Tethered Dimers as NAD Synthetase Inhibitors with Antibacterial Activity

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The solution-phase parallel synthesis of tethered dimers was employed to identify lead inhibitors of bacterial NAD synthetase. Active dimers contained two aromatic end groups joined by a polymethylene linker, with one end group containing a permanent positive charge. Effective inhibitors of NAD synthetase also inhibited the growth of Gram-positive (but not Gram-negative) bacteria, including antibiotic-resistant strains. The desmethyl precursors of active inhibitors lacked a permanent positive charge and were inactive as either enzyme inhibitors or antibacterial agents. Similarly, a close structural analogue of the most active inhibitors contained two additional ether oxygens in the tether and was inactive in both assays. These results are consistent with the premise that NAD synthetase inhibition is responsible for the antibacterial actions and support further studies on NAD synthetase as a new target for antibacterial agents.

Antibiotics have played a major role in the field of medicine by providing effective cures for life threatening bacterial infections.[1] However, during the past decade, antibiotic-resistant bacteria have emerged.[2] For example, approximately 50−80% of *Staphylococcus aureus* strains are resistant to methicillin and other antibiotics, about 30% of *Streptococcus pneumoniae* are resistant to penicillin,[3] and vancomycin-resistant *Enterococci* have become a significant problem. These issues are compounded by decreasing introductions of new antibiotics, particularly as compared to the period 1947−70 when a variety of new antibacterials with new structures were discovered.[2a] Bacterial and other infectious diseases have also received major attention because of their potential for use in biological warfare and bioterrorism,[4,5] emphasized by recent attacks using *Bacillus anthracis* (anthrax) in the United States.[6] Obviously, the development of new antibacterial agents must be a high priority.

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 (NADS) belongs to the amidotransferase family[7] and catalyzes the last step in the biosynthesis of NAD, transforming nicotinic acid adenine dinucleotide (NaAD) into the amide product NAD via a two-step process (Figure 1).[8] The inhibition of bacterial NADS appeared likely to provide antibacterial actions since NAD plays an important role in energy metabolism and numerous biochemical transformations such as DNA repair, DNA recombination, and protein-ADP ribosylation.[9] Additionally, this enzyme is required for the outgrowth of spore forming bacteria into the vegetative cell.[10]

We utilized the protein crystal structure of *Bacillus subtilis* NAD synthetase in complex with the natural substrates to guide the design of potential inhibitors.[11] As a first approach we attempted to bias design toward the NaAD binding subsite with the goal of obtaining inhibitors that were chemically simpler than NaAD. We thus based early libraries on simple tethered dimers containing aromatic end groups and a polymethylene linker of varying size (Figure 2). This approach resulted in low micromolar inhibitors of *B. subtilis* NAD synthetase that also exhibited effective antibacterial actions against the growth of Gram-positive bacteria.

Results and Discussion

The crystal structure of *B. subtilis* NAD synthetase in complex with substrate/substrate analogues has been published.[11] NAD synthetase is a homodimer with molecular weight around 60 000 and NaAD binds to a subsite at the homodimer interface in an extended conformation. To mimic this bound conformation of NaAD, we designed chemically simple tethered dimers containing aromatic rings at each end of a polymethylene linker (Figure 2).

For example, two aromatic "end groups" that were initially utilized are 5-nitroindole (**1**) and nicotinic acid (Scheme 1). Such compounds offered potentially straightforward synthetic approaches to attach a polymethylene tether via the indole NH and the carboxylate of nicotinic acid. To explore the potential of this approach, the individual synthesis of seven tethered dimers (**6a**−**g**) was initially proposed (Scheme 1).

As shown in Scheme 1, 5-nitroindole (**1**) was alkylated with bromo alcohol acetates (**2a**−**g**) using NaH in DMF to obtain the *N*-alkylated products **3a**−**g** in 75−83% yield. The acetate group of **3a**−**g** was then removed by treatment with aq. K_2CO_3 in MeOH to obtain the alcohols **4a**−**g** in 82−90% yield. These were converted to the corresponding esters **5a**−**g** in 80−90% yield by

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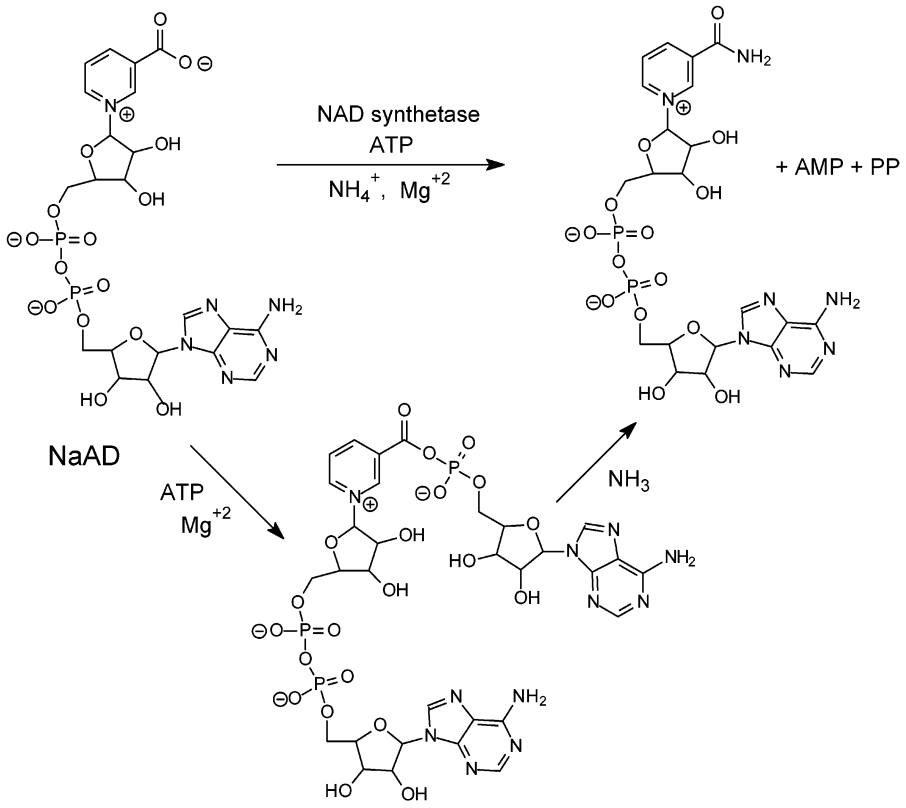


Figure 1. The reaction catalyzed by NAD synthetase.

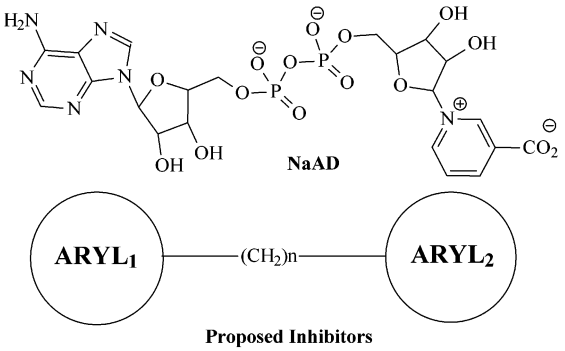


Figure 2. Similarity between the structure of the substrate NaAD and the proposed biaryl tethered dimer inhibitors.

treatment with nicotinic acid in CH_2Cl_2 in the presence of DCC and DMAP. A positive charge on the N atom of the pyridine ring (found also in NAD) was then introduced by the reaction of **5a**−**g** with MeI in DME to afford the pure *N*-methylpyridinium salts **6a**−**g** in 74−84% yield.

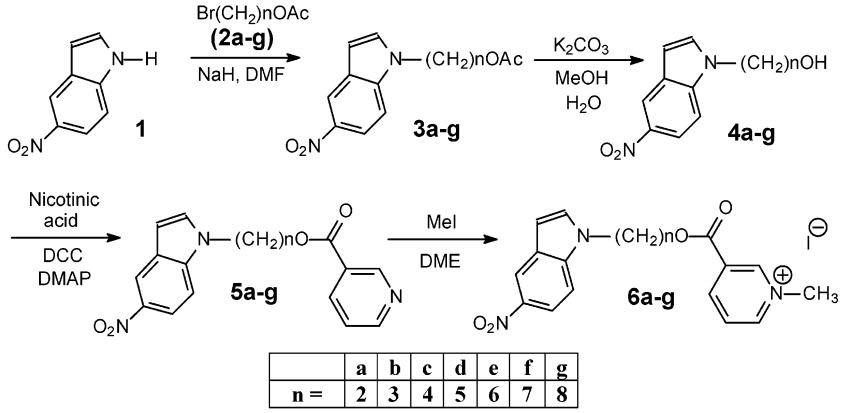
These initial tethered dimers were next evaluated as inhibitors of purified *B. subtilis* NAD synthetase. The IC_{50} values for **5a**−**g** and **6a**−**g** are given in Table 1. Since moderate enzyme inhibition ($IC_{50} = 0.5$ mM) was observed for longer tether lengths, although only when the pyridine N contained a positive charge, the synthesis of additional tethered dimers using parallel methods was proposed.

The four-step synthesis of dimers **6a**−**g** utilized only one chromatographic purification step, following the esterification using DCC/DMAP. We modified this esterification procedure to one more suitable for parallel synthesis. We found that conversion of the alcohol to a mesylate and treatment with nicotinic acid in the presence of K_2CO_3 in DMF appeared promising for parallel synthesis without requiring column chromatography. A library of 66 tethered dimers was then designed (Scheme 2).

The library was composed of three different indoles, 5-methoxyindole (**I_a**), 5-benzyloxyindole (**I_b**), and 5-nitroindole (**I_c**). Six different tether lengths were provided by using bromo alcohol acetates containing from four to nine carbons. Four different aromatic amino acids were also incorporated.

All reactions were carried out and worked up in parallel. As summarized in Scheme 2, the indoles (**I_{a-c}**) were alkylated with the bromo alcohol acetates in the presence of NaH in DMF at room temperature. Small amounts of unreacted bromoacetates were removed by treatment with an alkyl halide sequestering resin, polymer bound tris(2-aminoethyl)amine (4.1 mmol N/g). Filtration of the resin and removal of the solvent on a speedvac gave 18 alkylated products (**$II_{(a-c)n}$**). These were treated with aqueous K_2CO_3 in methanol to remove the acetate groups, cleanly providing 18 alcohols (**$III_{(a-c)n}$**), which were converted to the corresponding mesylates (**$IV_{(a-c)n}$**). Four different carboxylic acids, namely, nicotinic acid, 3-(*N*,*N*-dimethylamino)benzoic acid, 4-(*N*,*N*-dimethylamino)benzoic acid, and 4-(*N*,*N*-dimethylamino)phenylacetic acid, were chosen for the next esterification step. The MeO- and BnO-substituted intermediates (12 compounds) were then treated with each of the four carboxylic acids in the presence of K_2CO_3 in DMF. The remaining six mesylates (NO_2 derivatives) were also esterified with the above acids, except for nicotinic acid (previously prepared). This procedure afforded the 66 esters (**$V_{(a-c)nQ1}$**), which were reacted with MeI in DME to obtain 66 final products (**$VI_{(a-c)nQ2}$**). These were purified by parallel chromatog-

Scheme 1



Scheme 2

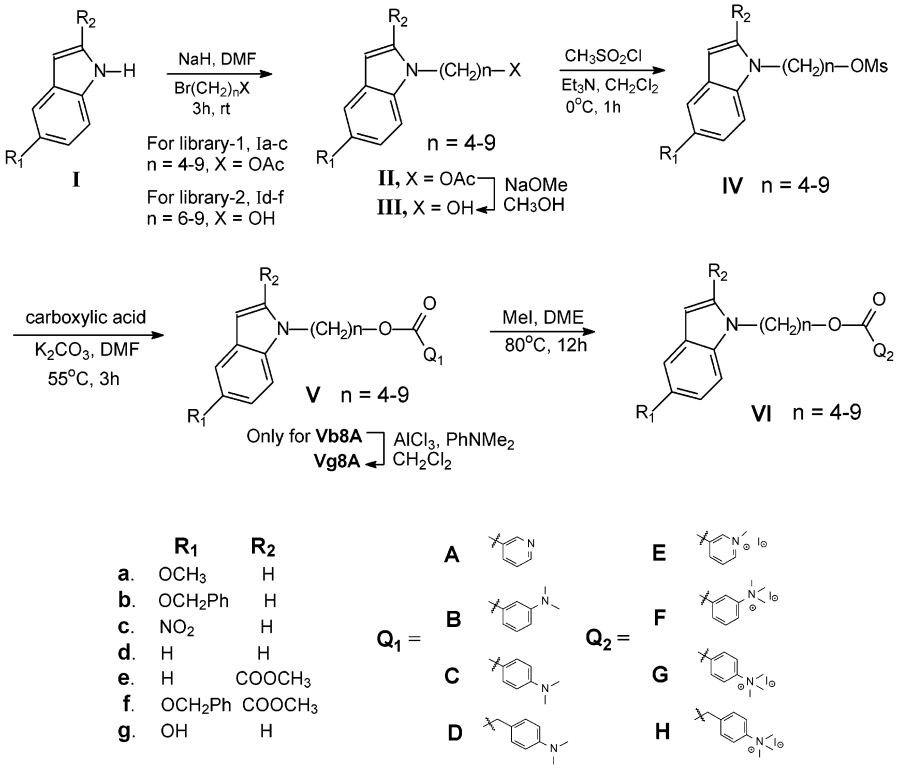


Table 1. Inhibition of *B. subtilis* NAD Synthetase by Initial Tethered Dimers

	5a	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**
n =	2	3	4	5	6	7	8	2	3	4	5	6	7	8
IC_{50} (mM)	>1	>1	>1	>1	>1	>1	>1	>1	>1	>1	1	0.5	-	-

raphy in silica-packed syringes, using a vacuum manifold, and had a purity >90% as determined by HPLC-MS. All of the products $\mathbf{V_{(a-c)nQ1}}$ and $\mathbf{VI_{(a-c)nQ2}}$ given in Scheme 2 were evaluated as inhibitors of purified *B. subtilis* NAD synthetase. IC_{50} values for the most active inhibitors (IC_{50} < 100 μM) are given in Table 2.

From the results in Table 2 it is observed that 5-benzyloxyindole consistently provides the best enzyme inhibition when present at one end of the dimer, and *N*-methylnicotinic acid and 4-(*N*,*N*,*N*-trimethylammonio)phenylacetic acid were most effective at the other end. The most effective linker sizes were $n = 6-9$.

To evaluate the effect of moderately polar substituents on the indole ring, an additional library was then designed (Scheme 2). Three different indoles, namely, unsubstituted indole ($\mathbf{I_d}$), methyl indole-2-carboxylate ($\mathbf{I_e}$), and methyl 5-benzyloxyindole-2-carboxylate ($\mathbf{I_f}$), were chosen for this library. Four different linker sizes ($n = 6-9$) were included. Two different end groups, nicotinic acid and 4-(*N*,*N*-dimethylamino)phenylacetic acid, were also selected. In this case, all bromo alcohols were used directly as the free alcohol. As shown in Scheme 2, the indoles ($\mathbf{I_{d-f}}$) were alkylated with four different bromo alcohols in the presence of NaH in DMF to obtain 12 long chain alcohols ($\mathbf{III_{(d-f)n}}$). These were converted to the corresponding mesylates ($\mathbf{IV_{(d-f)n}}$), which were esterified with nicotinic acid and 4-(*N*,*N*-dimethylamino)phenylacetic acid in the presence of K_2CO_3 in DMF. Aqueous workup afforded 24 different esters ($\mathbf{V_{(d-f)nQ1}}$), which were reacted with MeI in DME to obtain 24 final products ($\mathbf{VI_{(d-f)nQ2}}$). These were

Table 2. Structures and IC_{50} Values (most active) for Compounds Defined in Library 1 (Scheme 2)

compd	R_1	R_2	*n*	Q_2	IC_{50} (μM)	compd	R_1	R_2	*n*	Q_2	IC_{50} (μM)
VI$_{a4E}$	OCH_3	H	4	E	-	**VI$_{a7E}$**	OCH_3	H	7	E	24
VI$_{b4E}$	OCH_2Ph	H	4	E	89	**VI$_{b7E}$**	OCH_2Ph	H	7	E	-
VI$_{a4F}$	OCH_3	H	4	F	-	**VI$_{a7F}$**	OCH_3	H	7	F	-
VI$_{b4F}$	OCH_2Ph	H	4	F	-	**VI$_{b7F}$**	OCH_2Ph	H	7	F	-
VI$_{c4F}$	NO_2	H	4	F	-	**VI$_{c7F}$**	NO_2	H	7	F	-
VI$_{a4G}$	OCH_3	H	4	G	-	**VI$_{a7G}$**	OCH_3	H	7	G	-
VI$_{b4G}$	OCH_2Ph	H	4	G	-	**VI$_{b7G}$**	OCH_2Ph	H	7	G	-
VI$_{c4G}$	NO_2	H	4	G	-	**VI$_{c7G}$**	NO_2	H	7	G	-
VI$_{a4H}$	OCH_3	H	4	H	-	**VI$_{a7H}$**	OCH_3	H	7	H	-
VI$_{b4H}$	OCH_2Ph	H	4	H	84	**VI$_{b7H}$**	OCH_2Ph	H	7	H	30
VI$_{c4H}$	NO_2	H	4	H	89	**VI$_{c7H}$**	NO_2	H	7	H	-
VI$_{a5E}$	OCH_3	H	5	E	-	**VI$_{a8E}$**	OCH_3	H	8	E	-
VI$_{b5E}$	OCH_2Ph	H	5	E	94	**VI$_{b8E}$**	OCH_2Ph	H	8	E	20
VI$_{a5F}$	OCH_3	H	5	F	-	**VI$_{a8F}$**	OCH_3	H	8	F	-
VI$_{b5F}$	OCH_2Ph	H	5	F	-	**VI$_{b8F}$**	OCH_2Ph	H	8	F	-
VI$_{c5F}$	NO_2	H	5	F	-	**VI$_{c8F}$**	NO_2	H	8	F	-
VI$_{a5G}$	OCH_3	H	5	G	-	**VI$_{a8G}$**	OCH_3	H	8	G	-
VI$_{b5G}$	OCH_2Ph	H	5	G	-	**VI$_{b8G}$**	OCH_2Ph	H	8	G	-
VI$_{c5G}$	NO_2	H	5	G	-	**VI$_{c8G}$**	NO_2	H	8	G	-
VI$_{a5H}$	OCH_3	H	5	H	-	**VI$_{a8H}$**	OCH_3	H	8	H	-
VI$_{b5H}$	OCH_2Ph	H	5	H	60	**VI$_{b8H}$**	OCH_2Ph	H	8	H	20
VI$_{c5H}$	NO_2	H	5	H	70	**VI$_{c8H}$**	NO_2	H	8	H	-
VI$_{a6E}$	OCH_3	H	6	E	-	**VI$_{a9E}$**	OCH_3	H	9	E	-
VI$_{b6E}$	OCH_2Ph	H	6	E	29	**VI$_{b9E}$**	OCH_2Ph	H	9	E	-
VI$_{a6F}$	OCH_3	H	6	F	-	**VI$_{a9F}$**	OCH_3	H	9	F	-
VI$_{b6F}$	OCH_2Ph	H	6	F	-	**VI$_{b9F}$**	OCH_2Ph	H	9	F	-
VI$_{c6F}$	NO_2	H	6	F	-	**VI$_{c9F}$**	NO_2	H	9	F	-
VI$_{a6G}$	OCH_3	H	6	G	-	**VI$_{a9G}$**	OCH_3	H	9	G	-
VI$_{b6G}$	OCH_2Ph	H	6	G	-	**VI$_{b9G}$**	OCH_2Ph	H	9	G	-
VI$_{c6G}$	NO_2	H	6	G	-	**VI$_{c9G}$**	NO_2	H	9	G	-
VI$_{a6H}$	OCH_3	H	6	H	-	**VI$_{a9H}$**	OCH_3	H	9	H	-
VI$_{b6H}$	OCH_2Ph	H	6	H	69	**VI$_{b9H}$**	OCH_2Ph	H	9	H	-
VI$_{c6H}$	NO_2	H	6	H	84	**VI$_{c9H}$**	NO_2	H	9	H	24

Table 3. Structures and IC_{50} Values (most active) for Compounds Defined in Library 2 (Scheme 2)

compd	R_1	R_2	*n*	Q_2	IC_{50} (μM)	compd	R_1	R_2	*n*	Q_2	IC_{50} (μM)
VI$_{d6E}$	H	H	6	E	-	**VI$_{d8E}$**	H	H	8	E	-
VI$_{e6E}$	H	$COOCH_3$	6	E	-	**VI$_{e8E}$**	H	$COOCH_3$	8	E	44
VI$_{f6E}$	OCH_2Ph	$COOCH_3$	6	E	37	**VI$_{f8E}$**	OCH_2Ph	$COOCH_3$	8	E	10
VI$_{d6H}$	H	H	6	H	-	**VI$_{d8H}$**	H	H	8	H	63
VI$_{e6H}$	H	$COOCH_3$	6	H	24	**VI$_{e8H}$**	H	$COOCH_3$	8	H	14
VI$_{f6H}$	OCH_2Ph	$COOCH_3$	6	H	12	**VI$_{f8H}$**	OCH_2Ph	$COOCH_3$	8	H	20
VI$_{d7E}$	H	H	7	E	95	**VI$_{d9E}$**	H	H	9	E	48
VI$_{e7E}$	H	$COOCH_3$	7	E	34	**VI$_{e9E}$**	H	$COOCH_3$	9	E	34
VI$_{f7E}$	OCH_2Ph	$COOCH_3$	7	E	18	**VI$_{f9E}$**	OCH_2Ph	$COOCH_3$	9	E	27
VI$_{d7H}$	H	H	7	H	-	**VI$_{d9H}$**	H	H	9	H	54
VI$_{e7H}$	H	$COOCH_3$	7	H	19	**VI$_{e9H}$**	H	$COOCH_3$	9	H	10
VI$_{f7H}$	OCH_2Ph	$COOCH_3$	7	H	9	**VI$_{f9H}$**	OCH_2Ph	$COOCH_3$	9	H	18

purified by parallel silica chromatography as before and had a purity >90% as determined by HPLC-MS.

All of the products **V$_{(d–f)nQ1}$** and **VI$_{(d–f)nQ2}$** in Scheme 2 were evaluated as inhibitors of purified *B. subtilis* NAD synthetase. The IC_{50} values of the most potent inhibitors identified from this library are given in the Table 3. As shown, the methoxycarbonyl group appears to be beneficial for inhibition, even in the absence of the benzyloxy group.

Several of the most active NAD synthetase inhibitors were selected for MIC determination against different strains of Gram-positive and Gram-negative bacteria. For comparison purposes, identical MIC determinations were performed with rifampin and methicillin. The results are given in Table 4.

As shown in Table 4, the selected NAD synthetase inhibitors are very effective inhibitors of the growth of Gram-positive bacteria, but are inactive against Gram-negative bacteria (e.g., *Pseudomonas* and *Salmonella*). Some compounds are similar in effectiveness to rifampin and methicillin. Of particular interest is the observation that methicillin-resistant strains of *S. aureus* also remain susceptible.

Other Studies. A few additional compounds were prepared individually in order to investigate the effect of specific structural changes on activity. The phenolic analogue of **VI$_{b8E}$** (IC_{50} = 20 μM), compound **VI$_{g8E}$**, was synthesized to confirm the importance of the hydrophobic benzyl group for enzyme activity (Scheme 2). Compound **V$_{b8A}$** (R = $PhCH_2O$, *n* = 8) in Scheme 2 was synthesized individually starting from **I$_b$** as shown in Scheme 2. Compound **V$_{b8A}$** was debenzylated using $AlCl_3$ in the presence of *N*,*N*-dimethylaniline to obtain phenol **V$_{g8A}$** in 75% yield, and methylation with MeI in DME gave the ammonium salt **VI$_{g8E}$** in 74% yield. The enzyme inhibition assay for **VI$_{g8E}$** revealed that it was inactive (IC_{50} > 200 μM), illustrating the importance of the hydrophobic benzyl group.

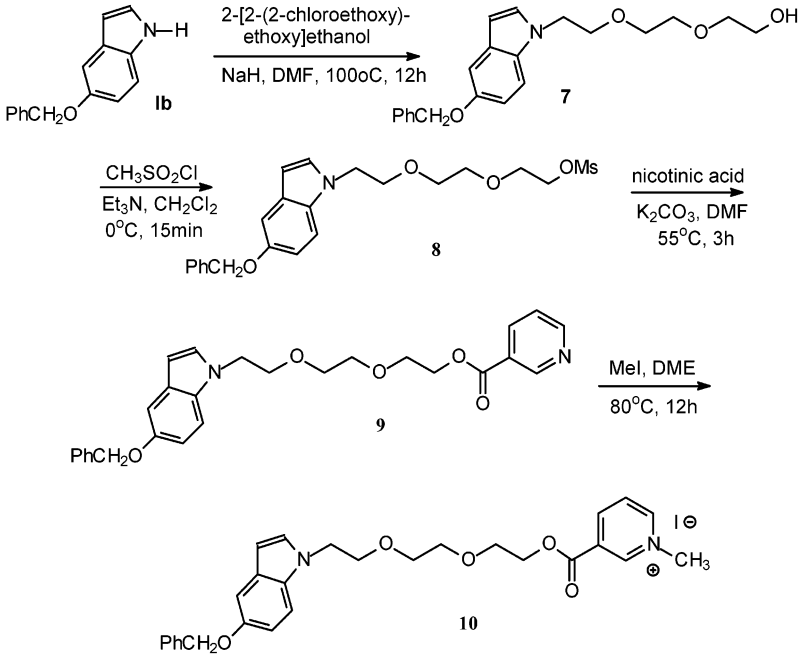
A second analogue of **VI$_{b8E}$** was also synthesized, but in this case more polar functionality was placed in the

Table 4. Antibacterial Activities (MIC, μM) for Selected NAD Synthetase Inhibitors

compound	*Bacillus subtilis* ATCC# 14289	*Staphylococcus aureus* ATCC# 29213	*Staphylococcus aureus* (MRSA[a]) ATCC# 33591	*Staphylococcus aureus* (MRSA[a]) ATCC# 33592	*Staphylococcus aureus* (MRSA[a]) ATCC# 33593	*Pseudomonas aeruginosa* ATCC# 27853	*Salmonella enteritidis* ATCC# 13076
10[c]	>50	>50[b]					
VI$_{b8E}$[c]	6.2	1.5	12.5	6.2	3.1	>50	>50
VI$_{b8H}$[c]	1.5	3.1				>50	>50
VI$_{f7E}$[c]	6.2	3.1				>50	>50
VI$_{f8E}$[c]	12.5	6.2				>50	>50
VI$_{e7H}$[d]	1.5	12.5				>50	>50
VI$_{e8H}$[d]	6.2	3.1				>50	>50
VI$_{e9H}$[d]	3.1	1.5				>50	>50
VI$_{f7H}$[d]	1.5	1.5	6.2	3.1	3.1	>50	>50
VI$_{f9H}$[d]	1.5	6.2				>50	>50
rifampin	1						
methicillin		1	32	32	32		
gentamicin						2	
trimethoprin							0.5

[a] MRSA = methicillin-resistant *Staphylococcus aureus*. [b] Determined to be inactive in a separate experiment for several *S. aureus* strains: ATCC# 1117, 1120, and 1152. [c] These compounds were synthesized individually and fully characterized by spectroscopic and combustion analysis. [d] These compounds were evaluated as the initial library synthesis product.

Scheme 3



hydrophobic linker. As shown in Scheme 3, target **10** was synthesized starting from 5-benzyloxyindole (**I$_b$**), using the approach previously described in Scheme 2 but incorporating 2-[2-(2-chloroethoxy)ethoxy]ethanol in the first step. Interestingly, although compound **10** is a close structural analogue of the best inhibitors, it was inactive in the enzyme inhibition assay (IC_{50} >200 μM). As shown in Table 4, compound **10** was also inactive in the antibacterial assays. Consistent with this observation, selected intermediate pyridines and anilines in Scheme 1 (compounds **5a**−**g**) and Scheme 2 (intermediates **V$_{(a-f)nQ1}$**) were evaluated and found to be inactive as enzyme inhibitors (IC_{50} > 200 μM), and all of these were also inactive as antibacterial agents against the vegetative growth of *Bacillus subtilis* (MIC > 50 μg/mL; data not shown). These results suggest that (1) a permanent positive charge on the pyridine or aniline nitrogen is required for enzyme inhibition and antibacterial activities, and (2) correspondence between the inhibition of NAD synthetase and antibacterial effects for structurally similar compounds is consistent with the premise that NAD synthetase is the antibacterial target for these agents.

The possibility that the antibacterial actions of these inhibitors might be wholly or partly due to nonspecific effects must be considered. This question is raised by (1) the detergent-like properties expected for cationic inhibitors, and (2) the range of tether lengths providing good activity. Here we have partly addressed the former concern, since two cationic analogues (**VI$_{g8E}$** and **10**) were not active in either the enzyme inhibition or antibacterial assays. Additionally, it appears unlikely that antibacterial actions observed at such a low concentration, in some cases near 1 μg/mL, would result from what should be relatively modest detergent/surfactant properties for these compounds. Ongoing studies have been designed to further address these issues.

Conclusions

We have used solution-phase parallel synthesis to identify a simple class of tethered dimers that inhibit NAD synthetase and are effective antibacterial agents. The antibacterial actions were selective for Gram-positive bacteria, including antibacterial resistant strains, and no significant effects were observed against selected Gram negative strains. Close structural analogues of the best inhibitors, resulting from incorporation of a polyether linkage or by omission of the *N*-methyl group and the permanent positive charge, were inactive as enzyme inhibitors and as antibacterial agents, consistent with the premise that effects on NAD synthetase result in the antibacterial actions. Further studies to better understand the mechanism of enzyme inhibition, to validate the target, and to improve antibacterial potency are being pursued.

Experimental Section

Cloning, Overexpression, and Purification of NAD Synthetase (NADS). The *OutB* gene (1-kb) encoding *Bacillus subtilis* NAD synthetase (NADS) was cloned into a prokaryotic expression vector pET 21 (Novagen Inc.). After transformation in *E. coli* BL21(DH3) plysS cells, single colonies were propagated overnight at 37 °C in LB media containing 50 μg/mL ampicillin and 34 μg/mL chroramphenicol. Large scale cultures (4−6 L) were grown in LB media to 0.8 O.D. (600 nm) and induced with 1 mM isopropylthio-D-galactoside (IPTG) for 4 h with constant shaking at 37 °C. Cells were harvested by centrifugation at 4500 rpm/15 min, and the pellet was stored at −80 °C until use.

The frozen cells were thawed and resuspended in buffer A (50 mM Tris, 1 mM EDTA, 0.1 mM PMSF, 1 mM DTT, pH 7.5). The cell suspension was treated with DNAse (1 mg/mL) for 30 min or subjected to a sonication step. After centrifugation at 30 000 rpm for 30 min, clear lysate was loaded onto a preparative Superdex-75 column (26 × 60 cm, Pharmacia) supported by a FPLC system using buffer A with 1% glycerol. NADS peak fractions were combined and further purified by ion-exchange chromatography (Bio-Q column, BioRad) using a linear gradient of 0−0.5 M NaCl. Finally, Amicon-concentrated samples (using 10K-ultrafiltration membrane) were resolved on a Superdex-75 column as above. This three-step protocol rendered ~30 mg NADS per liter with >95% electrophoretic purity. The enzyme concentration was determined using an extinction coefficient of 26 930 M^{-1} cm^{-1} (280 nm). Purified NADS samples (5−6 mg/mL) were stored in 30% glycerol at −80 °C, and assayed for enzyme activity prior to use.

Enzyme Inhibition Assay. This coupled assay monitors the production of NAD in the enzyme reaction through its conversion to NADH in the presence of ethanol and alcohol dehydrogenase (ADH) from bakers' yeast (Sigma).

(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. While 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.

The assay system at pH 8.5 contained 58.5 mM HEPPS buffer, 18.5 mM NH_4Cl, 19.5 mM KCl, 9.75 mM $MgCl_2$, 1% (v/v) EtOH, 0.1 mM NaAD, 0.3% BOG, 40 μg/mL ADH, 2.0 μg/mL NAD synthetase, 0.2 mM ATP, and inhibitor at several concentrations (with 2.5% (v/v) final DMSO concentration). Under these conditions the NaAD and ATP concentrations exceed their respective Michaelis−Menton constants; $K_{m,NaAD}$= 70 μM and $K_{m,ATP}$ = 100 μM. The enzyme inhibition assay was carried out in 96-well microtiter plates with a total reaction volume of 200 μL.

Two detection methods were simultaneously employed, UV absorbance at 340 nm and fluorescence emission at 460 nm, both resulting from the NADH produced in the reaction. OD was read on a SpectraMax *Pro* microplate reader (Molecular Devices), and fluorescence was read on a PolarStar microplate reader (BMG-LabTechnologies). Because of potential absorbance and fluorescence emission by the compounds at the assay detection wavelengths, background readings were recorded before initiating the reaction by addition of ATP. In the HTS screen, for each well, after adding 5 μL of DMSO with variable amount of compounds and 170 μL assay buffer containing everything except ATP, readings of OD at 340 and 500 nm and fluorescence with excitation at 320 nm and emission at 460 nm were recorded as background signal, I_{well}(background). After initiation by adding 25 μL of ATP solution, the reaction was allowed to proceed for 10 min and stopped by adding 50 μL of 6 M guanidine·HCl. The readings of OD and fluorescence were recorded as sample signal, I_{well}(sample). The OD and fluorescence intensity readings of each plate were normalized by using the 12 wells of the first row to calculate the percentage inhibition for each well,

$$\%\ \mathrm{inhibition_{well}} = \Delta I_{well}/\langle \Delta I_{control} \rangle \times 100\%$$

where $\Delta I_{well} = I_{well}$ (sample) − I_{well} (background) and $\langle \Delta I_{control} \rangle$ is the maximum intensity calculated as an average of the 12-point reading with background subtraction. Then % $\mathrm{inhibition_{well}}$ data were used to calculate the percentage inhibition data for each compound at six concentrations. The IC_{50} value was determined by plotting the percentage inhibition vs compound concentration.

Antibacterial Assays. Minimum inhibitory concentrations (MIC) were determined by MicroBioTest, Inc., of Sterling, VA. Bacteria were subcultured from stock cultures onto the appropriate agar and incubated overnight at 37 ± 2 °C in ambient air. At least five colonies from the overnight cultures were inoculated into 3 mL of the appropriate broth and thoroughly mixed. A portion (0.1 mL) of this suspension was diluted to 10 mL with the appropriate broth and incubated on a shaking incubator at 37 ± 2 °C for 5−6 h. Each suspension of the bacterial broth was adjusted to contain approximately 5×10^8 CFU/mL.

All test substances were supplied as 5 mg/mL stock solutions in 100% DMSO. For each MIC determination, the test substance was diluted 1:100 with broth medium into two tubes to give a total volume of 4 mL each (containing 1% DMSO final concentration) and a starting concentration of 50 μg/mL. For each MIC determination, two sets of nine tubes, each containing 2 mL of the appropriate broth medium (and containing 1% DMSO), were prepared. Serial doubling dilutions were performed for each duplicate set of 10 tubes by transferring 2 mL of material from the first tube to the second, thoroughly mixing, and then transferring 2 mL to the next tube, as before, until the 10th tube. From the 10th tube, 2 mL of material was discarded. Each tube was then inoculated with the challenge microorganism broth (0.01 mL), and the tubes were incubated at 37 ± 2 °C for 20 h. The MIC was defined as the lowest concentration of test substance that completely inhibited visible growth of the microorganism. Controls were included for viability, sterility, and reproducible MIC values for known antibiotics.

Synthetic Chemistry. General. Melting points were determined using an Electrothermal 9100 apparatus and are uncorrected. IR spectra were recorded 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. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and the results indicated by symbols for the elements were within ±0.4% of theoretical values. 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 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) mounted on an orbital shaker (purchased from VWR). Parallel evaporation of solvents was carried out in 20 mL wide mouth vials using a Savant SC-210 speedvac. Parallel filtrations were performed using the outer tubes of 5 mL plastic disposable syringes packed with cotton mounted on a Burdick & Jackson 24-port manifold (purchased from VWR). Preparative parallel chromatography was similarly performed on Baker flash silica gel packed in the outer tubes of 10 mL plastic disposable syringes using the same 24-port manifold.

General Procedure for *N*-Alkylation of Indoles. This procedure is illustrated for the preparation of 2-(5-nitro-*1H*-indol-1-yl)ethyl acetate (**3a**).

To a solution of 5-nitroindole (**1**) (1.01 g, 6.23 mmol) in anhydrous DMF (10 mL) was added NaH (0.274 g, 6.85 mmol, 60% dispersion in oil), and the mixture was stirred at room temperature for 30 min. A solution of 2-bromoethyl acetate (**2a**) (0.75 mL, 6.8 mmol) in DMF (2 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 h. The reaction was then quenched with saturated NH_4Cl (25 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were combined and washed with water (3 × 20 mL) and brine (2 × 20 mL). Removal of solvent from the dried (Na_2SO_4) extract afforded the crude product, which was purified by flash chromatography on silica gel using hexane/acetone (5:1) to give the pure alkylated product 2-(5-nitro-*1H*-indol-1-yl)ethyl acetate **3a** (1.21 g, 78.3%).

2-(5-Nitro-1*H*-indol-1-yl)ethyl Acetate (3a). Oil; ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 4.43 (s, 4H), 6.72 (d, 1H), 7.27 (d, 1H), 7.40 (d, 1H), 8.14 (dd, 1H), and 8.59 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.5, 45.3, 62.6, 104.4, 109.1, 117.1, 118.1, 127.6, 131.1, 138.9, 141.5, and 170.3; IR (neat) 1740 cm^{-1}; MS (ES) *m*/*z* 249 (M + H).

3-(5-Nitro-1*H*-indol-1-yl)-1-propyl Acetate (3b). Yield 81%; mp 65−66 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.19 (p, 2H), 4.06 (t, 2H), 4.29 (t, 2H), 6.70 (d, 1H), 7.26 (d, 1H), 7.36 (d, 1H), 8.13 (dd, 1H), and 8.59 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.5, 28.8, 43.1, 60.8, 103.9, 108.8, 116.8, 117.8, 127.4, 130.8, 138.5, 141.1, and 170.5; IR (KBr) 1740 cm^{-1}; MS (ES) *m*/*z* 263 (M + H); Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

4-(5-Nitro-1*H*-indol-1-yl)-1-butyl Acetate (3c). Yield 83%; mp 87−89 °C; ^{1}H NMR ($CDCl_3$) δ 1.59−1.71 (m, 2H), 1.85−1.99 (m, 2H), 2.05 (s, 3H), 4.09 (t, 2H), 4.25 (t, 2H), 6.68 (d, 1H), 7.26 (d, 1H), 7.35 (d, 1H), 8.10 (dd, 1H), and 8.57 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.7, 25.8, 26.6, 46.1, 63.3, 103.9, 109.0, 116.9, 118.0, 127.5, 130.8, 138.5, 141.2, and 170.8; IR (KBr) 1735 cm^{-1}; MS (ES) *m*/*z* 277 (M + H); Anal. ($C_{14}H_{16}N_2O_4$) C, H, N.

5-(5-Nitro-1*H*-indol-1-yl)-1-pentyl Acetate (3d). Yield 91%; mp 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 1.35−1.45 (m, 2H), 1.63−1.74 (m, 2H), 1.86−1.97 (m, 2H), 2.00 (s, 3H), 4.06 (t, 2H), 4.25 (t, 2H), 6.71 (d, 1H), 7.27 (d, 1H), 7.37 (d, 1H), 8.14 (dd, 1H), and 8.61 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.6, 22.9, 27.8, 29.5, 46.3, 63.6, 103.5, 108.9, 116.6, 117.7, 127.3, 130.8, 138.4, 141.0, and 170.7; IR (KBr) 1732 cm^{-1}; MS (ES) *m*/*z* 291 (M + H); Anal. ($C_{15}H_{18}N_2O_4$) C, H, N.

6-(5-Nitro-1*H*-indol-1-yl)-1-hexyl Acetate (3e). Yield 79%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.30−1.45 (m, 4H), 1.55−1.66 (m, 2H), 1.81−1.92 (m, 2H), 2.03 (s, 3H), 4.03 (t, 2H), 4.17 (t, 2H), 6.68 (d, 1H), 7.25 (d, 1H), 7.35 (d, 1H), 8.11 (dd, 1H), and 8.59 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.6, 25.2, 26.2, 28.1, 29.8, 46.4, 63.9, 103.5, 108.9, 116.6, 117.8, 127.3, 130.8, 138.5, 141.1, and 170.8; IR (KBr) 1734 cm^{-1}; MS (ES) *m*/*z* 305 (M + H); Anal. ($C_{16}H_{20}N_2O_4$) C, H, N.

7-(5-Nitro-1*H*-indol-1-yl)-1-heptyl Acetate (3f). Yield 75%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.29−1.39 (m, 6H), 1.54−1.65 (m, 2H), 1.81−1.92 (m, 2H), 2.04 (s, 3H), 4.03 (t, 2H), 4.17 (t, 2H), 6.68 (d, 1H), 7.20 (d, 1H), 7.35 (d, 1H), 8.12 (dd, 1H), and 8.60 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.9, 25.7, 26.7, 28.4, 28.7, 30.1, 46.8, 64.3, 103.8, 109.1, 117.0. 118.2, 127.6, 130.9, 138.7, 141.4, and 171.1; IR (neat) 1730 cm^{-1}; MS (ES) *m*/*z* 319 (M + H).

8-(5-Nitro-1*H*-indol-1-yl)-1-octyl Acetate (3g). Yield 73%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.21−1.35 (m, 8H), 1.52−1.69 (m, 2H), 1.79−1.92 (m, 2H), 2.03 (s, 3H), 4.03 (t, 2H), 4.15 (t, 2H), 6.67 (d, 1H), 7.25 (d, 1H), 7.34 (d, 1H), 8.10 (dd, 1H), and 8.58 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 20.8, 25.6, 26.6, 28.3, 28.8 (2C), 30.0, 46.7, 64.3, 103.6, 109.1, 116.8, 118.0, 127.5, 130.9, 138.6, 141.2, and 171.1; MS (ES) *m*/*z* 333 (M + H); Anal. ($C_{18}H_{24}N_2O_4$) C, H, N.

General Procedure for Deacetylation. This procedure is illustrated for the preparation of 2-(5-nitro-*1H*-indol-1-yl)-ethanol (**4a**).

To a solution of **3a** (0.493 g, 1.98 mmol) in MeOH (25 mL) was added K_2CO_3 (0.823 g, 5.96 mmol) followed by the dropwise addition of water (8 mL). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated to dryness, and the residue was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with water (2 × 10 mL) and brine (1 × 10 mL) and dried (Na_2SO_4). Removal of the solvent from the dried extract gave the crude product, which was purified by flash column chromatography (20 × 3 cm) over silica gel using EtOAc/hexanes (3:2) to afford the pure alcohol **4a** (0.348 g, 85.0%).

2-(5-Nitro-1*H*-indol-1-yl)ethanol (4a). Oil; ^{1}H NMR ($CDCl_3$) δ 1.78 (t, 1H, OH), 4.01 (q, 2H), 4.33 (t, 2H), 6.69 (d, 1H), 7.33 (d, 1H), 7.39 (d, 1H), 8.08 (dd, 1H), and 8.54 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 48.8, 61.4, 103.8, 109.2, 116.8, 117.9, 127.5, 131.6, 138.9, and 140.8; MS (ES) *m*/*z* 207 (M + H).

3-(5-Nitro-1*H*-indol-1-yl)-1-propanol (4b). Yield 95%; mp 86.2−87.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 1H, OH), 2.06 (p, 2H), 3.59 (dt, 2H), 4.32 (t, 2H), 6.66 (d, 1H), 7.26 (d, 1H), 7.39 (d, 1H), 8.08 (dd, 1H), and 8.56 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 32.4, 42.9, 58.7, 103.8, 109.2, 116.8, 118.0, 127.4, 131.2, 138.8, and 141.0; IR (KBr) 3300 cm^{-1}; MS (ES) *m*/*z* 221 (M + H); Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

4-(5-Nitro-1*H*-indol-1-yl)-1-butanol (4c). Yield 98%; mp 63−64 °C, ^{1}H NMR ($CDCl_3$) δ 1.42 (bs, 1H, OH), 1.51−1.66 (m, 2H), 1.91−2.03 (m, 2H), 3.62−3.71 (m. 2H), 4.22 (t, 2H), 6.68 (d, 1H), 7.26 (d, 1H), 7.36 (d, 1H), 8.10, (dd, 1H) 8.59 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 26.5, 29.4, 46.4, 61.7, 103.7, 109.1, 116.7, 117.9, 127.4, 130.9, 138.6, and 141.0; IR (neat) 3384 cm^{-1}; MS (ES) *m*/*z* 235 (M + H); Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

5-(5-Nitro-1*H*-indol-1-yl)-1-pentanol (4d). Yield 90%; mp 93−94 °C, ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 1H, OH), 1.35−1.47 (m 2H), 1.55−1.71 (m, 2H), 1.85−1.96 (m, 2H), 3.64 (q, 2H), 4.19 (t, 2H), 6.69 (d, 1H), 7.26 (d, 1H), 7.36 (d, 1H), 8.12 (dd, 1H), and 8.60 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 22.9, 29.7, 31.8, 46.5, 62.1, 103.6, 109.0, 116.7, 117.9, 127.4, 130.9, 138.5, and 141.0; IR (KBr) 3742 cm^{-1}; MS (ES) *m*/*z* 249 (M + H); Anal. ($C_{13}H_{16}N_2O_3$) C, H, N.

6-(5-Nitro-1*H*-indol-1-yl)-1-hexanol (4e). Yield 91%; mp 78−79 °C, ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 1H, OH), 1.30−1.48 (m, 4H), 1.50−1.61 (m, 2H), 1.82−1.94 (m, 2H), 3.63 (q, 2H), 4.18 (t, 2H), 6.68 (d, 1H), 7.25 (d, 1H), 7.36 (d, 1H), 8.12 (dd, 1H), and 8.60 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.1, 26.4, 29.9, 32.2, 46.5, 62.3, 103.6, 109.1, 116.8, 118.0, 127.4, 130.9, 138.6, and 141.1; IR (KBr) 3400 cm^{-1}; MS (ES) *m*/*z* 263 (M + H); Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

7-(5-Nitro-1*H*-indol-1-yl)-1-heptanol (4f). Yield 82%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.31−1.39 (m, 9H, including OH), 1.50−158 (m, 2H), 1.82−1.90 (m, 2H), 3.59−3.64 (m, 2H), 4.16 (t, 2H), 6.67 (d, 1H), 7.24 (d, 1H), 7.34 (d, 1H), 8.10 (dd, 1H), and 8.58 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.4, 26.6, 28.7, 29.9, 32.3, 46.6, 62.5, 103.6, 109.1, 116.8, 118.0, 127.4, 130.9, 138.6, and

141.1; IR (neat) 3407 cm^{-1}; MS (ES) *m/z* 277 (M + H); Anal. ($C_{15}H_{20}N_2O_3$) C, H, N.

8-(5-Nitro-1*H*-indol-1-yl)-1-octanol (4g). Yield 61%; mp 74−75 °C, ^{1}H NMR ($CDCl_3$) δ 1.25−1.36 (m, 8H), 1.41−1.60 (m, 3H including OH), 1.78−1.90 (m, 2H), 3.61 (t, 2H), 4.15 (t, 2H), 6.65 (d, 1H), 7.24 (d, 1H), 7.33 (d, 1H), 8.08 (dd, 1H), and 8.56 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.7, 29.0, 29.1, 30.1, 32.5, 46.7, 62.7, 103.7, 109.1, 116.9, 118.1, 127.5, 130.9, 138.7, and 141.2; IR (neat) 3318 cm^{-1}; MS (ES) *m/z* 291 (M + H); Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

General Procedure for Esterification. This procedure is illustrated for the preparation of [2-(5-nitro-*1H*-indol-1-yl)-ethyl] nicotinate (**5a**).

To a solution of alcohol **4a** (0.20 g, 0.97 mmol) in CH_2Cl_2 (15 mL) were added nicotinic acid (0.143 g, 1.16 mmol), DCC (0.221 g, 1.07 mmol), and DMAP (0.012 g, 0.099 mmol) and stirred at room temperature for 24 h. Precipitation of a white solid occurred. This was filtered and washed on the filter with CH_2Cl_2 (15 mL). The combined filtrate was washed with water (2 × 10 mL) and brine (2 × 10 mL). Removal of solvent from the dried (Na_2SO_4) extract gave the crude product. It was purified by flash column chromatography (20 × 3 cm) over silica gel using acetone/hexanes (3:7) to afford the pure ester **5a** (0.26 g, 87%).

2-(5-Nitro-1*H*-indol-1-yl)ethyl Nicotinate (5a). Oil; ^{1}H NMR ($CDCl_3$) δ 4.52 (t, 2H), 4.64 (t, 2H), 6.66 (d, 1H), 7.22−7.38 (m, 3H), 8.02−8.09 (m, 2H), 8.53 (d, 1H), 8.71 (dd, 1H), and 9.05 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 45.2, 48.9, 104.6, 108.9, 117.2, 118.1, 123.3, 125.1, 127.6, 130.9, 137.0, 138.8, 141.5, 150.4, 153.4, and 164.5; MS (ES) *m/z* 312 (M + H).

3-(5-Nitro-1*H*-indol-1-yl)-1-propyl Nicotinate (5b). Yield 74%; mp 118−119 °C, ^{1}H NMR ($CDCl_3$) δ 2.29 (p, 2H), 4.28 (t, 2H), 4.31 (t, 2H), 6.62 (d, 1H), 7.19−7.34 (m, 3H), 8.10 (dd, 1H), 8.20 (dt, 1H), 8.57 (d, 1H), 8.81 (dd, 1H), and 9.18 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 28.9, 43.4, 62.1, 104.1, 108.8, 117.0, 118.0, 123.1, 125.3, 127.5, 130.8, 136.7, 138.4, 141.2, 150.4, 153.4, and 164.7; IR (neat) 1718 cm^{-1}; MS (ES) *m/z* 326 (M + H); Anal. ($C_{17}H_{15}N_3O_4$) C, H, N.

4-(5-Nitro-1*H*-indol-1-yl)-1-butyl Nicotinate (5c). Yield 100%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.66−1.76 (m, 2H), 1.91−2.02 (m, 2H), 4.18 (t, 2H), 4.29 (t, 2H), 6.61 (d, 1H), 7.17−7.34 (m, 3H), 8.01 (dd, 1H), 8.17 (dt, 1H), 8.49 (d, 1H), 8.70 (dd, 1H), and 9.10 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.3, 25.9, 45.5, 63.8, 103.3, 108.5, 116.2, 117.3, 122.6, 125.2, 126.9, 130.4, 136.3, 137.9, 140.5, 149.7, 152.4, and 164.2; MS (ES) *m/z* 340 (M + H).

5-(5-Nitro-1*H*-indol-1-yl)-1-pentyl Nicotinate (5d). Yield 60%; mp 84−85 °C, ^{1}H NMR ($CDCl_3$) δ 1.42−1.52 (m, 2H), 1.78−1.87 (m, 2H), 1.91−1.99 (m, 2H), 4.21 (t, 2H), 4.34 (t, 2H), 6.68 (d, 1H), 7.25 (d, 1H), 7.36−7.41 (m, 2H), 8.10 (dd, 1H), 8.23 (dt, 1H), 8.58 (d, 1H), 8.87 (dd, 1H), and 9.19 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 23.0, 27.9, 29.5, 46.3, 64.5, 103.7, 108.9, 116.8, 117.9, 123.0, 125.7, 127.4, 130.8, 136.6, 138.5, 141.1, 150.5, 153.1, and 164.9; IR (KBr) 1717 cm^{-1}; MS (ES) *m/z* 354 (M + H); Anal. ($C_{19}H_{19}N_3O_4 \cdot 0.3H_2O$) C, H, N.

6-(5-Nitro-1*H*-indol-1-yl)-1-hexyl Nicotinate (5e). Yield 90%; mp 64−65 °C, ^{1}H NMR ($CDCl_3$) δ 1.32−1.52 (m, 4H), 1.70−1.81 (m, 2H), 1.82−1.93 (m, 2H), 4.17 (t, 2H) 4.31 (t, 2H), 6.65 (d, 1H), 7.23−7.41 (m, 3H), 8.07 (dd, 1H), 8.26 (dt, 1H), 8.55 (d, 1H), 8.76 (dd, 1H), and 9.19 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.4, 28.4, 30.0, 46.6, 65.1, 103.8, 109.1, 117.0, 118.1, 123.2, 126.1, 127.5, 130.8, 136.8, 138.6, 141.3, 150.7, 153.3, and 165.1; IR (KBr) 1721 cm^{-1}; MS (ES) *m/z* 368 (M + H); Anal. ($C_{20}H_{21}N_3O_4$) C, H, N.

7-(5-Nitro-1*H*-indol-1-yl)-1-heptyl Nicotinate (5f). Yield 73%; mp 78−79 °C, ^{1}H NMR ($CDCl_3$) δ 1.28−1.49 (m, 6H), 1.71−1.80 (m, 2H), 1.82−1.92 (m, 2H), 4.17 (t, 2H), 4.33 (t, 2H), 6.68 (d, 1H), 7.25 (d, 1H), 7.33−7.61 (m, 2H), 8.11 (dd, 1H), 8.29 (dt, 1H), 8.59 (d, 1H), 8.78 (dd, 1H), and 9.22 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.8, 26.7, 28.5, 28.7, 30.1, 46.8, 65.2, 103.9, 109.1, 117.1, 118.3, 123.3, 126.2, 127.7, 130.9, 137.0, 138.8, 141.5, 150.8, 153.4, and 165.3; IR (neat) 1720 cm^{-1}; MS (ES) *m/z* 382 (M + H); Anal. ($C_{21}H_{23}N_3O_4$) C, H, N.

8-(5-Nitro-1*H*-indol-1-yl)-1-octyl Nicotinate (5g). Yield 69%; mp 67−68 °C, ^{1}H NMR ($CDCl_3$) δ 1.28−1.47 (m, 8H), 1.71−1.80 (m, 2H), 1.80−1.92 (m, 2H), 4.17 (t, 2H), 4.34 (t, 2H), 6.68 (d, 1H), 7.24−7.42 (m, 3H), 8.10 (dd, 1H), 8.29 (dt, 1H), 8.58 (d, 1H), 8.79 (dd, 1H), and 9.22 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 25.7, 28.4, 28.9 (2C), 30.1, 46.8, 65.3, 103.8, 109.1, 117.0, 118.1, 123.2, 126.2, 127.6, 130.9, 136.9, 138.7, 141.3, 150.8, 153.3, and 166.2; IR (KBr) 1717 cm^{-1}; MS (ES) *m/z* 396 (M + H); Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

General Procedure for Quaternization. This procedure is illustrated for the preparation of 2-(5-nitro-*1H*-indol-1-yl)-ethyl *N*-methylnicotinate, iodide (**6a**).

To a solution of the ester **5a** (0.156 g, 0.502 mmol) in DME (8 mL) was added iodomethane (0.32 mL, 5.1 mmol), and the mixture was heated at 80 °C for 12 h. This was cooled to room temperature, and the solvent was completely removed. The residue was purified by column chromatography (10 × 2 cm) using MeOH/$CHCl_3$ (1:19) to obtain the pure pyridinium salt **6a** (0.192 g, 84.0%).

2-(5-Nitro-1*H*-indol-1-yl)ethyl *N*-Methylnicotinate, Iodide (6a). Oil; ^{1}H NMR (DMSO-d_6) δ 4.41 (s, 3H), 4.74 (s, 4H), 6.73 (d, 1H), 7.79 (d, 1H), 7.83 (d, 1H), 8.00 (dd, 1H), 8.24 (dd, 1H), 8.53 (d, 1H), 8.83 (d, 1H), 9.19 (d, 1H), and 9.43 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 44.8, 48.2, 65.4, 103.9, 110.3, 116.4, 117.4, 127.4, 127.8, 128.8, 132.8, 138.8, 140.8, 144.5, 146.6, 149.0, and 161.1; MS (ES) *m/z* 326 (M + H); Anal. ($C_{17}H_{16}IN_3O_4 \cdot 0.5H_2O$) C, H, N.

3-(5-Nitro-1*H*-indol-1-yl)-1-propyl *N*-Methylnicotinate, Iodide (6b). Yield 88%; mp 182−183 °C, ^{1}H NMR (DMSO-d_6) δ 2.29 (p, 2H), 4.34 (t, 2H), 4.43 (s, 3H), 4.52 (t, 2H), 6.76 (d, 1H), 7.72−7.78 (m, 2H), 7.99 (dd, 1H), 8.23 (dd, 1H), 8.53 (d, 1H), 8.83 (d, 1H), 9.19 (d, 1H), and 9.44 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.5, 43.0, 48.3, 63.9, 103.8, 110.4, 116.4, 117.5, 127.3, 127.7, 129.1, 132.6, 138.7, 140.6, 144.7, 146.5, 148.8, and 161.5; IR (neat) 1735 cm^{-1}; MS (ES) *m/z* 340 (M + H); Anal. ($C_{18}H_{18}IN_3O_4$) C, H, N.

4-(5-Nitro-1*H*-indol-1-yl)-1-butyl *N*-Methylnicotinate, Iodide (6c). Yield 60%; Oil; ^{1}H NMR ($CDCl_3$) δ 1.81−1.93 (m, 2H), 2.05−2.19 (m, 2H), 4.32 (t, 2H), 4.44 (t, 2H), 4.80 (s, 3H), 6.69 (d, 1H), 7.39 (d, 1H), 7.50 (d, 1H), 8.08 (dd, 1H), 8.21 (dd, 1H), 8.55 (d, 1H), 8.90 (d, 1H), 9.44 (d, 1H), and 9.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.9, 26.9, 46.4, 50.2, 66.7, 104.1, 109.6, 117.1, 118.2, 127.7, 128.2, 130.5, 131.3, 138.7, 141.4, 145.3, 146.4, 148.4, and 160.7; MS (ES) *m/z* 354 (M + H); Anal. ($C_{19}H_{20}IN_3O_4 \cdot 0.75H_2O$) C, H, N.

5-(5-Nitro-1*H*-indol-1-yl)-1-pentyl *N*-Methylnicotinate, Iodide (6d). Yield 74%; mp 107−108 °C; ^{1}H NMR ($CDCl_3$) δ 1.40−1.58 (m, 2H), 1.80−2.0 (m, 4H), 4.28 (t, 2H), 4.42 (t, 2H), 4.82 (s, 3H), 6.67 (d, 1H), 7.34 (d, 1H), 7.47 (d, 1H), 8.05 (dd, 1H), 8.26 (t, 1H), 8.54 (d, 1H), 8.88 (d, 1H), 9.51 (s, 1H), and 9.55 (d, 1H); ^{13}C NMR (MeOH-d_4) δ 24.1, 29.2, 30.9, 47.4, 48.3, 67.7, 104.8, 111.1, 117.7, 118.8, 129.3, 129.4, 132.1, 133.2, 140.5, 142.6, 146.3, 148.2, 149.8, and 162.7; IR (KBr) 1729 cm^{-1}; MS (ES) *m/z* 368 (M + H); Anal. ($C_{20}H_{22}IN_3O_4$) C, H, N.

6-(5-Nitro-1*H*-indol-1-yl)-1-hexyl *N*-Methylnicotinate, Iodide (6e). Yield 64%; mp 127−128 °C, ^{1}H NMR ($CDCl_3$) δ 21.31−1.55 (m, 4H), 1.74−1.83 (m, 2H), 1.83−1.97 (m, 2H), 4.21 (t, 2H), 4.39 (t, 2H), 4.78 (s, 3H), 6.67 (d, 1H), 7.40 (d, 1H), 7.47 (d, 1H), 8.05 (dd, 1H), 8.29 (dd, 1H), 8.54 (d, 1H), 8.89 (d, 1H), 9.44 (s, 1H), and 9.64 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.4, 28.3, 30.0, 46.8, 50.3, 67.2, 103.8, 109.4, 117.1, 118.1, 127.6, 128.5, 130.4, 131.1, 138.7, 141.2, 145.1, 146.1, 148.8, and 160.8; IR (KBr) 1721 cm^{-1}; MS (ES) *m/z* 382 (M + H); Anal. ($C_{21}H_{24}IN_3O_4$) C, H, N.

7-(5-Nitro-1*H*-indol-1-yl)-1-heptyl *N*-Methylnicotinate, Iodide (6f). Yield 82%; mp 97−98 °C, ^{1}H NMR ($CDCl_3$) δ 1.30−1.45 (m, 6H), 1.73−1.80 (m, 2H), 1.81−1.90 (m, 2H), 4.16 (t, 2H), 4.38 (t, 2H), 4.78 (s, 3H), 6.66 (d, 1H), 7.24 (d, 1H), 7.36 (d, 1H), 8.08 (dd, 1H), 8.31 (t, 1H), 8.56 (d, 1H), 8.91(d, 1H), 9.04 (s, 1H), and 9.70 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.6, 26.6, 28.3, 28.6, 30.1, 46.8, 50.4, 67.4, 103.9, 109.3, 117.1, 118.2, 127.6, 128.5, 130.6, 131.1, 138.8, 141.4, 145.1, 146.0, 148.9, and 160.8; MS (ES) *m/z* 396 (M + H); Anal. ($C_{22}H_{26}IN_3O_4$) C, H, N.

8-(5-Nitro-1*H*-indol-1-yl)-1-octyl *N*-Methylnicotinate, Iodide (6g). Yield 60%; mp 85−86 °C, ^{1}H NMR ($CDCl_3$) δ 1.23−1.41 (m, 8H), 1.65−1.79 (m, 2H), 1.79−1.84 (m, 2H), 4.15 (t, 2H), 4.37 (t, 2H), 4.77 (s, 3H), 6.64 (s, 1H), 7.24 (s, 1H), 7.35 (d, 1H), 8.04 (dd, 1H), 8.30 (t, 1H), 8.51 (d, 1H), 8.86 (d, 1H), 9.37 (s, 1H), and 9.69 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.6, 28.3, 28.8, 28.9, 30.0, 46.8, 50.5, 67.4, 103.9, 109.3, 117.0, 118.1, 127.7, 128.6, 130.6, 131.0, 138.9, 141.5, 145.0, 146.0, 149.0, and 160.9; IR (KBr) 1721 cm^{-1}; MS (ES) *m*/*z* 410 (M + H). Anal. ($C_{23}H_{28}IN_3O_4$) C, H, N.

8-(5-Benzyloxy-1*H*-indol-1-yl)-1-octanol (III_{b8}). This was prepared by using a procedure similar to that described for **3a**, starting from 5-benzyloxyindole (I_b, 0.70 g, 3.1 mmol) and 8-bromo-1-octanol (0.73 g, 3.5 mmol) to give the alcohol III_{b8} (1.1 g, 99%). Oil; ^{1}H NMR ($CDCl_3$) δ 1.18−1.38 (m, 9H), 1.43−1.61 (m, 3H), 1.78 (bs, 1H. OH), 3.57 (t, 2H), 4.04 (t, 2H), 5.08 (s, 2H), 6.38 (d, 1H), 6.94 (dd, 1H), 7.04 (d, 1H), 7.16 (d, 1H), and 7.19−7.51 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.8, 29.1, 29.2, 30.2, 32.6, 46.5, 62.8, 70.8, 100.3, 104.0, 110.0, 112.3, 127.5, 127.6, 128.3, 128.4, 128.7, 131.4, 137.7, and 152.9; IR (neat) 3383 cm^{-1}; MS (ES) *m*/*z* 352 (M + H); Anal. ($C_{23}H_{29}NO_2 \cdot 0.2H_2O$) C, H, N.

8-(5-Benzyloxy-1*H*-indol-1-yl)-1-octyl Methanesulfonate (IV_{b8}). This was prepared by using a procedure similar to that described for **8** (Scheme 3), starting from III_{b8} (1.10 g, 3.13 mmol) to yield the mesylate IV_{b8} (1.34 g, 100%). ^{1}H NMR ($CDCl_3$) δ 1.21−1.41 (m, 8H), 1.61−1.74 (m, 2H), 1.74−1.85 (m, 2H), 2.94 (s, 3H), 4.05 (t, 2H), 4.17 (t, 2H), 5.08 (s, 2H), 6.38 (d, 1H), 6.93 (dd, 1H), 7.04 (d, 1H), 7.16 (d, 1H), and 7.19−7.50 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 25.2, 26.7, 28.7, 28.9, 29.0, 30.1, 37.2, 46.4, 70.0, 70.8, 100.3, 104.1, 109.9, 112.3, 127.4, 127.6, 128.2, 128.4, 128.7, 131.4, 137.7, and 153.0; MS (ES) *m*/*z* 430 (M + H).

8-(5-Benzyloxy-1*H*-indol-1-yl)-1-octyl Nicotinate (V_{b8A}). This was prepared by using a procedure similar to that described for **9** (Scheme 3), starting from IV_{b8} (1.13 g, 2.63 mmol) and nicotinic acid (0.65 g, 5.3 mmol) to obtain the ester V_{b8A} (1.05 g, 87.0%); ^{1}H NMR ($CDCl_3$) δ 1.32−1.48 (m, 8H), 1.69−1.88 (m, 4H), 4.05 (t, 2H), 4.32 (t, 2H), 5.08 (s, 2H), 6.38 (d, 1H), 6.94 (d, 1H), 7.04 (d, 1H), 7.15−7.50 (m, 8H), 8.26 (dd, 1H), 8.75 (d, 1H), and 9.21 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.7, 26.8, 28.4, 28.9, 29.0, 30.1, 46.4, 65.3, 70.7, 100.3, 104.0, 109.9, 112.3, 123.1, 126.2, 127.4, 127.6, 128.2, 128.4, 128.7, 131.3, 136.9, 137.6, 150.7, 152.9, 153.2, and 165.2; MS (ES) *m*/*z* 457 (M + H); Anal. ($C_{29}H_{32}N_2O_3$) C, H, N.

8-(Hydroxy-1*H*-indol-1-yl)-1-octyl Nicotinate (V_{g8A}). To a solution of V_{b8A} (0.20 g, 0.44 mmol) and *N*,*N*-dimethylaniline (0.159 g, 1.31 mmol) in anhydrous CH_2Cl_2 (20 mL) was added anhydrous $AlCl_3$ (0.233 g, 1.75 mmol), and the mixture was stirred at room temperature for 1 h. TLC (50% EtOAc in hexanes) showed that reaction was complete. This was quenched with water (10 mL), extracted with CH_2Cl_2 (2 × 20 mL), and washed with water (2 × 10 mL) and brine (1 × 10 mL). Removal of solvent from the dried (Na_2SO_4) extract gave the crude product, which was purified by column chromatography over silica gel (20 × 2 cm) using EtOAc/hexanes (1:1) to afford the pure debenzylated product V_{g8A} (0.12 g, 74%); ^{1}H NMR ($CDCl_3$) δ 1.20−1.44 (m, 8H), 1.65−1.85 (m, 4H), 4.04 (t, 2H), 4.32 (t, 2H), 6.32 (d, 1H), 6.81 (dd, 1H), 7.05 (dd, 2H), 7.17 (d, 1H), 7.41 (dd, 1H), 8.32 (dt, 1H), 8.78 (dd, 1H), and 9.22 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 25.8, 26.7, 28.5, 29.0, 29.1, 30.2, 46.3, 65.5, 99.8, 105.3, 109.8, 111.4, 123.5, 126.5, 128.3, 129.1, 131.3, 137.4, 149.7, 150.4, 152.8, and 165.1; IR (neat) 3547−3080, 1722 cm^{-1}, MS (ES) *m*/*z* 367 (M + H); Anal. ($C_{22}H_{26}N_2O_3$) C, H, N.

8-(5-Hydroxy-1*H*-indol-1-yl)-1-octyl *N*-Methylnicotinate, Iodide (VI_{g8E}). This was prepared by using a procedure similar to that described for **6a** (Scheme 3), starting from V_{g8A} (0.08 g, 0.22 mmol) to obtain the pyridinium salt VI_{g8E} (0.083 g, 74%); ^{1}H NMR (DMSO-d_6) δ 1.16−1.34 (m, 6H), 1.34−1.44 (m, 2H), 1.65−1.78 (m, 4H), 4.07 (t, 2H), 4.37 (t, 2H), 4.43 (s, 3H), 6.20 (d, 1H), 6.62 (dd, 1H), 6.83 (d, 1H), 7.20−7.24 (m, 1H), 8.25 (dd, 1H), 8.64 (s, 1H), 8.95 (d, 1H), 9.18 (d, 1H), and 9.51 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.1, 26.2, 27.9, 28.5, 28.6, 29.8, 45.5, 48.3, 66.4, 99.2, 104.2, 110.0, 111.1, 127.8, 128.7, 128.8, 129.3, 130.3, 144.7, 146.6, 148.8, 150.5, and 161.7; MS (ES) *m*/*z* 381 (M + H); Anal. ($C_{23}H_{29}IN_2O_3$) C, H, N.

2-{2-[2-(5-Benzyloxy-1*H*-indol-1-yl)ethoxy]ethoxy}-ethanol (7). To a solution of 5-benzyloxyindole (I_b, 0.250 g, 1.12 mmol) in anhydrous DMF (5 mL) under N_2 was added NaH (0.037 g, 1.2 mmol, 80% dispersion in mineral oil), and the mixture was stirred at room temperature for 30 min. 2-[2-(2-Chloroethoxy)ethoxy]ethanol (0.250 g, 1.49 mmol) was added dropwise, and the reaction mixture was heated at 100 °C for 12 h. TLC (50% EtOAc in hexanes) showed that the reaction was complete. It was diluted with EtOAc (20 mL) and quenched with sat. NH_4Cl (10 mL). The organic layer was separated, and the aqueous layer was extracted with 10 mL of EtOAc. The combined EtOAc extracts were washed with water (3 × 15 mL) and brine (2 × 15 mL). It was dried (Na_2SO_4) and filtered, and solvent was removed in a vacuum to obtain the crude product. The crude product was purified by column chromatography over silica gel (20 × 2 cm) using EtOAc/hexanes (1:1) followed by 100% EtOAc to afford the pure alcohol **7** (0.26 g, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.47 (bs, 1H), 3.45−3.61 (m, 6H), 3.61−3.71 (m, 2H), 3.75 (t, 2H), 4.22 (t, 2H), 5.08 (s, 2H), 6.38 (d, 1H), 6.93 (dd, 1H), and 7.09−7.51 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 46.2, 61.5, 70.0, 70.2, 70.6, 70.7, 72.3, 100.7, 104.1, 109.9, 112.3, 127.4, 127.6, 128.3, 128.7, 128.9, 131.4, 137.6, and 153.1; IR (neat) 3427 cm^{-1}; MS (ES) *m*/*z* 355 (M + H).

2-{2-[2-(5-Benzyloxy-1*H*-indol-1-yl)ethoxy]ethoxy}-ethyl Methanesulfonate (8). To a solution of alcohol **7** (0.26 g, 0.73 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added Et_3N (0.20 mL, 1.5 mmol) followed by CH_3SO_2Cl (0.13 g, 1.1 mmol). The reaction mixture was stirred at 0 °C for 15min. TLC examination (50% EtOAc in hexanes) showed that the reaction was complete. 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). This was dried over Na_2SO_4 and filtered, and solvent was completely removed in a vacuum to obtain the mesylate **8** (0.29 g, 91%). As this mesylate was not very stable it was used directly for the next step without further purification and characterization.

2-{2-[2-(5-Benzyloxy-1*H*-indol-1-yl)ethoxy]ethoxy}-ethyl Nicotinate (9). To a solution of the mesylate **8** (0.288 g, 0.665 mmol) in anhydrous DMF (10 mL) were added nicotinic acid (0.164 g, 1.33 mmol) and K_2CO_3 (0.092 g, 0.67 mmol). The resulting mixture was heated at 50−55 °C for 3 h, during which the reaction mixture turned gelatinous. TLC examination (50% EtOAc in hexanes) showed 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). It was dried over Na_2SO_4 and filtered, and solvent was removed in a vacuum to obtain the crude product, which was purified by column chromatography over silica gel (20 × 4 cm) using EtOAc/hexanes (1:1) to afford the ester **9** (0.21 g, 69%); ^{1}H NMR ($CDCl_3$) δ 3.50−3.53 (m, 2H), 3.57−3.60 (m, 2H), 3.69−3.78 (m, 4H), 4.22 (t, 2H), 4.41 (t, 2H), 6.36 (d, 1H), 6.92 (dd, 1H); 7.12 (dd, 1H), 7.20−7.47 (m, 8H), 8.26 (dd, 1H), 8.75 (dd, 1H), and 9.2 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 46.3, 64.3, 68.9, 70.1, 70.5, 70.7 (2 peaks), 100.7, 104.0, 109.9, 112.3, 123.1, 125.8, 127.4, 127.6, 128.3, 128.7, 128.9, 131.4, 137.0, 137.6, 150.8, 153.0, 153.4, and 165.0; IR (neat) 1730 cm^{-1}; MS (ES) *m*/*z* 461 (M + H); Anal. ($C_{27}H_{28}N_2O_5$) C, H, N.

{2-{2-[2-(5-Benzyloxy-1*H*-indol-1-yl)ethoxy]ethoxy}-ethyl (*N*-Methylnicotinate)}, Iodide (10). This was prepared using a procedure similar to that described for **6a**, starting from **9** (0.213 g, 0.463 mmol), to obtain product **10** (0.183 g, 65.6%). ^{1}H NMR ($CDCl_3$) δ 3.38−3.60 (m, 4H), 3.73−3.79 (m, 4H), 4.14−4.21 (m, 2H), 4.40−4.46 (m, 5H), 5.05 (s, 2H), 6.26 (d, 1H), 6.80−7.50 (m, 9H), 7.99 (dd, 1H), 8.65 (d, 1H), and 9.10−9.27 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 46.2, 49.7, 65.9, 68.3, 69.9, 70.3, 70.5, 70.6, 100.6, 103.9, 110.1, 112.1, 127.4, 127.7, 128.0, 128.3, 128.4, 129.0, 129.4, 131.3, 137.4,

144.5, 145.6, 148.1, 152.7, and 160.7; IR (neat) 1734 cm^{-1}; MS (ES) *m*/*z* 475 (M + H); Anal. ($C_{28}H_{31}IN_2O_5$) C, H, N.

Procedure for Library 1 (Scheme 2). 1. Synthesis of Acetates ($II_{(a-c)n}$). To the solution of indoles ($I_{(a-c)}$) (0.5 mmol) and bromoacetates (1.1 equiv) in anhydrous DMF (5 mL) in screw cap vials (10 mL capacity) was added NaH (1.1 equiv, 60% dispersion in mineral oil). The reaction tubes were flushed with N_2, capped, and stirred with orbital shaking (225 rpm) at room temperature for 3 h. The reaction mixtures were transferred to 40 mL capacity tubes, diluted with EtOAc (10 mL), and quenched with water (10 mL). The tubes were shaken thoroughly and allowed to settle. The organic layer from each tube was separated into another set of 40 mL capacity tubes. The aqueous layer in each tube was once again extracted with EtOAc (5 mL) and combined with the earlier extract. The extracts in each tube were then washed with water (3 × 5 mL) and brine (1 × 5 mL). Na_2SO_4 (~1 g) was added to each tube and allowed to stand for 1 h. These solutions were filtered in parallel into 20 mL vials. The collected filtrates were evaporated in parallel using the speedvac at its medium temperature setting to obtain the product acetates ($II_{(a-c)n}$). The products were dissolved in MeOH (5 mL) and halide sequestering resin, polymer bound tris(2-aminoethyl)amine (4.1 mmol N/g, 0.25 g), was added and shaken for 3 h. Then these were filtered and washed with MeOH (2 × 5 mL). This solution of acetates was used without further purification in the next step.

2. Synthesis of Alcohols ($III_{(a-c)n}$). To the solution of acetates ($II_{(a-c)n}$) in MeOH contained in 20 mL screw cap vials was added a catalytic amount of NaH. This mixture was shaken at room temperature for 3 h. The reaction tubes were quenched with 1 N HCl (1 mL), and solvent was completely removed under vacuum in parallel on a speedvac. The residue was dissolved in EtOAc (15 mL) and transferred to 40 mL tubes. The content of each tube was then washed with water (2 × 5 mL) and brine (1 × 5 mL). Na_2SO_4 (~1 g) was added to each tube and allowed to stand for 1 h. These were filtered in parallel into 20 mL vials. The filtrates were collected in 20 mL vials and evaporated in parallel on a speedvac to obtain the product alcohols ($III_{(a-c)n}$).

3. Synthesis of Mesylates ($IV_{(a-c)n}$). The alcohols ($III_{(a-c)n}$) were dissolved in CH_2Cl_2 (6 mL) contained in 20 mL screw cap vials and cooled to 0 °C. 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. They were then diluted with CH_2Cl_2 (8 mL) and transferred into 40 mL tubes and washed with 1 N HCl (3 × 5 mL), water (2 × 5 mL), and brine (1 × 5 mL). Na_2SO_4 (~1 g) was added to each tube and allowed to stand for 1 h. These solutions were filtered in parallel into 20 mL vials. Evaporation of solvent from the filtrates on a speedvac afforded the mesylates ($IV_{(a-c)n}$).

4. Synthesis of Esters ($V_{(a-c)nQ1}$). The mesylates ($IV_{(a-c)n}$) were dissolved in anhydrous DMF (5 mL) in 10 mL screw cap vials, and the carboxylic acid (2 equiv) was added to each vial. K_2CO_3 (1 equiv) was added and the reaction mixtures were capped and heated at 55 °C with orbital shaking (225 rpm) for 12 h. They were allowed to attain room temperature and transferred to 40 mL capacity tubes. To this was added EtOAc (10 mL) and water (5 mL). The organic layers were separated, and the aqueous layers were extracted with 5 mL additional EtOAc. The combined organic extracts for each reaction were then washed with sat. $NaHCO_3$ (2 × 5 mL), water (2 × 5 mL), and brine (5 mL). Na_2SO_4 (~1 g) was added to each tube and allowed to stand for 1 h. These solutions were filtered in parallel into 20 mL vials. The filtrates were evaporated on a speedvac to afford the esters ($V_{(a-c)nQ1}$).

5. Synthesis of the Quaternary Salts ($VI_{(a-c)nQ2}$). To a solution of the esters ($V_{(a-c)nQ1}$) in anhydrous DME (6 mL) in screw cap vials (10 mL capacity) was added MeI (30 equiv). The reaction mixtures were capped and heated with orbital shaking (225 rpm) at 80 °C for 12h. They were allowed to cool to room temperature and solvent was completely removed using a speedvac at its medium temperature setting. The crude products were purified using parallel chromatography over silica gel columns (5 × 1 cm). The columns were eluted with CH_2Cl_2 (30 mL) and EtOAc (30 mL) to remove less polar impurities, and then with 5% MeOH in CH_2Cl_2 (30 mL). Fractions collected were checked by TLC (1:9 MeOH/$CHCl_3$), and the fractions containing the required compound were combined and evaporated on a speedvac to obtain the quaternary salts ($VI_{(a-c)nQ2}$).

Procedure for Library 2 (Scheme 2). 1. Synthesis of Alcohols ($III_{(d-f)n}$). To the solution of indoles ($I_{(d-f)}$) (0.50 mmol) and bromo alcohols (1.1 equiv) in anhydrous DMF (5 mL) in screw cap vials (10 mL capacity), was added NaH (1.1 eq, 60% dispersion in mineral oil). The reaction tubes were flushed with N_2, capped, and stirred with orbital shaking (225 rpm) for 3 h. Each reaction was diluted with EtOAc (10 mL), quenched with 10 mL of water, and transferred into tubes of 40 mL capacity. These tubes were shaken thoroughly and allowed to settle. The organic layer from each tube was transferred into another set of 40 mL capacity tubes. The aqueous layer in each tube was once again extracted with EtOAc (5 mL), which was combined with the earlier extract. The combined EtOAc extracts in each tube were then washed with water (3 × 5 mL) and brine (1 × 5 mL). Na_2SO_4 (~1 g) was added to each tube for and allowed to stand for 1 h. These solutions were filtered in parallel into 20 mL vials. The filtrates were then treated with halide sequestering resin, polymer bound tris(2-aminoethyl)amine (4.1 mmol N/g, 0.25 g), for 3 h. Resin was removed by parallel filtration, and filtrates were collected in 20 mL vials and evaporated on a speedvac to obtain the product alcohols ($III_{(d-f)n}$).

3. Synthesis of Mesylates ($IV_{(d-f)n}$). Alcohols ($III_{(d-f)n}$) were converted to corresponding mesylates ($IV_{(d-f)n}$) following a similar procedure described for the preparation of mesylates $IV_{(a-c)n}$ (Library 1).

4. Synthesis of Esters ($V_{(d-f)nQ1}$). Mesylates ($IV_{(d-f)n}$) were converted to their corresponding esters ($V_{(d-f)nQ1}$) following a similar procedure described for the preparation of esters $V_{(a-c)nQ1}$ (Library 1).

5. Synthesis of the Quaternary Salts ($VI_{(d-f)nQ2}$). Esters ($V_{(d-f)nQ1}$) were converted to the corresponding quaternary salts ($VI_{(d-f)nQ2}$) following a similar procedure described for the preparation of quaternary salts $VI_{(a-c)nQ2}$ (Library 1).

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