SAR Studies for a New Class of Antibacterial NAD Biosynthesis Inhibitors

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A new lead class of antibacterial drug-like NAD synthetase (NADs) inhibitors was previously identified from a virtual screening study. Here a solution-phase synthetic library of 76 compounds, analogs of the urea-sulfonamide **5838**, was synthesized in parallel to explore SAR on the sulfonamide aryl group. All library members were tested for enzyme inhibition against NADs and nicotinic acid mononucleotide adenylyltransferase (NaMNAT), the last two enzymes in the biosynthesis of NAD, and for growth inhibition in a *Bacillus anthracis* antibacterial assay. Most compounds that inhibited bacterial growth also showed inhibition against one of the enzymes tested. While only modest enhancements in the enzyme inhibition potency against NADs were observed, of significance was the observation that the antibacterial urea-sulfonamides more consistently inhibited NaMNAT.

Introduction

As the threat of bioterrorism increases[1,2] and the incidence of drug-resistant bacteria multiplies,[3] the need for new antibiotics that act at novel targets becomes more pressing. Our earlier studies[4−6] revealed that inhibition of one such target, the amidotransferase enzyme nicotinamide adenine dinucleotide (NAD) synthetase (NADs),[7] which catalyzes the final step in the biosynthesis of NAD, could hinder both spore outgrowth and vegetative growth of *Bacillus anthracis*, which would provide antibacterial action at two different steps in this bacterium's life cycle.[8,9] These were the first reported inhibitors of NADs and consisted of a general template of two aromatic end groups linked by a polymethylene tether of 6−8 carbons.[4,5] However, the permanent positive charge required on one end and the resulting detergent-like properties of this class of compounds were considered liabilities for further drug development, and we, therefore, sought to discover new, more drug-like lead classes of NADs inhibitors. A virtual screening study[10,11] led to the identification of 4 new lead inhibitors with antibacterial activity, and their structures and activities are shown in Table 1.

In this work, we noted that compounds closely related to the urea-sulfonamides **5617** and **5824**, but containing a reverse sulfonamide, were not widely available commercially. We therefore sought to determine if the reverse sulfonamide analog of **5824** would maintain activity and if the position of the nitro group was important for activity. Thus the synthesis of three **5824** analogs was proposed.

The syntheses of these parent compounds were performed in parallel according to Scheme 1. The ureas **3**{*1*−*3*} were formed on a 100 mg scale according to a literature procedure,[12] coupling *p*-phenylenediamine (**1**) to the appropriate 2-, 3-, and 4-nitrophenylisocyanates **2**{*1*−*3*}. Reactions of these urea-anilines **3**{*1*−*3*} with 3,4-dichlorobenzenesulfonyl chloride (**4**) gave chemset **5**. Compounds **5**{*1*−*3*} were characterized and were >90% pure by mass spectrometry, ^{1}H and ^{13}C NMR, and melting point determination.

As shown in Table 2, the biological activities of **5**{*1*} (4-nitro substituent) and **5824** were similar, while compounds **5**{*2*} and **5**{*3*} were less active. We next proposed SAR studies on the two most active leads, **5824** and **5**. Here we describe an SAR study with **5**, which we pursued first since we had on hand from another study a large group of substituted benzenesulfonyl chlorides suitable for analog synthesis.

Since many of the active NADs inhibitors from our previous virtual screening study[10,11] contained nitro groups, as a first approach, we decided to maintain the mononitro functionality on the urea *N*-aryl group in each of the 3 positions of **5** and to explore several different commercially available R-groups on the sulfonamide phenyl ring (Figure 2).

To this end, larger quantities of compounds **5** were prepared for use in the sulfonamide library synthesis. Though the literature procedure used for the small scale urea reactions called for refluxing conditions, at that temperature we observed a diurea byproduct resulting from the reaction of each of the desired products **3**{*1*−*3*} with another molecule of **2**{*1*−*3*}. Thus, subsequent reactions were performed at 0 °C; these byproducts could not be completely avoided,

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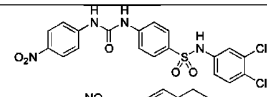
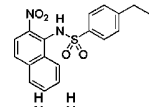
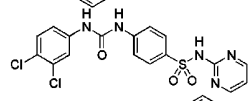
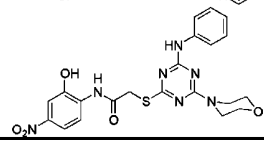
Table 1. Antibacterial Inhibitors of NADs Identified by Virtual Screening[10,11]

Cmpd ID	*Structure*	*NADs IC_{50} (μM)*	*LB[a] B.a. MIC*	*MH[b] B.a. MIC*
5824		6.4	1.9 μM (0.9 μg/mL)	2.8 μM (1.3 μg/mL)
5599		168	6.9 μM (2.4 μg/mL)	3.8 μM (1.3 μg/mL)
5617		77.5	22.1 μM (9.7 μg/mL)	3.8 μM (1.7 μg/mL)
5833		78.3	15 μM (7.2 μg/mL)	7.5 μM (3.6 μg/mL)

[a] Luria−Bertani broth. [b] Mueller-Hinton broth.

Scheme 1. Synthesis of Parent Compounds

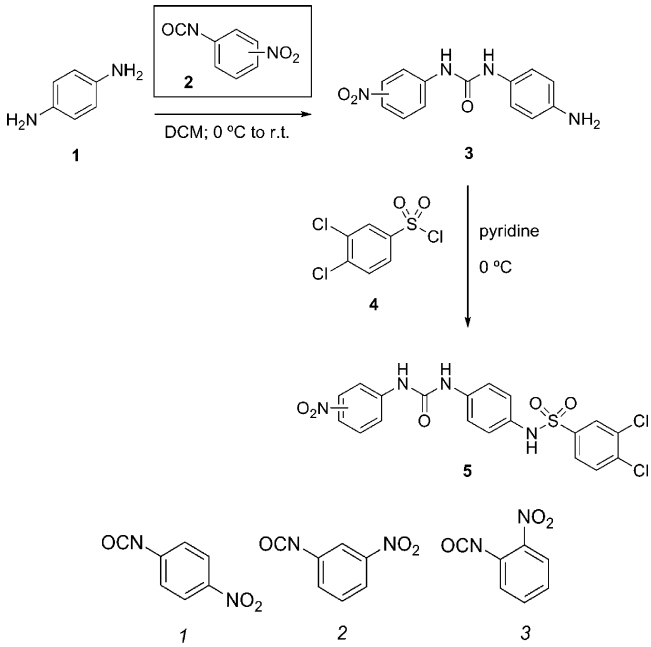


Figure 1. Diversity reagents **2**{*1−3*}.

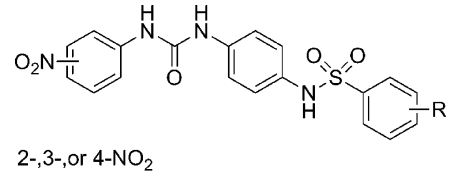
however, resulting in improved, yet moderate to poor yields, especially in the case of **5**{*2*} (see Experimental Section).

Next, the parallel sulfonamide-forming reactions were carried out in 10-mL reaction vials with pyridine as solvent at 0 °C according to a literature procedure (Scheme 2).[13] After workup and solvent removal, unreacted starting materials were removed by partially dissolving the crude reaction mixtures in 6% *i*-PrOH in $CHCl_3$ and filtering off the nearly pure product **7**{*1−3,1−22*}. In a few instances, parallel

Table 2. Reverse Sulfonamide Analogs of **5824** and Their Biological Activities

ID	NADs IC_{50} (μM)	LB *B. anthracis* MIC (μM [μg/mL])	MH *B. anthracis* MIC (μM [μg/mL])
5824	6.4	1.9 [0.9]	2.8 [1.3]
5{*1*}	32.7	2.8 [1.3]	3.8 [1.8]
5{*2*}	95.5	>240 [>115]	-[a]
5{*3*}	443	>240 [>115]	-[a]

[a] Compounds which showed no inhibition in the LB MIC assay were not included in the MH assay.



R	
2- or 3-Me	4-OPh
2-,3-,or 4-F	2-,3-,or 4-CN
2-,3-,or 4-Cl	4-NHAc
2-,3-,or 4-CF_3	3- or 4-OMe
4-Et	3- or 4-COOH
4-Ph	2-,3-,or 4-CH_2NH_2
4-NH_2	

Figure 2. SAR library proposed around parent compounds **5**{*1−3*}.

column chromatography carried out in silica-packed 10-mL syringes was required to obtain sufficiently pure products. No product was obtained for chemset members **7**{*1,15*} and **7**{*2,15*}. Compound identities were supported by mass spectroscopy and the purity of all but two library members was determined to be at least 80% by HPLC/MS. Purities were confirmed for 20 library members (see Table 3) using ^{1}H NMR with an internal standard (hexamethyldisiloxane). Two final compounds (**7**{*2,4*} and **7**{*2,17*}) were found to be less than 80% pure by LC/MS but were tested in that form with the intention of purifying further those which showed significant biological activity.

While most library products could be synthesized in one step from compounds **3**{*1−3*}, a few required additional steps. After the nitrile products **7**{*1−3,18−20*}were obtained, small amounts of each were reduced with 1 M BH_3−THF according to a literature procedure[14] to obtain the corresponding aminomethyl compounds **8**{*1−3,18−20*}, as shown is Scheme 3. Nitrile **7**{*1,18*} was not obtained in sufficient quantity to carry forward to the reduction step. The other nitriles provided reduction products in at least 80% purity (as confirmed by HPLC/MS) after parallel column chromatography using silica-packed 10-mL syringes.

We were also interested in preparing the aniline derivatives of the *N*-acetyl sulfonamide products; however, only the 2-nitro derivative **7**{*3,15*} was obtained successfully from

Scheme 2. Parallel Sulfonamide Library Synthesis

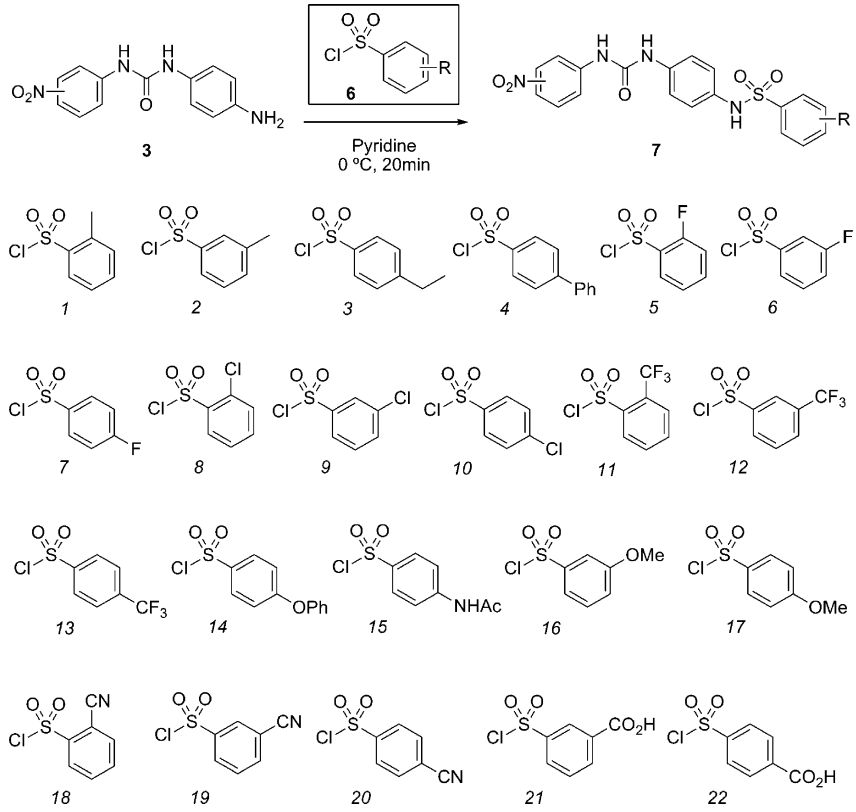


Figure 3. Diversity reagents **6**{*1*−*22*}

the parallel sulfonamide reactions. Therefore, a small amount of **7**{*3*,*15*} was hydrolyzed with hydrochloric acid in methanol according to a literature procedure[15] to give the unprotected aniline **9** (Scheme 4). Isolation of the crude reaction product gave a pale yellow oil, which was confirmed to be greater than 80% pure by HPLC/MS and was, therefore, not purified further.

All of the 75 final products, including the three parents, were first tested as inhibitors of *B. anthracis* NAD synthetase in an HPLC assay and were evaluated for antibacterial activity against *B. anthracis* according to our previously reported procedure.[11] Only compounds that showed some activity in the Luria−Bertani (LB) broth antibacterial assay were assayed again using Mueller Hinton (MH) broth as specified by the Clinical and Laboratory Standards Institute MIC broth microdilution protocol.[16] Subsequently, these same compounds were evaluated as inhibitors of NaMNAT (see below). The structural and biological data are shown in Table 5.

For NAD synthetase, the enzyme inhibition data in Table 5 suggests a few SAR trends. For example, as with the parent compounds, the 2-nitro is the least active of the three nitro positions, with few exceptions (**9**). Interestingly, most changes made on the sulfonamide end of the library members resulted in much poorer NADs enzyme activity. Only 13 compounds exhibited NADs inhibition at or below 300 μM, and none of those significantly inhibited bacterial growth. While the 4-CF_3 group (library members **7**{*1*−*3*,*13*}) gave moderate activity, the only substituents with activity comparable to the parent **5**{*1*} were the aminomethyl and aniline derivatives. At physiological pH the aminomethyl compounds (though not the aniline) should be protonated, bearing a striking resemblance to our earlier class of tethered dimer inhibitors that require a terminal permanent positive charge for enzyme inhibition.[4,6] These results suggest that additional examples of the urea-sulfonamides containing basic groups should be explored, as well as additional H-bond donors and acceptors. The aminomethyl derivatives, unlike **5**{*1*}, were not antibacterial, possibly because of much poorer entry of the protonated amine into the bacterial cell.

A striking observation about the biological activity of this library is the lack of correlation between NADs inhibition and antibacterial activity. We also observed this trend in previous virtual screening studies,[10,11] although our tethered dimer NADs inhibitors exhibited a good correlation between NADs inhibition and antibacterial action.[6] Several possibilities may reasonably explain the lack of antibacterial actions for some NADs inhibitors (e.g., may not permeate into the bacterial cell; may be removed by efflux pumps; may undergo metabolism by bacteria). On the other hand, there are several compounds (**7**{*1*,*1*}, **7**{*1*,*6*}, **7**{*1*,*9*}, **7**{*1*,*12*}, **7**{*1*,*16*}, etc.) that are antibacterial, but which do not inhibit NAD synthetase - a behavior also exhibited by select compounds in previous studies. These compounds must be inhibiting bacterial growth by some mechanism other than NADs inhibition.

In an attempt to explore the latter, all library members were assayed against the enzyme which immediately precedes NADs in the NAD biosynthetic pathway, nicotinic acid mononucleotide adenylyltransferase (NaMNAT).[17] While NaMNAT contains a smaller catalytic site than NADs, both enzymes share ATP as substrate and bind to an *N*-ribosylated

Table 3. Yield and Purity Data for Chemset **7**

chemset **7**	yield (%)	LCMS purity	NMR purity
{*1,1*}	36	87	93
{*2,1*}	54	82	*a*
{*3,1*}	70	82	*a*
{*1,2*}	45	90	94
{*2,2*}	38	82	*a*
{*3,2*}	66	89	*a*
{*1,3*}	70	87	*a*
{*2,3*}	58	86	*a*
{*3,3*}	61	88	96
{*1,4*}	50	80	*a*
{*2,4*}	49	69	*a*
{*3,4*}	57	88	*a*
{*1,5*}	40	93	99
{*2,5*}	50	100	94
{*3,5*}	26	88	*a*
{*1,6*}	29	86	91
{*2,6*}	60	83	*a*
{*3,6*}	75	88	*a*
{*1,7*}	33	85	*a*
{*2,7*}	65	100	98
{*3,7*}	61	90	*a*
{*1,8*}	63	85	*a*
{*2,8*}	45	98	96
{*3,8*}	56	94	*a*
{*1,9*}	68	91	*a*
{*2,9*}	57	85	*a*
{*3,9*}	66	98	96
{*1,10*}	70	94	*a*
{*2,10*}	26	84	*a*
{*3,10*}	65	80	96
{*1,11*}	61	90	91
{*2,11*}	47	84	*a*
{*3,11*}	54	86	*a*
{*1,12*}	46	79	*a*
{*2,12*}	55	87	*a*
{*3,12*}	56	88	91
{*1,13*}	51	84	*a*
{*2,13*}	58	81	*a*
{*3,3*}	75	90	99
{*1,14*}	60	84	*a*
{*2,14*}	63	78	*a*
{*3,14*}	74	88	96
{*1,15*}	*b*		
{*2,15*}	*b*		
{*3,15*}	29	87	*a*
{*1,16*}	79	83	*a*
{*2,16*}	60	100	94
{*3,16*}	51	85	*a*
{*1,17*}	63	100	96
{*2,17*}	56	59	*a*
{*3,17*}	76	88	*a*
{*1,18*}	17	100	*a*
{*2,18*}	71	93	*a*
{*3,18*}	45	94	*a*
{*1,19*}	70	92	*a*
{*2,19*}	49	100	93
{*3,19*}	70	89	*a*
{*1,20*}	66	88	*a*
{*2,20*}	60	100	97
{*3,20*}	36	89	*a*
{*1,21*}	41	88	*a*
{*2,21*}	48	81	*a*
{*3,21*}	13	95	*a*
{*1,22*}	69	92	94
{*2,22*}	52	84	90
{*3,22*}	21	93	*a*

[a] Data not obtained. [b] No product obtained.

nicotinic acid. Thus some small molecule inhibitors designed for NADs might reasonably inhibit NaMNAT. The NaMNAT inhibition data given in Table 5 confirms this supposition.

As with the NADs SAR, there are thus far no clear structural trends among the NaMNAT enzyme inhibitors. In general, the 4-NO_2 position is the most favorable for

Table 4. Yield and Purity Data for Chemset **8**

chemset **8**	aminomethyl position	yield (%)	LCMS purity
{*2,18*}	2	42	81
{*3,18*}	2	14	82
{*1,19*}	3	23	88
{*2,19*}	3	58	82
{*3,19*}	3	12	93
{*1,20*}	4	17	98
{*2,20*}	4	10	100
{*3,20*}	4	63	94

NaMNAT inhibition (as seen with compounds **7**{*1*−*3,11*}). The remaining SAR trends cannot be explained solely by hydrophobicity or electron-donating/withdrawing properties of the substituents on the sulfonamide terminus. The 4 most active NaMNAT inhibitors contain R groups that vary from methoxy, to ethyl, to aminomethyl, to trifluoromethyl, representing 4 very different substituent types. The only group which is not tolerated in the NaMNAT series is the nitrile. Of the 17 NaMNAT inhibitors ($IC_{50} \leq 100\ \mu M$), the position for the R group consistently giving the best activity is the 3-position, with 8 inhibitors (almost half) containing this substitution pattern. Unlike the NADs inhibition data, a number of different substituents give good NaMNAT inhibition, and there is a relationship between NaMNAT inhibition and antibacterial activity. Of the 20 antibacterial library compounds (MH MIC $\leq 30\ \mu M$), 15 (75%) have *B. anthracis* NaMNAT $IC_{50} \leq 50\ \mu M$. Likewise, among the 19 NaMNAT inhibitors with $IC_{50} < 100\ \mu M$ (including two parent compounds), 16 (or 84%) also inhibited bacterial growth below 30 μM, though the direct correlation between IC_{50} and MIC is modest ($R^2 = 0.29$).

This SAR study provided very few low micromolar inhibitors of NADs, making it difficult at this time to draw conclusions related to the antibacterial efficacy of good inhibitors from this structural class, although our earlier work strongly supported the utility of this target. The current study does, however, support NaMNAT as a promising target for the urea-sulfonamides.

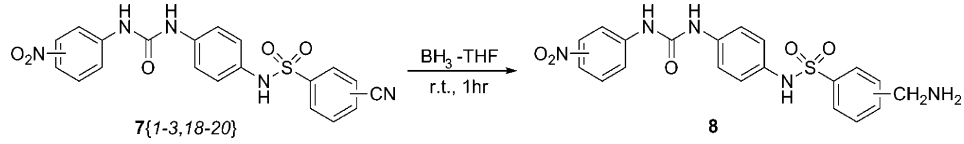
Conclusions

We have utilized parallel solution-phase synthetic chemistry to begin exploring the SAR of a new class of drug-like NAD synthetase inhibitors. Seventy-five new compounds were synthesized and tested in our NADs and NaMNAT enzyme inhibition and *B. anthracis* antibacterial assays. Though we have found no direct correlation between either NADs or NaMNAT IC_{50} and MIC, all but 3 antibacterial compounds from this compound library inhibited at least one of the enzymes. Thus far the hydrophobicity and electronic properties of aryl substituents do not appear to predict the enzyme activity for this class of compounds. Further SAR data appears warranted and is being pursued.

Experimental Section

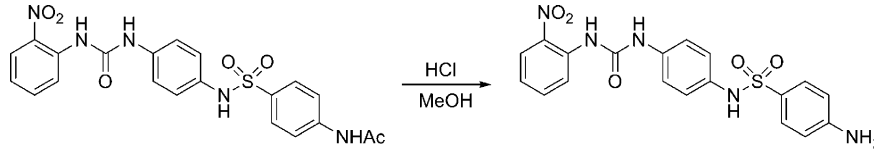
LC/MS Purity Assessment. HPLC analysis was performed using an HP1100 series system with diode array detection coupled with a MicroMass Platform LCZ mass spectrometer. A Phenomenex Luna 5 μm, C18, 100Å, 100 × 4.60 mm column was used for separations. The mobile

Scheme 3. Reduction of Nitriles **7** to the Corresponding Aminomethyls



7{1-3,18-20} 8

Scheme 4. Hydrolysis of *N*-Acetyl **7**{*3*,*15*} to the Corresponding Aniline



7{3,15} 9

phase was (A) H_2O (0.05% formic acid) and (B) acetonitrile (0.05% formic acid). The gradient is listed in Table 6. The flow rate was 0.7 mL/min and diode array detection from 190 − 600 nm was used for each 10 μL injection. The mass spectrometer was equipped with an electrospray ionization (ESI) probe and was operated in both the ESI(+) and ESI(−) mode. Peak height estimation for each analyte was based on baseline integration of peaks observed by the diode array detector.

NMR Internal Standard Purity Assessment. A selection of 20 library members was also examined for purity via an internal standard NMR purity assessment. The stock NMR solution was created by combining $CDCl_3$ and MeOH-d_4 in a 1:1 ratio; 10% DMSO-d_6 was added to aid in solubility, and hexamethyldisiloxane (HMDSO; NMR grade, Aldrich) was added to yield a final HMDSO concentration of 12 μM. A known amount (between 5 and 10 mg) of compound was dissolved into 0.5 mL of the NMR solvent, and the 1H NMR spectrum was recorded using a 400 MHz Bruker spectrometer. Peaks were integrated and calibrated according to a known peak area (methyl, when available; otherwise, a urea NH). Compound purity was determined by comparing the calculated weight based on HMDSO peak integration to the actual weight measured upon sample preparation.

NADs HPLC Enzyme Assay. Library compounds were tested for activity against the enzyme using a HPLC assay that we developed and described previously.[10,11] Briefly, the assay was carried out in two steps: sample preparation and sample analysis. The preparation of sample plates was performed using a BioMek FX liquid handling system. The standard reaction volume was 200 μL. The reaction mixture contained 60 mM HEPPS, pH 8.5, 0.5 mM NH_4Cl, 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM NaAD, 0.2 mM ATP, 6 μg/mL purified *B. anthracis* NADs, 2.5% (v/v) DMSO, 0.3% BOG, and inhibitors at various concentrations. Compounds were assayed beginning at 600 μM and at doubling dilutions down to 0.6 μM. The reaction was initiated by adding 0.2 mM ATP, and quenched after 10 min by adding 50 μL of 6 M guanidine-HCl. The plates were sealed by aluminum tape, and centrifuged at 2500 rpm for 10 min to pellet any precipitation that may have been caused by the inhibitors. Plates were stored at 4 °C prior to the HPLC analysis.

The HPLC procedure utilized a Gilson 215 Liquid Handler, two Gilson 306 pumps, and a Gilson 170 diode array detector. A Phenomenex Luna 5 μm, C5, 100 Å, 100 × 4.60 mm column was used for separations. The mobile phase was (A) 20 mM NaH_2PO_4 pH 6.90 and (B) acetonitrile. The gradient was 100% A from 0 to 3 min and 5% A/95% B from 3 to 4 min for each 20 μL injection. The flow rate was 1.0 mL/min and diode array detection was from 190−400 nm. Peak height estimation for NAD was based on baseline integration. The % inhibition at each inhibitor concentration was calculated by the difference in peak height of NAD compared to reactions without inhibitor. The IC_{50} was determined from the plot of NAD peak height versus inhibitor concentration, and is defined as the concentration of inhibitor required to produce NAD peak height at 50% of the uninhibited reaction. In the development of this assay, peak areas were also used to calculate the IC_{50} for selected active compounds, and similar results were obtained. Each compound was tested in duplicate, and the IC_{50} is reported as the average of duplicate runs.

NaMNAT HPLC Enzyme Assay. This assay monitors the production of NaAD in the enzymatic reaction by separating the reactants and products on an HPLC system. The assay system at pH 7.5 contained 50 mM HEPES, 10 mM $MgCl_2$, 25 μM nicotinic acid mononucleotide (NaMN), 44 μM ATP, 0.3% BOG, 0.25 μg/ml *B. anthracis* NaMNAT, and inhibitors at eleven different concentrations (with 2.5% v/v final DMSO concentration). Under these conditions, the NaMN and ATP concentrations were the same as their Michaelis−Menten constants, which we reported previously.[17] The enzymatic inhibition assay was carried out in 96-well microtiter plates with a total reaction volume of 200 μL.

In each well, 5 μL of DMSO with variable amount of compounds and 170 μL assay buffer containing everything except ATP were first incubated at room temperature for 10 min. The reaction was then initiated by adding 25 μL of ATP solution, and it was allowed to proceed for 10 min. Addition of 50 μL of 6 M guanidine−HCl stopped the reaction. The reaction mixture was next separated on a 4.6 mm × 100 mm Synergi Polar-RP column, using a Shimadzu (Columbia, MD) liquid chromatography system consisting of two pumps, a temperature controlled autosampler with a 12-plate rack changer, a column oven and a photo diode assay (PDA) detector. Separation of NaAD from the other component was achieved in less than 5 min by isocratic elution using 50 mM sodium phosphate as the running buffer at a flow rate of 1.0 mL/min. The peak area at 260 nm was

Table 5. Structures and Biological Activities of Final Library Members

cmpd ID	nitro position	R	NADs IC_{50} (μM)	NaMNAT IC_{50} (μM)	LB MIC (μM [μg/mL])	MH MIC (μM [μg/mL])
5824			10	2	1.9 [0.9]	2.8 [1.3]
5{*1*}	4	3,4,-Cl_2	32.7	7.3	2.8 [1.3]	3.8 [1.8]
5{*2*}	3	3,4,-Cl_2	95.5	382	>240 [>115]	*a*
5{*3*}	2	3,4,-Cl_2	443	>600	>240 [>115]	
7{*1,1*}	4	2-Me	600	16.3	15 [6.4]	7.5 [3.2]
7{*2,1*}	3	2-Me	513	>150	>240 [>102]	
7{*3,1*}	2	2-Me	538	>150	>240 [>102]	
7{*1,2*}	4	3-Me	321	19.9	15 [6.4]	7.5 [3.2]
7{*2,2*}	3	3-Me	>600	>150	>240 [>102]	
7{*3,2*}	2	3-Me	569	>150	>240 [>102]	
7{*1,3*}	4	4-Et	492	>150	>240 [>106]	
7{*2,3*}	3	4-Et	330	12	7.5 [3.3]	<3.8[<1.7]
7{*3,3*}	2	4-Et	>600	>150	>240 [>106]	
7{*1,4*}	4	4-Ph	427	>150	>240 [>117]	
7{*2,4*}	3	4-Ph	480	>150	>240 [>117]	
7{*3,4*}	2	4-Ph	>600	>150	>240 [>117]	
7{*1,5*}	4	2-F	422	22.2	30 [12.9]	15 [6.5]
7{*2,5*}	3	2-F	494	>150	>240 [>103]	
7{*3,5*}	2	2-F	>600	>150	240 103	30 [12.9]
7{*1,6*}	4	3-F	>600	35.8	15 [6.5]	7.5 [3.2]
7{*2,6*}	3	3-F	535	>150	30 [12.9]	15 [6.5]
7{*3,6*}	2	3-F	>600	36.1	30 [12.9]	15 [6.5]
7{*1,7*}	4	4-F	353	370	15 [6.5]	15 [6.5]
7{*2,7*}	3	4-F	463	>150	>240 [>103]	
7{*3,7*}	2	4-F	>600	>150	>240 [>103]	
7{*1,8*}	4	2-Cl	423	17.3	30 [13.4]	7.5 [3.3]
7{*2,8*}	3	2-Cl	>600	20.4	30 [13.4]	7.5 [3.3]
7{*3,8*}	2	2-Cl	>600	>150	>240 [>107]	
7{*1,9*}	4	3-Cl	>600	19.3	7.5 [3.3]	7.5 [3.3]
7{*2,9*}	3	3-Cl	414	17.5	7.5 [3.3]	7.5 [3.3]
7{*3,9*}	2	3-Cl	>600	>150	>240 [>107]	
7{*1,10*}	4	4-Cl	336	>150	>240 [>107]	
7{*2,10*}	3	4-Cl	287	>150	>240 [>107]	
7{*3,10*}	2	4-Cl	>600	>150	>240 [>107]	
7{*1,11*}	4	2-CF_3	505	13.3	15 [7.2]	15 [7.2]
7{*2,11*}	3	2-CF_3	>600	>150	>240 [>115]	
7{*3,11*}	2	2-CF_3	>600	>150	>240 [>115]	
7{*1,12*}	4	3-CF_3	>600	26.7	7.5 [3.6]	7.5 [3.6]
7{*2,12*}	3	3-CF_3	293	>150	>240 [>115]	
7{*3,12*}	2	3-CF_3	298	>150	>240 [>115]	
7{*1,13*}	4	4-CF_3	168	>150	>240 [>115]	
7{*2,13*}	3	4-CF_3	143	150	>240 [>115]	
7{*3,13*}	2	4-CF_3	560	150	>240 [>115]	
7{*1,14*}	4	4-OPh	327	>150	>240 [>121]	
7{*2,14*}	3	4-OPh	362	225	30 [15]	>60 [30]
7{*3,14*}	2	4-OPh	>600	>150	>240 [>121]	
7{*3,15*}	2	4-NHAc	370	>150	>240 [>113]	
7{*1,16*}	4	3-OMe	600	25.0	15 [6.6]	15 [6.6]
7{*2,16*}	3	3-OMe	>600	>150	60 [26]	15 [6.6]
7{*3,16*}	2	3-OMe	>600	>150	>240 [>106]	
7{*1,17*}	4	4-OMe	>600	10.1	30 [13]	7.5 [3.3]
7{*2,17*}	3	4-OMe	>600	>150	>240 [>106]	
7{*3,17*}	2	4-OMe	>600	>150	>240 [>106]	
7{*1,18*}	4	2-CN	526	>150	>240 [>105]	
7{*2,18*}	3	2-CN	>600	>150	>240 [>105]	
7{*3,18*}	2	2-CN	590	>150	>240 [>105]	
7{*1,19*}	4	3-CN	546	>150	>240 [>105]	
7{*2,19*}	3	3-CN	>600	>150	>240 [>105]	
7{*3,19*}	2	3-CN	>600	>150	>240 [>105]	
7{*1,20*}	4	4-CN	>600	>150	60 [26]	15 [6.6]
7{*2,20*}	3	4-CN	>600	>150	>240 [>105]	
7{*3,20*}	2	4-CN	>600	>150	>240 [>105]	
7{*1,21*}	4	3-CO_2H	537	>150	>240 [>109]	
7{*2,21*}	3	3-CO_2H	274	>150	>240 [>109]	
7{*3,21*}	2	3-CO_2H	456	150	>240 [>109]	
7{*1,22*}	4	4-CO_2H	498	>150	>240 [>109]	
7{*2,22*}	3	4-CO_2H	>600	>150	>240 [>109]	
7{*3,22*}	2	4-CO_2H	432	150	>240 [>109]	
8{*2,18*}	3	2-CH_2NH_2	>600	>150	>240 [>106]	
8{*3,18*}	2	2-CH_2NH_2	>600	>150	>240 [>106]	
8{*1,19*}	4	3-CH_2NH_2	33.2	74.6	60 [26]	
8{*2,19*}	3	3-CH_2NH_2	30	>200	120 [53]	
8{*3,19*}	2	3-CH_2NH_2	245	>150	>240 [>106]	
8{*1,20*}	4	4-CH_2NH_2	26	12.1	>240 [>106]	
8{*2,20*}	3	4-CH_2NH_2	15.3	51.2	>240 [>106]	
8{*3,20*}	2	4-CH_2NH_2	24.5	>150	>240 [>106]	
9	2	4-NH_2	17.1	>150	120 [53]	

[a] Data not obtained because of poor LB MIC.

used to quantify NaAD. Percent inhibition was calculated based on the difference in NaAD production between controls (DMSO only) and samples containing the compounds. The IC_{50} value was determined by plotting % inhibition versus

Table 6. Linear Gradient Used for Purity Estimation of Library Members by LC/MS

time (minutes)	A (%)	B (%)
0.00	80.0	20.0
10.00	10.0	90.0
11.00	10.0	90.0
11.50	80.0	20.0

compound concentrations and is reported as the average of duplicate runs.

Antibacterial Assay. All library members were also screened against *Bacillus anthracis Sterne* in an antibacterial assay, which we also previously reported.[10,11] Briefly, *B. anthracis Sterne* spores were subcultured from stock cultures into Luria−Bertani (LB) broth and incubated for 2−3 h at 37 °C in ambient air until the OD_{600} measurement reached 0.5 to 0.6 when the bacteria are in midlog phase. The cultures were diluted 1:1 into LB Broth with an absorbance at 600 nm measuring 0.25 to 0.3, then were added to plates containing 240 μM samples of the compounds to be tested. Compounds were tested at a final DMSO concentration of 1%. The plates were incubated at 37 °C, and absorbance at 600 nm was read at 0 h and every hour for 5 h. Any compounds which inhibited growth of the vegetative cell (as compared to the control containing only DMSO) were screened in the full MIC determination starting at 240 μM and creating doubling dilutions down to 7.5 μM in quadruplicate wells. A plot of cell density versus time yields inhibition of growth results, and the MIC is defined as the lowest concentration of compound required to completely inhibit growth (100% inhibition). MIC is reported as the average of the four data points acquired for each compound. Controls for each assay measured sterility, *B. anthracis Sterne* viability, and MIC_{100} for the clinical antibiotic ciprofloxacin hydrochloride (from MP Biomedicals).

All compounds which showed antibacterial action in the LB assay were then assayed according to the Clinical and Laboratory Standards Institute MIC broth microdilution protocol,[16] which standardizes the number of bacteria used in the inoculum as 5×10^5 cfu/mL, using cation-adjusted Mueller−Hinton (MH) broth, except that measurements were taken at 5 h, as opposed to 20 h.

Synthetic Chemistry. General. Melting points were determined using a Mel-Temp Electrothermal 1201-D apparatus and are uncorrected. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz (^{1}H) spectrometer using tetramethylsilane (TMS) as internal standard. Reactions were monitored by TLC (Whatmann silica gel, UV254, 25 μm plates), and flash column chromatography utilized Baker silica gel (40 μm) in the solvent system indicated. Anhydrous solvents used for reactions were purchased in SureSeal bottles from Aldrich Chemical Co. Other reagents were purchased from Aldrich, Alfa Aesar or Acros chemical companies and used as received. Parallel reactions were carried out in 10 mL screw-cap vials and were agitated by hand. Parallel workups were carried out in 50 mL conical Falcon tubes, were concentrated in 15 mL glass vials using a Savant SpeedVac Plus SC210A, and where indicated, were purified by parallel silica gel chromatography (gravity) in 10 mL disposable syringes.

Preparation of Lead Compounds 5{*1*−*3*} (Scheme 1). *N*-(4-Aminophenyl)-*N*′-(4-nitrophenyl)urea (3{*1*}). *p*-Phenylenediamine (**1**) (12 g, 0.11 mol) was partially dissolved in anhydrous CH_2Cl_2 (60 mL) under a nitrogen atmosphere, and the reaction vessel was submerged in an ice bath. A solution of 4-nitrophenylisocyanate **2**{*1*} (22 g, 0.13 mol) in anhydrous CH_2Cl_2 (60 mL) was added slowly to the cooled reaction vessel via an addition funnel over a course of 20 min with vigorous mechanical stirring, resulting in immediate precipitation of product. Once the addition was complete, the ice bath was removed, and the reaction continued with stirring at room temperature for an additional 20 min. TLC (15% *i*-PrOH in $CHCl_3$) showed that the diamine and isocyanate starting materials were gone; there was one new product spot (reaction with ninhydrin confirmed the presence of an amine), and one baseline spot corresponding to the diurea byproduct. Solvent was removed under vacuum to obtain a mixture of the two products (crude weight 29 g, 95% yield), which were then stirred in hot acetone (2 L). The diurea byproduct remained insoluble and was filtered off. Solvent was removed under vacuum, and the pure product **3**{*1*} was obtained as a dense yellow powder (21 g, 71%): mp 221−223 °C (decomposed). ^{1}H NMR (DMSO-d_6) δ 9.26 (s, 1H, NH), 8.39 (s, 1H, NH), 8.16 (dd, 2H, J = 9.33, 3.06 Hz), 7.66 (dd, 2H, J = 9.39, 3.06 Hz), 7.09 (dd, 2H, J = 8.79, 3.06 Hz), 6.52 (dd, 2H, J = 8.76, 3.09 Hz), 4.85 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 152.18, 146.88, 144.66, 140.59, 127.66, 125.15, 121.18, 117.10, 114.08. MS (ES^+): *m*/*z* 273 (M + H); MS (ES^-): *m*/*z* 271 (M − H).

The following were also prepared by this method:

***N*-(4-Aminophenyl)-*N*′-(3-nitrophenyl)urea (3{*2*}).** From **2**{*2*} (12 g, 0.11 mol) was obtained **3**{*2*} (8.2 g, 27%) as a pure yellow powder: mp 212−214 °C (decomposed). ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H, NH), 8.55 (t, 1H, J = 2.21), 8.31 (s, 1H, NH), 7.78 (m, 1H), 7.67, (m, 1H), 7.53 (t, 1H, J = 8.15 Hz), 7.09 (dd, 2H, J = 8.57, 3.06 Hz), 6.52 (dd, 2H, J = 8.56, 3.03 Hz), 4.84 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 152.76, 148.16, 144.53, 141.55, 129.97, 127.90, 124.01, 121.26, 115.78, 114.08, 111.81. MS (ES^+): *m*/*z* 273 (M + H); MS (ES^-): *m*/*z* 271 (M − H).

***N*-(4-Aminophenyl)-*N*′-(2-nitrophenyl)urea (3{*3*}).** From **2**{*3*} (12 g, 0.11 mol) was obtained **3**{*3*} (14 g, 48%) as a bright orange powder: mp 192−194 °C (decomposed). ^{1}H NMR (DMSO-d_6) δ 9.51 (s, 1H, NH), 9.36 (s, 1H, NH), 8.34 (d, 1H, J = 8.49), 8.08 (dd, 1H, J = 8.37, 1.42 Hz), 7.67 (td, 1H, J = 7.83, 1.48 Hz), 7.15 (td, 1H, J = 7.81, 1.21 Hz), 7.11 (d, 2H, J = 8.57 Hz), 6.53 (d, 2H, J = 8.55 Hz), 4.88 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 151.98, 144.75, 136.98, 135.66, 135.04, 127.75, 125.40, 122.14, 121.62, 121.25, 114.11. MS (ES^+): *m*/*z* 273 (M + H); MS (ES^-): *m*/*z* 271 (M − H).

3,4-Dichloro-(*N*-(4-(((4-nitrophenyl)amino)carbonyl)aminophenyl))benzenesulfonamide (5{*1*}). To a solution of *N*-(4-aminophenyl)-*N*′-(4-nitrophenyl) urea **3**{*1*} (1.5 g, 5.5 mmol) in anhydrous pyridine (15 mL) at 0 °C was slowly added 3,4-dichlorobenzenesulfonyl chloride (**4**) (1.0 mL, 1.6 g, 6.6 mmol). The reaction was stirred under a nitrogen atmosphere for 40 min and was diluted with EtOAc (100 mL). The reaction was quenched by adding 2 N HCl (50

mL), and the layers were separated; the organic layer was washed further with 2 N HCl (2 × 50 mL), water (100 mL), and brine (75 mL) and was dried over anhydrous Na_2SO_4. The drying agent was filtered, and the solvent was removed under reduced pressure. The residue (2.1 g, 84%) was taken up in hot methanol (300 mL) and was decolorized with activated charcoal, boiling for 30 min. The decolorizing agent was removed by gravity filtration, the filtrate was reduced to 150 mL, and the pure product crystallized to give **5**{*1*} as an off-white solid (1.2 g, 47%): mp 207−209 °C. ^{1}H NMR (DMSO-d_6) δ 10.25 (s, 1H, NH), 9.41 (s, 1H, NH), 8.91 (s, 1H, NH), 8.18 (dd, 2H, J = 9.29, 3.03 Hz), 7.89 (d, 1H, J = 2.10 Hz), 7.85 (d, 1H, J = 8.45 Hz), 7.66 (dd, 2H, J = 9.38, 3.11 Hz), 7.63 (dd, 1H, J = 8.45, 2.16 Hz), 7.38 (dd, 2H, J = 8.96, 2.96 Hz), 7.02 (dd, 2H, J = 8.95, 2.97 Hz). ^{13}C NMR (DMSO-d_6) δ 151.99, 146.40, 141.09, 139.77, 136.45, 136.04, 132.20, 131.78, 131.36, 128.49, 126.93, 125.25, 122.73, 119.60, 117.56. MS (ES$^-$): *m*/*z* 479 (M − H).

The following compounds were prepared by this method with minor changes in purification as noted:

3,4-Dichloro-(*N*-(4-(((3-nitrophenyl)amino)carbonyl)aminophenyl))benzenesulfonamide (5{*2*}). From **3**{*2*} (30 mg, 0.11 mmol) was obtained **5**{*2*} (26 mg, 49%): mp 210.5−212 °C (MeOH). Pure product was obtained by recrystallization from the decolorization solvent MeOH. ^{1}H NMR (DMSO-d_6) δ 10.22 (s, 1H, NH), 9.18 (s, 1H, NH), 8.81 (s, 1H), 8.53 (s, 1H, NH), 7.88 (s, 1H), 7.82 (m, 2H), 7.65 (m, 2H), 7.54 (t, 1H, J = 8.12 Hz), 7.37 (d, 2H, J = 8.60), 7.01 (d, 2H, J = 8.58 Hz). MS (ES$^-$): *m*/*z* 479 (M − H).

3,4-Dichloro-(*N*-(4-(((2-nitrophenyl)amino)carbonyl)aminophenyl))benzenesulfonamide (5{*3*}). From **3**{*3*} (50 mg, 0.18 mmol) was obtained **5**{*3*} (32 mg, 37%): mp 206.5−208 °C (MeOH). Pure product was obtained by recrystallization from the decolorization solvent MeOH. ^{1}H NMR (DMSO-d_6) δ 10.22 (s, 1H, NH), 9.83 (s, 1H, NH), 9.56 (s, 1H, NH), 8.26 (d, 1H, J = 8.48 Hz), 8.09 (dd, 1H, J = 8.32, 1.25 Hz), 7.89 (d, 1H, J = 2.13 Hz), 7.85 (d, 1H, J = 8.45 Hz), 7.69 (t, 1H, J = 7.86 Hz), 7.62 (dd, 1H, J = 8.44, 2.12 Hz), 7.39 (d, 2H, J = 8.80 Hz), 7.20 (td, 1H, J = 7.80, 1.14 Hz), 7.02 (d, 2H, J = 8.84 Hz). MS (ES$^-$): *m*/*z* 479 (M − H).

Preparation of Library Members 7 (Scheme 2). Procedure for Parallel Sulfonamide Synthesis. The starting urea-amines (**3**) (0.55 mmol) were partially dissolved in pyridine (1.5 mL) in 10-mL, screw-cap vials, and the reaction vials were placed in a rack and submerged in an ice bath. The appropriate sulfonyl chlorides (1.2 equiv) were added to each vial; the vials were capped and the entire apparatus was shaken manually at 0 °C for 20 min. The vials were removed from the ice bath; the reactions were quenched with 1 N HCl (1 mL), extracted with EtOAc (3 × 2 mL), and the organic layers were transferred to 50-mL Falcon tubes. The combined EtOAc extracts were again washed with 1 N HCl (2 × 2 mL) and water (2 × 2 mL). Carboxylic acid products (**70**−**75**) were extracted into saturated $NaHCO_3$ (2 × 3 mL); the aqueous layers were combined, acidified to pH 3 with concentrated HCl, and extracted with EtOAc (3 × 5 mL). All products were dried over Na_2SO_4, and the solutions were filtered in parallel into 15-mL screw-cap vials. Evaporation of the solvent using the high temperature setting of a speedvac afforded the crude sulfonamide products. All residues were triturated with 6% *i*-PrOH in $CHCl_3$ (∼2 mL) to dissolve any unreacted starting materials, and the products were suction filtered to afford the library members, **7** (13−79% yield). LC/MS of these products revealed that most met the 80% purity criteria; those that did not were further purified in parallel by passing through a short silica plug (5 × 1 cm) using 10-mL syringes and 6% *i*-PrOH in $CHCl_3$ as eluent.

Preparation of Aminomethyl Compounds (8) (Scheme 3). Procedure for Parallel Nitrile Reduction. To the starting nitriles **7**{*1*−*3*,*18*−*20*} (0.080−0.26 mmol) in anhydrous THF (final concentration of **V** = 1.0 M) in 5-mL vials was added BH_3 (1.0 M in THF; 1.3 equiv) at room temperature. The vials were capped, and the reactions stirred under nitrogen for 1 h. Concentrated HCl was added to quench the excess hydride present in the reaction, and all solvents were removed using the high temperature setting of the speedvac. To the residue was added 2 N NaOH (1 mL), and the amines were extracted into EtOAc (3 × 2 mL). The organic extracts were combined, washed with water (2 mL) and brine (2 mL), dried over Na_2SO_4, and filtered in parallel into 15-mL vials. The solvent was again removed via the speedvac; residues were taken up in minimal amounts of $CHCl_3$/*i*-PrOH (3:1) and purified by silica gel, eluting first with $CHCl_3$, then gradually increasing polarity to 1:1 $CHCl_3$/*i*-PrOH. Column fractions appearing to be at least 80% pure by TLC were combined into 15-mL vials and concentrated to dryness via a speedvac to yield library members **8** (10−63% yield).

Preparation of Aniline 9 (Scheme 4). 4-Amino-(*N*-(4-(((2-nitrophenyl)amino)carbonyl)aminophenyl))benzenesulfonamide (9). The *N*-acetyl sulfonamide product **7**{*3*,*15*} (32 mg, 0.068 mmol) was dissolved in MeOH (1 mL), and concentrated HCl (0.32 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature and was quenched with 2 N NaOH (0.5 mL). The product was extracted into EtOAc (3 × 1 mL); the combined extracts were washed with brine (1 mL) and dried over Na_2SO_4. After filtration, the solvent was removed under vacuum, and TLC (15% *i*-PrOH in $CHCl_3$) revealed one major new spot. No further purification was pursued, and **9** was obtained as an oil (9.4 mg, 32%). MS (ES$^+$): *m*/*z* 428 (M + H).

Supporting Information Available. Proton spectra of selected library members. This material is available free of charge via the Internet at http://pubs.acs.org.

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References and Notes

(1) (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) Friedlander, A. M. *Nature (London)* **2001**, *414*, 160–161. (d) Cohen, J. *Science* **2001**, *294*, 500.

(2) (a) Smiley, S. T. *Adv. Exp. Med. Biol.* **2007**, *603*, 376. (b) Santic, M.; Molmeret, M.; Klose, K. E.; Abu Kwaik, Y. *Trends Microbiol.* **2006**, *14*, 37. (c) Knight, J. *Nature (London)* **2001**, *414*, 837–838.

(3) Walsh, C. T.; Wright, G. D. *Chem. Rev.* **2005**, *105*, 391–394.

(4) (a) Velu, S. E.; Cristofoli, W.; Garcia, G. J.; Brouillette, C. G.; Pierson, M.; Luan, C.-H.; DeLucas, L. J.; Brouillette, W. J. *J. Med. Chem.* **2003**, *46*, 3371–3381. (b) Velu, S. E.; Luan, C.-H.; DeLucas, L. J.; Brouillette, C. G.; Brouillette, W. J. *J. Comb. Chem.* **2005**, *7*, 898–904. (c) Velu, S. E.; Mou, L.; Luan, C.-H.; DeLucas, L. J.; Brouillette, C. G.; Brouillette, W. J. *J. Med. Chem.* **2007**, *50*, 2612–2621.

(5) (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.

(6) Moro, W. B. The Design and Synthesis of Antibacterial Inhibitors of NAD Synthetase. Ph. D. Dissertation, University of Alabama, Birmingham, AL, 2007, Chapter 1, pp 23−92.

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

(8) Sutherland, S. *Drug Discovery Today* **2003**, *8*, 335–336.

(9) Nessi, C.; Albertini, A. M.; Speranza, M. L.; Galizzi, A. *J. Biol. Chem.* **1995**, *270*, 6181–6185.

(10) Moro, W. B.The Design and Synthesis of Antibacterial Inhibitors of NAD Synthetase. Ph. D. Dissertation, University of Alabama, Birmingham, AL, 2007, Chapter 2, pp 93−122.

(11) Moro, W. B.; Yang, W.; Kane, T.; Brouillette, C. G.; Brouillette, W. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2001−2005.

(12) Lewis, F. D.; Delos Santos, G. B.; Liu, W. *J. Org. Chem.* **2005**, *70*, 2974–2979.

(13) Natarajan, A.; Guo, Y.; Harbinski, F.; Fan, Y.-H.; Chen, H.; Luus, L.; Diercks, J.; Aktas, H.; Chorev, M.; Halperin, J. A. *J. Med. Chem.* **2004**, *47*, 4979–4982.

(14) Brown, H. C.; Subba Rao, B. C. *J. Am. Chem. Soc.* **1960**, *82*, 681–686.

(15) Van Meter, C. T.; Lowy, A. *J. Am. Chem. Soc.* **1941**, *63*, 1330–1331.

(16) (a) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard. In *The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS)*, 7th ed.; January 2006, *26* (2), M7−A7. (b) Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. In *The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS)*; January 2008, *28* (1), M100-S18(replaces 27 (1), M100-S17).

(17) Lu, S.; Smith, C. D.; Yang, Z.; Pruett, P. S.; Nagy, L.; McCombs, D.; Delucas, L. J.; Brouillette, W. J.; Brouillette, C. G. *Acta Crystallogr., Sect. F* **2008**, *64*, 893–898.

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