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Discovery of Selective Menaquinone Biosynthesis Inhibitors against *Mycobacterium tuberculosis*†

Joy Debnath,‡ Shajila Siricilla,‡ Bajoie Wan,§ Dean C. Crick,∥ Anne J. Lenaerts,∥ Scott G. Franzblau,§ and Michio Kurosu*,‡

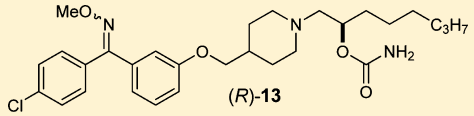
‡Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, 881 Madison, Memphis, Tennessee 38163, United States

§Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, United States

∥Department of Microbiology, Immunology and Pathology, Colorado State University, 200 West Lake Street, Fort Collins, Colorado 80523, United States

Supporting Information

ABSTRACT: Aurachin RE (**1**) is a strong antibiotic that was recently found to possess 1,4-dihydroxy-2-naphthoate prenyltransferase (MenA) and bacterial electron transport inhibitory activities. Aurachin RE is the only molecule in a series of aurachin natural products that has the chiral center in the alkyl side chain at C9′-position. To identify selective MenA inhibitors against *Mycobacterium tuberculosis*, a series of chiral molecules were designed based on the structures of previously identified MenA inhibitors and **1**. The synthesized molecules were evaluated in in vitro assays, including MenA enzyme and bacterial growth inhibitory assays. We could identify novel MenA inhibitors that showed significant increase in potency of killing nonreplicating *M. tuberculosis* in the low oxygen recovery assay (LORA) without inhibiting other Gram-positive bacterial growth even at high concentrations. The MenA inhibitors reported here are useful new pharmacophores for the development of selective antimycobacterial agents with strong activity against nonreplicating *M. tuberculosis*.



Selective antimycobacterial MenA inhibitor

MICs: 2.31 μg/mL (*M. tuberculosis*, MABA)
0.85 μg/mL (*M. tuberculosis*, LORA)
>60 μg/mL (*S. aureus*)

MABA: microplate alamar blue assay
LORA: Low-oxygen recovery assay

INTRODUCTION

Mycobacterium tuberculosis (Mtb) causes tuberculosis (TB) and is responsible for nearly two million deaths annually.[1,2] Moreover, the emergence of multidrug-resistant (MDR) strains of Mtb seriously threatens TB control and prevention efforts. One-third of the 42 million people living with HIV/AIDS worldwide are coinfected with Mtb.[3] Recent studies have shown that infection with Mtb enhances replication of HIV and may accelerate the progression of HIV infection to AIDS. There are significant problems associated with treatment of AIDS and Mtb coinfected patients.[4] Rifampicin and isoniazid (key components of the local directly observed treatment strategy) induce the cytochrome P450 3A4 enzyme, which shows significant interactions with anti-HIV drugs such as protease inhibitors. In addition, rifampicin strongly interacts with non-nucleoside reverse transcriptase and protease inhibitors for HIV infections. Therefore, clinicians avoid starting highly active antiretroviral therapy (HAART), which consists of three or more highly potent reverse transcriptase inhibitors and protease inhibitors, until the TB infection has been cleared.[5,6] *M. tuberculosis* is recognized to lie in a nonreplicating state (dormancy), particularly in the caseous pulmonary nodules where the lesions have little access to oxygen, and can survive for many years in the host by entering a dormant state. About 10% of patients with latent Mtb are reactivated, causing the risk of fatal diseases.[7−10] Thus, in addition to the necessity of drugs for the treatment of MDR-Mtb, the development of drugs that kill Mtb in any state is very important. However, no current TB drugs are effective in killing the dormant form of Mtb in vivo. Therefore, ideal novel antituberculosis compounds should show: (1) compatibility and lack of cross-resistance with other anti-Mtb agents because combination therapy remains mandatory to combat Mtb, (2) antimicrobial spectrum focused against Mtb, (3) strong growth inhibitory activity against nonreplicating Mtb, and (4) no interactions with HAART.

Function of ubiquinone (coenzyme Q_{10}) as a component of the mitochondrial respiratory chain in human is well established ("the chemiosmotic theory", Mitchell, 1978).[11−13] In prokaryotes, especially in Gram-positive bacteria, menaquinone transfers two electrons in a process of either aerobic or anaerobic respiration (Figure 1). On the other hand, a majority of Gram-negative organisms utilize ubiquinone under aerobic conditions and menaquinone under anaerobic conditions in their electron transport systems.[14−16] Therefore, inhibitors of menaquinone biosynthesis or specific inhibitors of enzymes associated with electron transport systems have great potential

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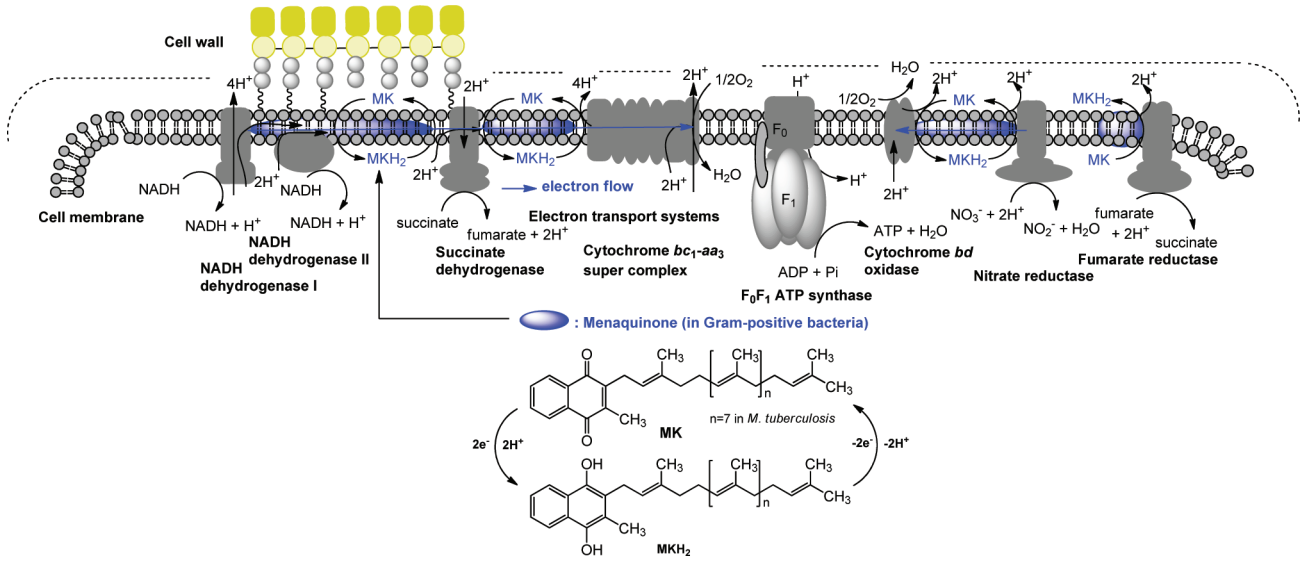

Figure 1. The electron flow system of *M. tuberculosis*. Menaquinone (MK) mediated energetic pathway (blue).

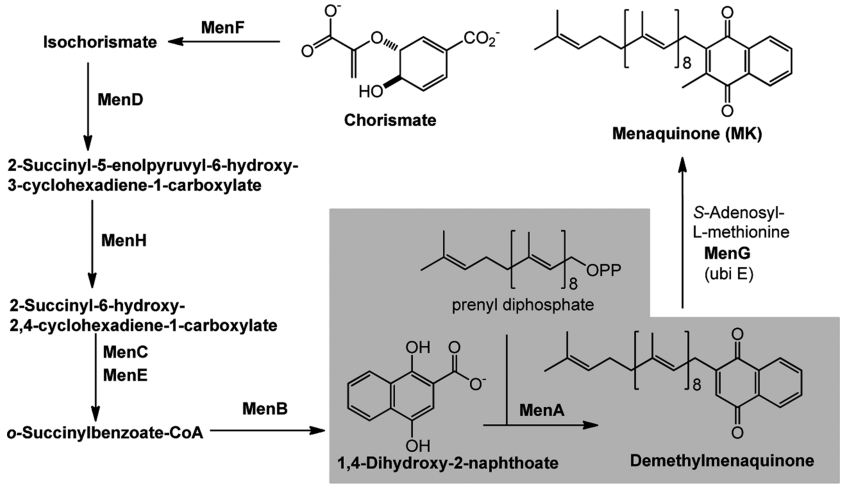

Figure 2. Biosynthesis of menaquinone from chorismate (classic pathway).

for the development of novel and selective drugs against MDR Gram-positive bacteria.[17] The electron transport system couples with ATP synthase to produce ATP through oxidative phosphorylation. Bacterial ATP synthase, F_1F_0-ATPase, is a viable target for treatment of MDR Mtb infections. A diarylquinolone, a phase II clinical drug, is an inhibitor of Mtb ATP synthase that exhibited a remarkable activity against Mtb.[18] However, only few studies have investigated the electron transport system for development of new antibacterial drugs.[17] Weinstein and co-workers reported the inhibitors of type II NADH:menaquinone oxidoreductase that effectively killed Mtb in vitro, and they concluded that type II NADH dehydrogenase could be a unique and interesting antimicrobial target.[19] We have reported that inhibition of 1,4-dihydroxy-2-naphthoate prenyltransferase (MenA), which catalyzes a formal decarboxylative prenylation of 1,4-dihydroxy-2-naphthoate (DHNA) to form demethylmenaquinone (DMMK) in menaquinone biosynthesis (Figure 2), showed significant growth inhibitory activities against drug resistant Gram-positive bacteria including *M. tuberculosis*.[20,21] In menaquinone biosynthesis, 2-succinyl-5-enoylpyruvyl-6-hydroxy-3-cyclohexane-1-carboxylic acid synthase (MenD), MenE (an acyl-CoA synthase), and 1,4-dihydroxynaphthoyl-CoA synthase (MenB) have recently been studied for the development of novel drug lead for Gram-positive pathogens including *M. tuberculosis*.[22−25]

In an attempt at finding a new pharmacophore for MenA inhibitors, we recently identified a new quinolone natural product, aurachin RE (**1**, Figure 3),[26] which exhibited MenA enzyme inhibitory activity as well as a wide antimicrobial spectrum against Gram-positive bacteria. As there is an overall structural resemblance between aurachin RE and our first generation MenA inhibitors (e.g., **10** in Figure 4) or menaquinone, aurachin RE's antibacterial activity could be attributed to a synergistic effect of respiratory chain and menaquinone biosynthesis inhibitory activities. The identification of aurachin RE's MenA enzyme inhibitory activity encouraged us to redesign and synthesize chiral MenA inhibitor molecules, in which *primary* or *secondary* alcohol was introduced in the side chain of the first-generation MenA inhibitor molecules.[20,21] To date, we have synthesized over 400 molecules with >95% purity either in solution or on polymer-support, and these molecules were evaluated in an enzymatic assay in vitro (IC_{50}) against MenA and in bacterial growth inhibitory assays (MIC). Figure 3 illustrates our assay scheme

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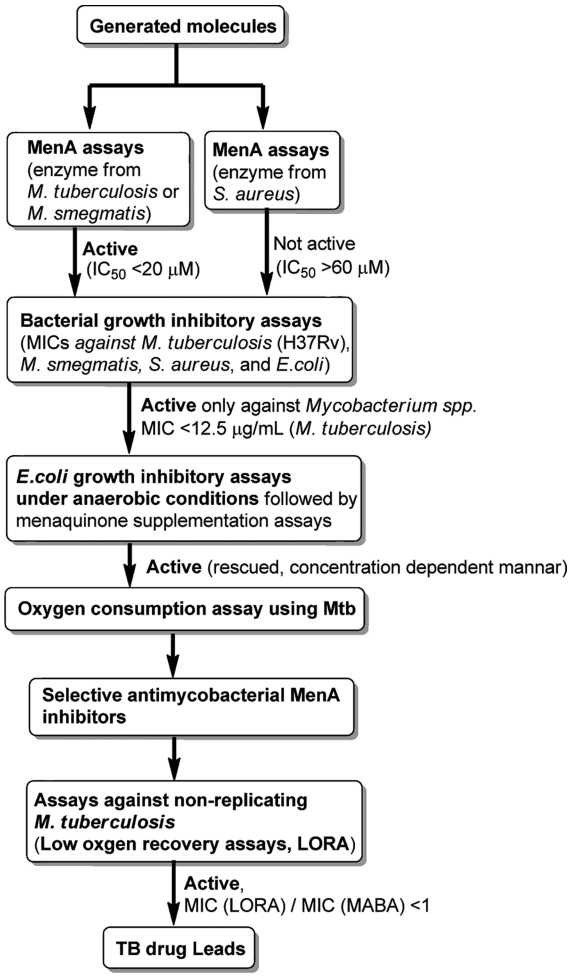

Figure 3. Assay to identify selective MenA inhibitors against *M. tuberculosis*.

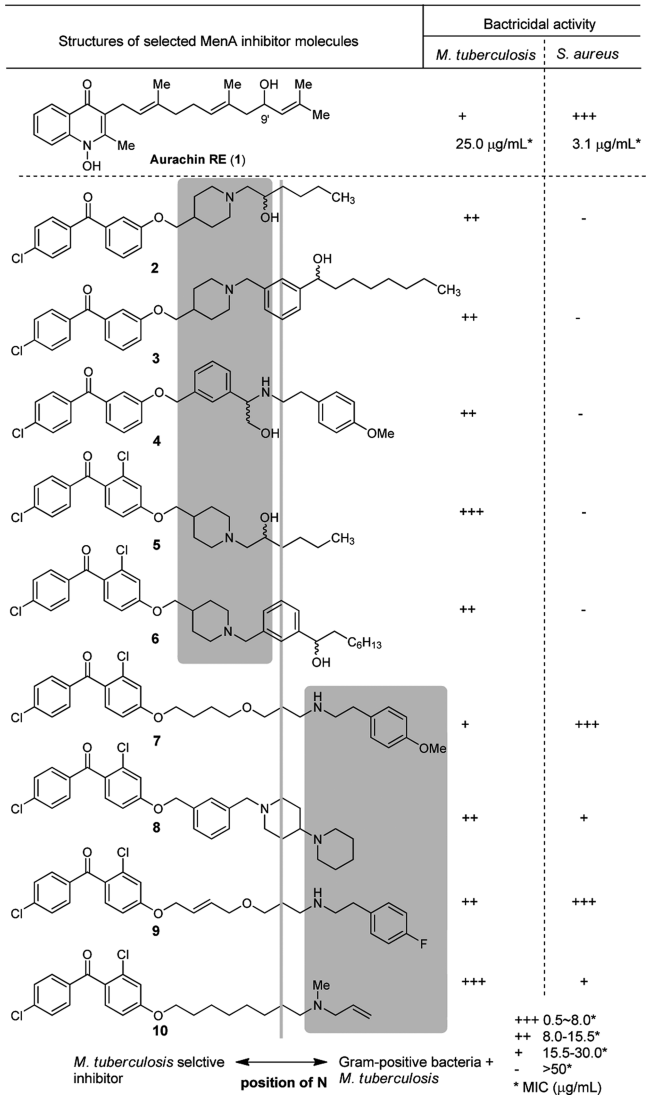

Figure 4. Rational of selective antimycobacterial MenA inhibitors.

to identify selective MenA inhibitors against *M. tuberculosis*. In these molecules, 26 compounds were identified to exhibit the in vitro biological activities which met the assay criteria summarized in Figure 3. On the basis of obtained SAR from a 400-membered library, it became evident that the topology of the *N* atom in the inhibitor molecules plays an important role in selectivity of the MenA enzymatic and bactericidal activities (Mtb vs *Staphylococcus aureus*). As summarized in Figure 4, selective mycobactericidal molecules (**2**–**6**) possess the *secondary* or *tertiary* amine in the near center of the molecules (highlighted moieties in **1**–**6** in Figure 4), whereas the topology of the *N* atom of the molecules possessing antibacterial activities against both Mtb and *S. aureus* (**7**–**10**) locates the right half of the molecules (highlighted moieties in **7**–**10** in Figure 4). We have identified selective antimycobacterial MenA inhibitors in their racemic forms. To obtain insight into the effect of chirality of new MenA inhibitors (**2**–**6**), we commenced syntheses of the optically active forms of the identified inhibitors. Herein we report the synthesis and in vitro biological activity evaluation of optically active molecules of **2**–**6** and their derivatives. The results disclosed in this article identify novel chiral antimycobacterial MenA inhibitors with significant activity in killing nonreplicating Mtb.

RESULTS AND DISCUSSION

Selective *M. tuberculosis* MenA Inhibitors: Assay Strategy. Antimicrobial spectrum focused against Mtb (selective antimycobacterial agent) is preferable for TB chemotherapy.[27] We realized that the peptide sequences of the *Mtb menA* and *S. aureus menA* gene products are only 32% identity and 50% similarity in the BLAST experiment.[28] Indeed, we have identified several molecules that exhibit selective MenA enzyme and bacterial growth inhibitory activities against Mtb, more than a 10-fold higher inhibitory activity against Mtb than *S. aureus*. To develop MenA inhibitors which are selective against Mtb, molecules generated in this program were first evaluated in MenA enzyme inhibitory assays. Only molecules exhibited Mtb MenA activity over *S. aureus* MenA (IC_{50} < 20 μM, >60 μM against *Mtb MenA* and *S. aureus MenA*, respectively) were evaluated in bacterial growth inhibitory assays (MICs) using Mtb, *S. aureus*, and *Escherichia coli* (Figure 3). The molecules exhibited good activity only against Mtb (MICs for Mtb, *S. aureus*, and *E. coli* are <12.5, >60, and >125 μg/mL, respectively) were evaluated in *E. coli* growth inhibitory assays under anaerobic conditions followed by menaquinone supplementation assays (*E. coli* utilize only menaquinone in

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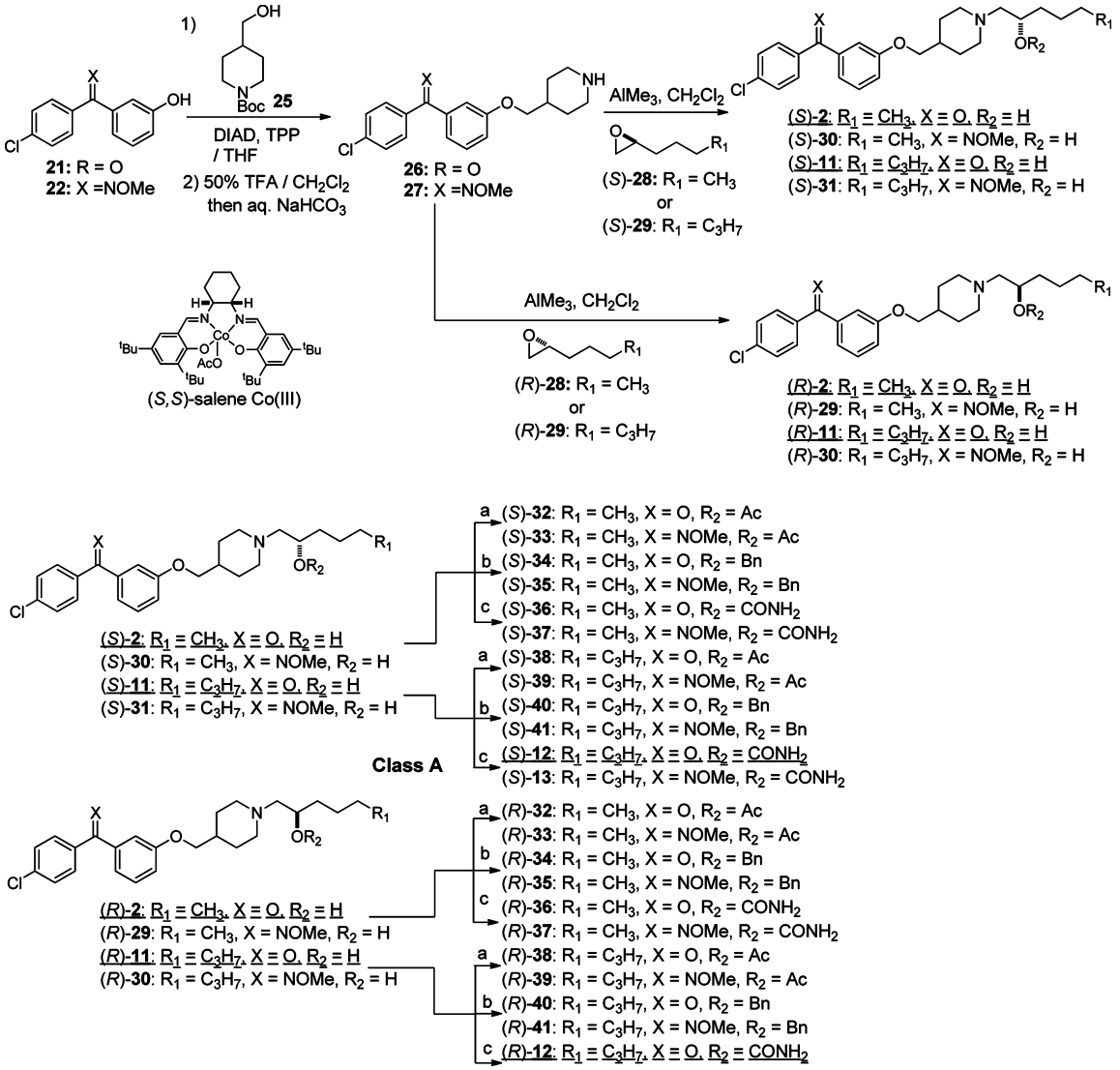
Scheme 1. Syntheses of Optically Active MenA Inhibitors, Class A[a]



[a]Reagents and conditions: (a) Ac_2O/Py. (1:1), room temperature; (b) BnBr, NaH, DMF/THF (4:1), 0 °C; (c) TMSNCO, DMAP, CH_2Cl_2, room temperature.

their electron transport system under anaerobic conditions).[29,30] This assay confirmed that the molecules killed bacteria by targeting MenA enzyme or electron transport systems (vide infra). Oxygen consumption assay using Mtb identifies electron transport system inhibitors;[31,32] in this assay, selective MenA inhibitors should not show activity at concentrations below the MIC against Mtb. Selective antimycobacterial MenA inhibitors confirmed via *E. coli* growth inhibitory (under anaerobic conditions), and oxygen consumption assays were evaluated for their activity against nonreplicating Mtb via the low oxygen recovery assays (LORA).[33]

Chemistry. As mentioned above, we have identified several antimycobacterial MenA inhibitors in their racemic forms. To obtain insight into the effect of chirality of **2**–**6** (Figure 4) and functional groups around the chiral center on biological activity, we synthesized both enantiomers of **2**–**6** and their analogues in which the *secondary* alcohols were functionalized with acyl, carbamate, and benzyl groups. Benzophenone functional group shows a moderate electrophilicity and thus may require chemical modification to improve its physicochemical property in early stage of drug development. In this study, we have introduced benzophenone *O*-methyl oxime derivatives to obtain preliminary SAR and their cytotoxicity data.

Syntheses of optically active molecules of **2**–**6** and their analogues are summarized in Schemes 1–5. The Cl-substituted benzophenone derivatives, **21** and **23**, were synthesized according to the previously reported procedures.[20] *O*-Methyl oxime derivatives **22** and **24** were prepared by the treatment of **21** or **23** with $MeONH_2$·HCl in pyridine at 105 °C. The phenolic alcohol of **21** was subjected to the Mitsunobu reaction with Boc-protected piperidin-4-ylmethanol **25** to provide the piperidinyl ethers **26** in over 90% yield after deprotection of the Boc group.[34,35] Optically active (2*R*)- or (2*S*)-alkyloxiranes, **28** and **29**, were synthesized via Jacobsen's kinetic resolutions of the racemic epoxides.[36] Opening of the epoxides (*S*)-**29** with the piperidine derivatives **26** was achieved by using a stoichiometric amount of $AlMe_3$ at room temperature to yield the amino-alcohols (*S*)-**11**. The generated optically active alcohol (*S*)-**11** was functionalized with Ac_2O, BnBr, and

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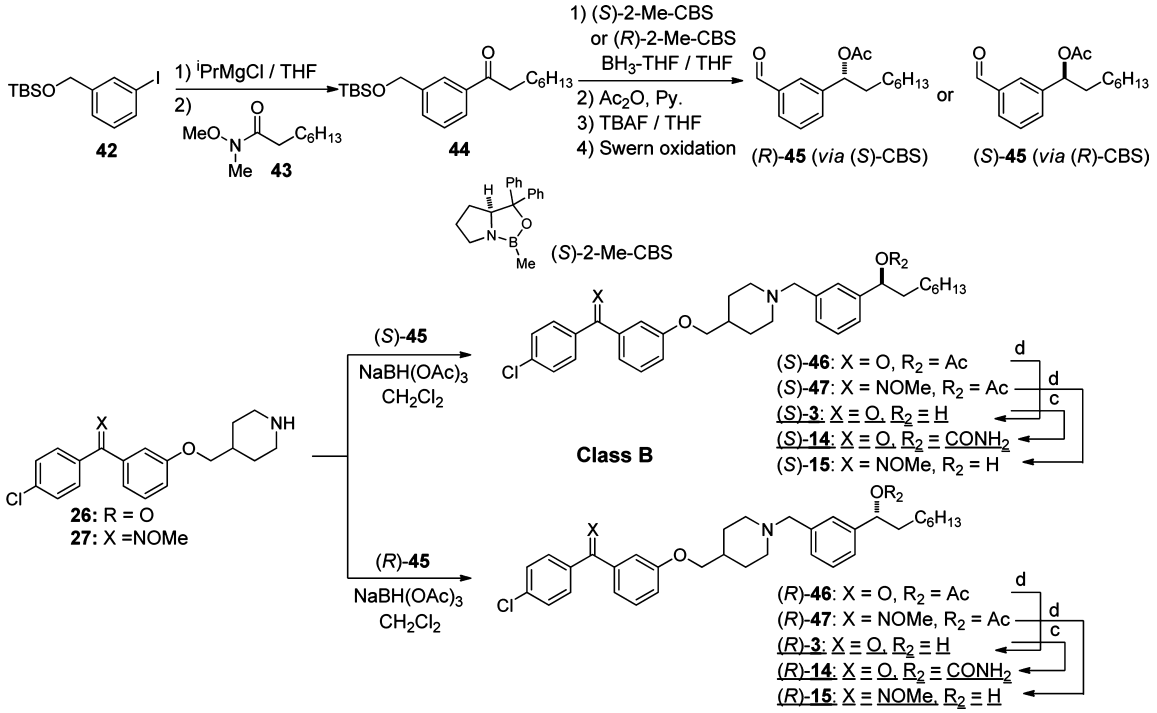
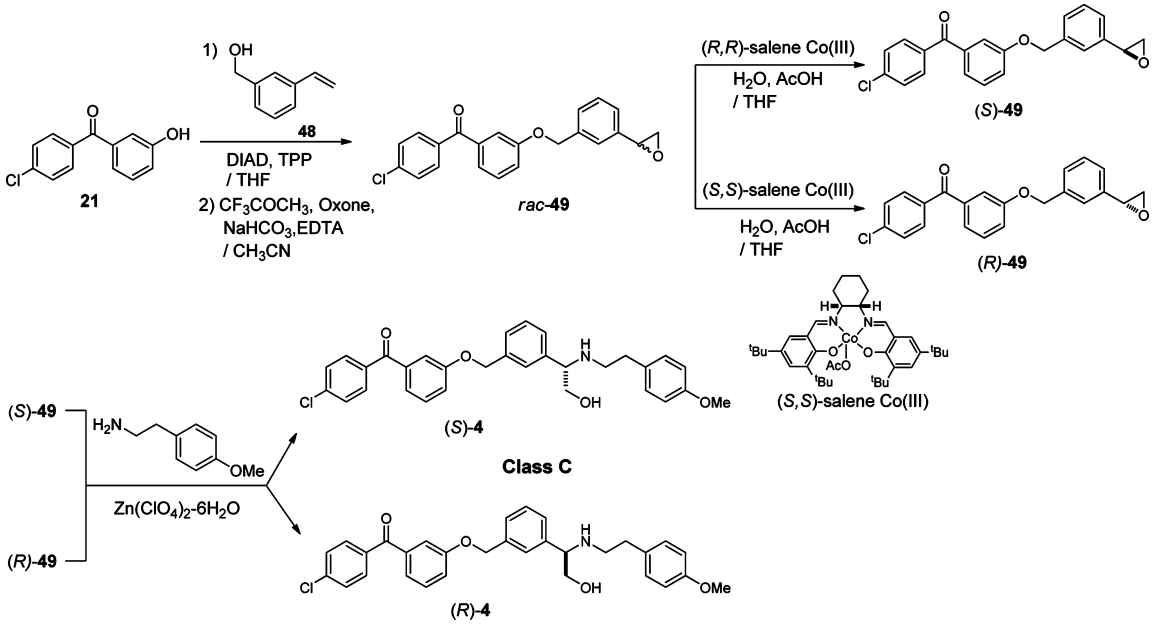
Scheme 2. Syntheses of Optically Active MenA Inhibitors, Class B[a]



[a]Reagents and conditions: (c) TMSNCO, DMAP, CH_2Cl_2, room temperature; (d) 1N NaOH, CH_3CN, 0 °C.

Scheme 3. Syntheses of Optically Active MenA Inhibitors, Class C



TMSNCO to afford the corresponding acetate (*S*)-**38**, benzyl ether (*S*)-**40**, and carbamate (*S*)-**12** (class **A** molecules, Scheme 1). To synthesize optically active piperidinyl-benzyl alcohol derivatives **3** (class **B** molecules), (*S*)- and (*R*)-benzaldehyde derivatives **45** were synthesized in four steps from **44** via CBS reductions (Scheme 2).[37] The starting material **44** was readily synthesized via a Grignard reaction of **42** with the Weinreb amide **43**.[38] Reductive aminations of **26** with (*S*)-**45** was achieved with $NaBH(OAc)_3$ in the absence of acid to provide the desired *tertiary* amine (*S*)-**46** without contamination of the diphenylmethanol byproduct. Deacetylation of (*S*)-**46** afforded the *secondary* alcohol (*S*)-**3**, whose alcohol was functionalized with TMSNCO to afford the corresponding carbamate (*S*)-**14**. Optically active amino-alcohols (*S*)- and (*R*)-**4** (class **C** molecules) were synthesized via the resolution of *rac*-epoxide **49** followed by $Zn(ClO_4)_2$-catalyzed selective opening of epoxide with the *primary* amine (Scheme 3).[39] The other optically active *secondary* alcohols (class **D** and **E** molecules) were successfully synthesized via the same synthetic procedures developed for class **A** and **B** molecules (Schemes 4 and 5). Syntheses of the molecules having (*R*)-configuration were also achieved by using the antipodes of the building blocks utilized

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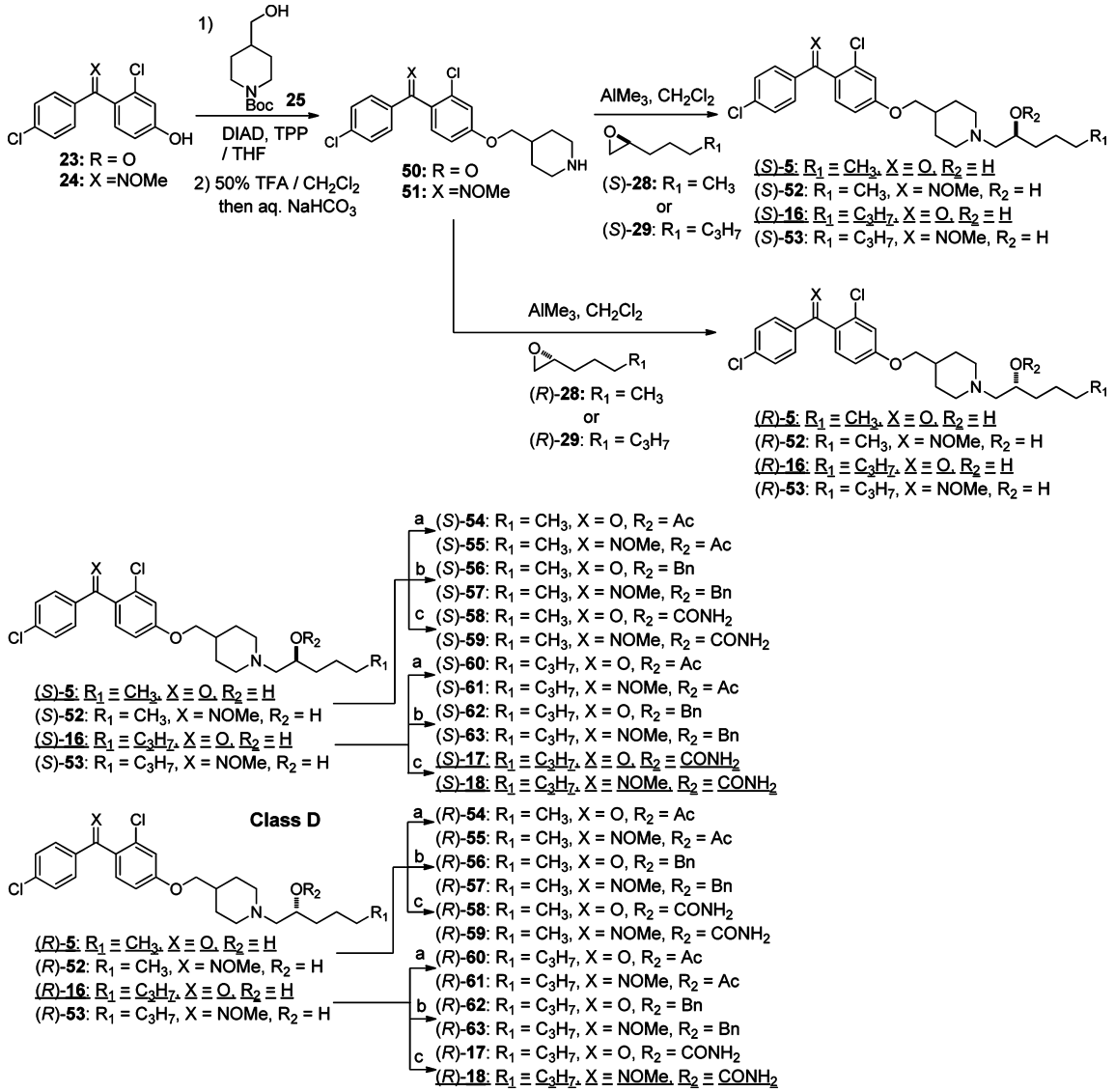
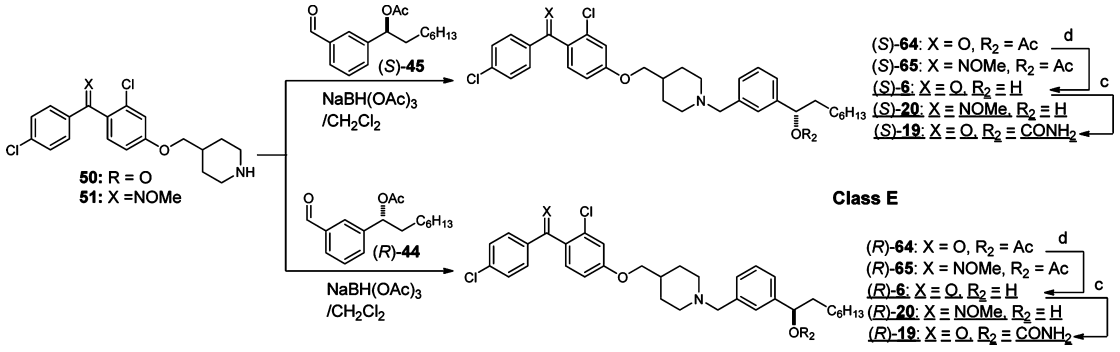
Scheme 4. Syntheses of Optically Active MenA Inhibitors, Class D[a]



[a]Reagents and conditions: (a) Ac_2O/Py (1:1), room temperature; (b) BnBr, NaH, DMF/THF (4:1), 0 °C; (c) TMSNCO, DMAP, CH_2Cl_2, room temperature.

Scheme 5. Syntheses of Optically Active MenA Inhibitors, Class E[a]



[a]Reagents and conditions: (c) TMSNCO, DMAP, CH_2Cl_2, room temperature; (d) 1N NaOH, CH_3CN, 0 °C.

for the synthesis for the (*S*)-configuration molecules. Similarly, syntheses of optically active benzophenone *O*-methyl oxime derivatives **13**, **15**, **18**, **19**, **59**, **63**, and **65** were successfully achieved. Thus, we have synthesized both (*S*)- and (*R*)- forms of **2**−**6** and their derivatives in short number of steps. Optical purity and purity of each molecule were established via HPLC

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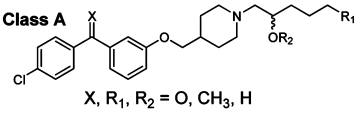
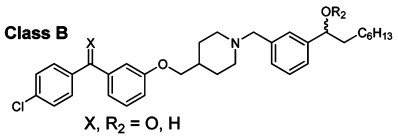
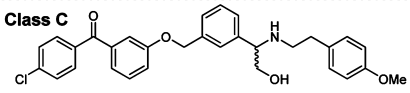
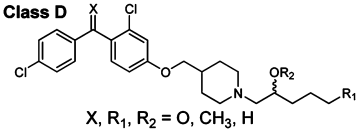
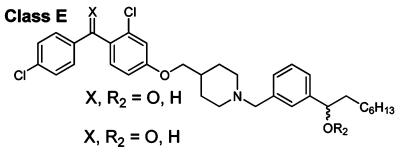
Table 1. Antibacterial Activities against *M. tuberculosis* and *S. aureus*, and MenA Enzyme Inhibitory Activities of the Selected Molecules

Compounds	Structure	MIC (μg/mL)[a]			MenA Inhibition		
	X, R_1, R_2	*S. aureus*	*M. tuberculosis* (MABA)[b]	*M. tuberculosis* (LORA)[c]	*S. aureus* (at 100 μM)	*M. tuberculosis* (at 100 μM)	IC_{50} (μM)
2	Class A  X, R_1, R_2 = O, CH_3, H	>60	12.5	5.2	-	+	15.0
(*S*)-**2**	X, R_1, R_2 = O, CH_3, H	>60	12.5	5.59	-	+	7.5
(*R*)-**2**	X, R_1, R_2 = O, CH_3, H	>60	6.2	4.91	-	+	17.0
(*S*)-**11**	X, R_1, R_2 = O, C_3H_7, H	>60	12.5	4.91	-	+	16.0
(*R*)-**11**	X, R_1, R_2 = O, C_3H_7, H	>60	6.2	5.59	-	+	9.0
(*S*)-**12**	X, R_1, R_2 = O, C_3H_7, $CONH_2$	>60	12.5	2.94	-	+	9.0
(*R*)-**12**	X, R_1, R_2 = O, C_3H_7, $CONH_2$	>60	**2.31**	**0.85**	-	+	**1.5**
(*R*)-**13**	X, R_1, R_2 = NOMe, C_3H_7, $CONH_2$	>60	**2.31**	**0.85**	-	+	**1.2**
3	Class B  X, R_2 = O, H	>60	12.5	1.88	-	+	15.0
(*S*)-**3**	X, R_2 = O, H	>60	12.5	1.88	-	+	25.0
(*R*)-**3**	X, R_2 = O, H	>60	12.5	1.46	-	+	20.0
(*S*)-**14**	X, R_2 = O, $CONH_2$	>60	12.5	4.93	-	+	20.0
(*R*)-**14**	X, R_2 = O, $CONH_2$	>60	12.5	4.93	-	+	15.0
(*R*)-**15**	X, R_2 = NOMe, OH	>60	6.25	1.43	-	+	15.0
4	Class C 	>60	12.5	3.00	-	+	15.0
(*S*)-**4**		>60	12.5	3.00	-	+	17.0
(*R*)-**4**		>60	12.5	3.00	-	+	14.0
rac-**5**	Class D  X, R_1, R_2 = O, CH_3, H	>60	6.25	2.82	-	+	15.0
rac-**16**	X, R_1, R_2 = O, CH_3, $CONH_2$	>60	3.25	2.83	-	+	7.5
(*S*)-**5**	X, R_1, R_2 = O, CH_3, H	>60	**1.50**	**1.43**	-	+	**4.5**
(*R*)-**5**	X, R_1, R_2 = O, CH_3, H	>60	6.25	2.85	-	+	7.5
(*S*)-**16**	X, R_1, R_2 = O, C_3H_7, H	>60	**1.50**	**1.43**	-	+	**3.5**
(*R*)-**16**	X, R_1, R_2 = O, C_3H_7, H	>60	6.25	5.20	-	+	9.5
(*S*)-**17**	X, R_1, R_2 = O, C_3H_7, $CONH_2$	>60	**1.50**	**1.45**	-	+	**1.5**
(*S*)-**18**	X, R_1, R_2 = NOMe, C_3H_7, $CONH_2$	>60	**1.50**	**1.40**	-	+	**1.5**
rac-**6**	Class E  X, R_2 = O, H	>60	12.5	1.88	-	+	20.0
(*S*)-**6**	X, R_2 = O, H	>60	12.5	1.88	-	+	15.0
(*R*)-**6**	X, R_2 = O, H	>60	12.5	1.46	-	+	15.0
(*S*)-**19**	X, R_2 = O, $CONH_2$	>60	12.5	2.93	-	+	20.0
(*R*)-**19**	X, R_2 = O, $CONH_2$	>60	12.5	4.93	-	+	20.0
(*S*)-**20**	X, R_2 = NOMe, OH	>60	12.5	5.54	-	+	20.0
(*R*)-**20**	X, R_2 = NOMe, OH	>60	12.5	5.54	-	+	15.0
RFP[d]		-	0.2	1.47			
INH[e]		-	0.1	>128			
EMB[f]		-	0.78	>128			

[a]The agar plate dilution method was used. [b]MABA: microplate alamar blue assay. [c]LORA: Low-oxygen recovery assay. [d]RFP: rifampicin. [e]INH: isoniazid. [f]EMB: ethanbutol.

analyses of Mosher esters of *secondary* alcohols or alcohols using a chiral column.[40]

MenA Enzyme Inhibitory Assays. MenA enzymatic assays of generated molecules were originally performed with [^{3}H]rated farnesyl diphosphate and MenA containing membrane fraction.[41] This assay requires a significant effort to separate demethylmenaquinone (DMMK) from the reaction mixtures and includes the significant cost for [^{3}H]farnesyl

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diphosphate. To simplify the procedure and to reduce the cost of MenA enzyme inhibitory assays, we have recently developed a MenA assay using HPLC. In new MenA assay, the MenA product, demethylmenaquinone, can readily be quantitated via UV absorbance (DMMK: λ_2 325 nm).[42]

We performed a preliminary screening of the activity of compounds synthesized in Schemes 1−5 at the single concentrations of 100 μM. In these MenA enzyme inhibitory assays, the optically active molecules were assayed against *M. tuberculosis* and *S. aureus* MenA. IC_{50} values of the molecules which exhibited activity against *Mtb* MenA and inactivity against *S. aureus* MenA were calculated. Of 86 optically active molecules synthesized in Schemes 1−5, 26 molecules exhibited Mtb MenA inhibitory activity and were inactive against *S. aureus* MenA at 100 μM concentrations. Dose−response plots (DMMK formation vs concentrations of inhibitor) were obtained for 26 molecules to determine IC_{50} values. Significant effect of chirality in the MenA enzyme inhibitory activity was observed in the molecules in classes **A** and **D**. On the contrary, an obvious effect of chirality in MenA enzyme inhibitory activity of the molecules in classes **B**, **C**, and **E** was not observed; the racemic forms and each enantiomer did not show noticeable difference in MenA enzyme inhibitory activity. It is noteworthy that the carbamate analogue (*R*)-**12** group showed 6-fold better MenA enzyme inhibitory activity compared to the alcohol from (*R*)-**11**. Similar improvement of enzymatic inhibition by modification of the alcohol with the carbamate group is observed in the molecules in class **D** (Table 1).

Bacterial Growth Inhibitory Assays. All molecules that showed activity against MenA in a preliminary assay at 100 μM concentrations were evaluated for their mycobacterial growth inhibitory activity via the microplate alamar blue assay (MABA) and low oxygen recovery assay (LORA).[33,43,44] Briefly, the MABA assay is a colorimetric assay that uses the color change of rezasurin to evaluate *M. tuberculosis* (Mtb) growth inhibitory activity under aerobic conditions. On the other hand, the LORA assay evaluates potency against nonreplicating Mtb cells under low oxygen conditions using a luminescent stain. Significantly, in all cases the MIC values obtained from the LORA assays are lower than those from the MABA assays. To the best of our knowledge, it is the first observations that the molecules killed nonreplicating Mtb at concentration below the MIC against Mtb grown under aerobic conditions; in all cases the values of MIC_{LORA}/MIC_{MABA} were <1 (Table 1). In the class **A** molecules, (*R*)-**2** was 2-fold more potent than *rac*-**2** and (*S*)-**2** in the MABA assays. Similar to an observed trend in MenA enzyme inhibitory assays, the carbamate (*R*)-**12** could improve over 5-fold increase in mycobactericidal activity compared to *rac*-**2**. (*R*)-**12** exhibited a significant activity in the LORA assay; the MIC value of (*R*)-**12** is 1.7-fold more effective in killing nonreplicating bacteria than rifampicin (MIC_{LORA} 1.47 μg/mL). It is worth mentioning that (*R*)-**12** was the most active in killing nonreplicating Mtb in vitro among antimycobacterial drugs (approved by FDA) and preclinical drugs tested in our laboratory. Regardless of the stereochemistry of the chiral centers, the molecules in class **B**, **C**, and **E** did not show noticeable difference in antimycobacterial activity in the both MABA and LORA assays. The molecules in class **D** are regioisomers at the benzophenone moiety of the class **A** molecules. Similar to the molecules in class **A**, pronounced effect of the stereochemistry of *secondary* alcohol on antimycobacterial activity was observed in the molecules in class **D**. Contrary to the chirality effect observed in the molecules in class **A**, the molecules possessing *S*-configuration (in class **D**) exhibited better antimycobacterial activity than the corresponding *R*-configuration molecules. The effect of carbamate group on enzyme and bacterial growth inhibitory activities was not observed in the molecules in class **D**; the MABA MIC value of the carbamate (*S*)-**16** was equal to the corresponding alcohol (*S*)-**17**. Nonetheless, the MABA MIC for the *rac*-alcohol **5** could be improved 2-fold by the formation of carbamate; the MABA MICs of *rac*-**5** and *rac*-**16** were 6.25 and 3.25 μg/mL, respectively. Antimycobacterial activity was improved by increasing the hydrophobicity of the side chain (C8 vs C6) in the molecules of class **A**, whereas noticeable bactericidal effect by increasing hydrophobicity of the side chain was not observed in the molecules of class **D**. All molecules summarized in Table 1 did not exhibit bactericidal activity against *S. aureus* at 60 μg/mL concentrations; lack of antistaphylococcal activity of these molecules was well-correlated with their *S. aureus* MenA enzyme inhibitory activity (IC_{50} >60 μM against *S. aureus* MenA).[45]

***E. coli* Growth Inhibitory Assays under Anaerobic Conditions.** *M. tuberculosis* or *S. aureus* treated with the MenA inhibitors could not be rescued completely even at higher concentrations of exogenous menaquinone (VK_2). In contrast, growth inhibition of *E. coli* by the MenA inhibitor could be rescued by supplementation of VK_2 (50 μM) under "anaerobic conditions" (vide supra).[46] *E. coli* growth inhibition rescued by addition of VK_2 may be attributed to the degree of permeability of VK_2 through the cell envelop. Although lack of activity of MenA inhibitors against Gram-negative bacteria grown under aerobic conditions have been demonstrated, all MenA inhibitors identified in this program showed growth inhibition of *E. coli* at 5−10 μg/mL concentrations under anaerobic conditions, and the inhibitory effect of MenA inhibitor was rescued by supplementation of VK_2. Therefore, these convenient cell-based assays using *E. coli* under anaerobic conditions can be utilized to confirm that the inhibitor molecules kill Gram-positive bacteria including Mtb by targeting MenA biosynthesis or bacterial electron transport systems.

Oxygen Consumption Assays. The basic concept underlying bacterial oxygen consumption assay is that changes in the rate of oxygen uptake result in a change in the oxygen concentrations. The oxygen consumption by bacterial concentrations greater than 10^8 cfu/mL bacteria is proportional to the concentration of bacteria. Effect of the inhibitor molecule on electron transport by the quantitation of decolorization of methylene blue, which is a well-known redox dye, has been unambiguously demonstrated.[30] Oxygen-consumption assays of (*R*)-**12** and (*S*)-**17** showed decolorization of methylene blue at concentrations (12.5 μg/mL) above the MIC of each molecule (MICs 2.31 and 1.50 μg/mL, respectively) against Mtb. Thus, we have concluded that (*R*)-**12** and (*R*)-**17** are very weak (or are not) electron transport system inhibitors, and thus antimycobacterial activity of these molecules are attributed by targeting menaquinone biosynthesis.

Cytotoxicity of MenA Inhibitors. To obtain insight into potential toxicity of identified inhibitor molecules, all antimycobacterial MenA inhibitors were evaluated in in vitro cytotoxicity assays using Vero monkey kidney cells and HepG2 human hepatoblastoma cells.[47] Most MenA inhibitors possessing the benzophenone group showed IC_{50} of 1−6.5 μg/mL against two mammalian cell lines; selectivity indexes (IC_{50} in mammalian cells/MIC against Mtb) of a series of benzophe-

none MenA inhibitors identified in this program were less than 2.[48] On the other hand, *O*-methyl oxime derivatives in class **A** showed approximately 10-fold less cytotoxicity than the corresponding benzophenone derivatives in in vitro cytotoxicity assays (Figure 5); (*R*)-**13** showed an encouraging in vitro

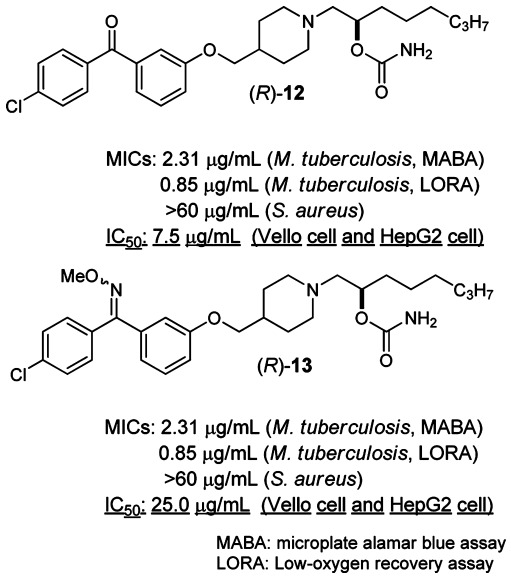

Figure 5. In vitro assay data for (*R*)-**12** and (*R*)-**13**.

activity/toxicity ratio (a SI value >10). It is believed that electrophilicity of the benzopheneone moiety of (*R*)-**12** can be diminished by *O*-methyl oxime formation of the benzophenone. Thus, the benzophenone *O*-methyl oxime may not be a good electron acceptor that interferes with redox systems of mammalian cells.

Antibacterial Activities of (*R*)-12 and (*R*)-13 against Drug-Resistant *M. tuberculosis* and other *Mycobacterium* Species. Two MenA inhibitors molecules (*R*)-**12** and (*R*)-**13** showed MIC values of 2.31 μg/mL against Mtb (H37Rv, a common laboratory strain). We resynthesized (*R*)-**12** and (*R*)-**13** and determined MICs against several other *Mycobacterium* species and drug-resistant Mtb. As summarized in Table 2, (*R*)-**12** and (*R*)-**13** showed mycobactericidal activity against rifampicin- and isoniazid-resistant strains at slightly lower concentrations (entries 2 and 3). (*R*)-**12** and (*R*)-**13** also killed other *Mycobacterium* species such as *Mycobacterium. bovis*, *Mycobacterium intracellulare*, and *Mycobacterium smegmatis* at

Table 2. MICs of (*R*)-12, (*R*)-13, and Representative Antimycobacterial Agents (Clinically Used) for *Mycobacterium* Species Including Drug-Resistant Strains

		MIC (μg/mL)[a]			
entry	species and strain	(*R*)-**12**	(*R*)-**13**	RFP[d]	INH[e]
1	*M. tuberculosis* H37Rv	2.31	2.31	0.07	0.03
2	*M. tuberculosis* H37Rv INHr[b]	2.91	1.52	0.03	>8
3	*M. tuberculosis* H37Rv RFPr[c]	2.91	1.51	>4	0.05
4	*M. bovis* BCG	3.00	2.95		
6	*M. intracellulare* ATCC15984	6.50	6.50		
7	*M. smegmatis*	6.50	6.50		

[a]The agar plate dilution method was used. [b]INH-resistant *M. tuberculosis*. [c]RFP-resistant *M. tuberculosis*. [d]RFP: rifampicin. [e]INH: isoniazid.

relatively low concentrations (3.0−6.5 μg/mL). Thus, we concluded that new MenA inhibitors identified in this program kills *Mycobacterium* species selectively and are especially effective in killing Mtb at low concentrations. MenA inhibitors (*R*)-**12** and (*R*)-**13** effectively inhibited growth of drug-resistant *Mycobacterium* species, indicating that MenA is a valid drug target to develop new drugs for MDR-*Mycobacterium tuberculosis*.

CONCLUSIONS

Through asymmetric synthesis of a series of optically active molecules followed by screening these molecules by the assay methods described here, we have identified new MenA (1,4-dihydroxy-2-naphthoate prenyltransferase) biosynthesis inhibitors that showed very weak (or no) inhibitory activities of bacterial electron transport systems. A series of MenA inhibitors identified in this program exhibited antimicrobial spectrum focused against Mtb. Selective activity against Mtb is ideal in TB drug discovery due to the fact that TB chemotherapy requires long regimen, so that broad-spectrum anti-TB agents may cause resistant to other bacteria during TB chemotherapy. The carbamates (*R*)-**12** and (*R*)-**13** in class **A** (Scheme 1) showed significant growth inhibitory activities against nonreplicating Mtb (MIC_{LORA}, 0.85 μg/mL) with the MIC_{LORA}/MIC_{MABA} value of 0.37 (MIC_{LORA}/MIC_{MABA} = 7.35 for rifampin). Effectiveness in nonreplicating Mtb was also confirmed via assays using a modified Wayne model.[49−51] Among antimycobacterial agents tested in our laboratories, the inhibitor (*R*)-**12** and (*R*)-**13** were the most active in killing nonreplicating Mtb in vitro. MenA inhibitors identified in this program strongly suggested that menaquinone biosynthesis is important in maintaining mycobacterial viability under conditions of restricted oxygen.[52] MenA inhibitors seem to be able to block the electron flow, consequently inhibiting the bacterial growth. It is conceptually very interesting that MenA inhibitors can be developed as indirect ATP synthesis inhibitors.[17] The assay data for the identified MenA inhibitors indicate that menaquinone biosynthesis is a unique and new antimycobacterial target. In addition, in tuberculosis, the key to shortening the long regimen lies in targeting the nonreplicating persistence subpopulation (vide supra). Thus, the discovery of molecules that kill nonreplicating Mtb at lower concentrations than MIC against Mtb under aerobic conditions is expected to be of significance in terms of discovering new lead molecules that can be developed into new drugs to kill Mtb in any state. Moreover, over the last several years, we are unable to isolate resistant bacteria to the MenA inhibitors in the mutation frequency studies. Therefore, we concluded that unlike the other known bacterial molecular targets *menA* shows extremely low mutation frequency.

In conclusion, robustness of our in vitro assay approaches to identify novel and selective MenA inhibitors against *M. tuberculosis* (summarized in Figure 3) has been demonstrated by the identification of a lead molecule (*R*)-**13** (MIC_{LORA}/MIC_{MABA} value of 0.37, SI >10). Further studies are underway to thoroughly characterize the activity of (*R*)-**13** against multidrug resistant strains of Mtb and to investigate activities of (*R*)-**13** and its related molecules against the other menaquinone biosynthesis enzymes.[53] The MenA inhibitors described here can be synthesized in short steps with high yield, and structural modifications to improve in vitro efficacy will be achieved by modifying the hydrophobic side chain and benzophenone *O*-methyl oxime moieties of (*R*)-**13**. Thorough

in vitro biological evaluation of (*R*)-**13** and its analogues and in vivo evaluation of promising menaquinone biosynthesis inhibitors will be reported elsewhere.[53]

EXPERIMENTAL SECTION

Chemistry. *General Information.* All glassware were oven-dried, assembled hot, and cooled under a stream of nitrogen before use. Reactions with air sensitive materials were carried out by standard syringe techniques. Commercially available reagents were used as received without further purification. Thin layer chromatography was performed using 0.25 mm silica gel 60 (F254, Merck) plates visualizing at 254 nm or developed with ceric ammonium molybdate or anisaldehyde solutions by heating with a hot-air gun. Specified products were purified by flash column chromatography using silica gel 60 (230−400 mesh, Merck). IR absorptions on NaCl plates were run on a Perkin-Elmer FT-IR 1600. ^{1}H NMR spectral data were obtained using Varian 300, 400, and 500 MHz instruments. The residual solvent signal was utilized as an internal reference. ^{13}C NMR spectral data were obtained using a Varian 100, 125 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS, using the middle resonance of $CDCl_3$ (77.0 ppm) as an internal standard. For all NMR spectra, δ values are given in ppm and J values in Hz. Mass spectra were obtained at University of Tennessee Central Instrument Facility. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. All compounds were purified by PTLC or reverse HPLC to be ≥95% purity whose purities were established by HPLC.

(4-Chlorophenyl)(3-methoxyphenyl)methanone. 4-Chlorobenzoyl chloride (5.00 g, 28.58 mmol) was dissolved in CH_2Cl_2 (240 mL) and cooled to 0 °C. *N,O*-Dimethylhydroxyl amine (3.07 g, 31.43 mmol) and triethylamine (6.36 g, 62.85 mmol) were added into the reaction mixture. After 4 h at rt, the reaction mixture was quenched with water and the organic phase was washed with 1N HCl. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo to afford crude 4-chloro-*N*-methoxy-*N*-methylbenzamide (6.41 g). This was used for further purification. To a stirred solution of 1-iodo-3-methoxybenzene (21.48 g, 91.80 mmol) in THF (10 mL) was added isopropyl magnesium chloride (2M, 1.50 mmol) at −20 °C. After 1 h at the same temperature, the reaction mixture was cooled to −78 °C and 4-chloro-*N*-methoxy-*N*-methylbenzamide (100 mg, 0.50 mmol) in THF (1 mL). The reaction mixture was warmed to 0 °C over 30 min. After 3 h at 0 °C, the reaction mixture was quenched with aq satd NH_4Cl. The water phase was extracted with EtOAc, and the combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (4:1, hexanes:EtOAc) afforded (4-chlorophenyl)(3-methoxyphenyl)methanone (97.0 mg, 79%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.88 (s, 3H), 7.16 (dd, J = 1.5, 8 Hz, 1H), 7.31−7.35 (m, 2H), 7.40 (t, J = 8 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.6, 114.4, 119.2, 122.8, 128.8, 129.5, 131.6, 136.1, 138.7, 139.1, 159.8, 195.4. LRMS (ESI) m/z: 247.0 $(M + H)^+$.

*(4-Chlorophenyl)(3-hydroxyphenyl)methanone (**21**).* To a stirred solution of (4-chlorophenyl)(3-methoxyphenyl)methanone (5.30 g, 21.5 mmol) in AcOH (15 mL) was added HBr (48% in water, 200 mL). The reaction mixture was gently refluxed at 125 °C for 36 h. The reaction mixture was cooled to rt, and all volatiles were distilled off. Purification by silica gel chromatography (4:1, hexanes:EtOAc) afforded **21** (3.84 g, 78%) as a white solid, and the unreacted starting material (15%) was recovered. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.84 (s, 1H), 7.02−7.04 (m, 1H), 7.19−7.21 (m, 1H), 7.25−7.29 (m, 2H), 7.37−7.39 (m, 2H), 7.67−7.69 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 116.6, 120.4, 122.9, 128.9, 129.9, 131.8, 135.8, 138.7, 139.4, 156.2, 195.9; LRMS (ESI) m/z: 233.0 $(M + H)^+$.

tert-Butyl 4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidine-1-carboxylate. To a stirred solution of (4-chlorophenyl)(3-hydroxyphenyl)methanone (**21**, 2.00 g, 8.71 mmol), *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (**25**, 3.75 g, 17.4 mmol), and TPP (3.42 g, 13.05 mmol) in THF (40 mL) was added DIAD (4.79 g, 13.05 mmol). After 3 h, all volatiles were evaporated in vacuo. Purification by silica gel chromatography (4:1, hexanes:EtOAc) provided *tert*-butyl 4-((3-(4-chlorobenzoyl)phenoxy)methyl)-piperidine-1-carboxylate (3.61 g, 97%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.64−1.25 (m, 2H), 1.39 (s, 9H), 1.75 (d, J = 13.5 Hz, 2H), 1.87−1.90 (m, 1H), 2.67 (bs, 2H), 3.77 (d, J = 6.5 Hz, 2H), 4.08 (bs, 2H), 7.04 (dd, J = 2, 8 Hz, 1H), 7.20−7.22 (m, 2H), 7.29 (t, J = 8 Hz, 1H), 7.37 (d, J = 8.5 Hz, 2H), 7.66−7.68 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.6, 29.0, 36.4, 72.7, 79.6, 115.0, 119.6, 122.8, 128.8, 129.5, 131.6, 136.0, 138.7, 139.1, 155.0, 159.2, 195.4. LRMS (ESI) m/z: 430.1 $(M + H)^+$.

*(4-Chlorophenyl)(3-(piperidin-4-ylmethoxy)phenyl)methanone (**26**).* *tert*-Butyl 4-((3-(4-chlorobenzoyl)phenoxy)methyl)piperidine-1-carboxylate (3.61 g, 8.50 mmol) was dissolved in 50% trifluoroacetic acid (TFA) in CH_2Cl_2 (35 mL) and stirred for 2 h at rt. All volatiles were evaporated in vacuo. The reaction mixture was dissolved in CH_2Cl_2 and washed with 1N NaOH (twice). The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (4:1, $CHCl_3$:MeOH) afforded **26** (2.66 g, 95%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24−1.32 (m, 2H), 1.79 (d, J = 13 Hz, 2H), 1.86−2.10 (m, 1H), 2.79−2.64 (m, 2H), 3.11 (d, J = 12.5 Hz, 2H), 3.78 (d, J = 6.5 Hz, 2H), 7.05 (dd, J = 2, 8 Hz, 1H), 7.20−7.23 (m, 2H), 7.30 (t, J = 8 Hz, 1H), 7.39 (d, J = 9 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.2, 29.9, 31.1, 36.3, 46.2, 73.2, 115.1, 119.7, 122.8, 128.8, 129.5, 131.6, 136.1, 138.7, 139.1, 159.4, 195.5. LRMS (ESI) m/z: 330.1 $(M + H)^+$.

*(S)-(4-Chlorophenyl)(3-((1-(2-hydroxyoctyl)piperidin-4-yl)-methoxy)phenyl)methanone ((S)-**11**).* To a stirred solution of **26** (100.0 mg, 0.31 mmol) in CH_2Cl_2 was added $Al(CH_3)_3$ (0.2 M in CH_2Cl_2, 0.43 mmol) at 0 °C. After 15 min, (*S*)-1,2-epoxyoctane (**29**, 60.0 mg, 0.43 mmol) was added. After 4 h at rt, the reaction mixture was quenched with aq $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (3:2, $CHCl_3$:MeOH) afforded (*S*)-**2** (103.0 mg, 73%) as a colorless oil. $[\alpha]^{20}_D$ = +13.9 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (s, 3H), 1.31−1.49 (m, 11H), 1.84−1.99 (m, 4H), 2.28−2.39 (m, 3H), 2.87 (d, J = 10.2 Hz, 1H), 3.10 (d, J = 10.2 Hz, 1H), 3.69 (s, 1H), 3087 (d, J = 3.9 Hz, 2H), 7.14 (d, J = 7.2 Hz, 1H), 7.29−7.49 (m, 5H), 7.77 (dd, J = 2.1, 8.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 22.0, 25.0, 28.5, 28.8, 28.9, 31.2, 34.5, 35.3, 51.3, 54.7, 64.0, 65.8, 72.3, 114.4, 118.8, 122.0, 128.1, 128.8, 130.8, 135.4, 138.1, 138.3, 158.7, 194.6. HRMS (ESI^+): m/z calcd for $C_{27}H_{36}ClNO_3$ $(M + H)^+$, 458.2405; found, 458.2403. The purity of (*S*)-**11** was determined to be >99% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**11** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 21.0 min; (*R*)-enantiomer, t_R = 26.7 min).

*(R)-(4-Chlorophenyl)(3-((1-(2-hydroxyoctyl)piperidin-4-yl)-methoxy)phenyl)methanone ((R)-**11**).* Colorless oil. $[\alpha]^{20}_D$ = −12.8 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (t, J = 7 Hz, 3H), 1.18−1.42 (m, 12H), 1.76−1.78 (m, 3H), 1.90 (t, J = 11 Hz, 1H), 2.17−2.28 (m, 3H), 2.77 (d, J = 11 Hz, 1H), 3.00 (d, J = 11.5 Hz, 1H), 3.58−3.62 (m, 1H), 3.78 (d, J = 6 Hz, 2H), 7.05 (dd, J = 2, 8 Hz, 1H), 7.19−7.23 (m, 2H), 7.30 (t, J = 8 Hz, 1H), 7.39 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ14.3, 14.3, 22.8, 22.9, 25.9, 29.3, 29.6, 29.7, 32.1, 32.1, 34.5, 35.3, 36.1, 52.0, 55.5, 64.7, 66.5, 73.0, 110.2, 115.1, 119.7, 122.8, 128.9, 129.6, 131.7, 136.2, 138.8, 139.1, 159.4, 195.5. HRMS (ESI^+): m/z calcd for $C_{27}H_{36}ClNO_3$ $(M + H)^+$, 458.2404; found, 458.2403. The purity of (*R*)-**11** was determined to be >99% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**11** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 21.0 min; (*R*)-enantiomer, t_R = 26.7 min).

*(S)-1-(4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidin-1-yl)-octan-2-yl carbamate ((S)-**12**).* To a stirred solution of (*S*)-**11** (10 mg, 0.02 mmol) and DMAP (5.0 mg, 0.04 mmol) in CH_2Cl_2 was added trimethylsilylisocyanate (TMSNCO) (7.0 mg, 0.06 mmol). After 4 h at rt, the reaction mixture was quenched with aq $NaHCO_3$, and extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (9:1, $CHCl_3$:MeOH) afforded (*S*)-**12** (7.0 mg, 63%) as a colorless oil. $[\alpha]^{20}_D$ = +9.4° (*c* 0.9 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (d, *J* = 7.2 Hz, 3H), 1.31−1.58 (m, 12H), 1.90 (d, *J* = 10.8 Hz, 3H), 2.09 (m, 1H), 2.37−2.44 (m, 3H), 2.96 (m, 1H), 3.15 (m, 1H)3.76−3.89 (m, 3H), 7.13−7.16 (m, 1H), 7.28−7.50 (m, 5H), 7.78(dd, *J* = 1.8, 8.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 22.0, 25.0, 28.1, 28.4, 28.9, 29.1, 31.2, 34.5, 35.1, 51.5, 54.7, 64.1, 65.7, 72.1, 114.5, 118.8, 122.1, 128.1, 128.8, 130.8, 135.4, 138.1, 138.4, 158.6, 194.6. MS (ESI$^+$): *m*/*z* calcd for $C_{28}H_{37}ClN_2O_4$ (M + H)$^+$, 501.2400; found, 501.2309. The purity of (*S*)-**12** was determined to be >99% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**12** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 18.0 min; (*R*)-enantiomer, t_R = 20.0 min).

*(R)-1-(4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidin-1-yl)-octan-2-yl carbamate ((R)-**12**).* A colorless oil. $[\alpha]^{20}_D$ = +-8.6 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88−0.97 (m, 3H), 1.28−1.49 (m, 13H), 1.93 (d, *J* = 10.8 Hz, 3H), 2.21 (s, 2H), 2.49 (s, 2H), 3.10 (s, 1H), 3.27 (s, 1H), 3.90 (d, *J* = 5.4 Hz, 2H), 7.13−7.15 (m, 1H), 7.25−7.50 (m, 5H), 7.76−7.79 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 22.0, 23.3, 24.9, 27.7, 28.8, 29.1, 31.2, 34.5, 34.9, 51.7, 54.7, 64.1, 65.6, 71.9, 114.5, 118.8, 122.1, 128.1, 128.8, 130.8, 138.2, 138.4, 158.5, 194.7. HRMS (ESI$^+$): *m*/*z* calcd for $C_{28}H_{37}ClN_2O_4$ (M + H)$^+$, 501.2400; found, 501.2309. The purity of (*R*)-**12** was determined to be >99% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**12** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 18.0 min; (*R*)-enantiomer, t_R = 20.0 min).

*1-(3-(((tert-Butyldimethylsilyl)oxy)methyl)phenyl)octan-1-one (**44**).* To a stirred solution of *tert*-butyl-(3-iodo-benzyloxy)-dimethylsilane (**42**, 0.70 g, 2.0 mmol) in THF (3 mL) was added isopropylmagnesium chloride (2 M in THF, 3.0 mmol) at 0 °C. After 2.5 h, the reaction mixture was cooled to −78 °C and *N*-methoxy-*N*-methyloctanamide (**43**, 0.12 g, 0.66 mmol) in THF (1 mL) was added. After 3 h at 0 °C, the reaction mixture was quenched with aq NH_4Cl. The water phase was extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (9.8:0.2, hexanes:EtOAc) afforded **44** (0.21 g, 91%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.11 (s, 6H), 0.88 (t, *J* = 7.5 Hz, 3H), 0.96 (s, 9H), 1.29−1.39 (m, 8H), 1.73 (d, *J* = 7 Hz, 2H), 2.95 (t, *J* = 7 Hz, 2H), 4.79 (s, 2H), 7.42 (t, *J* = 7 Hz, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.84 (d, *J* = 8 Hz, 1H), 7.91 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.3, 14.1, 18.4, 22.6, 24.5, 25.9, 29.1, 29.4, 31.7, 38.7, 64.5, 125.6, 126.7, 128.5, 130.4, 137.1, 142.0, 200.7. LRMS (ESI) *m*/*z*: 336.2 (M + H)$^+$.

(R)-1-(3-(((tert-Butyldimethylsilyl)oxy)methyl)phenyl)octan-1-ol. To a stirred solution of **44** (0.2 g, 0.57 mmol) in THF (2 mL) at −78 °C was added (*S*)-2-methyl-CBS (0.12 g, 0.43 mmol) and BH_3-Me_2S (43.0 mg, 0.57 mmol). The reaction mixture was kept at −15 °C for an additional 1.5 h and quenched with MeOH followed by water. The water phase was extracted with EtOAc. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (9:1, hexanes:EtOAc) afforded (*R*)-1-(3-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)octan-1-ol (180.0 mg, 89%) as a colorless oil. $[\alpha]^{20}_D$ = +35.1 (*c* 1 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.10 (s, 6H), 0.87 (t, *J* = 6.5 Hz, 3H), 0.94 (s, 9H), 1.25−1.31 (m, 9H), 1.37−1.42 (m, 1H), 1.67−1.83 (m, 3H), 4.66 (t, *J* = 6.5 Hz, 1H), 4.74 (s, 3H), 7.21−7.26 (m, 2H), 7.29−7.32 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.2, 14.1, 18.4, 22.6, 25.8, 26.0, 29.2, 29.5, 31.8, 39.1, 65.0, 74.8, 123.6, 124.5, 125.3, 128.3, 141.7, 144.9. LRMS (ESI) *m*/*z*: 338.2 (M + H)$^+$.

(S)-1-(3-(((tert-Butyldimethylsilyl)oxy)methyl)phenyl)octan-1-ol. Colorless oil. $[\alpha]^{20}_D$ = −34.8 (*c* 1 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.10 (s, 6H), 0.87 (t, *J* = 6.5 Hz, 3H), 0.94 (s, 9H), 1.25−1.31 (m, 9H), 1.37−1.42 (m, 1H), 1.67−1.83 (m, 3H), 4.66 (t, *J* = 6.5 Hz, 1H), 4.74 (s, 3H), 7.21−7.26 (m, 2H), 7.29−7.32 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ −5.2, 14.1, 18.4, 22.6, 25.8, 26.0, 29.2, 29.5, 31.8, 39.1, 65.0, 74.8, 123.6, 124.5, 125.3, 128.3, 141.7, 144.9. LRMS (ESI) *m*/*z*: 338.2 (M + H)$^+$.

(R)-1-(3-(Hydroxymethyl)phenyl)octyl Acetate. (*R*)-1-(3-(((*tert*-Butyldimethylsilyl)oxy)methyl)phenyl)octan-1-ol (70 mg, 0.2 mmol) was dissolved in pyridine (1 mL) and acetic anhydride (1 mL). After 5 h at rt, all volatiles were evaporated in vacuo. Purification by silica gel chromatography (9.5:0.5, hexanes:EtOAc) afforded (*R*)-1-(3-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)octyl acetate (78.4 mg, 100%) as a colorless oil. To a stirred solution of (*R*)-1-(3-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)octyl acetate (25 mg, 0.06 mmol) in THF (0.5 mL) was added TBAF (1 M in THF, 0.12 mmol). After 4 h at rt, the reaction was quenched with water. The water phase was extracted with EtOAc, and the combined extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel chromatography (7:3, hexane:EtOAc) afforded (*R*)-1-(3-(hydroxymethyl)phenyl)octyl acetate (14.0 mg, 79%) as a colorless oil. $[\alpha]^{20}_D$ = +38.0 (*c* 0.7 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7 Hz, 3H), 1.20−1.34 (m, 10H), 1.73−1.79 (m, 1H), 1.86−1.93 (m, 1H), 2.06 (s, 3H), 4.70 (s, 2H), 5.71 (t, *J* = 7 Hz, 1H), 7.24−7.29 (m, 2H), 7.32−7.35 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 21.3, 22.6, 25.6, 29.1, 29.3, 31.8, 36.3, 65.3, 76.2, 125.1, 125.8, 126.4, 128.7, 141.1, 141.3, 170.5. LRMS (ESI) *m*/*z*: 279.2 (M + H)$^+$.

(S)-1-(3-(Hydroxymethyl)phenyl)octyl Acetate. Colorless oil. $[\alpha]^{20}_D$ = −35.0 (*c* 0.8 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7 Hz, 3H), 1.20−1.34 (m, 10H), 1.73−1.79 (m, 1H), 1.86−1.93 (m, 1H), 2.06 (s, 3H), 4.70 (s, 2H), 5.71 (t, *J* = 7 Hz, 1H), 7.24−7.29 (m, 2H), 7.32−7.35 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 21.3, 22.6, 25.6, 29.1, 29.3, 31.8, 36.3, 65.3, 76.2, 125.1, 125.8, 126.4, 128.7, 141.1, 141.3, 170.5. LRMS (ESI) *m*/*z*: 279.2 (M + H)$^+$.

*(R)-1-(3-((4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidin-1-yl)-methyl)phenyl)octyl Acetate ((R)-**46**).* To a stirred solution of DMSO (113.0 mg, 1.44 mmol) in CH_2Cl_2 (2 mL) at −78 °C was added oxalyl chloride (91.0 mg, 0.72 mmol). After 30 min, (*R*)-1-(3-(hydroxymethyl)phenyl)octyl acetate (100.0 mg, 0.36 mmol) in CH_2Cl_2 (0.5 mL) was added. After 30 min at −78 °C, Et_3N (220.0 mg, 2.16 mmol) was added and the reaction mixture was warmed to rt over 1 h. The reaction mixture was quenched with water, and the water phase was extracted with CH_2Cl_2. The combined extract was washed with brine, dried over $Na_2S_2O_4$, and evaporated in vacuo. Purification by silica gel chromatography afforded the corresponding aldehyde (*R*)-1-(3-formylphenyl)octyl acetate ((*R*)-**45**, 95.0 mg) as a colorless oil. To a stirred solution of (*R*)-**45** (95.0 mg, 0.34 mmol) and (4-chlorophenyl)(3-(piperidin-4-ylmethoxy)phenyl)methanone (**26**, 98.7 mg, 0.30 mmol) in CH_2Cl_2 was added $NaBH(OAc)_3$ (150.0 mg, 0.72 mmol). After 8 h at rt, the reaction was quenched with aq satd $NaHCO_3$, and the water phase was extracted with CH_2Cl_2. The combined extract was washed with brine, dried over $Na_2S_2O_4$, and evaporated in vacuo. Purification by silica gel chromatography (3:7, hexanes:EtOAc) afforded (*R*)-**46**; (170.0 mg, 96%) as a colorless oil. $[\alpha]^{20}_D$ = −17.9 (*c* 0.8 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 7 Hz, 3H), 1.23−1.29 (m, 10H), 1.43−1.45 (m, 2H), 1.73−1.91 (m, 5H), 1.99−2.04 (m, 2H), 2.07 (s, 3H), 2.93 (d, *J* = 10 Hz, 2H), 3.53 (d, *J* = 1.5 Hz, 2H), 3.85 (d, *J* = 6 Hz, 2H), 5.72 (t, *J* = 7 Hz, 1H), 7.12 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.21−7.30 (m, 6H), 7.36 (t, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 21.4, 22.6, 25.6, 29.0, 31.8, 35.9, 36.4, 53.3, 53.3, 63.2, 72.9, 76.2, 114.9, 119.5, 122.5, 125.1, 127.3, 128.3, 128.6, 128.6, 129.3, 131.4, 135.9, 138.5, 138.9, 140.8, 159.2, 170.4, 195.3.

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HRMS (ESI$^+$): m/z calcd for $C_{36}H_{44}ClNO_4$ (M + H)$^+$, 590.3010; found, 590.3015.

*(R)-(4-Chlorophenyl)(3-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)methoxy)phenyl) Methanone ((R)-**3**).* To a stirred solution of (*R*)-**46** (20.0 mg, 0.03 mmol) in acetonitrile (1 mL) was added 1N NaOH (1 mL). After 2 h at rt, the reaction mixture was quenched with water and extracted with $CHCl_3$. The combined organic phase was washed with brine and $Na_2S_2O_4$ and evaporated in vacuo. Purification by silica gel chromatography (1:3, hexanes:EtOAc) afforded (*R*)-**3** (10.0 mg, 54%) as a colorless oil. $[\alpha]^{20}_D$ = +25.8 (*c* 0.8 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 7 Hz, 3H), 1.21−1.28 (m, 10H), 1.40−1.47 (m, 3H), 1.68−1.82 (m, 5H), 2.02 (t, *J* = 12 Hz, 2H), 2.94 (d, *J* = 11 Hz, 2H), 3.53 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.66 (t, *J* = 7 Hz, 1H), 7.12 (dd, *J* = 2.5, 8 Hz, 1H), 7.22−7.31 (m, 6H), 7.36 (t, *J* = 8 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.0, 29.2, 29.5, 31.8, 35.9, 39.2, 45.6, 53.3, 53.3, 63.3, 72.9, 74.7, 114.9, 119.5, 122.6, 124.6, 126.8, 128.3, 128.4, 128.6, 129.3, 131.4, 135.9, 138.5, 138.9, 145.0, 159.2, 195.3. HRMS (ESI$^+$): m/z calcd for $C_{34}H_{42}ClNO_3$ (M + H)$^+$, 548.2906; found, 548.2909. The purity of (*R*)-**3** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**3** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 15.0 min; (*R*)-enantiomer, t_R = 16.0 min).

*(S)-(4-Chlorophenyl)(3-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)methoxy)phenyl) Methanone ((S)-**3**).* Colorless oil, $[\alpha]^{20}_D$ = −25.6 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 7 Hz, 3H), 1.21−1.28 (m, 10H), 1.40−1.47 (m, 3H), 1.68−1.82 (m, 5H), 2.02 (t, *J* = 12 Hz, 2H), 2.94 (d, *J* = 11 Hz, 2H), 3.53 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.66 (t, *J* = 7 Hz, 1H), 7.12 (dd, *J* = 2.5, 8 Hz, 1H), 7.22−7.31 (m, 6H), 7.36 (t, *J* = 8 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.0, 29.2, 29.5, 31.8, 35.9, 39.2, 45.6, 53.3, 53.3, 63.3, 72.9, 74.7, 114.9, 119.5, 122.6, 124.6, 126.8, 128.3, 128.4, 128.6, 129.3, 131.4, 135.9, 138.5, 138.9, 145.0, 159.2, 195.3. HRMS (ESI$^+$): m/z calcd for $C_{34}H_{42}ClNO_3$ (M + H)$^+$, 548.2907; found, 548.3001. The purity of (*S*)-**3** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**3** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 15.0 min; (*R*)-enantiomer, t_R = 16.0 min).

*(R)-1-(3-((4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidin-1-yl)methyl)phenyl)octyl Carbamate ((R)-**14**).* To a stirred solution of (*R*)-**3** (20.0 mg, 0.04 mmol) and DMAP (100.0 mg, 0.08 mmol) in CH_2Cl_2 (0.5 mL) was added TMSNCO (100.0 mg, 0.09 mmol). After 4 h at rt, all volatiles were evaporated in vacuo. Purification by silica gel chromatography (9:1, $CHCl_3$:MeOH) afforded (*R*)-**14** (17.3 mg, 80%) as a colorless oil. $[\alpha]^{20}_D$ = +21.1 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, *J* = 7 Hz, 3H), 1.26 (m, 9H), 1.35−1.46 (m, 3H), 1.59−1.74 (m, 2H), 1.82 (d, *J* = 10.5 Hz, 3H), 2.02 (m, 2H), 2.94 (d, *J* = 8 Hz, 2H), 3.54 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.60 (q, *J* = 5 Hz, 1H), 7.11−7.12 (m, 1H), 7.18−7.19 (m, 2H), 7.24−7.29 (m, 4H), 7.36 (t, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 26.0, 29.0, 29.3, 29.5, 31.9, 35.9, 40.7, 53.2, 72.9, 75.1, 114.9, 119.4, 122.5, 126.8, 128.0, 128.6, 129.3, 131.4, 135.9, 138.5, 138.9, 159.2, 195.3. HRMS (ESI$^+$): m/z calcd for $C_{35}H_{43}ClN_2O_4$ (M + H)$^+$, 590.2901; found, 590.2898. The purity of (*R*)-**14** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**14** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 14.0 min; (*R*)-enantiomer, t_R = 15.0 min).

*(S)-1-(3-((4-((3-(4-Chlorobenzoyl)phenoxy)methyl)piperidin-1-yl)methyl)phenyl)octyl Carbamate (S)-**14**.* Colorless oil. $[\alpha]^{20}_D$ = −21.9 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, *J* = 7 Hz, 3H), 1.26 (m, 9H), 1.35−1.46 (m, 3H), 1.59−1.74 (m, 2H), 1.82 (d, *J* = 10.5 Hz, 3H), 2.02 (m, 2H), 2.94 (d, *J* = 8 Hz, 2H), 3.54 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.60 (q, *J* = 5 Hz, 1H), 7.11−7.12 (m, 1H), 7.18−7.19 (m, 2H), 7.24−7.29 (m, 4H), 7.36 (t, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 26.0, 29.0, 29.3, 29.5, 31.9, 35.9, 40.7, 53.2, 72.9, 75.1, 114.9, 119.4, 122.5, 126.8, 128.0, 128.6, 129.3, 131.4, 135.9, 138.5, 138.9, 159.2, 195.3. HRMS (ESI$^+$): m/z calcd for $C_{35}H_{43}ClN_2O_4$ (M + H)$^+$, 590.2901; found, 590.2899. The purity of (*S*)-**14** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**14** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 14.0 min; (*R*)-enantiomer, t_R = 15.0 min).

(4-Chlorophenyl)(3-((3-vinylbenzyl)oxy)phenyl)methanone. To a stirred solution of (4-chlorophenyl)(3-hydroxyphenyl)methanone (**21**, 26.0 mg, 1.13 mmol), (3-vinylphenyl)methanol (**48**, 230.0 mg, 1.7 mmol), and TPP (0.45 g, 1.7 mmol) in THF (10 mL) was added DIAD (0.62 g, 1.7 mmol). After 4 h at rt, all volatiles were evaporated in vacuo to afford the crude product. Purification by silica gel chromatography (4:1, hexanes:EtOAc) provided (4-chlorophenyl)(3-((3-vinylbenzyl)oxy)phenyl)methanone (334.3 mg, 85%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.12 (s, 3H), 5.28 (d, *J* = 10.5 Hz, 1H), 5.77 (d, *J* = 17.5 Hz, 1H), 6.73 (q, *J* = 11 Hz, 1H), 7.22 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.31−7.46 (m, 8H), 7.71−7.73 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 69.9, 70.1, 70.4, 114.3, 114.4, 114.5, 114.5, 114.5, 114.6, 115.3, 115.6, 119.8, 120.0, 122.7, 123.0, 125.2, 125.5, 125.8, 126.1, 126.7, 127.0, 128.5, 128.8, 129.0, 129.4, 129.6, 131.3, 131.5, 135.8, 136.5, 136.5, 136.8, 138.1, 138.5, 138.9, 158.7, 195.1. LRMS (ESI) m/z: 349.1 (M + H)$^+$.

*(4-Chlorophenyl)(3-((3-(oxiran-2-yl)benzyl)oxy)phenyl)-methanone (rac-**49**).* To a stirred solution of (4-chlorophenyl)(3-((3-vinylbenzyl)oxy)phenyl)methanone (250.0 mg, 0.70 mmol) in acetonitrile (60 mL) was added Na_2EDTA (0.09%, 8 mL), oxone (86.0 mg, 0.28 mmol), $NaHCO_3$ (0.056 g, 0.67 mmol), and trifluoroacetone (250.0 mg, 2.24 mmol) at 0 °C. After 1 h, an additional oxone (86.0 mg, 0.28 mmol) and $NaHCO_3$ (56.0 mg, 0.67 mmol) were added every 1 h; this process was repeated 8 times, during which the reaction temperature was kept at 0 °C. After completion of the reaction all volatiles were evaporated in vacuo. The residue was dissolved in water, and the water phase was extracted with CH_2Cl_2. The combined organic phase was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (9:1, hexane:EtOAc) afford *rac*-**49** (148.0 mg, 74%) as a colorless oil. *rac*-**49** was resolute via Jocobsen's kinetic resolution with (*S*,*S*)-salene Co(III) or (*R*,*R*)-salene Co(III) to afford (*R*)-**49** and (*S*)-**49**, respectively. (*R*)-**49**: $[\alpha]^{20}_D$ = +11.3° (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.79 (dd, *J* = 2.5, 5.5 Hz, 1H), 3.15 (t, *J* = 4 Hz, 1H), 3.88 (t, *J* = 3 Hz, 1H), 5.10 (s, 2H), 7.21 (dd, *J* = 4.5, 8 Hz, 1H), 7.26−7.27 (m, 1H), 7.33−7.41 (m, 6H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 51.5, 52.4, 70.2, 115.6, 120.0, 123.1, 124.7, 125.5, 127.5, 128.8, 129.1, 129.7, 131.6, 136.0, 137.1, 138.5, 138.8, 139.1, 158.8, 195.3. LRMS (ESI) m/z: 365.1 (M + H)$^+$. (*S*)-**49**: colorless oil; $[\alpha]^{20}_D$ = −9.3° (*c* 0.5 in $CHCl_3$).

*(R)-(4-Chlorophenyl)(3-((3-(2-hydroxy-1-((4-methoxyphenethyl)-amino)ethyl)benzyl)oxy)phenyl)methanone ((R)-**4**).* A mixture of (*R*)-**49** (700.0 mg, 0.2 mmol), *p*-methoxyphenethyl amine (30.0 mg, 0.20 mmol) and $Zn(ClO_4)_2{\cdot}6H_2O$ (10.0 mg) is heated at 90 °C for 1 h. Purification by silica gel chromatography (7:3, hexanes:EtOAc) afforded (*R*)-**4** (27.0 mg, 27%) as a colorless oil and the corresponding regiodisomer (15%). (*R*)-**4**: $[\alpha]^{20}_D$ = +20.3 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.68−2.76 (m, 3H), 2.84−2.95 (m, 3H), 3.78 (s, 3H), 4.72 (dd, *J* = 3.5, 9.5 Hz, 1H), 5.09 (s, 2H), 6.83 (d, *J* = 8 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 7.20 (dd, *J* = 1.5, 8 Hz, 1H), 7.32−7.44 (m, 9H), 7.72 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.9, 35.2, 50.7, 55.3, 56.8, 70.2, 71.2, 114.0, 115.5, 119.8, 122.9, 124.9, 125.7, 126.7, 128.6, 128.7, 129.5, 129.6, 131.4, 135.8, 136.6,

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138.5, 138.9, 143.0, 158.2, 158.7, 195.2. HRMS (ESI^+): m/z calcd for $C_{31}H_{30}ClNO_4$ $(M + H)^+$, 516.1905; found, 516.2101. The purity of (*R*)-**4** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**4** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 13.5 min; (*R*)-enantiomer, t_R = 15.0 min).

*(S)-(4-Chlorophenyl)(3-((3-(2-hydroxy-1-((4-methoxyphenethyl)-amino)ethyl)benzyl)oxy)phenyl)methanone ((S)-**4**).* A colorless oil. $[\alpha]^{20}_D$ = −19.5 (*c* 0.5 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 2.68−2.76 (m, 3H), 2.84−2.95 (m, 3H), 3.78 (s, 3H), 4.72 (dd, *J* = 3.5, 9.5 Hz, 1H), 5.09 (s, 2H), 6.83 (d, *J* = 8 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 7.20 (dd, *J* = 1.5, 8 Hz, 1H), 7.32−7.44 (m, 9H), 7.72 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.9, 35.2, 50.7, 55.3, 56.8, 70.2, 71.2, 114.0, 115.5, 119.8, 122.9, 124.9, 125.7, 126.7, 128.6, 128.7, 129.5, 129.6, 131.4, 135.8, 136.6, 138.5, 138.9, 143.0, 158.2, 158.7, 195.2. HRMS (ESI^+): m/z calcd for $C_{31}H_{30}ClNO_4$ $(M + H)^+$, 516.1895; found, 516.19051. The purity of (*S*)-**4** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**4** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 13.5 min; (*R*)-enantiomer, t_R = 15.0 min).

tert-Butyl 4-((3-Chloro-4-(4-chlorobenzoyl)phenoxy)methyl)-piperidine-1-carboxylate. Colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.29 (m, 2H), 1.48 (s, 9H), 1.83 (d, 12.5 Hz, 2H), 1.98 (s, 1H), 2.76 (bs, 2H), 3.86 (d, *J* = 6.5 Hz, 2H), 4.18 (bs, 2H), 6.87 (dd, *J* = 1, 8.5 Hz, 1H), 6.97 (d, *J* = 1.5 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.72 (d, *J* = 8.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.6, 28.9, 36.2, 72.9, 79.5, 113.2, 116.2, 128.9, 130.2, 131.4, 131.4, 133.3, 135.8, 139.8, 154.9, 161.3, 193.7. LRMS (ESI) m/z: 465.4 $(M + H)^+$.

*(2-Chloro-4-(piperidin-4-ylmethoxy)phenyl)(4-chlorophenyl)-methanone (**50**).* Colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.38−1.29 (m, 2H), 1.84 (s, 2H), 2.01−1.95 (m, 1H), 2.70 (m, 2H), 3.18 (d, *J* = 12 Hz, 2H), 3.87 (d, *J* = 6.5 Hz, 2H), 6.89 (dd, *J* = 2, 8.5 Hz, 1H), 6.99 (d, *J* = 2.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.2, 36.5, 46.4, 73.6, 113.3, 116.3, 129.0, 130.2, 131.5, 131.6, 133.5, 136.0, 140.0, 161.6, 193.9. LRMS (ESI) m/z: 364.3 $(M + H)^+$.

*(R)-(2-Chloro-4-((1-(2-hydroxyoctyl)piperidin-4-yl)methoxy)-phenyl)(4-chlorophenyl)methanone ((R)-**16**).* Colorless oil. $[\alpha]^{20}_D$ = +28.6 (*c* 1.0 in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 0.90 (bs, 3H), 1.31−1.56 (m, 12H), 1.88 (d, *J* = 10.5 Hz, 3H), 2.08 (bs, 1H), 2.36−2.44 (m, 3H), 2.94−3.18 (m, 2H), 3.75 (s, 1H), 3.88 (d, *J* = 4.5 Hz, 2H), 6.87−6.90 (m, 1H), 6.99 (t, *J* = 2.1 Hz, 1H), 7.34−7.46 (m, 3H), 7.73−7.76 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 8.3, 13.5, 22.0, 25.0, 28.1, 28.4, 28.9, 31.2, 34.5, 35.1, 45.4, 51.4, 54.6, 64.0, 65.7, 72.4, 112.5, 115.7, 128.3, 129.7, 130.7, 130.8, 132.7, 135.2, 139.2, 160.7, 194.0. MS (ESI^+): m/z calcd for $C_{27}H_{35}Cl_2NO_3$ $(M + H)^+$, 492.2063; found, 492.2068. The purity of (*R*)-**16** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**16** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 16.5 min; (*R*)-enantiomer, t_R = 17.5 min).

*(R)-1-(4-((3-Chloro-4-(4-chlorobenzoyl)phenoxy)methyl)-piperidin-1-yl)octan-2-yl Carbamate ((R)-**17**).* Colorless oil. $[\alpha]^{20}_D$ = +21.3 (*c* 1.0 in $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 0.9 (bs, 3H), 1.31−1.50 (m, 12H), 1.85−2.00 (m, 4H), 2.29−2.40 (m, 3H), 2.88 (d, *J* = 10.8 Hz, 1H), 3.11 (d, *J* = 10.8 Hz, 1H), 3.70 (s, 1H), 3.87 (d, *J* = 5.7 Hz, 2H), 6.87−6.99 (m, 2H), 7.28−7.46 (m, 3H), 7.73−7.76 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 22.0, 25.0, 28.4, 28.7, 28.9, 31.3, 34.5, 35.2, 51.3, 54.6, 64.0, 65.8, 72.5, 112.6, 115.7, 128.3, 129.7, 130.7, 130.8, 132.7, 135.3, 139.2, 160.8, 193.0. HRMS (ESI+): m/z calcd for $C_{28}H_{36}Cl_2N_2O_4$ $(M + H)^+$, 535.2106; found, 535.2109. The purity of (*R*)-**17** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**17** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 18.0 min; (*R*)-enantiomer, t_R = 19.5 min).

*(S)-(2-Chloro-4-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)-methoxy)phenyl)(4-chlorophenyl) Methanone ((S)-**6**).* Colorless oil. $[\alpha]^{20}_D$ = −31.7 (*c* 0.2 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.25−1.50 (m, 12H), 1.68−1.86 (m, 5H), 2.05 (t, *J* = 11.5 Hz, 3H), 2.97 (d, *J* = 11.5 Hz, 2H), 3.56 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.67 (t, *J* = 7 Hz, 1H), 6.86 (dd, *J* = 2, 8.5 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 7.24−7.26 (m, 2H), 7.29−7.35 (m, 3H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.73 (d, J = 9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 28.8, 29.3, 29.5, 31.8, 35.7, 39.2, 53.2, 53.2, 63.3, 73.1, 76.6, 113.1, 116.1, 124.7, 126.8, 128.4, 128.4, 128.8, 030.0, 131.3, 131.4, 133.2, 135.8, 139.7, 145.0, 161.4, 193.7. HRMS (ESI^+): m/z calcd for $C_{34}H_{41}Cl_2NO_3$ $(M + H)^+$, 582.2504; found, 582.2302. The purity of (*S*)-**6** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**6** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 14.5 min; (*R*)-enantiomer, t_R = 15.5 min).

*(R)-(2-Chloro-4-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)-methoxy)phenyl)(4-chlorophenyl) Methanone ((R)-**6**).* Colorless oil. $[\alpha]^{20}_D$ = +32.5 (*c* 0.2 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.25−1.50 (m, 12H), 1.68−1.86 (m, 5H), 2.05 (t, *J* = 11.5 Hz, 3H), 2.97 (d, *J* = 11.5 Hz, 2H), 3.56 (s, 2H), 3.85 (d, *J* = 6 Hz, 2H), 4.67 (t, *J* = 7 Hz, 1H), 6.86 (dd, *J* = 2, 8.5 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 7.24−7.26 (m, 2H), 7.29−7.35 (m, 3H), 7.42 (d, J = 8.5 Hz, 2H), 7.73 (d, *J* = 9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 28.8, 29.3, 29.5, 31.8, 35.7, 39.2, 53.2, 53.2, 63.3, 73.1, 76.6, 113.1, 116.1, 124.7, 126.8, 128.4, 128.4, 128.8, 030.0, 131.3, 131.4, 133.2, 135.8, 139.7, 145.0, 161.4, 193.7. HRMS (ESI^+): m/z calcd for $C_{34}H_{41}Cl_2NO_3$ $(M + H)^+$, 582.2508; found, 582.2509. The purity of (*R*)-**6** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**6** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 14.5 min; (*R*)-enantiomer, t_R = 15.5 min).

*(S)-1-(3-((4-((3-Chloro-4-(4-chlorobenzoyl)phenoxy)methyl)-piperidin-1-yl)methyl)phenyl)octyl Carbamate ((S)-**19**).* Colorless oil. $[\alpha]^{20}_D$ = −26.6 (*c* 0.2 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.29−1.42 (m, 13H), 1.68−1.88 (m, 6H), 2.17 (bs, 2H), 3.08−3.11 (m, 1H), 3.67 (bs, 1H), 3.87 (d, *J* = 6 Hz, 2H), 4.68 (q, *J* = 1.5 Hz, 1H), 6.85 (dd, *J* = 2.5, 9 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 7.28−7.43 (m, 7H), 7.71−7.74 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 16.6, 20.0, 22.6, 24.5, 25.9, 29.5, 31.8, 39.3, 113.0, 116.2, 128.9, 131.3, 131.4, 133.2, 135.6, 139.8, 159.7, 192.4, 193.7. HRMS (ESI^+): m/z calcd for $C_{35}H_{42}Cl_2N_2O_4$ $(M + H)^+$, 625.2508; found, 625.2510. The purity of (*S*)-**19** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**19** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 12.5 min; (*R*)-enantiomer, t_R = 14.0 min).

*(R)-1-(3-((4-((3-Chloro-4-(4-chlorobenzoyl)phenoxy)methyl)-piperidin-1-yl)methyl)phenyl)octyl Carbamate ((R)-**19**).* Colorless oil. $[\alpha]^{20}_D$ +26.3 (*c* 0.2 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.29−1.42 (m, 13H), 1.68−1.88 (m, 6H), 2.17 (bs, 2H), 3.08−3.11 (m, 1H), 3.67 (bs, 1H), 3.87 (d, *J* = 6 Hz, 2H), 4.68 (q, *J* = 1.5 Hz, 1H), 6.85 (dd, *J* = 2.5, 9 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 7.28−7.43 (m, 7H), 7.71−7.74 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 16.6, 20.0, 22.6, 24.5, 25.9, 29.5, 31.8, 39.3,

113.0, 116.2, 128.9, 131.3, 131.4, 133.2, 135.6, 139.8, 159.7, 192.4, 193.7. MS (ESI^+): m/z calcd for $C_{35}H_{42}Cl_2N_2O_4$ $(M + H)^+$, 625.2508; found, 625.2506. The purity of (*R*)-**19** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**19** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 12.5 min; (*R*)-enantiomer, t_R = 14.0 min).

*(4-Chlorophenyl)(3-hydroxyphenyl)methanone O-methyl Oxime (**22**).* To a stirred solution of **21** (800.0 mg, 3.5 mmol) in pyridine (30 mL) was added *O*-methylhydroxylamine hydrochloride (880 mg, 10.5 mmol). The reaction mixture was heated to 105 °C for 16 h. All volatiles were evaporated in vacuo. Purification by silica gel chromatography (7:3, hexanes:EtOAc) afforded **22** (780.0 mg, 86%) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 3.96 (s, 3H), 6.77−6.86 (m, 2H), 6.92−6.96 (m, 1H), 7.24−7.28 (m, 3H), 7.37−7.42 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 62.5, 62.5, 114.5, 116.1, 116.3, 116.8, 120.5, 121.3, 128.5, 128.5, 129.1, 129.6, 129.6, 130.8, 131.4, 134.2, 134.6, 135.0, 135.5, 137.3, 155.6, 155.6, 155.8, 155.8. LRMS (ESI) m/z: 262.026 $(M + H)^+$.

*(2-Chloro-4-hydroxyphenyl)(4-chlorophenyl)methanone O-methyl Oxime (**24**).* To a stirred solution of **23** (500.0 mg, 1.87 mmol) in pyridine (15 mL) was added *O*-methylhydroxylamine hydrochloride (470.0 mg, 5.60 mmol). The reaction mixture was heated to 105 °C for 16 h. All volatiles were evaporated in vacuo. Purification by silica gel chromatography (7:3, hexanes:EtOAc) afforded **24** (520.0 mg, 93%) as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$): δ 3.99 (s, 3H), 6.80 (dd, J = 2.5, 8.5 Hz, 1H), 6.95 (d, J = 2 Hz, 1H), 7.03 (d, J = 8 Hz, 1H), 7.29 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 62.7, 114.4, 116.8, 124.8, 128.2, 128.7, 130.7, 133.2, 133.6, 135.5, 153.4, 156.7. LRMS (ESI) m/z: 296.02 $(M + H)^+$.

tert-Butyl 4-((3-((4-Chlorophenyl)(methoxyimino)methyl)-phenoxy)methyl)piperidine-1-carboxylate. To a stirred solution of **22** (0.93 g, 3.59 mmol), *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (**25**, 1.55 g, 7.18 mmol), and TPP (1.41 g, 5.38 mmol) in THF (8 mL) was added DIAD (1.98 g, 5.38 mmol). After 4 h at rt, all volatiles were evaporated in vacuo. Purification by silica gel chromatography (9:1, hexanes:EtOAc) provided *tert*-butyl 4-((3-((4-chlorophenyl)(methoxyimino)methyl)phenoxy)methyl)piperidine-1-carboxylate (1.28 g, 78%; a mixture of isomers) as a yellow colored liquid. ^1H NMR (500 MHz, $CDCl_3$): δ 1.22−1.31 (m, 2H), 1.46 (s, 9H), 1.81 (d, J = 13 Hz, 2H), 1.93−1.95 (m, 1H), 2.74 (bs, 2H), 3.79 (dd, J = 2.5, 6.5 Hz, 2H), 3.98 (s, 3H), 4.15 (bs, 2H), 6.82−7.04 (m, 3H), 7.20−7.44 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 28.5, 28.9, 36.3, 62.5, 62.6, 72.3, 72.3, 79.4, 113.6, 115.0, 115.1, 120.6, 121.4, 128.4, 128.5, 129.0, 129.3, 129.4, 130.7, 131.5, 134.1, 134.7, 134.9, 135.4, 137.4, 154.9, 155.5, 158.8, 159.0. LRMS (ESI) m/z: 459.20 $(M + H)^+$.

*(4-Chlorophenyl)(3-(piperidin-4-ylmethoxy)phenyl)methanone O-methyl Oxime (**27**).* *tert*-Butyl 4-((3-((4-chlorophenyl)-(methoxyimino)methyl)phenoxy)methyl)piperidine-1-carboxylate (0.6 g, 1.3 mmol) was subjected to 50% TFA in CH_2Cl_2 (7 mL). After 1 h at rt, all volatiles were evaporated in vacuo. The residue was dissolved in $CHCl_3$ and washed with 1N NaOH. The combined $CHCl_3$ was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. Purification by silica gel chromatography (4:1, $CHCl_3$:MeOH) afforded **27** (0.56 g, 79%) as a yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 1.74(q, J = 12 Hz, 2H), 2.05 (t, J = 14 Hz, 3H), 2.95 (q, J = 11.5 Hz, 2H), 3.47 (d, J = 12.5 Hz, 2H), 3.83 (d, J = 5.5 Hz, 2H), 3.98 (s, 3H), 6.82−7.04 (m, 3H), 7.21−7.43 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.7, 34.4, 43.7, 62.6, 62.6, 71.2, 71.3, 113.4, 115.0, 115.1, 115.7, 121.0, 121.7, 128.5, 128.5, 129.0, 129.5, 130.7, 131.4, 134.2, 134.6, 134.9, 135.4, 137.5, 155.3, 155.3, 158.4, 158.6. LRMS (ESI) m/z: 459.14 $(M + H)^+$.

*(R)-1-(4-((3-((4-Chlorophenyl)(methoxyimino)methyl)phenoxy)-methyl)piperidin-1-yl)octan-2-yl Carbamate ((R)-**13**).* To a stirred solution of **27** (180 mg, 0.50 mmol) in CH_2Cl_2 was added $Al(CH_3)_3$ (0.2 M in CH_2Cl_2, 1.50 mmol). After 15 min at 0 °C, (*R*)-1,2-epoxyoctane ((*R*)-**29**, 220.0 mg, 1.75 mmol) in CH_2Cl_2 (0.5 mL) was added. After 4 h at 0 °C, the reaction mixture was quenched with aq satd $NaHCO_3$. The water phase was extracted with CH_2Cl_2, and the combined extract was dried over Na_2SO_4 and evaporated in vacuo. Purification by silica gel chromatography (4:1, $CHCl_3$:MeOH) afforded (*R*)-(4-chlorophenyl)(3-((1-(2-hydroxyoctyl)piperidin-4-yl)-methoxy)phenyl)methanone *O*-methyl oxime ((*R*)-**30**, 195.5 mg, 80%) as a colorless oil. To a stirred solution of (*R*)-(4-chlorophenyl)-(3-((1-(2-hydroxyoctyl)piperidin-4-yl)methoxy)phenyl)methanone *O*-methyl oxime ((*R*)-**30**, 40.0 mg, 0.08 mmol) and DMAP (33.0 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added TMSNCO (30.0 mg, 0.27 mmol). After 4 h at rt, all volatiles were evaporated in vacuo. Purification by silica gel chromatography (9:1, $CHCl_3$:MeOH) afforded (*R*)-**13** (30.0 mg, 69%) as a colorless oil. $[\alpha]^{20}_D$ +105 (*c* 0.2 in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (d, J = 6.8 Hz, 3H), 1.26−1.53 (m, 11H), 1.86 (d, J = 11.6 Hz, 2H), 2.06 (t, J = 9.6 Hz, 1H), 2.39 (t, J = 11.6 Hz, 2H), 2.96 (d, J = 8.4 Hz, 1H), 3.16 (d, J = 10 Hz, 1H), 3.74−3.80 (m, 3H), 3.97 (s, 2H), 1.82−7.04 (m, 2H), 7.13−7.44 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.6, 25.6, 26.5, 28.6, 28.9, 29.2, 29.4, 31.8, 35.0, 35.7, 52.0, 55.3, 60.5, 62.5, 62.6, 64.5, 66.1, 72.3, 113.6, 115.0, 115.1, 115.6, 120.6, 121.3, 127.8, 128.4, 128.5, 129.0, 129.3, 129.4, 130.7, 131.5, 134.1, 134.7, 134.8, 135.3, 137.4, 155.5, 155.5, 158.8, 159.0. MS (ESI^+): m/z calcd for $C_{29}H_{40}ClN_3O_4$ $(M + H)^+$, 530.2706; found, 530.2801. The purity of (*R*)-**13** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**13** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 20.5 min; (*R*)-enantiomer: t_R = 21.5 min).

*(R)-(4-Chlorophenyl)(3-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)methoxy)phenyl)methanone O-Methyl Oxime ((R)-**15**).* Colorless oil. $[\alpha]^{20}_D$ +30.5 (*c* 0.2 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 6.5 Hz, 3H), 1.24−1.43 (m, 12H), 1.67−1.80 (m, 5H), 1.99 (t, J = 11.5 Hz, 2H), 2.91 (d, J = 11.5 Hz, 2H), 3.51 (s, 2H), 3.77 (d, J = 4 Hz, 2H), 3.97 (s, 3H), 4.65 (t, J = 7 Hz, 1H), 6.81−7.03 (m, 3H), 7.19−7.43 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.0, 29.3, 29.5, 31.8, 35.9, 39.2, 53.3, 53.4, 62.5, 62.6, 63.4, 72.7, 74.6, 113.7, 115.0, 115.1, 115.6, 120.5, 121.2, 124.6, 126.8, 128.3, 128.4, 128.4, 128.5, 129.0, 129.3, 129.3, 130.7, 131.5, 134.1, 134.7, 134.8, 135.3, 137.4, 138.4, 145.0, 155.5, 155.6, 159.0, 159.1. HRMS (ESI^+): m/z calcd for $C_{35}H_{45}ClN_2O_3$ $(M + H)^+$, 577.3105; found, 577.3106. The purity of (*R*)-**15** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**15** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 20/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 20.5 min; (*R*)-enantiomer, t_R = 21.0 min)..

*(R)-1-(4-((3-Chloro-4-((4-chlorophenyl)(methoxyimino)methyl)-phenoxy)methyl)piperidin-1-yl)octan-2-yl Carbamate ((R)-**18**).* Colorless oil. $[\alpha]^{20}_D$ +21.5 (*c* 0.5 in $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, J = 7 Hz, 3H), 1.29−1.51 (m, 13H), 1.78−1.85 (m, 3H), 1.95−2.08 (m, 1H), 2.23−2.36 (m, 3H), 2.92 (bs, 1H), 3.67−3.75 (1H), 3.82 (t, J = 6 Hz, 2H), 3.98 (s, 3H), 6.88 (dd, J = 1, 8 Hz,, 1H), 7.01 (d, J = 2 Hz, 1H), 7.07 (dd, J = 3, 9 Hz, 1H), 7.29 (d, J = 8.5 Hz, 2H), 7.42 (d, J = 8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 14.1, 22.6, 22.7, 25.6, 25.7, 29.0, 29.1, 29.3, 29.4, 29.5, 30.9, 31.8, 31.9, 35.0, 35.8, 36.1, 51.7, 54.3, 54.4, 55.2, 62.7, 64.5, 65.8, 66.3, 70.6, 72.8, 73.1, 113.5, 113.5, 115.4, 124.6, 124.7, 128.1, 128.6, 130.5, 130.5, 133.2, 133.2, 133.8, 133.8, 135.4, 153.1, 153.2, 159.9, 160.0. HRMS (ESI^+): m/z calcd for $C_{29}H_{39}Cl_2N_3O_4$ $(M + H)^+$, 564.2308; found, 564.2310. The purity of (*R*)-**18** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**18** was determined to be >99% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 22/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 20.5 min; (*R*)-enantiomer, t_R = 21.0 min).

*(S)-(2-Chloro-4-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)-methoxy)phenyl)(4-chlorophenyl) Methanone O-Methyl Oxime ((S)-**20**).* Colorless oil. $[\alpha]^{20}_{D}$ −30.5 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7 Hz, 3H), 1.25−1.46 (m, 12H), 1.68−1.74 (m, 1H), 1.81 (d, *J* = 10.5 Hz, 4H), 2.02 (t, *J* = 9.5 Hz, 2H), 2.94 (d, *J* = 10.5 Hz, 2H), 3.53 (s, 2H), 3.81 (d, *J* = 6 Hz, 2H), 3.97 (s, 3H), 4.67 (t, *J* = 6.5 Hz, 1H), 6.86 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.00 (d, *J* = 2 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.23−7.32 (m, 6H), 7.41 (d, *J* = 8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.0, 29.3, 29.5, 31.8, 35.8, 39.2, 45.9, 53.3, 53.3, 62.7, 63.4, 72.9, 74.7, 113.5, 115.4, 124.6, 126.8, 128.1, 128.3, 128.4, 128.6, 130.5, 133.2, 133.8, 135.8, 145.0, 153.2, 160.0. HRMS (ESI$^+$): *m*/*z* calcd for $C_{35}H_{44}Cl_2N_2O_3$ (M + H)$^+$, 611.2712; found, 611.2717. The purity of (*S*)-**20** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*S*)-**20** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 25/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 20.0 min; (*R*)-enantiomer, t_R = 21.5 min).

*(R)-(2-Chloro-4-((1-(3-(1-hydroxyoctyl)benzyl)piperidin-4-yl)-methoxy)phenyl)(4-chlorophenyl) Methanone O-Methyl Oxime ((R)-**20**).* Colorless oil. $[\alpha]^{20}_{D}$ +29.5 (*c* 0.5 in $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7 Hz, 3H), 1.25−1.46 (m, 12H), 1.68−1.74 (m, 1H), 1.81 (d, *J* = 10.5 Hz, 4H), 2.02 (t, *J* = 9.5 Hz, 2H), 2.94 (d, *J* = 10.5 Hz, 2H), 3.53 (s, 2H), 3.81 (d, *J* = 6 Hz, 2H), 3.97 (s, 3H), 4.67 (t, *J* = 6.5 Hz, 1H), 6.86 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.00 (d, *J* = 2 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.23−7.32 (m, 6H), 7.41 (d, *J* = 8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 25.9, 29.0, 29.3, 29.5, 31.8, 35.8, 39.2, 45.9, 53.3, 53.3, 62.7, 63.4, 72.9, 74.7, 113.5, 115.4, 124.6, 126.8, 128.1, 128.3, 128.4, 128.6, 130.5, 133.2, 133.8, 135.8, 145.0, 153.2, 160.0. HRMS (ESI$^+$): *m*/*z* calcd for $C_{35}H_{44}Cl_2N_2O_3$ (M + H)$^+$, 611.2712; found, 611.2717. The purity of (*R*)-**20** was determined to be >95% by reverse HPLC analysis (Phenominex kinetex 2.6 μ C18 100A, 100 mm × 4.60 mm; CH_3CN/0.05%TFA in H_2O = 25/1). The optical purity of (*R*)-**20** was determined to be 95% by HPLC analyses (Daicel Chiralcel OD-H (0.46 cm × 25 cm; hexanes/tBuOH = 25/1 with flow rate = 1.0 mL/min and a UV detector at 245 nm; (*S*)-enantiomer, t_R = 20.0 min; (*R*)-enantiomer, t_R = 21.5 min).

Preparation of MenA Containing Membrane Fraction. *M. tuberculosis* was grown to midlog phase in Difco Middlebrook 7H9 nutrient broth (enriched with OADC), and then the cells were harvested by centrifugation at 4 °C followed by washing with 0.9% saline solution (thrice) through centrifugation. The washed cell pellets were suspended in homogenization buffer (containing 50 mM MOPS of pH = 8, 0.25 M sucrose, 10 mM $MgCl_2$ and 5 mM 2-mercaptoethanol) and disrupted by probe sonication on ice (10 cycles of 60s on and 90s off). The resulting suspension was then centrifuged at 15000*g* for 15 min at 4 °C. The pellet was discarded, and the supernatant was centrifuged again at 200000*g* at 4 °C for 1 h. The resulting supernatant contained the membrane bound MenA. *M. smegmatis* and *S. aureus* MenA containing membrane fractions were obtained by the same procedures.[40]

MenA Enzyme Inhibitory Assay (IC_{50}). The substrate 1,4-dihydroxy-2-napthanoic acid (DHNA) (500 μM; 20 μL), $MgCl_2$ (5 μM; 20 μL); CHAPS (0.1%; 20 μL), Tris-buffer (pH = 8; 20 μL), membrane fraction containing Men A (100 μL), inhibitors (0−60 μg/mL of DMSO), and fernesylpyrophosphate (200 μM, 40 μL) were mixed together and incubated for 2 h at 37 °C. After the incubation, the reaction mixture was quenched with 0.1 M AcOH in MeOH and the reaction mixture was extracted with hexanes (thrice). The organic portions were then concentrated and diluted with MeOH (300 μL). From each set of enzymatic reaction mixture, 20 μL was injected into the HPLC (CH_3CN:0.05%TFA in H_2O = 90:10, UV: 325 nm, flow rate: 1.5 mL/min) and the area of the peak for DMMK was quantified to obtain the IC_{50} value for different inhibitor molecules.

Minimum Inhibitory Concentration Assays (MABA and LORA). These were performed according to the published protocol. A preliminary screening was conducted at 12.5 μg/mL against *M. tuberculosis* H_{37}Rv (ATCC 27294), at 60 μg/mL against *S. aureus* Seattle 1945 (ATCC 25923), and at 125 mg/mL against *E. coli* Seattle 1946 (ATCC 25922). Compounds demonstrated at least 90% inhibitions in the preliminary screen were retested at lower concentrations to determine the MIC.

***E. coli* Growth Inhibitory Assays under Anaerobic Conditions.** *E. coli* was grown with inhibitor molecule (5 μg/mL) under anaerobic conditions in the modified *E. coli* growth media (see Supporting Information), and the growth curve was monitored photometrically by reading the optical density at 600 nm.[54,55] *E. coli* growth rescue studies were performed by supplementing VK_2 (50 μM).

Oxygen Consumption Assays. In sterile glass vial, 1.5 mL of *M. tuberculosis* or *M. smegmatis* (grown to 0.5 OD value) was placed. Inhibitor molecule (80 μL, total concentration ranged from 5 to 100 μg/mL) and 0.01% methylene blue (100 μL) was added into the culture solution. The culture vials were kept at 37 °C for 22 h. The effective concentration of the inhibitor molecule at which the electron transport systems was determined by comparing the color intensity with the control vial (no inhibitor).

Determination of IC_{50} in Vero and HepG2 cells. Selected molecules were tested for cytotoxicity (IC_{50}) in Vero and HepG2 cells at concentrations 10 times the MIC for *M. tuberculosis* H_{37}Rv. After 72 h of exposure of molecule to these cell lines, viability was assessed on the basis of cellular conversion of MTT into a formazan product. Selectivity index (SI = IC_{50}/MIC) was determined. SI >10 was considered to be significant in this program.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Some assay data, copies of NMR spectra, and HPLC chromatogram of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 901-448-1045. Fax: 901-448-6940. E-mail: mkurosu@uthsc.edu.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

†Dedicated to Professor Kitagawa on the occasion of his 82nd birthday

■ ABBREVIATIONS USED

Mtb, *Mycobacterium tuberculosis*; *S. aureus*, *Staphylococcus aureus*; MDR, multidrug resistant; VK_2, vitamin K_2 (menaquinone); MenA, 1,4-dihydroxy-2-naphthoate prenyltransferase; Q_{10}, coenzyme Q_{10} (ubiquinone); MIC, minimum inhibitory concentration; MABA, microplate alamar blue assay; ROLA,

low-oxygen recovery assay; MK, menaquinone; DMMK, demethylmenaquinone; SI, selectivity index; REF, rifampin; INH, isoniazid; EMB, ethanbutol a diarylquinolone TB drug lead ((1*R*,2*S*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-(naphthalen-1-yl)-1-phenylbutan-2-ol); Tet, tetracycline; DIAD, diisopropyl azodicarboxylate; TPP, triphenylphosphine; CBS, Corey–Bakshi–Shibata reduction; Py, pyridine; DMAP, dimethylaminopyridine

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■ NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published online April 6, 2012, a correction was made to Table 2. The corrected version was reposted April 10, 2012.