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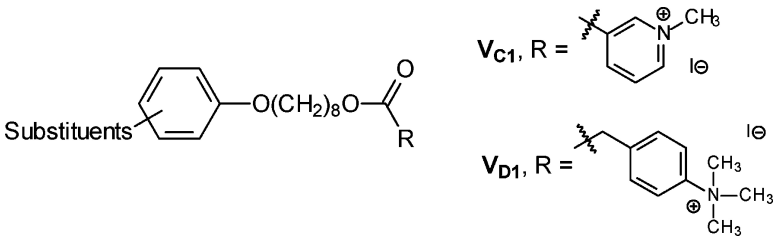

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Tethered Dimer Inhibitors of NAD Synthetase: Parallel Synthesis of an Aryl-Substituted SAR Library

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We previously reported that tethered dimers containing indoles on one end and a permanent positive charge on the other, using a 6−9 carbon polymethylene tether, provided NAD synthetase inhibitors with impressive antibacterial activities against Gram-positives. Here, we report that the phenyl ring is a good substitute for indole, and we utilize solution-phase parallel synthesis to explore structure−activity relationships for substituents on that ring. General conclusions are that nonpolar substituents are more effective than polar ones and that different positional isomers often have very different enzyme inhibition activities. This latter observation reveals that enzyme activity is sensitive to minor structural changes and suggests that nonspecific detergent actions are not important for the observed effects.

Introduction

For the past half century, antibiotics have played a major role in the field of medicine by providing effective cures for life-threatening infectious diseases.[1] However, during the past decade, we have observed significant emergence of antibiotic-resistant bacteria,[2] which in some cases results in untreatable infections. Furthermore, introductions of new antibiotics have been decreasing in recent years.[2a] Infectious diseases have also received major attention due to their potential for use in biological warfare and bioterrorism,[3] including pathogens such as *Bacillus anthracis* (the causative agent in anthrax).[4] The importance is readily apparent in the wake of recent anthrax attacks in different parts of United States.[5]

In an effort to identify new antibacterial compounds that act against novel targets, we have pursued inhibitors of the enzyme nicotinamide adenine dinucleotide (NAD) synthetase. NAD synthetase belongs to the amidotransferase family[6] and catalyzes the last step in the biosynthesis of NAD, transforming nicotinic acid adenine dinucleotide (NaAD) to the amide product via a two-step process.[7] NAD is a coenzyme that plays important roles in biochemical transformations such as DNA repair, DNA recombination, protein−ADP ribosylation,[8] and energy production. Thus, inhibition of prokaryotic NAD synthetase should reasonably lead to antibacterial actions.

The protein crystal structure of *Bacillus subtilis* NAD synthetase provided an attractive target for the structure-based design of compounds that are potential antibacterial agents.[9] We previously reported the solution-phase synthesis of two libraries that resulted in the identification of a new class of antibacterial agents that are effective inhibitors of NAD synthetase.[10,11] The most active compounds were tethered dimers with a substituted indole at one end and an aromatic group containing a positively charged N at the other end, connected by a polymethylene tether with optimum length of 6−9 carbons. Two lead compounds identified from these libraries were **1a** and **1b**[10,11] (Figure 1).

To study the SAR of lead compounds **1a** and **1b**, we sought ways to efficiently incorporate a variety of biologically friendly groups on different parts of the molecule. One goal was to modify the benzyloxyindole end by using solution-phase parallel synthesis to introduce a variety of substituents at different positions on the indole ring. But commercial availability of substituted indoles appeared limited. Alternatively, we explored the use of substituted phenols in place of the substituted indole, since phenols with a wide range of substituents are commercially available.

Results and Discussion

The first objective was to synthesize analogues of **1a** and **1b** containing a 4-benzyloxyphenol in the place of 5-benzyloxyindole and to confirm that this modification maintains good activity. These two compounds, $\mathbf{V}_{C1}$ and $\mathbf{V}_{D1}$, were synthesized individually as outlined in Scheme 1.

As shown, commercially available 4-benzyloxyphenol $\mathbf{I}_1$ was alkylated with 8-bromo-1-octanol in the presence of K_2CO_3 in refluxing acetone to obtain the long-chain alcohol $\mathbf{II}_1$. The alcohol $\mathbf{II}_1$ was then converted to the corresponding mesylate $\mathbf{III}_1$ by treatment with CH_3SO_2Cl in the presence of Et_3N in dichloromethane at 0 °C. Mesylate $\mathbf{III}_1$ was esterified with nicotinic acid in the presence of K_2CO_3 in

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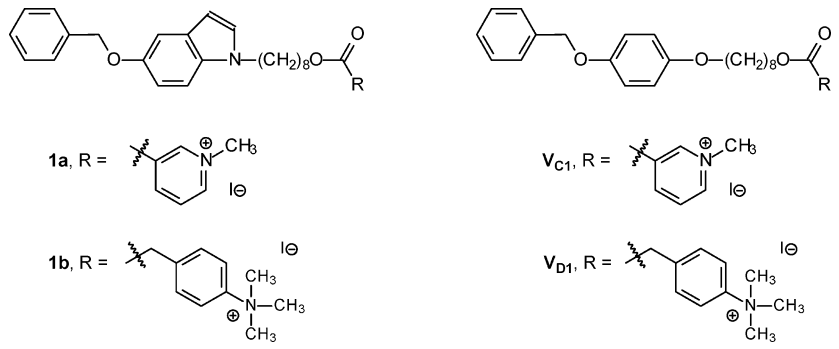
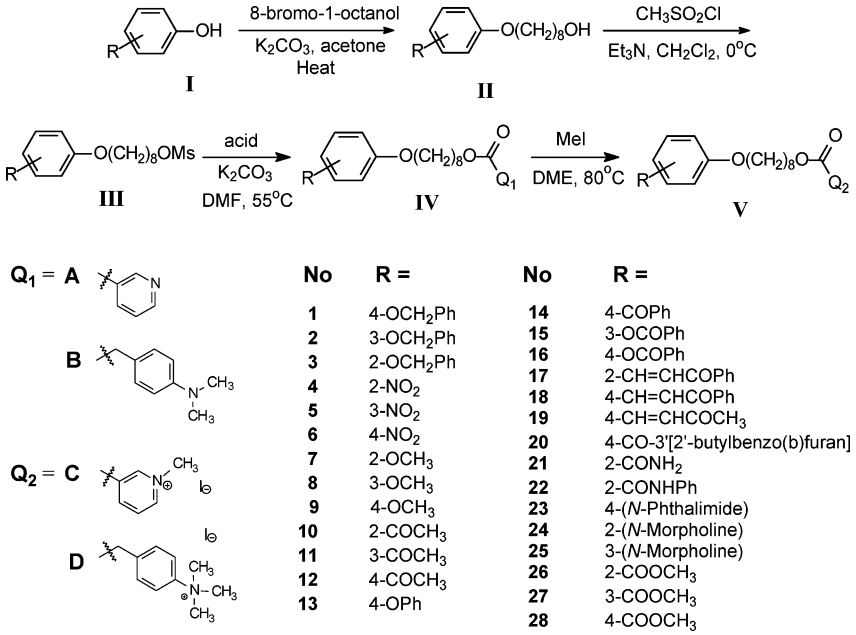


Figure 1.

Scheme 1



No	R =	No	R =
1	4-OCH_2Ph	14	4-COPh
2	3-OCH_2Ph	15	3-OCOPh
3	2-OCH_2Ph	16	4-OCOPh
4	2-NO_2	17	2-CH=CHCOPh
5	3-NO_2	18	4-CH=CHCOPh
6	4-NO_2	19	4-CH=CHCOCH$_3$
7	2-OCH_3	20	4-CO-3'[2'-butylbenzo(b)furan]
8	3-OCH_3	21	2-$CONH_2$
9	4-OCH_3	22	2-CONHPh
10	2-$COCH_3$	23	4-(*N*-Phthalimide)
11	3-$COCH_3$	24	2-(*N*-Morpholine)
12	4-$COCH_3$	25	3-(*N*-Morpholine)
13	4-OPh	26	2-$COOCH_3$
		27	3-$COOCH_3$
		28	4-$COOCH_3$

Table 1. Inhibition of *B. subtilis* NAD Synthetase by Selected Tethered Dimers

compd no.	**1a**	**1b**	V_{C1}	V_{D1}
IC_{50} (μM)	22	20	75	20

DMF to form the nicotinate ester $\mathbf{IV}_{A1}$. Methylation of $\mathbf{IV}_{A1}$ with iodomethane in dimethoxyethane afforded the positively charged pyridinium salt $\mathbf{V}_{C1}$. Esterification of mesylate $\mathbf{III}_1$ with 4-(*N*,*N*-dimethylamino)phenyl acetic acid in the presence of K_2CO_3 in anhydrous DMF gave ester $\mathbf{IV}_{B1}$, which upon quaternization with iodomethane in dimethoxyethane furnished the quaternary ammonium salt $\mathbf{V}_{D1}$.

Compounds $\mathbf{V}_{C1}$ and $\mathbf{V}_{D1}$ were then evaluated as inhibitors of purified *B. subtilis* NAD synthetase. The IC_{50} values of $\mathbf{V}_{C1}$ and $\mathbf{V}_{D1}$ as compared to **1a** and **1b** are given in Table 1.

As shown, both $\mathbf{V}_{C1}$ and $\mathbf{V}_{D1}$ effectively inhibit the enzyme. The inhibitor $\mathbf{V}_{D1}$ contains an *N*,*N*,*N*-trimethylammonio end group and is a better inhibitor than $\mathbf{V}_{C1}$, which contains an *N*-methyl nicotinate end group. Compound $\mathbf{V}_{D1}$ was then evaluated against different strains of Gram-positive and Gram-negative bacteria. The MIC values are given in Table 2.

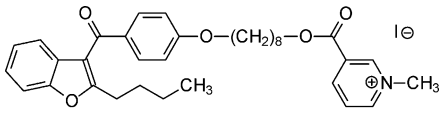
Compound $\mathbf{V}_{D1}$ exhibited MIC values comparable to indoles **1a** and **1b**. Results were also compared with the clinically used antibiotics rifampin and methicillin, and some of the tethered dimers had antibacterial activities with similar potencies. Of particular interest is the observation that $\mathbf{V}_{D1}$ (like **1a**) remains active against methicillin-resistant strains of *Staphylococcus aureus.*

Encouraged by these results, we designed a library to incorporate a variety of substituents on the phenyl ring of $\mathbf{V}_{C1}$ and $\mathbf{V}_{D1}$, as summarized in Scheme 1. Twenty-seven different substituted phenols were purchased as starting materials for this library. These phenols were selected on the basis of commercial availability and structural diversity. In the first step, the 27 phenols (**I**) were alkylated in parallel with 8-bromo-1-octanol in the presence of K_2CO_3 in anhydrous acetone to obtain 27 long-chain alcohols (**II**). The 27 alcohols thus obtained were converted to the corresponding mesylates (**III**) by treatment with CH_3SO_2Cl in the presence of Et_3N in CH_2Cl_2. The mesylates were then divided into two halves. One-half was esterified with nicotinic acid in the presence of K_2CO_3 in DMF at 55 °C to afford the 27 different esters $\mathbf{IV}_{A(2-28)}$. The other half was esterified with 4-(*N*,*N*-dimethylamino)phenylacetic acid in the presence of K_2CO_3 in DMF at 55°C to afford the 27 different esters $\mathbf{IV}_{B(2-28)}$. The esters $\mathbf{IV}_{A(2-28)}$ and $\mathbf{IV}_{B(2-28)}$ were then methylated using MeI in anhydrous DME to obtain 54 final products ($\mathbf{V}_{C(2-28)}$ and $\mathbf{V}_{D(2-28)}$). Final products were purified by parallel chromatography in silica-packed syringes. After

Table 2. Antibacterial Activities (MIC, μg/mL) of $\mathbf{V}_{D1}$, **1a**, **1b**, and $\mathbf{V}_{C20}$ in Comparison with Ampicillin, Methicillin, and Rifampin

compd	*B. subtilis* ATCC #9372	*S. aureus* ATCC #29213	*S. aureus* (MRSA[a]) ATCC #33592	*S. aureus* (MRSA[a]) ATCC #33593	*Pseudomonas aeruginosa* ATCC #27853	*Salmonella enteritidis* ATCC #13076
$\mathbf{V_{D1}}$	2	9.4	6.2	3.1	>50	>50
1a	6	1.5	6.2	3	>50	>50
1b	15.5	4.5	–	–	>50	>50
$\mathbf{V_{C20}}$	2.5	–[b]	–	–	–	–
Ampicillin	2	–		–	–	–
Methicillin	–	1.0	32	32	–	–
Rifampin	1.5	–	–	–	–	–

[a] Methicillin-resistant *S. aureus*. [b] Not tested.



Compound V_{C20}

Figure 2. Chemical structure of $\mathbf{V}_{C20}$, the most active compound identified from the current study.

sample loading, columns were first washed with CH_2Cl_2 and EtOAc to remove nonpolar impurities, and pure products were then eluted with 5% MeOH in CH_2Cl_2. The purity of all final products was determined using HPLC/MS, and ~80% of the final products had purity ≥80%. The purity of 20 final products was accurately determined by ^{1}H NMR using hexamethyl disiloxane as internal standard. The purity results obtained from NMR measurements were comparable to purities determined by HPLC. Mass spectral data, purity, and the yields of all final products in the library are given in Table 3.

All of the products $\mathbf{V}_{C-D(2-28)}$ and their desmethyl precursors $\mathbf{IV}_{C-D(2-28)}$ (Scheme 1) were evaluated as inhibitors of purified *B. subtilis* NAD synthetase. The IC_{50} values are given in Table 3. As shown, all of the desmethyl precursors $\mathbf{IV}_{C-D(2-28)}$ were inactive against NAD synthetase, emphasizing our earlier conclusion[10] that a permanent positive charge is important. Among the positively charged products, the 4-CO-3′-[2′-butylbenzo[*b*]furan] group ($\mathbf{V}_{C20}$ and $\mathbf{V}_{D20}$) was the most effective phenyl substituent for both of the quaternary ammonium end groups, suggesting that relatively large and hydrophobic substituents are favorable. This hypothesis is supported by good activities in both series for PhCO and PhCOCH═CH substituents, whereas small polar groups, such as NO_2 and MeO, were generally ineffective (although $\mathbf{V}_{D4}$ is an exception). The chemical structure of $\mathbf{V}_{C20}$, the most active compound identified from the library, is given in Figure 2.

Given the structural resemblance of the best inhibitors to detergents, it was of particular interest to determine if structurally similar (thus possessing similar detergent properties) compounds would reveal significant differences in enzyme activity. Indeed, this appears to be the case. For the PhCOCH═CH group in the $\mathbf{V}_C$ series, 2-susbstitution gave good enzyme inhibition activity, but the 4-substituent was inactive; for this group in the $\mathbf{V}_D$ series, both 2- and 4-substituents were similarly active. Other substituents that also exhibited very different activities for positional isomers include the acetyl and benzoyloxy groups in $\mathbf{V}_C$ and the nitro group in $\mathbf{V}_D$. Furthermore, the SAR trends were often different for the two different positively charged end groups.

The most active compound identified from the library, $\mathbf{V}_{C20}$, was then evaluated for antibacterial activity against *B. subtilis*. The MIC value was similar to those for compounds **1a**, $\mathbf{V}_{D1}$, and ampicillin and was better than that for **1b**. The results are summarized in Table 2.

These results are suggestive of specific interactions that give rise to enzyme inhibition, as opposed to nonspecific detergent or surfactant properties. Further studies to better understand the mechanism of enzyme inhibition and to improve the potency of lead compounds are being pursued.

Experimental Section

Enzyme Inhibition Assay. Procedures for cloning, overexpression, purification of NAD synthetase (NADS), and the NADS inhibition assay were described in an earlier publication from this laboratory.[10]

Briefly, NAD synthetase activity was evaluated by monitoring the production of NAD in the enzyme reaction through its conversion to NADH in the presence of ethanol and alcohol dehydrogenase (ADH) from baker's yeast.

(1) NAD synthetase reaction:

$$\mathrm{NaAD + ATP + NH_4^+ + Mg^{2+} \rightarrow NAD^+ + AMP + PPi + Mg^{2+}}$$

(2) Alcohol dehydrogenase reaction

$$\mathrm{NAD^+ + ethanol \rightarrow NADH + H^+ + acetaldehyde}$$

Excess ADH is used to ensure rapid conversion of NAD^+ to NADH so that the rate-limiting step in this system is the NAD synthetase reaction. Although excess ADH decreases the possibility that inhibition of ADH gives a false result, this assumption was verified by confirming that the best NADS inhibitors did not inhibit ADH.

More specifically, the enzyme inhibition assay was carried out in 96-well microtiter plates with a reaction volume of 200 μL. For each compound, six concentrations were prepared from a stock solution by serial dilution. Then 5 μL of each solution was transferred to assay plates with duplicate wells for each concentration, followed by the addition of 170 μL of assay solution containing NADS and all other reagents except ATP using a Biomek FX liquid handler (Beckman-Coulter). After 10 min, the enzyme reaction was initiated by the addition of 25 μL of ATP solution. The

Table 3. Structures, Mass Spectral Data, Purity, Yield, and IC_{50} Values of the Compounds Defined in the Library (Scheme 1)

compd no.	IC_{50} (μM)	compd no.	R =	mass (ES+)	purity % (HPLC)	overall yield %	IC_{50} (μM)
$\mathbf{IV}_{C2}$	>100	$\mathbf{V}_{C2}$	3-OCH_2Ph	448	97	47	27
$\mathbf{IV}_{C3}$	>100	$\mathbf{V}_{C3}$	2-OCH_2Ph	448	95	45	>100
$\mathbf{IV}_{C4}$	>100	$\mathbf{V}_{C4}$	2-NO_2	387	92	22	>100
$\mathbf{IV}_{C5}$	>100	$\mathbf{V}_{C5}$	3-NO_2	387	85	34	>100
$\mathbf{IV}_{C6}$	>100	$\mathbf{V}_{C6}$	4-NO_2	387	85	56	>100
$\mathbf{IV}_{C7}$	>100	$\mathbf{V}_{C7}$	2-OCH_3	372	83	51	>100
$\mathbf{IV}_{C8}$	>100	$\mathbf{V}_{C8}$	3-OCH_3	372	80	42	>100
$\mathbf{IV}_{C9}$	—[a]	$\mathbf{V}_{C9}$	4-OCH_3	—[a]	—[a]	—[a]	—[a]
$\mathbf{IV}_{C10}$	>100	$\mathbf{V}_{C10}$	2-$COCH_3$	384	85	60	>100
$\mathbf{IV}_{C11}$	>100	$\mathbf{V}_{C11}$	3-COCH3	384	84	52	49
$\mathbf{IV}_{C12}$	>100	$\mathbf{V}_{C12}$	4-$COCH_3$	384	79	54	>100
$\mathbf{IV}_{C13}$	>100	$\mathbf{V}_{C13}$	4-OPh	434	90	41	>100
$\mathbf{IV}_{C14}$	>100	$\mathbf{V}_{C14}$	4-COPh	446	82	59	>100
$\mathbf{IV}_{C15}$	>100	$\mathbf{V}_{C15}$	3-OCOPh	462	82	42	36
$\mathbf{IV}_{C16}$	>100	$\mathbf{V}_{C16}$	4-OCOPh	462	86	22	>100
$\mathbf{IV}_{C17}$	>100	$\mathbf{V}_{C17}$	2-CH=CHCOPh	472	78	51	17
$\mathbf{IV}_{C18}$	>100	$\mathbf{V}_{C18}$	4-CH=CHCOPh	472	95	57	>100
$\mathbf{IV}_{C19}$	>100	$\mathbf{V}_{C19}$	4-CH=$CHCOCH_3$	410	82	22	>100
$\mathbf{IV}_{C20}$	>100	$\mathbf{V}_{C20}$	4-CO-3′[2′-butylbenzo(*b*)furan]	542	94	66	9
$\mathbf{IV}_{C21}$	>100	$\mathbf{V}_{C21}$	2-$CONH_2$	385	40	22	>100
$\mathbf{IV}_{C22}$	>100	$\mathbf{V}_{C22}$	2-CONHPh	461	81	25	>100
$\mathbf{IV}_{C23}$	>100	$\mathbf{V}_{C23}$	4-(*N*-phthalimide)	487	91	57	36
$\mathbf{IV}_{C24}$	—[a]	$\mathbf{V}_{C24}$	2-(*N*-morpholine)	—[a]	—[a]	—[a]	—[a]
$\mathbf{IV}_{C25}$	—[a]	$\mathbf{V}_{C25}$	3-(*N*-Morpholine)	—[a]	—[a]	—[a]	—[a]
$\mathbf{IV}_{C26}$	>100	$\mathbf{V}_{C26}$	2-$COOCH_3$	400	94	42	>100
$\mathbf{IV}_{C27}$	>100	$\mathbf{V}_{C27}$	3-$COOCH_3$	400	98	48	>100
$\mathbf{IV}_{C28}$	>100	$\mathbf{V}_{C28}$	4-$COOCH_3$	400	96	51	>100
$\mathbf{IV}_{D2}$	>100	$\mathbf{V}_{D2}$	3-OCH_2Ph	504	97	37	12
$\mathbf{IV}_{D3}$	>100	$\mathbf{V}_{D3}$	2-OCH_2Ph	504	96	36	85
$\mathbf{IV}_{D4}$	>100	$\mathbf{V}_{D4}$	2-NO_2	443	93	29	31
$\mathbf{IV}_{D5}$	>100	$\mathbf{V}_{D5}$	3-NO_2	443	90	58	>100
$\mathbf{IV}_{D6}$	>100	$\mathbf{V}_{D6}$	4-NO_2	443	90	51	>100
$\mathbf{IV}_{D7}$	>100	$\mathbf{V}_{D7}$	2-OCH_3	428	88	49	>100
$\mathbf{IV}_{D8}$	>100	$\mathbf{V}_{D8}$	3-OCH_3	428	78	47	>100
$\mathbf{IV}_{D9}$	—[a]	$\mathbf{V}_{D9}$	4-OCH_3	—[a]	—[a]	—[a]	—[a]
$\mathbf{IV}_{D10}$	>100	$\mathbf{V}_{D10}$	2-$COCH_3$	440	82	54	68
$\mathbf{IV}_{D11}$	>100	$\mathbf{V}_{D11}$	3-$COCH_3$	440	88	61	>100
$\mathbf{IV}_{D12}$	>100	$\mathbf{V}_{D12}$	4-$COCH_3$	440	95	51	>100
$\mathbf{IV}_{D13}$	>100	$\mathbf{V}_{D13}$	4-OPh	490	84	44	21
$\mathbf{IV}_{D14}$	>100	$\mathbf{V}_{D14}$	4-COPh	502	95	50	40
$\mathbf{IV}_{D15}$	>100	$\mathbf{V}_{D15}$	3-OCOPh	518	60	38	37
$\mathbf{IV}_{D16}$	>100	$\mathbf{V}_{D16}$	4-OCOPh	518	80	34	52
$\mathbf{IV}_{D17}$	>100	$\mathbf{V}_{D17}$	2-CH=CHCOPh	528	91	64	27
$\mathbf{IV}_{D18}$	>100	$\mathbf{V}_{D18}$	4-CH=CHCOPh	528	85	52	40
$\mathbf{IV}_{D19}$	>100	$\mathbf{V}_{D19}$	4-CH=$CHCOCH_3$	466	96	42	>100
$\mathbf{IV}_{D20}$	>100	$\mathbf{V}_{D20}$	4-CO-3′[2′-butylbenzo(*b*)furan]	598	89	57	13
$\mathbf{IV}_{D21}$	>100	$\mathbf{V}_{D21}$	2-$CONH_2$	441	85	35	100
$\mathbf{IV}_{D22}$	>100	$\mathbf{V}_{D22}$	2-CONHPh	517	94	28	>100
$\mathbf{IV}_{D23}$	>100	$\mathbf{V}_{D23}$	4-(*N*-phthalimide)	543	88	50	>100
$\mathbf{IV}_{D24}$	>100	$\mathbf{V}_{D24}$	2-(*N*-morpholine)	483	37	44	>100
$\mathbf{IV}_{D25}$	>100	$\mathbf{V}_{D25}$	3-(*N*-morpholine)	483	43	52	>100
$\mathbf{IV}_{D26}$	>100	$\mathbf{V}_{D26}$	2-$COOCH_3$	456	92	44	81
$\mathbf{IV}_{D27}$	>100	$\mathbf{V}_{D27}$	3-$COOCH_3$	456	95	41	>100
$\mathbf{IV}_{D28}$	>100	$\mathbf{V}_{D28}$	4-$COOCH_3$	456	94	43	95

[a] The desired product was not formed.

reaction was allowed to proceed for 10 min before termination by 50 μL of 6 M GuaHCl. The amount of NADH produced in each well was read by UV absorbance at 340 nm (SpectraMax Pro microplate reader; Molecular Devices) and by fluorescence emission at 460 nm (PolarStar microplate reader; BMG-LabTechnologies). Each plate was read twice, once before the addition of ATP to record background signal and, second, after the addition of 6 M GuaHCl to record the sample signal. In each plate, there were 12 control wells containing no inhibitor. After subtracting the background signal from the sample signal, the reading for each plate was normalized by using the average signal of the 12 control wells to calculate the percentage inhibition at each concentration and the IC_{50} for each compound.

Antibacterial Assays. Minimum inhibitory concentrations (MIC) were determined by MicroBioTest, Inc., of Sterling, VA. We previously described the details of this procedure.[10]

Synthetic Chemistry. General. Melting points were determined using an Electrothermal 9100 apparatus and are uncorrected. IR spectra were taken with Brucker Vector-22 and Bomen MB-104 instruments. All ^1H and ^{13}C NMR spectra were recorded on a Brucker 300-MHz spectrometer

using TMS as internal standard. The values of chemical shifts (δ) are given in parts per million and coupling constants (J), in Hz. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and the results are within ±0.4% of theoretical values. Reactions were monitored by TLC (Whatmann silica gel, UV 254, 25 μM plates), and flash column chromatography was performed using Baker silica gel (40 μM) in the solvent systems indicated. Anhydrous solvents used for reactions were purchased in Sure-Seal bottles from Aldrich Chemical Co. Other reagents were purchased from Aldrich, Lancaster, or Acros Chemical Companies and used as received. Parallel reactions were carried out in 10-mL, screw-cap vials using a Digi-Block heater (purchased from Aldrich Scientific Company) mounted on an orbital shaker (purchased from VWR Scientific Company). Parallel evaporation of solvents was conducted in 20-mL, wide-mouthed vials using a Savant SC-210 Speedvac Plus instrument. Parallel filtrations were carried out using the outer tubes of 5-mL plastic disposable syringes packed with cotton mounted on a Burdick & Jackson 24-port manifold (purchased from VWR Scientific Company). Preparative parallel chromatography was performed on Baker flash silica gel packed in the outer tubes of 10-mL plastic disposable syringes using a Burdick & Jackson 24-port manifold.

8-(4-Benzyloxyphenoxy)-1-octanol ($\mathbf{II}_1$). To a solution of 4-benzyloxyphenol $\mathbf{I}_1$ (0.60 g, 3.0 mmol) in anhydrous acetone (30 mL), 8-bromo-1-octanol (0.69 g, 3.3 mmol) and K_2CO_3 (2.48 g, 17.9 mmol) were added, and the reaction mixture was refluxed for 18 h. It was then cooled to room temperature and filtered through Celite to remove the solid materials. Solvent was completely removed from the filtrate. The residue was then dissolved in EtOAc (50 mL) and washed with water (2 × 10 mL) and brine (1 × 10 mL). Removal of solvent from the dried (Na_2SO_4) organic layer afforded the product alcohol $\mathbf{II}_1$ (0.84 g, 85%): mp 94−95 °C. ^{1}H NMR ($CDCl_3$) δ 1.28−1.51 (m, 8H), 1.51−1.63 (m, 3H), 1.69−1.81 (m, 2H), 3.62 (t, 2H, J = 6.58 Hz), 3.88 (t, 2H, J = 6.52 Hz), 5.00 (s, 2H), 6.82 (d, 2H, J = 9.09 Hz), 6.89 (d, 2H, J = 9.21 Hz), and 7.26−7.45 (m, 5H).^{13}C NMR ($CDCl_3$) δ 25.6, 25.9, 29.3, 32.6, 62.9, 68.5, 70.6, 115.3, 115.7, 127.4, 127.8, 128.4, 137.2, 152.7, and 153.4. IR (KBr): 3303 cm^{-1}. MS (ES^+): m/z 329 (M + H). Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.64; H, 8.58.

8-(4-Benzyloxyphenoxy)-1-octyl Methanesulfonate ($\mathbf{III}_1$). To a solution of alcohol $\mathbf{II}_1$ (0.40 g, 1.2 mmol) in anhydrous CH_2Cl_2 (25 mL) at 0 °C was added Et_3N (0.33 mL, 2.5 mmol), followed by CH_3SO_2Cl (0.21 g, 1.8 mmol), and the mixture was stirred at 0 °C for 15 min. The reaction mixture was diluted with 10 mL of CH_2Cl_2 and washed with 1 N HCl (3 × 15 mL), water (2 × 15 mL), and brine (1 × 15 mL). It was dried over Na_2SO_4 and filtered, and the solvent was completely removed under vacuum to obtain the mesylate $\mathbf{III}_1$ (0.43 g, 88%). ^{1}H NMR ($CDCl_3$) δ 1.29−1.51 (m, 8H), 1.68−1.81 (m, 4H), 2.99 (s, 3H), 3.89 (t, 2H, J = 6.41 Hz), 4.22 (t, 2H, J = 6.52 Hz), 5.01 (s, 2H), 6.81 (d, 2H, J = 8.99 Hz), 6.89 (d, 2H, J = 9.09 Hz), 7.27−7.45 and (m, 5H). ^{13}C NMR ($CDCl_3$) δ 25.3, 25.9, 28.9, 29.06, 29.13, 29.2, 37.3, 68.4, 70.0, 70.6, 115.3, 115.7, 127.4, 127.8, 128.5, 137.2, 152.8, and 153.4. MS (ES^+) m/z 407(M + H). Anal. Calcd for $C_{22}H_{30}O_5S$: C, 65.00; H, 7.44. Found: C, 64.8; H, 7.47.

8-(4-Benzyloxyphenoxy)-1-octyl Nicotinate ($\mathbf{IV}_{A1}$). To a solution of the mesylate $\mathbf{III}_1$ (0.15 g, 0.37 mmol) in anhydrous DMF (6 mL) were added nicotinic acid (0.091 g, 0.74 mmol) and K_2CO_3 (0.051 g, 0.37 mmol), and the mixture was heated at 50−55 °C for 16 h. The reaction mixture turned into a gelatinous mass, although TLC examination (50% EtOAc in hexanes) revealed that the reaction was complete. It was diluted with 30 mL of EtOAc and quenched with sat. NH_4Cl (20 mL). The organic layer was separated, and the aqueous layer was extracted with more EtOAc (10 mL). The combined EtOAc extracts were washed with sat. $NaHCO_3$ (3 × 15 mL), water (2 × 15 mL), and brine (2 × 15 mL). This was dried over Na_2SO_4 and filtered, and the solvent was removed under vacuum to obtain the crude product, which was purified by column chromatography over Si gel (20 × 4 cm) using EtOAc/hexanes (1:1) to afford the ester $\mathbf{IV}_{A1}$ (0.12 g, 74%). ^{1}H NMR ($CDCl_3$) δ 1.29−1.52 (m, 8H), 1.69−1.84 (m, 4H), 3.89 (t, 2H, J = 6.49 Hz), 4.35 (t, 2H, J = 6.65 Hz), 5.01 (s, 3H), 6.81 (d, 2H, J = 9.18 Hz), 6.89 (d, 2H, J = 9.17 Hz), 7.27−7.44 (m, 6H), 8.29 (dt, 1H, J_1 = 7.96 Hz, J_2 = 1.92 Hz), 8.77 (dd, 1H, J_1 = 4.76 Hz, J_2 = 1.84 Hz), and 9.23 (d, 1H, J = 1.23 Hz). ^{13}C NMR ($CDCl_3$) δ 25.8, 25.9, 28.5, 29.1, 29.2, 29.3, 65.5, 68.4, 70.6, 115.3, 115.7, 123.2, 126.3, 127.4, 127.8, 128.4, 137.0, 137.3, 150.7, 152.7, 153.2, 153.4, and 165.2. IR (KBr) 1719 cm^{-1}. MS (ES^+) m/z 434 (M + H). Anal. Calcd for $C_{27}H_{31}NO_4$: C, 74.99; H, 7.21; N, 3.23. Found: C, 74.76; H, 7.30; N, 3.12.

8-(4-Benzyloxyphenoxy)-1-octyl *N*-Methylnicotinate Iodide ($\mathbf{V}_{C1}$). To a solution of the ester $\mathbf{IV}_{A1}$ (0.08 g, 0.18 mmol) in anhydrous DME (6 mL) was added iodomethane (0.34 mL, 5.45 mmol), and the mixture was heated at 80 °C for 12 h. This was cooled to room temperature, the solid product crystallized, and the precipitate was filtered and washed with 50% EtOAc in hexanes to obtain the pure quaternary salt $\mathbf{V}_{C1}$ (0.065 g, 61%). ^{1}H NMR ($CDCl_3$) δ 1.31−1.52 (m, 8H), 1.68−1.91 (m, 4H), 3.89 (t, 2H, J = 6.39 Hz), 4.42 (t, 2H, J = 6.74 Hz), 4.77 (s, 3H), 4.99 (s, 2H), 6.81 (d, 2H, J = 9.06 Hz), 6.89 (d, 2H, J = 9.03 Hz), 7.29−7.45 (m, 5H), 8.32 (t, 1H, J = 7.04 Hz), 8.89 (d, 1H, J = 8.09 Hz), 9.35 (s, 1H), and 9.72 (d, 1H, J = 5.88 Hz). ^{13}C NMR ($CDCl_3$) δ 25.6, 25.8, 28.3, 28.9, 29.1, 29.2, 50.4, 67.4, 68.4, 70.5, 115.3, 115.7, 127.4, 127.7, 128.4, 128.6, 130.4, 137.1, 144.8, 145.9, 149.1, 152.7, 153.3, and 160.9. IR (KBr): 1732 cm^{-1}. MS (ES^+) m/z 448 (M^+). Anal. Calcd for $C_{28}H_{34}INO_4$: C, 58.44; H, 5.95; N, 2.43. Found: C, 58.56; H, 5.89; N, 2.41.

8-(4-Benzyloxyphenoxy)-1-octyl 4-(*N*,*N*-Dimethylamino)phenylacetate ($\mathbf{IV}_{B1}$). Compound $\mathbf{IV}_{B1}$ was prepared by using a procedure similar to that described for the synthesis of $\mathbf{IV}_{A1}$, starting from $\mathbf{III}_1$ (0.15 g, 0.36 mmol) and 4-(*N*,*N*-dimethylamino)phenylacetic acid (0.13 g, 0.74 mmol) to obtain the ester $\mathbf{IV}_{B1}$ (0.14 g, 76%). ^{1}H NMR ($CDCl_3$) δ 1.26−1.36 (m, 6H), 1.36−1.48 (m, 2H), 1.52−1.66 (m, 2H), 1.67−1.79 (m, 2H), 2.91 (s, 6H), 3.50 (s, 2H), 3.88 (t, 2H,

J = 6.51 Hz), 4.06 (t, 2H, J = 6.68 Hz), 4.99 (s, 2H), 6.69 (d, 2H, J = 8.70 Hz), 6.81 (d, 2H, J = 9.17 Hz), 6.89 (d, 2H, J = 9.17 Hz), 7.14 (d, 2H, J = 8.67 Hz), and 7.26−7.44 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 25.7, 25.9, 26.1, 28.5, 29.1, 29.2, 29.3, 29.7, 40.4, 40.6, 64.7, 68.4, 70.6, 112.7, 115.3, 115.7, 122.0, 127.4, 127.8, 128.4, 129.7, 137.2, 149.6, 152.7, 153.4, and 172.3. IR (KBr): 1730 cm^{-1}. MS (ES^+) m/z 490 (M + H). Anal. Calcd for $C_{31}H_{39}NO_4$: C, 76.03; H, 8.03; N, 2.86. Found: C, 75.79; H, 7.91; N, 2.90.

8-(4-Benzyloxyphenoxy)-1-octyl 4-(*N,N,N*-Trimethylammonio)phenylacetate Iodide (V_{D1}). Compound $\mathbf{V}_{D1}$ was prepared using a procedure similar to that described for the synthesis of $\mathbf{V}_{C1}$, starting from $\mathbf{IV}_{B1}$ (0.080 g, 0.16 mmol) and iodomethane (0.31 mL, 4.9 mmol) to obtain the pure quaternary salt $\mathbf{V}_{D1}$ (0.071 g, 68%). ^{1}H NMR ($CDCl_3$) δ 1.27−1.39 (m, 6H), 1.39−1.51 (m, 2H), 1.57−1.69 (m, 2H), 1.69−1.83 (m, 2H), 3.67 (s, 2H), 3.89 (t, 2H, J = 6.44 Hz), 3.97 (s, 9H), 4.08 (t, 2H, J = 6.75 Hz), 5.01 (s, 2H), 6.82 (d, 2H, J = 9.15 Hz), 6.89 (d, 2H, J = 9.14 Hz), 7.29−7.45 (m, 5H), 7.52 (d, 2H, J = 8.86 Hz), and 7.96 (d, 2H, J = 8.93 Hz). ^{13}C NMR ($CDCl_3$) δ 25.6, 25.8, 28.4, 29.0, 29.1, 29.2, 40.2, 57.7, 65.4, 68.4, 70.6, 115.3, 115.7, 120.1, 127.4, 127.8, 128.4, 131.6, 137.1, 137.2, 145.9, 152.7, 153.3, and 170.5. IR (KBr): 1731 cm^{-1}. MS (ES^+) m/z 504 (M^+). Anal. Calcd for $C_{32}H_{42}NO_4I$: C, 60.83; H, 6.71; N, 2.22. Found: C, 60.66; H, 6.76; N, 2.29.

Procedure for Parallel Synthesis (Scheme 1). Synthesis of Alcohols (II). To a solution of the phenols (I) (1 mmol) and 8-bromo-1-octanol (1.1 equiv) in acetone (5 mL) contained in screw-cap vials (10-mL capacity), K_2CO_3 (6 equiv) was added. The reaction mixtures were flushed with N_2, capped, and heated with orbital shaking (225 rpm) at 70 °C on a digiblock heater for 36 h. They were allowed to attain room temperature and filtered into vials (20-mL capacity). The filtration was carried out in parallel through syringe tubes fitted with cotton and Celite using a 24-port filtration manifold. Each filter was washed twice with 5 mL of acetone. The filtrates were concentrated using the speedvac at its medium temperature setting. The residues obtained after the removal of acetone were dissolved in EtOAc (15 mL each), transferred into 40-mL capacity tubes, and washed with 1 N NaOH (2 × 5 mL) and water (2 × 5 mL). Then Na_2SO_4 (∼1 g) was added to each tube for drying the solution over ∼1 h. These solutions were filtered in parallel into 20-mL vials as before. The filtrates collected were then evaporated using the speedvac at its high temperature setting to obtain the product alcohols (II) (70−97% yield).

Synthesis of Mesylates (III). The alcohols (**II**) were dissolved in CH_2Cl_2 (6 mL) in 20-mL, screw-cap vials and cooled to 0 °C in parallel using a metal stand immersed in an ice bath. Then MsCl (1.5 equiv) was added to each vial, followed by the dropwise addition of Et_3N (2 equiv). The vials were capped and kept at 0 °C for 1 h with occasional shaking by hand. The reaction mixtures were then diluted with CH_2Cl_2 (8 mL), transferred into 40-mL tubes, and washed with 1 N HCl (3 × 4 mL), water (2 × 4 mL), and brine (1 × 4 mL). Each vial was dried using Na_2SO_4 (∼1 g) over 1 h. The solutions were filtered in parallel into 20-mL vials using 10-mL plastic syringe tubes, as before. Evaporation of solvent from the filtrates using the low temperature setting of the speedvac afforded the mesylates (**III**) (90−100% yield).

Synthesis of Esters (IV). The mesylates (**III**) were dissolved in DMF (6 mL) in 10-mL, screw-cap vials, and the appropriate carboxylic acid (2 equiv) was added to each. K_2CO_3 (1 equiv) was added, and the reaction mixtures were capped and heated with orbital shaking (225 rpm) at 55 °C on a digiblock heater mounted on an orbital shaker for 12 h. These were cooled to room temperature and transferred to 40-mL capacity tubes. During the transfer, they were diluted with EtOAc (10 mL) and quenched with water (5 mL). The organic layers were separated, and the aqueous layers were extracted with additional EtOAc (5 mL). The combined organic extracts were each washed with sat. $NaHCO_3$ (2 × 5 mL), water (2 × 5 mL), and brine (5 mL) and dried over Na_2SO_4 (∼1 g), as before. These solutions were filtered in parallel into 20-mL vials. Evaporation of solvent from the filtrates using the high temperature setting of the speedvac afforded the esters (**IV**) (59−91% yield).

Synthesis of the Quaternary Ammonium Salts (V). To a solution of the esters (**IV**) in DME (6 mL) in screw-cap vials (10 mL capacity) was added MeI (30 equiv). The reaction mixtures were capped, heated with orbital shaking (225 rpm) at 85 °C on a digiblock shaker for 36 h, and cooled to room temperature. The solvent was completely evaporated using a speedvac, and the crude products were purified by parallel chromatography over Si gel columns (5 × 1 cm). The columns were eluted sequentially with CH_2Cl_2 (20 mL), EtOAc (20 mL), and 10% MeOH in CH_2Cl_2 (30 mL) to obtain the quarternary salts (**V**) (40−92% yield).

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Supporting Information Available. ^{1}H and ^{13}C NMR spectral data. This material is available free of charge via the Web at http://pubs.acs.org.

References and Notes

(1) (a) Singh, M. P.; Peterson, P. J.; Weiss, W. J.; Kong, F.; Greenstein, M. *Antimicrob. Agents Chemother*. **2000**, *44*, 2154−2159. (b) Demain, A. L. *Nat. Biotechnol*. **1998**, *16*, 3−4.

(2) (a) Chopra, I.; Hodgson, J.; Metcalf, B.; Poste, G. *Antimicrob. Agents Chemother*. **1997**, *41*, 497−503. (b) Jack, D. B. *Mol. Med. Today* **1996**, *12*, 499−502. (c) Walsh, C. *Nature* **2000**, *406*, 775−781. (d) Novak, R.; Henriques, B.; Charpentier, E.; Normark, S.; Tuomanen, E. *Nature* **1998**, *399*, 590−593.

(3) (a) Marty, A. M. *Clin. Lab. Med.* **2001**, *21*, 421−434. (b) Marty, A. M.; Conran, R. M.; Kortepeter, M. G. *Clin. Lab. Med.* **2001**, *21*, 411−420. (c) Polgreen, P. M.; Helms, C. *Primary Care* **2001**, *28*, 807−821.

(4) Friedlander, A. M. *Nature* **2001**, *414*, 160−161.

(5) (a) Knight, J. *Nature* **2001**, *414*, 837−838. (b) Cohen, J.; Marshall, E. *Science* **2001**, *294*, 498−501.

(6) Zalkin, H. *Adv. Enzymol. Relat. Areas Mol. Biol*. **1993**, *66*, 203−309.

(7) Spener, R. L.; Preiss, J. *J. Biol. Chem*. **1967**, *242*, 385−392.

(8) (a) Tritz, G. J. NAD Biosynthesis and Recycling. In "*Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology*," Neidhardt, F. C., Ingraham, J. L., Brooks Low, K., Magasanik, B., Schaechter, M., Umbarger, H. E., Eds.; American Society for Microbiology: Washington, DC, 1987; Vol. 1, pp 557−563. (b) Foster, J. W.; Moat, A. G. *Microbiol. Rev.* **1980**, *44*, 83−105.

(9) (a) Rizzi, M.; Galizzi, A. *EMBO J.* **1996**, *15*, 5125−5134. (b) Rizzi, M.; Bolognesi, M.; Coda, A. *Structure* **1998**, *6*, 1129−1140. (c) Devedjiev, Y., Symersky, J.; Singh, R.; Jedrzejas, M.; Brouillette, C.; Brouillette, W.; Muccio, D.; DeLucas, L. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2001**, *57*, 806−812. (d) Symersky, J.; Devedjiev, Y.; Moore, K.; Brouillette, C.; DeLucas, L. *Acta Crystallogr. D: Biol. Crystallogr*. **2002**, *58*, 1138−1146.

(10) Velu, S. E.; Cristofoli, W.; Garcia, G. J.; Brouillette, C. G.; Pierson, M. C.; Luan, C.-H.; DeLucas, L. J.; Brouillette, W. J. *J. Med. Chem.* **2003**, *46*, 3371−3381.

(11) (a) Brouillette W. J.; Muccio, D.; Jedrzejas, M. J.; Brouillette, C. G.; Devedjiev, Y.; Cristofoli, W.; DeLucas, L. J.; Garcia, G. J.; Schmitt, L.; Velu, S. E. U.S. Patent 6,500,852 B1, 2002. (b) Brouillette, W. J.; Brouillette, C. G.; DeLucas, L. J. U.S. Patent 6,673,827, 2004. (c) Brouillette, W. J.; Muccio, D.; Jedrzejas, M. J.; Brouillette, C. G.; Devedjiev, Y.; Cristofoli, W.; DeLucas, L. J.; Garcia, G. J.; Schmitt, L.; Velu, S. E. U.S. Patent 6,727,237, 2004. (d) Brouillette, W. J.; DeLucas, L. J.; Brouillette, C. G.; Velu, S. E.; Kim, Y.-C.; Mou, L.; Porter, S. U.S. Patent 6,861,448, 2005.

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