single crude diamine following cleavage from resin. Results are reported in percent LCPS (luminescence count per second) and compared to EMB (100% of the intensity) at 3.1 μM. (B) Structures of EMB analogues produced within this sublibrary. (C) MIC_{99} of each analogue and EMB measured by broth microdilution in Middlebrook 7H9 media against *M. tuberculosis* H37Rv.

zontal and 1 vertical) per plate. Successful production of a compound was based on an appearance of a molecular ion of the calculated mass (see Table 3). In 73% of the cases in the ethylenic series, the predicted ions were observed, and therefore, the predicted compounds were believed to be produced. Thus, this procedure resulted in the formation of 40 781 diamines in pools of up to 10 components for assay (Table 4).

Synthesis of Libraries Containing Phenyl- and Alkyl-Substituted Ethylenediamines. To further enhance the structural diversity of our library of EMB analogues and to assess the influence of a modified linker on the activity of structurally diverse diamines against *M. tuberculosis*, we modified the synthetic scheme to incorporate phenyl and alkyl substituents in the bridging linker between the two amine components.

Synthesis of phenyl diamines was accomplished as shown in Scheme 1, substituting α-chloro-α-phenylacetyl chlorides

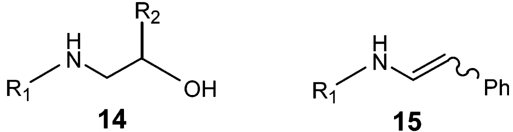


Figure 4. Side-products observed in alkyl- and phenylethylenediamine synthesis.

for the acylation. As expected, an introduction of the second amine into the molecule became more difficult because of steric hindrance at the reaction site. As a result, the formation of the corresponding alcohols **14**, R_2 = Ph (via competing hydrolysis followed by reduction), and in some cases, C1-2 unsaturated amines **15** (by subsequent H_2O elimination from **14**) were commonly observed (Figure 4). For example, compare the MS data for the simple and branched ethylenediamines from three resins with the same set of 10 amines (Table 3). In the first two cases, significantly more product

Table 2. General Structures of 288 Amines that Were Used for the Library Synthesis

Table 3. Mass Spectral Data for Synthetic Ethylenediamines[a]

R_1NH_2	[M+1]+ (morpholinopropyl linker)			[M+1]+ (alkyl linker)			[M+1]+ (4-methyl-1,4-diazepane linker)		
	R_2 = H	R_2=Ph	(14)[b] R_2=Ph	R_2 = H	R_2=Me	(14) R_2=Me	R_2 = H	R_2=Me	(14) R_2=Me
Tyramine	308	384	258	278	293	196	278	292	196
2-Adamantamine	321	398	272	293	307	210	292	306	210
cis-Myrtanylamine	324	400	274	295	309	212	294	308	212
3-Amino-1-propanol	246	322	196	217	231	134	216	230	134
L-Methioninol	305	382	256	277	291	194	276	290	194
Cyclooctylamine	298	374	248	269	284	186	268	282	186
(1S,2S)-2-Amino-1-phenyl-1,3-propandiol	337	414	288	309	323	226	308	322	226
1-Adamantane-methylamine	336	412	286	307	321	224	306	320	224
2,2-Diphenylethylamine	368	444	318	339	353	256	338	352	256
5-Amino-1-pentanol	274	350	224	245	259	162	244	258	162

[a] Ions that were observed in mass spectra are underlined. [b] Refers to the corresponding alcohol shown in Figure 4.

14 was observed with the alkyl ethlyene linker. In the third case, with a phenyl branched linker, the only products observed were due to this hydrolysis.

This was a problem, particularly when less-reactive amines were to be introduced onto the second position of the molecule. When utilizing hygroscopic amines, such as amino alcohols (phenylalaninol or 1-amino-2-propanol, for example), or more sterically challenging amines, such as isopinocampheylamine or 1-(1-naphthyl)-ethylamine, compounds **14** were often the only identifiable products formed, suggesting amine formation failed completely. On the basis of MS data, in the phenylethylene sublibrary only 31% of

Table 4. Summary of Synthetic Success and Hit Rate of EMB Libraries by Structural Type

EMB analogs	Predicted # Compounds	Synthesis success (% by MS)	Number of diamines actually produced	MICs (<12.5μM)	Hits in Lux assay
H N R$_1$ N R$_2$ R$_3$	51,792	73	37,808	160	165
H Me N R$_1$ N R$_2$ R$_3$	5,760	51	2,938	11	24
H Alk N R$_1$ N R$_2$ R$_3$	46,080	43	19,814	0	0
H Ph N R$_1$ N R$_2$ R$_3$	8,640	31	2,678	2	2

Scheme 2. Synthesis of Alkyl-Substituted EMB Analogs in Pools of 30 Compounds per Well

the anticipated diamines were obtained (Table 4). Therefore, out of 8640 compounds predicted, only 3456 were actually formed.

Alkyl-substituted ethylenediamines (**3**, R_2 = Me, Et, Bu) were prepared using essentially the same synthetic route, but with two different acylation reagents in the third step: α-bromo-α-alkylacetylbromides and pyridine or α-chloro-α-methylacetic acid and PyBrOP/EtN(*i*Pr)$_2$ (Scheme 2). Acylation with the α-bromo-α-alkylacetylbromides was carried out using a mixture of three acylating reagents, α-methyl-, α-ethyl-, and α-butyl-α-bromoacetylbromides, for every resin-attached amine so that the final alkyl substituted diamines were produced as mixtures of 30 compounds per well. MS data of the products showed that there were no substantial differences in the reactivities of methyl, ethyl or butyl bromides (data not shown). The outcome of the synthesis throughout this library, therefore, solely depended upon the nature and reactivity of the amines used.

Unfortunately, the total compound yield for this sublibrary of diamines again did not exceed 43%. One possible reason for this low success was that the α-alkyl-α-bromo amides may undergo competing base-catalyzed $\alpha-\beta$ (C2−3) dehydobromination under the conditions used for the N2 amine addition.[30] Of the predicted 46 080 compounds represented in this sublibrary, MS data suggests that only 18 432 compounds were actually produced (Table 4).

Better results were achieved when the α-branched linker was introduced under peptide coupling conditions. Synthesis of methyl-substituted diamines with α-chloro-α-methylacetic acid/PyBrOP/EtN(*i*Pr)$_2$ in pools of 10 compounds per well led to the formation of 51% of the desired products. Within this smaller sublibrary, therefore, 3456 members were produced of 5760 theoretical compounds (Table 4).

Amine Reactivity and Library Success. Throughout the library, chemical yields of the products synthesized in 96-well plates were lower than on the Quest, probably due to lack of efficient mixing and increased exposure to moisture. Many times the yields did not exceed 20% with respect to quoted resin loading capacities. There were several examples when compounds successfully made from amino alcohols on the Quest were barely obtained (or not obtained at all) on the reaction plates. The lower efficiency of compounds obtained was likely due to failure to adequately mix the reactions in 96-well format. Analysis of the mass spectral data permitted an estimate of the actual number of the compounds that had been synthesized and was also useful in understanding the influence of reactivity of the amines on chemical yield. Comparison of the results for different wells from the same plates (and, therefore, for the same resins) allowed some understanding of the relative reactivity of the amines for introduction into the second position.

Not surprisingly, throughout the library, reactive and unhindered amines, such as benzylamines and 2-substituted ethylamines, gave outstanding results. Upon introduction into the N1 or the N2 position of the molecule, these virtually always provided the desired products. Reaction of these amines with the Rink−chloride required no heating, and formation of side products during and after cleavage was minimal. When the reactions were performed on the Quest 210 synthesizer, chemical yields of the ethylenediamines

Table 5. The Top Hit Compounds Synthesized on Quest 210 Synthesizer

Cmpd #	Name	Structure	Yield, %	MIC, μM[a]
7	*N*-(1-Adamantylmethyl)- *N'*-(3,3-diphenylpropyl)ethane-1,2-diamine		22	0.5
10	*N*-(-)-*cis*-Myrtanyl-*N'*-(3,3-diphenylpropyl)ethane-1,2-diamine		11	1
14	*N*-(3,3-Diphenylpropyl)-*N'*-*exo*-(2-norbornyl)ethane-1,2-diamine		16	1
21	*N*-(3,3-Diphenylpropyl)-*N'*-(1*S*)-(1-ethylcyclohexane)-ethane-1,2-diamine		4	3
32	*N*-(2,2-Diphenylethyl)-*N'*-*(R)*-(+)-bornylethane-1,2-diamine		48	6
34	*N*-(2,2-Diphenylethyl)-*N'*-(1-methyladamantyl)ethane-1,2-diamine		6	1
37	*N*-(2,2-Diphenylethyl)-*N'*-(-)-*cis*-(myrtanyl)ethane-1,2-diamine		38	1[b]
38	*N*-(-)-*cis*-(Myrtanyl)-*N'*-(2,2-diphenylethyl)propyl -1,2-diamine		30	0.5
40	*N*-(2,2-Diphenylethyl)-*N'*-(1*R*, 2*R*, 3*R*, 5*S*)-(-)-isopinocampheylethane-1,2-diamine		23	1
47	*N*-(-)-*cis*-Myrtanyl-*N'*-(1*R*, 2*R*, 3*R*, 5*S*)-(-)-isopinocampheylethane-1,2-diamine		33	1[b]
52	*N*-(Cyclooctyl)-*N'*-(3,3-diphenylpropyl)ethane -1,2-diamine		18	3
55	*N*-(Cyclooctyl)-*N'*-(1-methyladamantyl)ethane -1,2-diamine		6	1[b]
57	*N*-(-)-*cis*-Myrtanyl-*N'*-(cyclooctyl)ethane-1,2-diamine		18	1
58	*N*-(Cyclooctyl)-*N'*-(2-adamantyl)ethane -1,2-diamine		23	6[b]
59	*N*-(Cyclooctyl)-*N'*-(1*R*, 2*R*, 3*R*, 5*S*)-(-)-isopinocampheylethane-1,2-diamine		14	1[b]
62	*N*-(-)-*cis*-Myrtanyl-*N'*-(1*S*)-(1-ethylcyclohexane)ethane-1,2-diamine		46	1

Table 5. The Top Hit Compounds Synthesized on Quest 210 Synthesizer

Cmpd #	Name	Structure	Yield, %	MIC, μM[a]
65	*N*-(1-Adamantyl)-*N'*-*trans*-(2-phenylcyclopropyl)ethane-1,2-diamine		16	5
66	*N*-(3,3-Diphenylpropyl)-*N'*-(1*R*, 2*R*, 3*R*, 5*S*)-(-)-isopinocampheylethane-1,2-diamine		2	3
109	*N*-Geranyl-*N'*-(2-adamanthyl)ethane-1,2-diamine		24	0.2[b]
111	*N*-[2-(*N'*-Geranyl)aminoethyl]-2-ethylpiperidine		24	1[b]
116	*N*-Geranyl-*N'*-allyl-*N'*-(cyclopentyl)ethane-1,2-diamine		42	1[b]
117	*N*-Geranyl-*N'*-(1,1-diphenyl-methyl)ethane-1,2-diamine		20	1
125	*N,N'*-bis-(-)-*cis*-Myrtanylpropane-1,2-diamine		35	0.2[b]
151	*N*-[2-(2-Methoxy)phenylethyl]-*N'*-(1*R*, 2*R*, 3*R*, 5*S*)-(-)-isopinocampheyl-ethane-1,2-diamine		60	5[b]

[a] In the same assay, the MIC of commercially available ethambutol was found to be 7 μM. [b] MICs for these compounds were also determined by BACTEC, the semi-automated method used widely in clinical microbiology laboratories that relies on release of radioactive carbon dioxide by lipid metabolism. Variation in these two methods for highly lipophilic compounds is common. The obtained values were **37**, 1.25 μM; **47**, 5.0 μM; **55**, 25.0 μM; **58**, 5.0 μM; **59**, 12.5 μM; **109**, 0.63 μM; **111**, 5.0 μM; **116**, 12.5 μM; **125**, 6.25 μM; **151**, 6.25 μM. The MIC for EMB by BACTEC was found to be 9 μM in the same assay.

varied from 30 to 59% (see Table 5 for selected examples). In contrast, sterically hindered amines, such as cyclooctylamine or isopinocampheylamine, resulted in the corresponding diamines, but typically in significantly lower yields. When used in the N1 position, loading these amines onto Rink−Cl always required higher temperatures (12 h at 45−50 °C). Generally, introduction of these amines into the second position of the molecule proceeded with better chemical yields. Our attempts to attach bulky amines, such as *tert*-octylamine or bornylamine, directly to the Rink−Cl resin failed, although the same amines yielded the desired products with 6−18% yield when they were introduced into the second position of the molecule.

Amino alcohols and amino acids were difficult synthons to introduce into the library. Comparing peak intensities in mass spectra, linear amino alcohols, when used for the first position, appeared to give the substituted resins in better yields than sterically hindered amino alcohols, but these were still in lower yield than alkylamines. When the amino alcohols were to be introduced into the second position, product yields achieved on the Quest 210 synthesizer were in the range of 23−48%. At the same time, chemical yields of the corresponding amino alcohol-containing diamines on the plates did not exceed 5%.

The formation of mono-amino alcohols **14** (R_2 = Me, Ph) was often a major competing reaction (Table 3). This process dominated when sterically hindered primary or secondary amines were used. Reactions with 1-methylhomopiperazine were good examples. While this amine yielded the desired products for the ethylenediamine series, none of the corresponding methyl-substituted ethylenediamines were formed from the same resins. The only signals present in the MS spectrum for these samples were identified as amino alcohols **14**.

Amino acid esters were used only in the 4th step of the synthetic scheme. Unfortunately, in our hands, while making the library in pools of 10 or 30 compounds, amino acids rarely gave the desired ethylenediamine products, although prolonged reaction time (heating at 75 °C for 72 h vs 24 h at the same temperature) improved the chemical yields somewhat. This may have been attributable to the poor

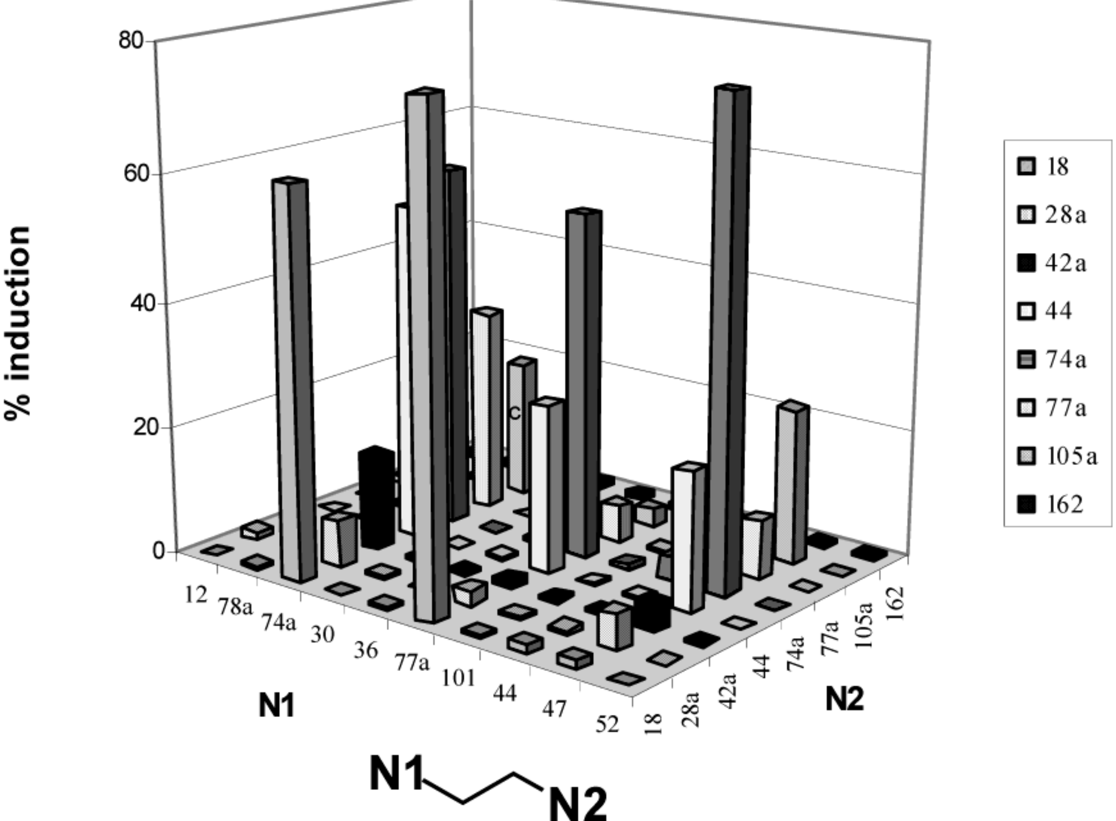


Figure 5. Bioassay-directed deconvolution of active compounds from pooled diamines. Eight representative deconvolutions are shown from a plate containing ten N1 amine-containing resins from eight active N2-containing wells. Assays were performed directly on samples synthesized from each of 10 individual archived resins reacted separately with the unique amine located in the active well. The data shown are from the luciferase gene induction assay. For example, the initially active well N2 = amine 18 deconvoluted to two resins containing amines 74a and 77a. In this case, deconvolution yielded two highly active molecules that trigger the reporter at <6.25 μM. Percent induction is expressed relative to that of the primary mixture of 10 compounds.

solubility of the hydrochloride salts in DMF, inefficient neutralization or mixing in the filter plates.

Biological Screening and Deconvolution of Active Compounds against *M. tuberculosis.* All four libraries of diamines were screened in vitro against *M. tuberculosis* in two assays: (i) a direct determination of the MIC and (ii) a bioluminescent reporter strain that produces light in response to inhibition of cell wall synthesis by EMB[31,32] (Lee, RE, Barry, CE, III, unpublished). Compounds were screened as pools of 10 or 30 following TFA cleavage from the resin in a series of 2-fold dilutions spanning from 50 μM to 50 nM (per each individual compound) in both assays. Wells were scored as active if they appeared to have an MIC of below 50 μM and induced bioluminescence at less than 50 μM. During screening, we assumed an ideal theoretical yield (100%) for every unpurified compound, and the compounds were compared to commercially available, pure EMB as an internal control in each assay (Sigma). In reality, we expected all MIC data to be better than they appeared, since the chemical yields of the compounds on the plates did not typically exceed 20%, presumably because of handling losses, which were uniform across all wells. On the basis of these screening results, ~2796 compound mixtures were found to exhibit anti-TB activity.

Active mixtures were deconvoluted by rereacting the 10 archived resins used to produce the assay plate containing each active mixture with the single amine used in the active well. Reduction and cleavage allowed the rapid production of all of the 10 individual components contained within each active well, and 2796 wells that possessed activity at <50 μM in the luciferase gene induction assay or with an MIC of <50 μM were deconvoluted. Figure 5 shows luciferase gene expression data for eight deconvolutions that show typical patterns. Axis N1 represents amines that were first introduced into the molecule (step 2 of the synthetic scheme), and the axis N2 represents the amines introduced into the second position (step 4). Often, the activity from the mixture was found to be attributable to a single active component, but occasionally, active mixtures contained more than one active molecule. For example, the active well containing N2 = amine 18 deconvoluted into two active compounds with N1 = 74a and 77a. In other cases, the activity did not cleanly deconvolute (e.g., the active well containing N2 = 28a) or deconvoluted to a less active well (e.g., the active well containing N2 = 42a). In some of these cases with high activity, new resins were synthesized to check if activity could be recovered. From the 2796 initial hit pools, 409 individual compounds were identified following deconvolution, with apparent MICs equivalent to or lower than 25 μM (Figure 6A), and 226 compounds were identified with luciferase gene-inducing ability at 25 μM or lower (Figure 6B).

Figure 6A and B summarizes the cumulative MIC and luciferase assay data for all discrete compounds with activity

Figure 6. Summary of biological assay data of the primary hits from library screening. (A) Number of hits with an MIC(99) of the indicated concentration as taken from the raw deconvolution data. These values are an underestimate of the actual MIC, since 100% yield was assumed for the calculation. (B) Number of hits with luciferase-inducing ability of at least 50% of maximal response at the indicated concentration.

at concentrations $\leq$50 μM. The luciferase assay graph (Figure 6B) includes all compounds that exhibited at least 10% activity at each concentration (compared to EMB). Analysis of the acquired data enabled us to conclude that at least 60 compounds were discovered to possess equal or better MICs than EMB, since the MICs were obtained for nonpurified samples (with chemical yields not higher than 20%). Additionally, at least 119 compounds were at least as potent as EMB at inducing expression of the reporter in the indicator strain of *M. tuberculosis* used. Only compounds with an MIC of <12.5 μM and reporter gene inducing ability at the same concentration were selected for large-scale synthesis.

Analysis of Hit Molecules and SAR. Sixty-nine of the most active molecules were resynthesized in larger quantities on the resin according to the same synthetic schemes and purified by semipreparative HPLC using a C18-column. The compounds were obtained in sufficient quantities (2−60 mg) for characterization by ^{1}H NMR and mass spectrometry and testing. The structures and MIC of the top 24 compounds are shown in Table 5. The MICs reported were determined by microbroth dilution assay, a standard laboratory method described in detail in the Experimental Section; however, considerable variation in MIC was observed with some alternative methods, such as BACTEC, a technique used widely in clinical laboratories. One potential source of such variation is the highly lipophilic nature of some of the lead molecules and their potential inhibitory effects on lipid metabolism. Nonetheless, highly active molecules, such as **109**, display submicromolar MICs in both microdilution (0.19 μM) and BACTEC (0.63 μM).

The vast majority of the active compounds appear to be derivatives of a diverse subset of ~30 amine monomers. Those amines appearing five times or more in either position in the deconvoluted hits from the library are shown in Figure 7. In addition to the expected monomers, such as 2-amino-1-butanol (amine 63), many simple alkyl (undecylamine, amine 266) and cycloalkylamines (e.g., 2,3-dimethylcyclohexylamine (11), cyclooctylamine (77.1), and (*S*)-cyclohexylethylamine (255)) appeared in this set. The most frequently occurring amines, however, contain diphenyl moieties (3,3-diphenylpropylamine (18) and 2,2-diphenylethylamine (47)) or, alternatively, tricyclic carbon skeletons similar to sub-

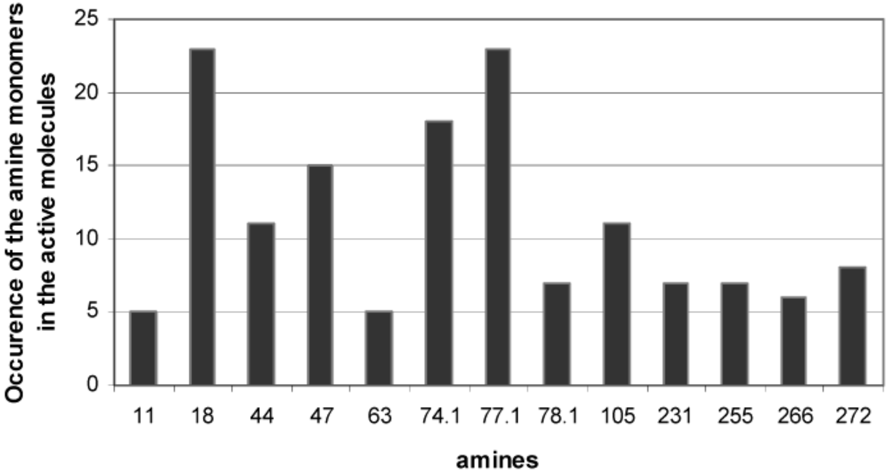


Figure 7. Frequency of occurrence of selected amine monomers in active molecules. Summation of occurrence of amines in all of the active deconvoluted wells from the primary libraries. Amines: 11, 2,3-dimethylcyclohexylamine; 18,, 3,3-diphenylpropylamine; 44, 1-adamantanemethylamine; 47, 2,2-diphenylethylamine; 63, (S)-2-amino-1-butanol; 74.1, (−)-*cis*-myrtanylamine; 77.1, cyclooctylamine; 78.1, 2-adamantanamine; 105a, (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylamine; 231, 2-methoxyphenethylamine; 255, (S)-cyclohexylethylamine; 266, undecylamine; and 272, geranylamine.

stituted adamantanes (1-adamantanemethylamine (44), *cis*-myrtanylamine (74.1), 2-adamantanamine (78.1), and (*1R*,*2R*,*3R*,*5S*)-(−)-isopinocamphylamine (105)). Finally, geranylamine (272) occurs eight times in hits from the library. Indeed, the two most active molecules synthesized, with MICs of 200 nM, all contain either 2-adamantanamine or (*1R*,*2R*,*3R*,*5S*)-(−)-isopinocamphylamine (compounds 109 and 125, Table 5). Compounds 109, 111, 116, and 117 all contain an isoprenylamine in one of the two positions of the diamine, and all have submicromolar MICs. This result may be significant in relation to the known substrate of the arabinosyltransferase target of EMB, a decaprenylarabinose substrate.[18] In any case, the frequency of occurrence of highly α-branched aliphatic moieties in the set of active compounds is consistent with previous results obtained by Wilkinson and colleagues at Lederle.[11]

At first glance, molecules containing tricyclic carbon frameworks, such as those identified here, would appear to have significant liabilities as drug candidates because of poor predicted absorption characteristics. However, detailed pharmacokinetic measurements of the oral bioavailability of several of these compounds in mice and rats confirm significant levels of oral absorption, and testing in animal models of tuberculosis has confirmed the potential of such compounds in the treatment of this disease (Slayden, R. A.; Kraus, C. N.; Barry, C. E., III. Unpublished work). In addition, it is worth noting that currently marketed, orally available pharmaceuticals, such as the tricyclic antiviral agents amantadine and rimantadine also contain such moieties.[33−35]

In summary, we have developed a general solid-phase scheme for the synthesis of ethylenediamines starting from Rink resin and using a diverse set of 288 commercially available amines. A library of 63 238 EMB analogues was prepared and screened for biological activity against *M. tuberculosis*. Screening was performed by monitoring bioluminescence of a gene reporter fusion identified through microarray analysis of the effects of EMB on TB (Voskuil et al., unpublished data). Although in some cases, MIC values correlated well with reporter gene activity, this was not generally true, suggesting that genomic information, such as microarray data and reporter fusions, may provide a useful surrogate for in vitro enzyme activity (Boshoff et al., in preparation). This library revealed 25 compounds with activity that equals or exceeds that of EMB (MIC = 7 μM), including many with unique structural features that suggest further routes for optimization. Preclinical evaluation of these molecules in both in vivo and in vitro assays is currently underway and will potentially identify novel therapeutic candidates for the treatment of this important human disease.

Experimental Section

All reagents were purchased from Sigma-Aldrich. Rink acid resin was obtained from NovaBiochem. Solvents acetonitrile, dichloromethane, dimethylformamide, ethylene dichloride, methanol, and tetrahydrofuran were purchased from Sigma-Aldrich and used as received. Solid-phase syntheses were performed on a Quest 210 synthesizer (Argonaut Technologies). Where indicated, resins produced in the Quest were combined and reacted in combinatorial 96-well-plate format using either Whatman Polyfiltronics or Robbins Scientific equipment. Evaporation of solvents was performed using a SpeedVac AES system (Savant). All necessary chromatographic separations were performed on a Waters' Alliance HT System. Analytical thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates. Mass spectra data were obtained by elecrospray ionization on a Perkin-Elmer/Sciex, API-300, TQMS equipped with an autosampler. NMR data were recorded in deuteriomethanol at 500 MHz on a Varian Inova NMR (University of Tennessee). Elemental analyses were obtained from Midwest Laboratory, Ohio.

Generating the Library/General Synthetic Procedure. Steps 1, 2, and 3 were carried out in 10-mL tubes on a Quest 210 synthesizer. Steps 4, 5, and 6 were carried out using 96-well (2-mL) chemically resistant plates.

Step 1: Activation of the Rink Acid Resin. A suspension of the Rink acid resin (4 g, up to 2.52 mmol, stated capacity

of 0.43−0.63 mmol/g), in 80 mL of a 2:1 mixture of dichloromethane and THF, was distributed into 10 tubes, 8 mL/tube; filtered; and washed twice with THF. A solution of triphenylphosphine (3.80 g, 14.5 mmol) in 30 mL of THF was added, 3 mL/tube, followed by the addition of a solution of hexachloroethane (3.39 g, 14.3 mmol) in 30 mL of THF, 3 mL/tube. After 6 h, the resins were washed with THF (2 × 8 mL) and dichloromethane (2 × 8 mL).

Step 2: Addition of the First Amine (N1). Each tube was charged with 3 mL of dichloroethane, $EtN(iPr)_2$ (0.3 mL, 1.74 mmol) and the corresponding amine (1 mmol). (When a selected amine was a solid, it was added as a solution or a suspension in DMF). Dichloroethane was added to each tube to a final volume of 8 mL. The reaction was carried for 8 h at 45 °C and 6−8 h at room temperature. The resins were filtered; washed with a 2:1 mixture of dichloromethane and methanol (1 × 8 mL), then with methanol (2 × 8 mL); and then were dried on internal frits under nitrogen.

Step 3A: Acylation with Chloroacetyl Chloride. The resins were prewashed with THF (2 × 8 mL) before each tube was charged with THF (8 mL), pyridine (0.3 mL, 3.67 mmol), and chloroacetyl chloride (0.2 mL, 2.5 mmol) and were allowed to stir for 8 h at 45 °C followed by 6−8 h at room temperature. After the reaction was complete, the resins were filtered, washed with a 2:1 mixture of dichloromethane and methanol (1 × 8 mL), methanol (2 × 8 mL), and THF, and the acylation was repeated using the same loads of the reagents, but shorter reaction times: 4 h at 45 °C and 2 h at RT. After the reaction, the resins were filtered, washed with a 2:1 mixture of dichloromethane and methanol (1 × 8 mL) and methanol (3 × 8 mL) and were dried by filtration. The resins were transferred into vials (one resin per vial), and dried in a desiccator under vacuum for 1 h. Anal. Found for resin **5**: N, 0.91%, 0.65 mmol/g; Cl, 3.02%, 0.85 mmol/g. For resin **6**: N, 0.81%, 0.58 mmol/g; Cl, 2.05%, 0.58 mmol/g. For resin **7**: N, 0.82%, 0.59 mmol/g; Cl, 2.24%, 0.63 mmol/g. For resin **8**: N, 1.30%, 0.92 mmol/g; Cl, 4.63%, 1.3 mmol/g. For resin **9**: N, 2.97%, 2.12 mmol/g; Cl, 5.44%, 1.53 mmol/g. For resin **10**: N, 0.98%, 0.7 mmol/g; Cl, 3.23%, 0.91 mmol/g. For resin **11**: N, 1.82%, 1.30 mmol/g; Cl, 3.48%, 0.98 mmol/g. For resin **12**: N, 1.66%, 1.18 mmol/g; Cl, 4.41, 1.24 mmol/g. The same procedure was applied for the synthesis of 1-phenyl-ethylene-1,2-diamines (Figure 2, R_2 = Ph) using α-phenylacetyl chloride, and 1-alkyl-ethylene-1,2-diamines (Figure 2, R_4 = Me, Et, Bu) using an equimolar mixture of α-methyl-, α-ethyl-, and α-butylacetyl bromides. Or alternatively, step 3B.

Step 3B. Acylation with α-Chloro-α-methylacetic Acid. The resins were prewashed twice with dichloromethane. Each tube was charged with 3 mL of a solution of PyBrOP (0.29 g, 0.62 mmol) in dichloromethane, a solution of the acid (0.095 g, 0.62 mmol) in 3 mL of DMF, and $EtN(iPr)_2$ (0.2 mL, 1.2 mmol) and allowed to react for 16−18 h at room temperature. After the reaction was complete, the resins were filtered, washed with dichloromethane (2 × 8 mL) and methanol (2 × 8 mL), and the acylation was repeated. At the end of the reaction, the resins were filtered, washed with dichloromethane (2 × 8 mL) and methanol (3 × 8 mL), and dried under nitrogen with vacuum aspiration. The resins were transferred into vials (one resin per vial) and dried in a desiccator under vacuum for 1 h.

Step 4. Addition of the Second Amine (N2). Each of 10 prepared resins from the first three steps (0.3 g each) were pooled together, leaving 0.050−0.1 g of each resin, which was stored at −20 °C, for deconvolution. A suspension of the resin mixture (3.0−3.3 g) in 100 mL of a 2:1 mixture of dichloromethane/THF was distributed into two 96-well filterplates, and the solvent was removed using a filtration manifold. The reaction plates were transferred into combiclamps, and a 10% solution of $EtN(iPr)_2$ in DMF was added, 0.2 mL per well (0.21 mmol of $EtN(iPr)_2$ per well), followed by the addition of a 1.0 M solution of the appropriate amine from the corresponding master plate, 0.1 mL per well (0.1 mmol of the amine per well). The reaction plates were sealed and kept in an oven at 70−75 °C for 16 h. After cooling to room temperature, the resins were filtered, washed with a 1:1 mixture of dichloromethane and methanol (1 × 1 mL) and methanol (2 × 1 mL) and dried in a desiccator under vacuum for 2 h.

Step 5. Reduction with Red-Al. The reaction plates were sealed with combiclamps before adding a 1:6 mixture of Red-Al (65+ wt % in toluene)/THF (0.6 mL per well, resulting in 0.28 mmol of Red-Al per well). After 4 h, the resins were filtered, washed with THF (2 × 1 mL) and methanol (3 × 1 mL) and dried in the filtration manifold.

Step 6. Cleavage. The reaction plates were placed on the top of the collection plates in a cleavage manifold and charged with 0.5 mL per well of a 10:85:5 mixture of TFA, dichloromethane, and methanol. After 15 min, the solutions were filtered and collected into the collection plate. This procedure was repeated once to improve the yield. Solvents were evaporated, and the residual samples were used directly for testing.

Solid-Phase Synthesis of the Compounds Using a Quest 210 Synthesizer. EMB. The same solid-phase protocol was applied to the synthesis of EMB, with the exception that all 6 steps were carried out using the Quest instrument. Starting from 0.2 g of the Rink acid resin in 5-mL reaction vessels using the above protocol gave crude EMB that was analyzed by NMR and ESI-MS. The sample was purified on HPLC (C18 column, 4 mL/min, 30-min run, with a linear gradient from 5% AcOH/MeOH to acetonitrile (100%)) to provide 16.0 mg of EMB as the AcOH salt, 48% yield, >90% purity by NMR.

Resynthesis of Library Hits. The same solid-phase protocol was applied to the resynthesis of desired hit compounds, with the exception that a larger amount of resin was used (0.45 g) in two 10-mL reaction vessels. The same protocol and HPLC purification procedure provided purified individual compounds.

MIC Determination. MIC assays were performed in 96-well round-bottom plates (Costar no. 3799, Corning Inc., NY) for seven-days. Each diamine analogue was suspended in DMSO in a final concentration of 10 mM and diluted 100-fold into 7H9-OADC liquid medium containing 0.05% Tween-80. This 100 μM stock was diluted by 2-fold serial dilutions in the same medium in microtiter wells, resulting

in 50 μL total volume in each well. In addition to a control lacking drug, the lowest concentration of drug assayed was 0.04 μM (11 dilutions total). *M. tuberculosis* H37Rv was grown to an optical density$_{600nm}$ 0.5 and diluted 1:10 before adding 50 μL to each individual well of the 96-well plates. The final optical density$_{600nm}$ was 0.025, and there was no visible growth within the wells of the microtiter plate. Upon incubation for 7 days at 37 °C, growth became apparent, and the MIC was determined to be the lowest concentration that prevents visible growth. BACTEC assays were performed as described.[36]

***N*-(3,3-Diphenylpropyl)-*N*′-(1-adamantylmethyl)ethane-1,2-diamine (7).** 28 mg, 22% yield. ^{1}H NMR (500 MHz) δ: 7.23 (m, 10H), 3.95 (t, J = 7.6 Hz, 1H), 2.91 (d, J = 1.2 Hz, 4H), 2.70 (dd, J = 7.6 Hz, 1.2 Hz, 2H), 2.40 (s, 2H), 2.32 (q, J = 8.0 Hz, 2H), 1.98 (br d, J = 1.7 Hz, 3H), 1.72 (d, J = 12.2 Hz, 3H), 1.62 (d, J = 12.2 Hz, 3H), 1.51 (br s, 6H). Mass spectrum (ESI) m/z (MH)$^+$ 403.6.

***N*-(−)-*cis*-Myrtanyl-*N*′-(3,3-diphenylpropyl)ethane-1,2-diamine (10)**. 14 mg, 11% yield. ^{1}H NMR (500 MHz) δ: 7.23 (m, 10H), 3.95 (m, 1H), 2.87 (m, 4H), 2.76 (t, J = 8 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.38 (m, 1H), 2.29 (m, 3H), 1.90 (m, 5H), 1.42 (m, 1H), 1.19 (s, 3H), 0.95 (s, 3H), 0.90 (m, 1H). Mass spectrum (ESI) m/z (MH)$^+$ 391.3.

***N*-(3,3-Diphenylpropyl)-*N*′-*exo*-(2-norbornyl)ethane-1,2-diamine (14).** 17 mg, 16% yield. ^{1}H NMR (500 MHz) δ: 7.23 (m, 10H), 3.95 (t, J = 7.9 Hz, 1H), 2.86 (m, 4H), 2.73 (dd, J = 8.0 Hz, 3.3 Hz, 1H), 2.64 (t, J = 7.6 Hz, 2H), 2.29 (m, 4H), 1.45−1.63 (m, 4H), 1. 30 (dt, J = 4.0 Hz, 13.5 Hz, 1H), 1.19 (m, 1H), 1.10 (m, 2H). Mass spectrum (ESI) m/z (MH)$^+$ 349.1.

***N*-(3,3-Diphenylpropyl)-*N*′-(1*S*)-(1-ethylcyclohexyl)ethane-1,2-diamine (21).** 5 mg, 4% yield.: Mass spectrum (ESI) m/z (MH)$^+$ 365.5.

***N*-(2,2-Diphenylethyl)-*N*′-*(R)*-(+)-bornylethane-1,2-diamine (32).** 58 mg, 48% yield. ^{1}H NMR (500 MHz): δ 7.23 (m, 10H), 4.19 (t, J = 6.8 Hz, 1H), 3.34 (d, J = 8 Hz, 2H), 3.02 (m, 4H), 2.94 (m, 1H), 2.11 (m, 1H), 1.67 (m, 1H), 1.40 (m, 2H), 1.21 (m, 2H), 1.07 (m, 1H), 0.82 (s, 6H), 0.78 (s, 3H). Mass spectrum (ESI) m/z (MH)$^+$ 377.2.

***N*-(2,2-Diphenylethyl)-*N*′-(adamantylmethyl)ethane-1,2-diamine (34).** 6.8 mg, 6% yield. ^{1}H NMR (500 MHz) δ: 7.23 (m, 10H), 4.13 (t, J = 7.6 Hz, 1H), 3.24 (dd, J = 7.9 Hz, 1.2 Hz, 2H), 2.79 (t, J = 6.5 Hz, 2H), 2.74 (t, J = 6.0 Hz, 2H), 2.25 (s, 2H), 1.95 (m, 3H), 1.69 (d, J = 12.5 Hz, 3H), 1.59 (d, J = 11.9 Hz, 3H), 1.40 (s, 6H). Mass spectrum (ESI) m/z (MH)$^+$ 389.0.

***N*-(2,2-Diphenylethyl)-*N*′-(−)-*cis*-myrtanylethyl-1,2-diamine (37).** 54 mg, 38% yield. ^{1}H NMR (500 MHz): δ 7.23 (m, 10H), 4.13 (t, J = 7.6 Hz, 1H), 3.26 (d, J = 7.6 Hz, 2H), 2.86 (dd, J = 4.3 Hz, 8.0 Hz, 4H), 2.76 (dd, J = 7.6 Hz, 12.2 Hz, 2H), 2.37 (ddd, J = 1.8 Hz, 9.0 Hz, 12.5 Hz, 1H), 2.12 (dq, J = 1.8 Hz, 7.6 Hz, 1H), 1.98−1.84 (m, 5H), 1.39 (ddd, J = 2.4 Hz, 4.0 Hz, 6.1 Hz, 1H), 1.18 (s, 3H), 0.93 (s, 3H), 0.90 (dd, J = 9.7 Hz, 4.2 Hz, 1H). Mass spectrum (ESI) m/z (MH)$^+$ 377.2.

***N*-(−)-*cis*-Myrtanyl-*N*′-(2,2-diphenylethyl)propane-1,2-diamine (38).** 39 mg, 30% yield. ^{1}H NMR (500 MHz) δ: 7.23 (m, 10H), 4.13 (t, J = 8.0 Hz, 1H), 3.26 (d, J = 7.5 Hz, 2H), 2.96 (m, 1H), 2.82 (m, 2H), 2.69 (m, 1H), 2.58 (m, 1H), 2.36 (m, 1H), 2.21 (m, 1H), 1.96(s, 1H), 1.88 (m, 4H), 1.33 (m, 1H), 1.16 (s, 6H), 0.93 (s, 3H), 0.90 (dd, J = 9.7 Hz, 4.2 Hz, 1H). Mass spectrum (ESI) m/z (MH)$^+$ 391.0.

***N*-(2,2-Diphenylethyl)-*N*′-(1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylethane-1,2-diamine (40).** 33 mg, 23% yield. ^{1}H NMR (500 MHz): δ 7.23 (m, 10H), 4.13 (t, J = 7.5 Hz, 1H), 3.27 (d, J = 8.0 Hz, 2H), 3.14 (dt, J = 6.0 Hz, 10 Hz, 1H), 2.91 (m, 4H), 2.36 (qd, J = 2.0 Hz, 6.0 Hz, 2H), 1.98 (m, 3H), 1.82 (dt, J = 2.0 Hz, 6.0 Hz, 1H), 1.72 (ddd, J = 2.5 Hz, 5.5 Hz, 13.5 Hz, 1H), 1.22 (s, 3H), 1.09 (d, J = 7.0 Hz, 3H), 0.91 (s, 3H). Mass spectrum (ESI) m/z (MH)$^+$ 377.3.

***N*-(−)-*cis*-Myrtanyl-*N*′-(1*R*,2*R*,3*R*, *S*)-(−)-isopinocampheylethane-1,2-diamine (47).** 42 mg, 33% yield. ^{1}H NMR (500 MHz): δ 3.28 (m, 1H), 3.25 (m, 4H), 2.93 (dd, J = 4.6 Hz, 2.0 Hz, 2H), 2.40 (m, 4H), 2.02 (m, 3H), 1.93 (m, 3H), 1.87 (m, 2H), 1.79 (m, 1H), 1.51 (ddd, J = 4.6 Hz, 10.0 Hz, 13.0 Hz, 1H), 1.23 (s, 3H), 1.19 (s, 6H), 1.03 (d, J = 10.3 Hz, 1H), 0.98 (s, 3H), 0.94 (d, J = 9.8 Hz, 1H), 0.94 (s, 3H). Mass spectrum (ESI) m/z (MH)$^+$ 333.6.

***N*-(3,3-Diphenylpropyl)-*N*′-cyclooctylethane-1,2-diamine (52).** 20 mg, 18% yield. ^{1}H NMR (500 MHz): δ 7.23 (m, 10H), 3.96 (t, J = 7.9 Hz, 1H), 3.00 (m, 1H), 2.90 (dd, J_1 = J_2 = 5.5 Hz, 2H), 2.84 (dd, J_1 = J_2 = 5.0 Hz, 2H), 2.61 (t, J = 7.3 Hz, 2H), 2.27 (q, J = 7.6 Hz, 2H), 1.83 (m, 2H), 1.74 (m, 2H), 1.58 (m, 10H). Mass spectrum (ESI) m/z (MH)$^+$ 365.5.

***N*-(1-Adamantylmethyl)-*N*′-cyclooctylethane-1,2-diamine (55).** 6.7 mg, 6% yield. ^{1}H NMR (500 MHz): δ 3.05 (m, 1H), 3.01 (m, 2H), 2.94 (m, 2H), 2.36 (s, 2H), 1.98 (m, 3H), 1.89 (m, 2H), 1.73 (m, 3H), 1.67 (m, 2H), 1.64 (m, 3H), 1.54 (m, 10H), 1.52 (m, 6H). Mass spectrum (ESI) m/z (MH)$^+$ 319.5.

***N*-(−)-*cis*-Myrtanyl-*N*′-(cyclooctyl)ethane-1,2-diamine (57).** 18 mg, 18% yield. ^{1}H NMR (500 MHz) δ: 3.01 (m, 1H), 3.00 (m, 1H), 2.74 (m, 2H), 2.36 (m, 1H), 2.27 (m, 1H), 1.91 (m, 5H), 1.87 (m, 2H), 1.75 (m, 2H), 1.55 (m, 10H), 1.41 (m, 1H), 1.18 (s, 3H), 0.97 (s, 3H), 0.91 (d, J = 9.8 Hz, 1H). Mass spectrum (ESI) m/z (MH)$^+$ 307.5.

***N*-(2-Adamantyl)-*N*′-cyclooctylethane-1,2-diamine (58).** 25 mg, 23% yield. ^{1}H NMR (500 MHz): δ 3.06 (m, 1H), 3.00 (t, J = 6.1 Hz, 2H), 2.93 (t, J = 5.5 Hz, 2H), 2.83 (br s, 1H), 1.85 (m, 7H), 1.75 (m, 5H), 1.51 (m, 16H). Mass spectrum (ESI) m/z (MH)$^+$ 305.1.

***N*-(Cyclooctyl)-*N*′-(1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylethane-1,2-diamine (59).** 15 mg, 14% yield. ^{1}H NMR (500 MHz): δ 3.48 (dt, J = 6.0 Hz, 10.0 Hz, 1H), 3.30 (m, 1H), 3.20 (m, 4H), 2.44 (tq, J = 2.0 Hz, 10.0 Hz, 1H), 2.36 (dtd, J = 2.0 Hz, 6.0 Hz, 10.0 Hz, 1H), 2.09 (dq, J = 2.0 Hz, 7.2 Hz, 1H), 1.95 (m, 2H), 1.81 (m, 2H), 1.72 (m, 2H), 1.49 (m, 11H), 1.17 (s, 6H), 1.16 (d, J = 7.2 Hz, 1H), 0.90 (s, 3H). Mass spectrum (ESI) m/z (MH)$^+$ 307.4.

***N*-(−)-*cis*-Myrtanyl-*N*′-(1*S*)−(1-ethylcyclohexyl)ethane-1,2-diamine (62).** 48 mg, 46% yield. ^{1}H NMR (500 MHz): δ 2.96 (m, 3H), 2.76 (m, 2H), 2.39 (m, 1H), 2.28 (quintet, J = 8.5 Hz, 1H), 1.95 (m, 5H), 1.78 (m, 2H), 1.69 (m, 3H), 1.48 (m, 2H), 1.23 (m, 4H), 1.20 (s, 3H), 1.13 (d, J = 6.7 Hz, 3H), 1.05 (dd, J = 12 Hz, 3 Hz, 3H), 0.98 (s, 3H), 0.93

(d, $J = 9.7$ Hz, 1H). Mass spectrum (ESI) *m/z* $(MH)^+$ 306.9.

***N-trans*-(2-Phenylcyclopropyl)-*N′*-(1-adamanthyl)ethane-1,2-diamine (65).** 18 mg, 16% yield. 1H NMR (300 MHz): δ 7.26 (m, 5H), 3.54 (m, 2H), 3.42 (m, 2H), 3.06 (m, 2H), 2.52 (m, 1H), 2.23 (m, 1H), 1.96 (s, 6H), 1.75 (m, 9H), 1.60 (m, 1H), 1.42 (m, 1H), 1.25 (m, 1H). Mass spectrum (ESI) *m/z* $(MH)^+$ 311.3.

***N*-(3,3-Diphenylpropyl)-*N′*-(1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylethane-1,2-diamine (66).** 2 mg, 2% yield. 1H NMR (500 MHz) δ: 7.26 (m, 10H), 3.96 (t, $J = 7.6$ Hz, 1H), 3.09 (m, 1H), 2.92 (m, 1H), 2.84 (m, 2H), 2.62 (m, 2H), 2.35 (m, 4H), 1.97 (s, 3H), 1.82 (m, 1H), 1.68 (m, 1H), 1.21 (s, 3H), 1.12 (d, $J = 7.3$ Hz, 3H), 1.01 (m, 1H), 0.92 (s, 3H). Mass spectrum (ESI) *m/z* $(MH)^+$ 391.4.

***N*-Geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine (109).** 27 mg, 24% yield. ^{1}H NMR (500 MHz): δ 5.21 (t, $J = 7.2$ Hz, 1H), 5.00 (br t, 1H), 3.63 (d, $J = 7.6$ Hz, 2H), 3.34 (m, 1H), 3.19 (m, 4H), 2.08 (m, 2H), 2.03 (m, 3H), 1.89 (m, 6H), 1.82 (m, 2H), 1.75 (m, 4H), 1.67 (s, 3H), 1.64 (s, 1H), 1.57 (s, 3H), 1.50 (s, 3H). Mass spectrum (ESI) *m/z* $(MH)^+$ 331.2.

***N*-Geranyl-*N′*-(2-ethylpiperidine)ethane-1,2-diamine (111).** 44 mg, 24% yield. ^{1}H NMR (500 MHz): δ 5.23 (t, $J = 6.1$ Hz, 1H), 5.04 (m, 1H), 3.52 (d, $J = 7.3$ Hz, 2H), 2.95 (m, 4H), 2.65 (m, 1H), 2.43 (m, 2H), 2.08 (m, 4H), 1.69 (s, 3H), 1.65 (s, 3H), 1.61 (m, 2H), 1.55 (s, 3H), 1.45 (q, 2H), 1.40 (br m, 4H), 0.89 (t, $J = 7.3$, 3H). Mass spectrum (ESI) *m/z* $(MH)^+$ 292.5.

***N*-Geranyl-*N′*-allyl-*N′*-(cyclopentyl)ethane-1,2-diamine (116).** 45 mg, 42% yield. ^{1}H NMR (500 MHz): δ 5.86 (ddd, $J = 10.0$ Hz, 16.1 Hz, 6.7 Hz, 1H), 5.27 (m, 3H), 5.05 (m, 1H), 3.59 (d, $J = 7.3$ Hz, 2H), 3.29 (br d, $J = 6.4$ Hz, 2H), 3.16 (quintet, $J = 8.2$ Hz, 1H), 3.05 (m, 2H), 2.90 (m, 2H), 2.09 (m, 4H), 1.85 (m, 2H), 1.70 (m, 2H), 1.67 (s, 3H), 1.65 (s, 3H), 1.54 (s, 3H), 1.50 (m, 2H), 1.45 (m, 2H). Mass spectrum (ESI) *m/z* $(MH)^+$ 305.3.

***N*-Geranyl-*N′*-diphenylmethylethane-1,2-diamine (117).** 24 mg, 20% yield. ^{1}H NMR (500 MHz): δ 7.37 (d, $J = 7.2$ Hz, 4H), 7.29 (t, $J = 7.3$ Hz, 4H), 7.21 (t, $J = 7.0$ Hz, 2H), 5.15 (t, $J = 7.5$, 1H), 5.01 (m, 1H), 4.89 (br s, 1H), 3.43 (d, $J = 7.0$ Hz, 2H), 2.95 (m, 2H), 2.92 (m, 2H), 2.05 (m, 4H), 1.64 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H). Mass spectrum (ESI) *m/z* $(MH)^+$ 363.3.

***N*,*N′*-bis-(−)-*cis*-Myrtanylpropane-1,2-diamine (125).** 82 mg, 35% yield. ^{1}H NMR (500 MHz): δ 3.62 (m, 1H), 3.18 (dd, $J = 13.7$ Hz, 3.7 Hz, 1H), 3.06 (dt, $J = 11.5$ Hz, 7.5 Hz, 2H), 2.98 (m, 1H), 2.87 (dt, $J = 12.2$ Hz, 7.3 Hz, 2H), 2.40 (m, 4H), 1.94 (m, 10H), 1.50 (m, 2H), 1.36 (d, $J = 6.7$ Hz, 3H), 1.20 (s, 6H), 0.97 (s, 6H), 0.94 (dd, $J = 10.1$ Hz, 2H). Mass spectrum (ESI) *m/z* $(MH)^+$ 346.9.

***N*-[2-(2-Methoxy)phenylethyl]-*N′*-(1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheyl-ethane-1,2-diamine (151).** 67 mg, 60% yield. ^{1}H NMR (500 MHz): δ 7.22 (t, $J = 5.8$ Hz, 1H), 7.12 (dd, $J = 5.8$ Hz, 1.8 Hz, 1H), 6.87 (m, 2H), 3.81 (s, 3H), 3.14 (m, 1H), 3.04 (m, 6H), 2.89 (t, $J = 7.0$ Hz, 2H), 2.37 (m, 2H), 1.98 (m, 2H), 1.83 (dt, $J = 6.0$ Hz, 2.0 Hz, 1H), 1.72 (ddd, $J = 2.5$ Hz, 5.5 Hz, 13.5 Hz, 1H), 1.22 (s, 3H), 1.13 (d, $J = 7.3$ Hz, 3H), 0.99 (d, $J = 10.1$ Hz, 1H), 0.93 (s, 3H). Mass spectrum (ESI) *m/z* $(MH)^+$ 331.5.

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Supporting Information Available. (1) Complete list of amines used in construction of the primary library. (2) Additional 1H NMR and MS data for resynthesized individual diamine hits. This material is available free of charge via the Internet at http://pubs.acs.org.

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