DNA Binding Ligands Targeting Drug-Resistant Bacteria: Structure, Activity, and Pharmacology

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We describe the lead optimization and structure−activity relationship of DNA minor-groove binding ligands, a novel class of antibacterial molecules. These compounds have been shown to target A/T-rich sites within the bacterial genome and, as a result, inhibit DNA replication and RNA transcription. The optimization was focused on N-terminal aromatic heterocycles and C-terminal amines and resulted in compounds with improved in vivo tolerability and excellent in vitro antibacterial potency (MIC ≥ 0.031 μg/mL) against a broad range of Gram-positive pathogens, including drug-resistant strains such as methicillin-resistant *Stapylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus faecalis* (VRE). In a first proof-of-concept study, a selected compound (**35**) showed in vivo efficacy in a mouse peritonitis model against methicillin-sensitive *S. aureus* infection with an ED_{50} value of 30 mg/kg.

Introduction

The recently observed emergence of bacterial resistance to commonly used antibiotics is a severe health care problem and has revived the search for new classes of antibacterial agents.[1] In particular, antibiotics with a novel mode of action are needed to overcome cross-resistance to known drugs. Since the discovery of the quinolones roughly 40 years ago, only *one* novel class of antibiotics, the oxazolidinones, has been introduced to the market.[2] Currently, the oxazolidinone linezolid is used as the last line of defense against vancomycin-resistant bacterial infections. However, resistance to linezolid has already emerged and resistant clinical isolates of *Staphylococcus aureus* and *Enterococcus faecium* have been described.[3,4]

The increasing knowledge of microbial genomes and the rapidly expanding repertoire of genomic and proteomic analysis techniques have certainly facilitated the search for essential bacterial proteins as potential drug targets.[5,6] However, the transition from such targets to novel therapeutic agents with broad antibacterial spectrum, high in vivo efficacy, and good tolerability has been extremely difficult and the overall success has been disappointing so far.[1] The bacterial genome itself has been recognized as a potential target for antibiotics: a number of natural products (e.g., distamycin A[7] or actinomycin D[8]) have been isolated and later shown to interact with bacterial DNA. This interaction was consequently postulated to cause the antibacterial activity by interference with DNA replication, DNA processing enzymes, and/or RNA synthesis.[9] DNA minor-groove binding molecules that are structurally related to distamycin A have been shown to exhibit potent in vitro antibacterial activity against clinically relevant drug-resistant pathogens.[10,11]

We have reported a first generation of potent DNA minor-groove binding antibacterials that were designed based on the natural product distamycin A as well as crescent shaped molecules[12−14] that bind within the minor-groove of DNA.[11] An early prototype was mechanistically investigated and has been shown to block DNA replication and RNA synthesis.[15] Inhibition of these processes is likely responsible for the antibacterial activity. The compound has demonstrated bactericidal activity against clinical isolates of *Streptococci* and *Staphylococci*, but was static against *Enterococci*. As these small molecules ($M_w \approx 550$ to 750) bind at multiple functionally relevant sites within the bacterial genome, the development of resistance by single point mutations (target alteration) is predicted to be extremely difficult. More importantly, this novel mode of action renders these compounds active against Gram-positive bacteria with various resistance profiles, including isolates of methicillin-resistant and vancomycin-intermediate *S. aureus* (MRSA and VISA), penicillin-resistant *Pneumococcus*, as well as vancomycin-resistant *Enterococcus* (VRE).

The in vivo pharmacological behavior of such DNA minor-groove binding ligands has not been well reported. As a next step in the development of this class of molecules for therapeutic applications, we started to improve the drug-like properties, mainly focusing on tolerability. First indications were that distamycin A and two DNA binding ligands representing the previously reported generation of antibacterials were acutely toxic in mice at dose levels that might be required for antibacterial efficacy (vide infra).

Herein, we describe the iterative optimization process leading to a second generation of DNA minor-groove binding ligands with an improved tolerability profile and excellent in vitro activity against various drug-sensitive and -resistant Gram-positive bacterial strains. In this process, we focused on structural modifications of the end groups of prototypic lead molecules and used a whole-cell antibacterial activity screen as the primary assay. We reasoned that this approach would have several advantages as compared to an in vitro target-

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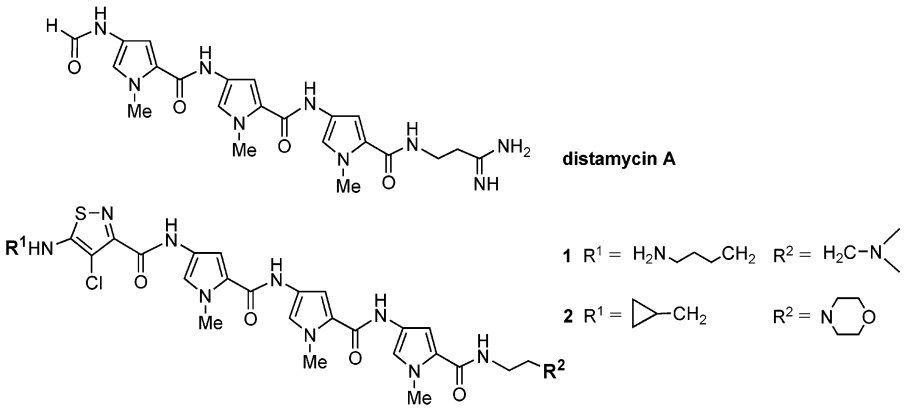


Figure 1. Structure of distamycin A and two prototypic DNA binding antibacterials.

based primary assay (DNA): it is well documented that cellular uptake and/or efflux mechanisms are challenging parameters influencing the antibacterial whole cell activity.[16] In addition, the functionally optimal DNA target sequence within the bacterial genome is not known with certainty and therefore would render such an assay questionable as a selection criterion. Therefore, the DNA binding property was only analyzed for a subset of advanced compounds. Similarly, the in vivo tolerability was studied for selected compounds that showed promising activities in the primary in vitro antibacterial screen.

Results and Discussion

The acute tolerability of distamycin A and two representative early lead molecules **1** and **2** (Figure 1, see ref 11 for more details) was tested in mice. In this study, mice were administered 50 mg/kg via intravenous bolus and observed for 48−72 h. Lethality or abnormal behavior such as lethargy were monitored during the experiment and blood samples were analyzed post mortem. While the dibasic compound **1** led to mortality within minutes after injection, the mice survived the treatment with the morpholine analogue **2**. However, the blood sample analysis indicated a trend toward mild hepatotoxicity, as evidenced by 2-fold elevated ALT (alanine amino transferase) and LDH (lactate dehydrogenase) levels. The animals showed marked lethargy upon treatment with distamycin A.

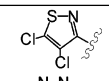
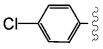
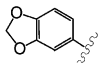
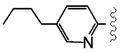
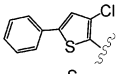
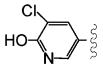
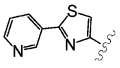
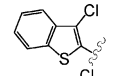
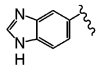
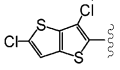
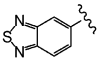
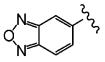
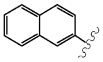
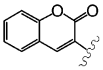
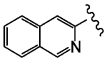
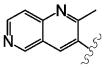
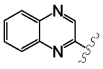
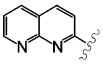
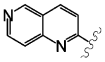
This initial study suggested that the monobasic morpholino compound **2** was better tolerated than the dibasic **1** or the amidine distamycin A, indicating that the basicity of the compound had an effect on tolerability. However, the hepatotoxicity of **2** was still limiting and had to be improved. Subsequently, we set out to identify alternative N-terminal units while conserving the Py_3-ethyl morpholine element (Py = *N*-methyl pyrrolecarboxamide): about 150 molecules bearing modified N-terminal heterocyclic moieties were synthesized and tested for in vitro antibacterial activity. The selection included a diverse range of mono- and bicyclic aromatic and heteroaromatic N-termini with various electron donating and withdrawing substituents, with the goal of retaining DNA binding properties and improving solubility. A set of representative compounds is shown in Table 1. In an iterative process, the compounds with novel N-terminal end caps that demonstrated good in vitro activity were modified further. A subset of the most promising compounds was then selected for a mouse tolerability screen.

Chemistry. The medicinal chemistry efforts were centered around structural modifications at either the N- or the C-terminus of the crescent shaped molecules. For compounds with modified N-terminal units, we first prepared the trimeric amine **7** in a linear fashion from the known pyrrole building blocks **3** and **6**[17] (Scheme 1). Coupling of either an aromatic acid chloride or an activated ester to this intermediate gave the final compounds, which were purified by preparative HPLC. The yields were relatively low and ranged between 10 and 30%, as we collected only the fractions of highest purity during the HPLC purification. Notably, compound **35** has been scaled up in solution (50 g scale) with a total yield of 31% (seven steps) and a 57% yield for the final coupling reaction. All compounds were characterized by ^{1}H NMR, mass spectroscopy, and analytical HPLC (UV detection). Most compounds showed clean NMR spectra and an analytical purity in excess of 95%. A few compounds were slightly less pure according to analytical HPLC (92−94%). As we have not seen differences in MIC values for specific molecules within this purity range in the past, these compounds were still analyzed for in vitro potency (highlighted in the tables), but not used for in vivo studies. The analytical approach outlined above has proven reliable for multiple compounds that have been scaled up and characterized more extensively.

The majority of the carboxylic acids or acid chlorides used in this "small-library approach" were commercially available or were prepared according to known procedures. For known protocols, the literature is referenced in the Experimental Section. To complement the understanding of the structure−activity relationship, we additionally synthesized some novel building blocks as outlined in Scheme 2.

The 3-chloro-5-phenyl-thiophene **9** was prepared from the amino ester **8**: conversion of the amine **8** to the corresponding diazonium salt, treatment of this intermediate with CuCl under Sandmeyer conditions, and saponification gave the 3-chlorothiophene-2-carboxylic acid **9**. Similarly, the 3-amino-thiophene **10** was converted to the 3-cyano-thiophene **11** via the corresponding diazonium salt. Subsequent saponification of the ester **11** gave the acid **12** in good yield. The bicyclic imidazo[2,1-*b*]thiazole **16** and the imidazo[1,2-*a*]pyridine **19** were both synthesized by treatment of the aryl-

Table 1. Influence of End Caps on in Vitro Antimicrobial Activity against ATCC Strains (Scheme 1)

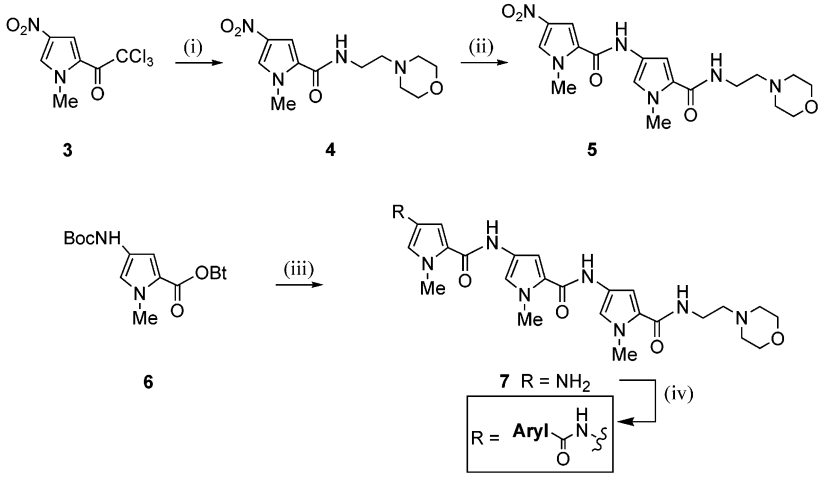
	R	[a]*MRSA* 27660	[a]*VSEF* 29212	[a]*PISP 49619*		R	[a]*MRSA 27660*	[a]*VSEF* 29212	[a]*PISP 49619*
31		0.5	0.125	0.062	**51**		32	32	16
32		32	4	1	**52**		2	0.5	0.25
33		16	8	2	**53**		8	16	2
34		>32 [b]	>32	>32	**54**		16	>32	>32
35		2–4	1	0.125	**55**		>32	>32	4
36		>32	>32	32	**56**		>32	32	2
37		32	32	1	**57**		18	8	1
38		>32	>32	8	**58**		8	32	2
39		16	>32	2	**59**		8	>32	0.5
40		>32	>32	2	**60**		2	0.5	0.25
41		0.25	0.25–1	0.125	**61**		>32	>32	>8
42[c]		1	16	0.125	**62**		8	1	0.5
43		1	1	0.125	**63**		8	8	8
44		2	4	0.5	**64**		8	4	4
45		8	16	2	**65**		4	16	0.5
46		8	8	2	**66**		0.5	0.25	0.061
47		4	32	8	**67**		>32	>32	>32
48		2	0.5	0.031	**68**[d]		16	>32	>32
49		2	2	0.031	**69**		>32	>32	2
50		8	8	0.5	**70**		8	4	0.25

[a] MIC values in [μg/mL]. MRSA: methicillin-resistant *S. aureus*. VSEF: vancomycin-susceptible *E. faecalis*. PISP: penicillin-intermediate *S. pneumoniae*. [b] Tested for activity against MSSA 13709 instead of MRSA. [c] 94% purity as determined by HPLC−UV. [d] 92% purity as determined by HPLC−UV.

amines **13** and **17**, respectively, with ethyl bromopyruvate, followed by saponification of the resulting esters **15** and **18**.[18] Refluxing the cinnamic acid **20** and $SOCl_2$ in chlorobenzene yielded the 3-chloro-benzothiophene **21** as the acid chloride. The chloro-benzothiophene **22** has been prepared in a similar fashion according to a reported procedure.[19] Conversion of this acid chloride to the methyl ester **23**, hydrogenolytic dechlorination (→ **24**), followed by saponification gave the benzothiophene-2-carboxylic acid **25** in an overall yield of 76% (three steps).

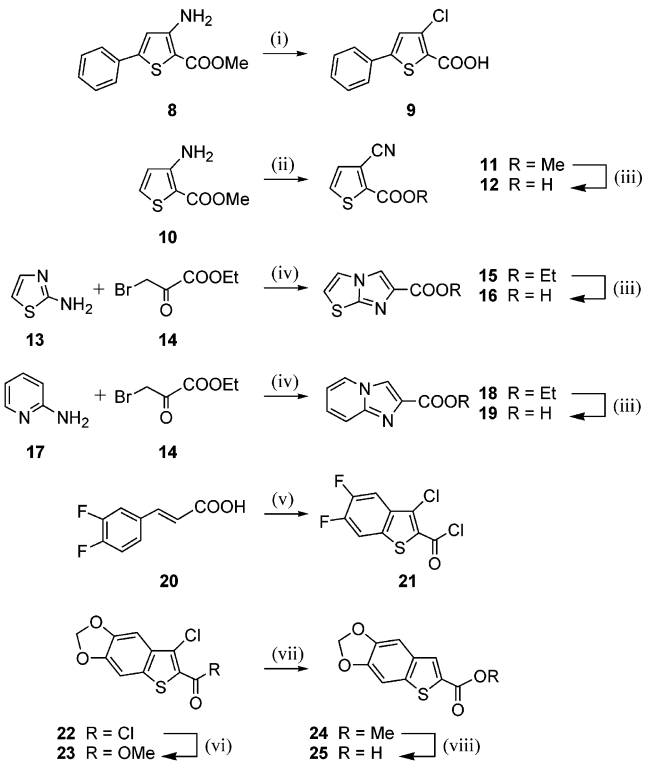
Compounds with modified C-termini were prepared from the known trimer **26** (Scheme 3).[20] Coupling of this trimeric amine and 3-chloro-thiophene-2-carboxylic acid **27** under standard conditions, followed by saponification of the intermediary formed ester gave the tetrameric acid **29**. This tetramer served as a starting material for the preparation of a series of N-terminal 3-chlorothiophene analogues with diverse C-termini: in situ activation of the acid **29** with BopCl and addition of an aliphatic amine gave the final compounds. As mentioned previously, all compounds were purified by preparative HPLC and characterized by ^{1}H NMR, mass spectroscopy, and analytical HPLC−UV. The tetrameric isoquinoline intermediate **30** and a series of N-terminal isoquinoline compounds were synthesized by the same route.

Scheme 1. Synthesis of Final Compounds with Modified *N*-Termini[a]



[a] Reagents: (i) 4-(2-aminoethyl)morpholine, THF, 2 h, 25−50 °C, 88%; (ii) H_2, Pd/C, THF, 2 h, 25 °C, then **3**, NMP, 35 min, 25 °C, 71%; (iii) H_2, Pd/C, EtOH, 16 h, 70 °C, then **6**, THF, 6 h, 80 °C, then HCl (g), 10 min, 80 °C, 64%; (iv) Aryl-COOH, HBTU, DMF, DIEA, or Aryl-COCl, DMF, DIEA, 12−16 h, 25−37 °C, then preparative HPLC.

Scheme 2. Synthesis of the Building Blocks[a]



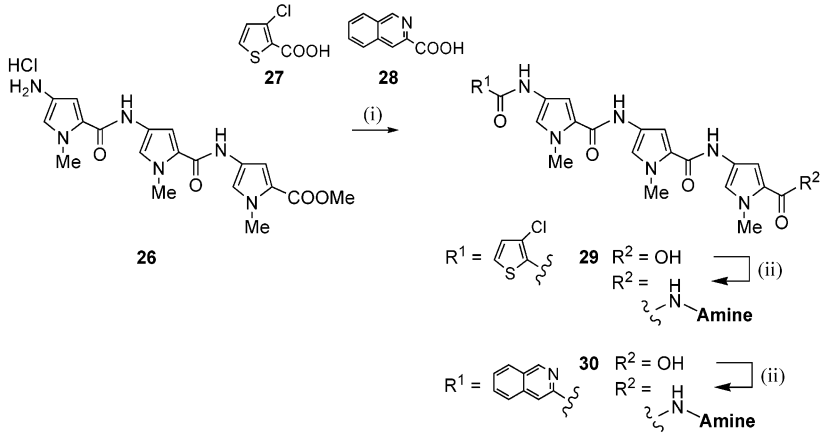
[a] Reagents: (i) $NaNO_2$, HCl, H_2O, 0−4 °C, then CuCl, HCl, 90 min, 25 °C, then KOH, EtOH, H_2O, 14 h, 25 °C, 40%; (ii) $NaNO_2$, HCl, H_2O, 0−4 °C, then CuCN, NaCN, 1 h, 25 °C, 75%; (iii) KOH, EtOH, H_2O, for **12** (90%), for **16** (64%), for **19** (45%); (iv) THF, 25 °C, for **15** (54%), for **18** (63%); (v) $SOCl_2$, cat. pyridine, PhCl, reflux, 65 h, 20%; (vi) MeOH, Et_3N, 30 min, reflux, 95%; (vii) H_2, Pd, MeOH, AcOEt, Et_3N, 72 h, 80 °C, 92%; (viii) NaOH, EtOH, H_2O, 2 h, 60 °C, 87%.

Microbiology. In the primary in vitro antimicrobial screen, all compounds were studied for antimicrobial activity against MRSA, penicillin-intermediate *Streptococcus pneumoniae* (PISP), and vancomycin-susceptible *Enterococcus faecalis* (VSEF). For this class of antibiotics, no significant differences in MIC values (minimum inhibitory concentration) for drug-susceptible vs -resistant strains has been observed.[11] The in vitro susceptibility data are shown in Tables 1−3 for a representative selection of molecules. Promising compounds were advanced into a more rigorous activity screen, as shown in Table 5. As a first development criterion, we aimed for MIC values of ≤ 1 μg/mL against each of these bacterial strains.

As many compounds with an N-terminal isothiazole cap have exhibited potent antibacterial activity (the structure of two representative members of the first generation is shown in Figure 1),[11] five-membered heterocycles containing nitrogen and/or sulfur were investigated first. The 4,5-dichloro-isothiazole **31** was very potent but unfortunately turned out to be acutely toxic (Table 5). Therefore, isothiazoles were not pursued further. Various thiadiazoles and thiazoles showed insufficient activity (**32**−**34**). The 3-chloro-thiophene **35** contained the first novel end cap that led to good activity and was profiled further, even though its MIC value against MRSA was only moderate. Various furans (**37**, **38**) as well as the tetra pyrrole **36** were only marginally active. Most compounds typically showed slightly better activity against *S. pneumoniae* as compared to the other Gram-positive strains. Biaryl compounds such as **39** and **40** showed at best only moderate potency. In contrast, several fused ring systems with a five-membered ring bearing the carboxamido function were promisingly active: the most potent compound was the 3-chlorobenzothiophene **41**. The benzoisothiazole **43**, the imidazo[2,1-b]thiazole **48**, and the imidazo[1,2-a]pyridine **49** were only slightly less active, whereas the benzofurans **44**/**45** and the indoles **46**/**47** exhibited only moderate potency.

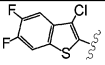
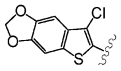
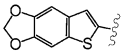
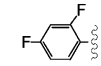
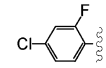
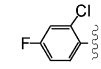
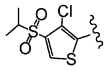
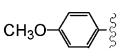
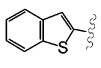
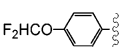
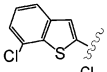
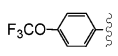
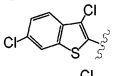
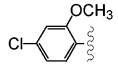
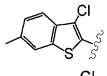
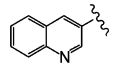
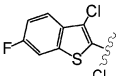
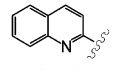
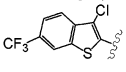
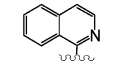
In the series of six-membered aromatic end groups halogenated, alkylated, and/or hydroxy/alkoxy-substituted benzene, pyridine and pyrazine rings were investigated. The pyridines (**54**−**56**) were not active enough, irrespective of the position of the ring nitrogen. Introduction of substituents at the pyridyl moiety did not greatly improve activity (**57**−**59**). The benzamide **51** was almost inactive, while its more lipophilic 4-chloro derivative **52** passed the critical 1 μg/mL limit for VSEF and PISP. The pyrazine **60** was as potent as **52**. In the series of the fused ring systems with a six-membered ring attached to the linking carboxamido group, there

Scheme 3. Synthesis of Final Compounds with Modified C-Termini[a]



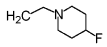
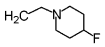
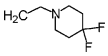
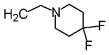
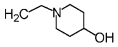
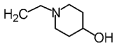
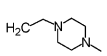
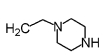
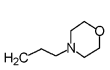
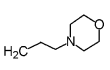
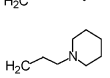
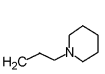
[a] Reagents: (i) HBTU, NMP, DIEA, then KOH, EtOH, H_2O, for **29** (92%), for **30** (43%); (ii) BopCl, DMF, DIEA, R−NH_2, 12−16 h, 37 °C, then preparative HPLC.

Table 2. Optimization of Thiophenes, Benzothiophenes, Benzamides, and Isoquinoline Analogues (Scheme 1)

	R	[a]*MRSA* 27660	[a]*VSEF* 29212	[a]*PISP* *49619*		R	[a]*MRSA* *27660*	[a]*VSEF* 29212	[a]*PISP* *49619*
71		32	16	2	**89**[b]		1	8	0. 5
72		4	>32	0.5	**90**		0.031	0.031	0.031
73		4	1	0.25	**91**		0.5	1	0.25
74		16	16	4	**92**		32	16	32
75		4	2	0.5	**93**		8	8	8
76		32	>32	>32	**94**		8	0.5	1
77		>32	>32	>32	**95**		32	32	16
78[b]		4	8	0.125	**96**		16	8	2
79		4	2	2	**97**		2	1	0.125
80		16	>32	8	**98**		2	0.5	0.25
81		8	8	4	**99**		16[c]	8	4
82		4	2-4	0.25–1	**100**		8	>32	0.125
83		4	32	1	**101**		2	4	0.062
84		8	>32	2	**102**		8	>32	1
85		4	32	0.25	**103**		16	8	0.5
86		1	8	0.25	**104**		>32	>32	16
87		0.5	0.25	0.125	**105**		2	32	1
88		8	8	4	**106**		16	4	1

[a] MIC values in [μg/mL]. MRSA: methicillin-resistant *S. aureus*. VSEF: vancomycin-susceptible *E. faecalis*. PISP: penicillin-intermediate *S. pneumoniae*. [b] 94% purity as determined by HPLC−UV. [c] Tested for activity against MSSA 13709 instead of MRSA.

Table 3. Modification of C-Terminal Amines (Figure 2)

R^1: 	R^2	[a] *MRSA* 27660	[a] *VSEF* 29212	[a] *PISP* 49619	R^1: 	R^2	[a] *MRSA* 27660	[a] *VSEF* 29212	[a] *PISP* 49619
107		1	0.5	0.125	**119**		0.125	0.25	0.031
108		0.5	0.5	0.25	**120**		0.25	0.062	0.031
109		1	1	0.031	**121**		0.25	0.125	0.031
110 [b]		2	2	0.5	**122**		0.5	0.25	0.125
111		4	4	2	**123**		2	2	1
112		4	32	2	**124**		16	>32	>32
113		8	16	2	**125**		0.5	2	0.062
114		8	4	2	**126**		2	2	2
115		8	4	4	**127** [b]		4	4	0.25
116		8	16	4	**128**		4	4	1
117		2	2	2	**129**		1	1	1
118		>32	4	1	**130**		16	16	16

[a] MIC values in [μg/mL]. MRSA: methicillin-resistant *S. aureus*. VSEF: vancomycin-susceptible *E. faecalis*. PISP: penicillin-intermediate *S. pneumoniae*. [b] 94% purity as determined by HPLC−UV.

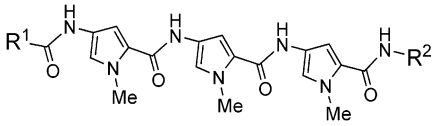


Figure 2. General structure for Table 3.

Table 4. DNA Binding Properties and Calculated pK_a Values

compd	K_d (app)[a] [nM] 5′-ACAA-TTAA-3′	pK_a[b] (calc)	compd	K_d (app)[a] [nM] 5′-ACAA-TTAA-3′	pK_a[b] (calc)
35	20	7.16 ± 0.12	**113**	2	9.93 ± 0.20
107	<1	7.14 ± 0.20	**114**	71	7.57 ± 0.10
108	5	9.20 ± 0.20	**115**	50	9.64 ± 0.20
109	5	7.48 ± 0.40	**116**	5	9.49 ± 0.28
110	50	5.77 ± 0.40	**117**	100	5.44 ± 0.12

[a] Determined by DNase I footprint titration. [b] Calculated by ACD[21] for the corresponding acetylated C-terminal alkylamine (e.g., using *N*-(2-morpholin-4-yl-ethyl)-acetamide for **35**).

was one promising hit: the isoquinoline **66**. The closely related naphthalene **64** and various naphthyridine derivatives (**67**−**70**) did not show sufficient potency. Similarly, the benzimidazole **61**, the benzothiadiazole **62**, and the benzoxazole **63** were not potent enough.

This initial screening study successfully identified several new end caps that could serve as alternatives to the isothiazole N-terminus. Next, the structure−activity relationship of thiophene, benzothiophene, benzamide, and isoquinoline derivatives was investigated in more detail. The results of this study are summarized in Table 2.

For the thiophenes, the 3-chloro substituent proved to be essential for good antibacterial activity; replacement of this chloride by a hydrogen (**71**), fluoride (**72**), bromide (**73**), methyl (**74**), cyano (**75**), methoxy (**76**), hydroxy (**77**), or a methyl thio substituent (**78**) resulted in various degrees of loss of potency. Whereas fluoride, bromide, and cyano substituents caused the least change in MIC values, replacement of the C(3)-chloride by the strongly electron donating methoxy or hydroxy groups resulted in a significant loss of activity.

This particular dataset was subjected to a QSAR analysis using the MIC value as biological datapoint and the following substituent parameters: (i) $\sigma_{m,p}$ (Hammett constant for the electronic influence), (ii) π (lipophilicity parameter), and (iii) σ_v (Charton's v size values). The following equation was used to determine optimal constants by multiple linear regression to extract the most important constants contributing to the observed MICs:

$$\log(\mathrm{MIC}) = a\sigma_{m,p} + b\pi + c\sigma_v + d$$

Due to the small dataset, the evaluation has limited accuracy, but can be used to qualitatively assess the importance of the selected parameters. The results of this analysis can be summarized as follows for MRSA and PISP: Good correlations were achieved with the Hammett constant $\sigma_{m,p}$ as the most important parameter, contributing roughly 75% to the calculated MIC values. The constant σ_m was consistently producing better fits as compared to σ_p. The second constant of

Table 5. Advanced Compounds: Antimicrobial Activity against Vancomycin-Resistant *E. faecalis*, Penicillin-resistant *S. pneumoniae*, *B. cereus*, and *C. albicans*, DNA Binding, and Tolerability Data

	M_w	VREF[a] 51559	PRSP[a] 51422	*B. Cereus*[a] 11778	*C. albicans*[a] 38247	K_d (app)[b] [nM] 5′-ACAA-TTAA-3′	tolerability in mice[c] (50 mg/kg, IV bolus)
31	677	0.25	N/A	0.25	>32	N/A	3/4 lethality, ↑ ALT, AST, BUN
35	641	1	0.125	1	>32	20	no adverse effects
41	691	0.5	0.031	0.5	>32	0.2	no adverse effects
48	646	1	0.25	2	>32	N/A	1/4 lethality, lethargy, 2x ↓ TG[d]
60	616	4	0.25	1	>32	N/A	5/5 lethality
66	651	0.5	0.125	0.5	>32	N/A	no adverse effects
87	709	0.5	0.062	0.25	>32	N/A	no adverse effects
90	735	0.031	0.031	0.031	>32	<0.1	N/A: lack of solubility
91	700	0.5	0.5	2	>32	N/A	2/4 lethality
98	653	0.5	0.062	0.5	>32	0.1	5/5 lethality
107	657	2	0.062	0.5	>32	<1	no adverse effects
108	639	2	2	0.5	32	5	5/5 lethargy
109	657	0.5	0.125	1	>32	5	1/5 lethality
114	655	8	8	8	>32	70	3−4x ↑ ALT, AST
115	653	16	16	8	>32	50	3/5 lethality
119	667	0.125	0.062	0.25	>32	1	no adverse effects

[a] MIC values in [μg/mL]. VREF: vancomycin-resistant *E. faecalis*. PRSP: penicillin-resistant *S. pneumoniae*. *Bacillus cereus*, *Candida albicans*. [b] Determined by DNase I footprinting titration. [c] Tolerability end points: survival, body weight (only noted if abnormal), clinical observations, serum chemistry. [d] Triglycerides.

importance was the lipophilicity parameter π, whereas the constant σ_v for substituent size could be omitted without a major impact on the predictive accuracy of the model within the dataset. Compounds carrying substituents at C(3) with high (positive) σ_m values and high lipophilicity (π) were the most active, an observation that was reflected in the unique value of halogens (especially chloride and bromide) at this position. The importance of the halide at this particular position was further demonstrated by a different placement of the chloride or the introduction of multiple chloride groups: both modifications reduced the antibacterial activity (**79**, **80**). As outlined for the thiophene derivatives, the 3-chloro substituent played a similarly important role in the benzothiophene series: removal of the chloride in **41** or altering the position of the chloride resulted in a substantial loss of biological activity (→ **83**, **84**).

The nature of the substituents at C(5) and C(6) of the benzothiophene group clearly influenced the antibacterial potency as well. The same semi-quanitative analysis as outlined above for the C(3)-position was performed for the C(6)-position, using a dataset of eight compounds (including compounds with 6-bromo-, 6-(dimethylamino)-, and 6-nitro-substituents, respectively; data not shown). The pattern for this position was quite different with almost no electronic influence and a major contribution from size (σ_v) and lipophilicity (π). Consequently, highly polar (hydrophilic) substituents of limited size were preferred at C(6) of the 3-chloro-benzothiophene group. This is exemplified by sterically more demanding, lipophilic substituents such as a chloride (**85**), a methyl group (**86**), or a trifluoromethyl group (**88**); all groups dramatically reduced the potency relative to **41**. The small and more polar fluoride substituent (compound **87**), however, did not significantly affect the antibacterial activity, even though the electronic influence is quite different from hydrogen. Introduction of a second fluoride at C(5) of benzothiophene moiety reduced potency by 2−16-fold (**89**). In contrast, the 5,6-dioxymethylene compound **90** exhibited excellent antibacterial activity against all strains. The dechlorinated derivative **91** was roughly 8-fold less potent than **90**, but still passed the MIC selection criteria of 1 μg/mL.

Thiophene and benzene rings have been regarded as bioisosteres due to their similar geometry. In this study, replacement of the thiophene end cap by a benzene moiety retained activity; however, the structure−activity relationship of the two scaffolds revealed some differences. For instance, the 4-chloro-benzamide **52** and the 3-chloro-thiophene **35** showed comparable good activities, whereas the 2-chloro-benzamide **92** — the direct benzene analogue of **35** — was less potent. The 3-chloro-benzene **93** was not sufficiently active. All mono-fluorinated benzamides were only moderately potent. As the 2-fluoro benzamide **94** was more potent than its isomers **95** and **96**, and the 4-chloro benzamide was the most potent within its series, the 2-fluoro-4-chlorobenzamide **98** was prepared next. Indeed, compound **98** achieved good antibacterial potency. Reversing the position of the chloride and fluoride substituents in **98** reduced the antibacterial activity (**99**), whereas replacement of chloride by fluoride at C(4) did not affect potency. Phenolic end groups or alkylated versions were significantly less potent than their halogenated analogues (e.g., **100**−**103**).

Several isomers of the isoquinoline **66** were studied; the position of the ring nitrogen as well as the carboxamido group turned out to be essential for good activity: the isomers **104**, **105**, and **106** were significantly less potent than the originally identified **66**. In fact, the isoquinoline **66** is one of few active compounds lacking a halogen substituent. The reasons for the enhanced antibacterial activity of halogenated compounds versus their nonhalogenated analogues are not fully understood and might be complex; a halogen substituent generally adds lipophilicity and might facilitate cellular uptake of the antibacterial compound. In addition, a halogen atom could also influence the DNA target interaction as a result of conformational, steric, and/or electronic changes within the molecule. For instance, the 3-chlorobenzothiophene **41** showed a K_d value of 0.2 nM for the sequence 5′-ACAATTAA-3′, while its dechlorinated ana-

logue **83** bound the same DNA site only at 100 times higher concentrations (K_d = 20 nM).

The influence of the C-terminal amine on the antimicrobial activity was studied for two series: the N-terminal 3-chloro-thiophenes and the isoquinolines (Table 3). Six-membered tertiary amines that are attached by an ethylene group to the adjacent pyrrole carboxamide have generally shown good in vitro potencies: for instance, the thiomorpholines **107** and **119**, the piperidines **108** and **120**, and the 4-fluoro-piperidines **109** and **121** passed the 1 μg/mL criterion. The 4,4-difluoropiperidine showed MIC values well below 1 μg/mL for the isoquinoline **122**, but was slightly less active for the 3-chloro-thiophene **110**. The piperazines **112** and **124** and the five-membered pyrrolidines **113** and **125** were somewhat less potent than their corresponding piperidine analogues. Similarly, the amines with a propyl linkage to the pyrrole carboxamide significantly lost potency as compared to their ethylene analogues (e.g., **114** vs **35**, **126** vs **66**, **115** vs **108**, **127** vs **120**). Substitution of the six-membered amines by pyridines also decreased the antibacterial potency (**117**, **118**), irrespective of linker length. In summary, the ethylene-bridged, six-membered tertiary amines showed optimal antibacterial activity.

The DNA binding properties of subset of 3-chlorothiophenes bearing various C-terminal amines was analyzed by quantitative DNase I footprint titrations (Table 4).

As reported previously,[11] we used a 352-bp DNA fragment containing the sequence 5′-ACAATTAA-3′, a site proximal to the σ70 RNA polymerase subunit binding site within the *Escherichia coli* Trc promoter. At different concentrations, all compounds showed DNA binding at this site. The compounds that were most active against bacteria showed low nanomolar DNA binding (**107**−**109**), while the less active propyl compounds **114** and **115** bound DNA only at higher concentrations. The morpholine **35** exhibited good DNA binding properties with a K_d = 20 nM. However, this correlation of DNA binding and whole-cell activity has not generally been observed, e.g., the pyrrolidine **113** and the dimethylaminopropyl compound **116** had low nanomolar DNA binding affinities, but were only moderately potent antibacterials. Notably, even though a strict correlation is not observed, inactive compounds consistently bound this target site poorly (≥100 nM). These data support the expectation that apart from the target interaction, other parameters such as cellular uptake and efflux mechanisms contribute to cellular activity. Bacterial cell penetration could be influenced by the basicity of the C-terminal amino group via the level of protonation or charge under physiological conditions. Weakly basic amines such as the morpholine **35** might penetrate the bacterial cell wall more readily than more basic compounds such as **115** or **116**. On the other hand, the weakly basic pyridine **117** showed reduced antibacterial potency. Even if this compound has excellent penetration properties, the reduced DNA binding affinity (K_d = 100 nM) would not be sufficient for high potency. In addition, the conformational flexibility of the C-terminal unit might influence cellular uptake: compounds in which the amino group is tethered by an ethylene linker to the adjacent pyrrole consistently show higher potency as compared to those tethered by a propylene group (e.g., **35** vs **114**; **108** vs **115**; **66** vs **126**; **120** vs **127**). Thus, a higher degree of conformational flexibility might reduce cellular uptake.

Pharmacology. The most promising compounds resulting from this optimization process and some control compounds were tested for acute tolerability in a mouse model (single IV bolus dose at 50 mg/kg). The results of this study, as well as additional microbiology data against vancomycin-resistant *E. faecium*, penicillin-resistant *S. pneumoniae*, *B. cereus*, and *C. albicans* are shown in Table 5. Clearly, these compounds showed in vitro antibacterial activity against clinically relevant Gram-positive strains, irrespective of their susceptibility to known drugs. Generally, these compounds were not active against *C. albicans*.

The 4,5-dichloro-isothiazole **31** was poorly tolerated with partial lethality (3/4) and greatly increased BUN (blood urea nitrogen), ALT, and AST (aspartate aminotransferase) levels indicating kidney and liver toxicity. In contrast, the thiophene **35**, the benzothiophenes **41** and **87**, and the isoquinoline **66** did not cause any adverse effects under these conditions. The methylenedioxy benzothiophene **90** exhibited excellent MIC values (0.031 μg/mL) against all Gram-positive strains, but its poor solubility did not allow formulation at the concentration required for in vivo evaluation. The dechlorinated analogue **91** was roughly 16-fold less potent than **90** but had improved aqueous solubility; however, only two out of four animals survived. Treatment of the animals with the imidazo[2,1-b]thiazole **48** resulted in lethargy and partial lethality. Out of the thiophene and isoquinoline compounds bearing various C-terminal amines, only the thiomorpholine **107** was well tolerated in addition to the morpholine compounds. The lethality or lethargic behavior observed for this series of compounds seemed to partially parallel the basicity of the C-terminal amines. Such a correlation, however, is purely based on experimental observations and most likely additional parameters contribute to the overall safety profile. Moreover, multiday toxicity studies with compound **35** indicated that daily IV administrations of doses ≥30 mg/kg had no adverse effect on any endpoint (body or organ weights, behavior, clinical chemistries, blood hematology, survival). In addition, a complete genotoxicity characterization in vitro (Ames assays and CHO cell mutagenesis or chromosomal aberration analysis) and in vivo (mouse bone marrow micronucleus testing) with compounds such as **35** indicated that the inherent mechanism of action, i.e., reversible DNA binding, is not genotoxic (data not shown). A comprehensive summary of in vivo profiling has been submitted for publication.[22]

The thiophene **35**, a prototypic lead molecule, was further advanced into an initial proof-of-concept efficacy study. The compound was tested in a mouse peritoneal sepsis model for efficacy against *S. aureus* (MSSA, ATCC 13709). Briefly, mice were infected intraperitoneally with a lethal dose of *S. aureus* (inoculum of 5−10× LD_{50}) and treated IV 1 and 5 h post infection with positive control, test compound, or vehicle alone. Survival of animals, the primary endpoint of this study, was monitored for 5 days. In this efficacy screen, **35** effectively protected mice from the lethal *S. aureus*

infection with an approximate ED_{50} of 30 mg/kg. This in vivo study demonstrated that this compound class has the potential to protect lethally infected mice at well-tolerated doses. Notably, the ED_{50} value of compound **35** against MRSA (ATCC 27660) is greater than 50 mg/kg. This is not surprising given that this particular MRSA strain is more virulent than the MSSA strain used in the model. A more comprehensive study directed at optimization of DNA minor-groove binding antibacterials for in vivo efficacy against MRSA will be reported in due course.

Conclusion

In an iterative process, DNA minor-groove binding ligands were optimized for in vitro antibacterial activity and in vivo tolerability by modification of their terminal groups. This study has demonstrated that both of these biological effects are highly sensitive to small structural changes at the termini of the molecules; for instance, a semiquantitative SAR analysis of a family of molecules bearing various substituents at C(3) of an N-terminal thiophene unit revealed that a strongly electron withdrawing group is required at this position for good antibacterial potency. Several compounds bearing novel N-terminal units showed excellent in vitro potency and passed an IV acute tolerability screen in mice. In a first proof-of-concept study, the 3-chloro-thiophene **35** showed in vivo efficacy in a mouse peritonitis model with an ED_{50} of 30 mg/kg against MSSA. These data suggest that such reversible DNA binding ligands have potential as novel antibacterial therapeutics. Structural optimization leading to compounds with improved in vivo efficacy will be reported in due course.

Experimental Section

General Synthetic Procedures. Commercially available reagents and solvents were purchased from Sigma-Aldrich Inc. and used without further purification, unless otherwise noted. Final coupling reactions were typically performed on a scale of 0.1−0.2 mmol. All final compounds were purified by preparative HPLC (Hamilton PRP-1 column, 250 × 21.5 mm, A: 0.5% AcOH in H_2O, B: CH_3CN, 0% to 60% B in 60 min, 20 mL/min, UV detection at 310 nm). The HPLC fractions were analyzed by UV detection (310 nm) and mass spectroscopy. Pure fractions (MS) containing the desired material were combined and lyophilized. The ^1H NMR spectra were recorded in DMSO-d_6 on a Varian Unity 400 MHz spectrometer. Purity analysis was performed by RP-HPLC (YMC Pro C18, S3μm, 120 Å, 2.0 × 50 mm, A: 0.05% HCOOH in H_2O, B: 0.05% HCOOH in MeOH/^{i}PrOH (90/10), 20−95% B, 13 min, 0.2 mL/min, UV detection at 310 nm). Electrospray ionization mass spectra were recorded on a Finnigan LCQ mass spectrometer.

General Procedure (A) for the Synthesis of Final Compounds Using HBTU. A mixture of the carboxylic acid (1.2 equiv) and HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 1.14 equiv) in DMF (1 mL/100 mg **7**) and DIEA (0.1 mL/100 mg **7**) was stirred for 30 min at 25 °C, treated with a solution of trimer **7** (1.0 equiv) in DMF (1 mL/100 mg **7**) and DIEA (0.1 mL/100 mg **7**), stirred for 12−16 h at 25−37 °C, and diluted with aqueous AcOH (50%, 10 mL). The solution was washed with Et_2O (3 × 3 mL), diluted to a volume of 15 mL and subjected to preparative HPLC purification.

General Procedure (B) for the Synthesis of Final Compounds Using an Acid Chloride. A mixture of the acid chloride (1.2 equiv) and trimer **7** (1.0 equiv) in DMF (1 mL/100 mg **7**) and DIEA (0.1 mL/100 mg **7**) was stirred for 5−12 h at 25 °C and diluted with aqueous AcOH (50%) to a total volume of 15 mL. HPLC purification.

General Procedure (C) for the Modification of the C-Terminus. A mixture of the tetrameric carboxylic acid (100 mg, 1 equiv) and BopCl (bis(2-oxo-3-oxazolidinyl)-phosphinic chloride, 1.1 equiv), in DMF (1 mL) and DIEA (0.1 mL) was stirred for 30 min at 25 °C, treated with the corresponding amine (5−10 equiv), stirred for 12−16 h at 37 °C, and diluted with 50% aqueous AcOH to a volume of 15 mL. HPLC purification.

4-[2-(1-Methyl-4-nitro-2-pyrrolecarboxamido)-ethyl]morpholine (4). A solution of 4-(2-aminoethyl)-morpholine (65 g, 0.50 mol) in THF (600 mL) was treated at 25 °C with the pyrrole **3** (135 g, 0.50 mol). The reaction temperature reached 50 °C within 3 min (exothermic). The mixture was stirred for 2 h without external heating, concentrated in vacuo, and treated with Et_2O (300 mL). The resulting solids were filtered, washed with Et_2O, and dried to give **4** a light yellow solid (136 g, 97%). Recrystallization of a portion of the crude material (68 g) from AcOEt (400 mL) gave **4** as a white solid (62 g, 88%).

4-{2-[1-Methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (5). A solution of nitro pyrrole **4** (50.0 g, 0.177 mol) in THF (500 mL) was treated with Pd/C (10%, 2.5 g) in a 2 L autoclave under N_2. The mixture was stirred for 2 h at 25 °C under H_2 atmosphere (125 psi) and filtered through Celite. The filtrate was concentrated in vacuo, treated with a solution of trichloroacetyl pyrrole **3** (48.0 g, 0.177 mol) in NMP (100 mL), and stirred. The reaction was exothermic, and the mixture solidified within 5 min. After cooling of the solids to 25 °C (30 min), MeOH (500 mL) was added and the mixture was heated at reflux for 30 min and cooled to 25 °C. The solids were collected by filtration, washed with cold MeOH (3 × 30 mL), and dried to give **5** (51 g, 71%).

4-{2-[1-Methyl-4-(1-methyl-4-(4-amino-1-methyl-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (7). A solution of dimer **5** (40 g, 0.10 mol) in EtOH (800 mL) was treated with Pd/C (10%, 2.5 g) in a 2 L autoclave under N_2. The mixture was stirred at 70 °C under H_2 atmosphere (125 psi) for 16 h and filtered through Celite. The filtrate was concentrated in vacuo, treated with a solution of the ester **6** (36 g, 0.10 mol) in THF (400 mL), heated to reflux for 6 h, cooled to 0 °C, and saturated with HCl (g). The mixture was slowly heated to reflux for 10 min, cooled to 25 °C, and added to Et_2O (2 L) under vigorous stirring. A white solid precipitated, which was collected by filtration, washed with Et_2O (3 × 100 mL), and dried to give **7** as the double HCl salt (37 g, 64%).

3-Chloro-5-phenyl-thiophene-2-carboxylic Acid (9). At 0−4 °C, a suspension of the thiophene **8** (500 mg, 2.14 mmol, Maybridge) in H_2O (7 mL) and concen-

trated HCl (37% in H_2O, 3 mL) was treated dropwise with a solution of $NaNO_2$ (185 mg, 2.68 mmol) in H_2O (1 mL) over 5 min (→ clear solution). The mixture was stirred for 20 min and poured into a cold solution (0 °C) of CuCl (800 mg, 8.08 mmol) in conc HCl (37%, 20 mL). The solution was stirred at 25 °C for 1.5 h, diluted with ice water (100 mL), and extracted with Et_2O (4 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to give a yellow solid (365 mg) that was purified by flash chromatography (hexane → hexane/AcOEt 1:1, 237 mg). A solution of this product in EtOH (2 mL) and aqueous KOH (2M, 2 mL) was stirred at 25 °C for 14 h, diluted with H_2O (40 mL), washed with AcOEt (25 mL), acidified to pH 1.6 (4 M HCl in H_2O), and extracted with AcOEt (3 × 25 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to give the acid **9** as a white solid (206 mg, 40%, two steps).

Methyl 3-cyano-thiophene-2-carboxylate (11). At 0−4 °C, a mixture of the 3-amino-thiophene **10** (1.00 g, 6.36 mmol) and conc HCl (37%, 5 mL) in H_2O (10 mL) was treated dropwise with a solution of $NaNO_2$ (0.55 g, 7.97 mmol) in H_2O (1.5 mL) → clear solution after 20 min. The mixture was treated with small portions of Na_2CO_3 over 1 h until pH = 7 was reached, diluted with ice water (50 mL), and added dropwise to a solution of CuCN (0.855 g, 9.55 mmol) and NaCN (0.50 g, 10.2 mmol) in H_2O (100 mL). After stirring of the sample at 25 °C for 1 h, the mixture was extracted with Et_2O (3×). The combined organic phases were dried ($MgSO_4$) and evaporated to give the cyano thiophene **11** as an orange solid (800 mg, 75%).

3-Cyano-thiophene-2-carboxylic Acid (12). At 0 °C, a solution of the ester **11** (200 mg, 1.20 mmol) in EtOH (5 mL) was treated with aqueous 2 M KOH (1 mL) → color change to dark brown. After 45 min, the mixture was diluted with H_2O (80 mL), washed with AcOEt (3×), acidified to pH = 2.4 (3 M HCl), and extracted with AcOEt (2×). The organic layers were dried ($MgSO_4$) and evaporated to give acid **12** as a yellow solid (164 mg, 90%).

Ethyl Imidazo[2,1-b]thiazole-6-carboxylate (15). To a suspension of 2-aminothiazole **13** (2.00 g, 20.0 mmol) in THF (200 mL, dried over 4 Å MS) was added ethyl bromopyruvate **14** (4.33 g, 20.2 mmol, 90% pure from Aldrich). After stirring of the sample for 20 h at 25 °C the solids were filtered, washed with THF, and treated with EtOH (100 mL). The mixture was heated at reflux for 4 h, concentrated to a volume of ca. 50 mL, and left at −20 °C for 12 h. The resulting white crystals were collected by filtration (**15**, 1.42 g). Evaporation of the mother liquor and recrystallization from hot ethanol gave additional **15** (0.69 g, total yield 54%).

Imidazo[2,1-b]thiazole-6-carboxylic Acid (16). A suspension of **15** (1.00 g, 6.10 mmol) in EtOH (5 mL) and H_2O (5 mL) was treated with KOH (0.50 g, 12.5 mmol), stirred at 25 °C for 80 min, diluted with H_2O (10 mL), and acidified to pH = 4.9 (1 M HCl). The resulting precipitate was collected by filtration and dried to give **16** as white solids (0.545 g, 64%).

Ethyl Imidazo[1,2-a]pyridine-2-carboxylate (18). A solution of 2-aminopyridine **17** (2.50 g, 26.6 mmol) in THF (60 mL, dried over 4Å MS) was treated with ethyl bromopyruvate **14** (5.16 g, 26.5 mmol) at 25 °C and heated at reflux for 16 h. The resulting white precipitate was collected by filtration and washed with THF. Recrystallization of the solids from boiling EtOH (100 mL) gave the ester **18** (3.20 g, 63%).

Imidazo[1,2-a]pyridine-2-carboxylic Acid (19). A suspension of the ester **18** (1.00 g, 5.26 mmol) in EtOH (5 mL) and H_2O (5 mL) was treated with KOH (0.50 g, 8.9 mmol), stirred at 25 °C for 90 min, diluted with H_2O (10 mL), acidified to pH = 4.9 (6 M HCl), and evaporated. The resulting white solids were washed with H_2O (2 × 2 mL) and dried to give the acid **19** (380 mg, 45%).

5,6-Difluoro-benzo[*b*]thiophene-2-carbonyl Chloride (21). A mixture of the cinnamic acid **20** (5.18 g, 0.028 mol), thionyl chloride (17.3 g, 0.144 mol), pyridine (0.29 g, 0.004 mol) in chlorobenzene (30 mL) was refluxed for 65 h and evaporated. Recrystallization of the solids from boiling hexane gave the acid chloride **21** as pale yellow crystals (1.47 g, 20%).

Methyl 7-Chloro-1,3-dioxa-5-thia-s-indacene-6-carboxylate (23). A solution of the chloride **22** (15.0 g, 54.7 mmol) in MeOH (150 mL) and Et_3N (10 mL) was refluxed for 30 min and cooled to 0 °C. The resulting precipitate was collected by filtration and washed with H_2O (3 × 30 mL), MeOH (2 × 30 mL), Et_2O (20 mL), and dried to give the ester **23** (14.0 g, 95%).

Methyl 1,3-Dioxa-5-thia-s-indacene-6-carboxylate (24). A suspension of the ester **23** (3.50 g, 13.0 mmol) and Pd (black, 400 mg) in MeOH (200 mL), AcOEt (100 mL), and Et_3N (2 mL) was stirred at 80 °C for 72 h under H_2 (1 atm) and filtered through Celite. Evaporation of the filtrate gave compound **24** as a solid (2.8 g, 92%).

1,3-Dioxa-5-thia-s-indacene-6-carboxylic Acid (25). A mixture of the ester **24** (1.00 g, 4.23 mmol) in EtOH (15 mL) and 2 M aqueous NaOH (15 mL) was stirred at 60 °C for 2 h and poured into acidic ice−water (400 mL, 3 M HCl). The resulting precipitate was collected by filtration, washed with H_2O, and dried to give the acid **25** as a yellow solid (0.82 g, 87%).

1-Methyl-4-{1-methyl-4-[1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}-2-pyrrolecarboxylic Acid (29). A mixture of the thiophene **27** (0.90 g, 5.53 mmol) and HBTU (1.99 g, 5.25 mmol) in NMP (5 mL) and DIEA (1 mL) was stirred for 2 h at 45 °C, treated with a solution of trimer **26** (2.00 g, 4.60 mmol) in NMP (5 mL) and DIEA (1 mL), stirred for 8 h at 25 °C, and added dropwise to ice−water (400 mL). The resulting precipitate was collected by filtration, washed with H_2O (50 mL), and lyophilized. The solids were suspended in EtOH (50 mL) and treated with a solution of KOH (5 g) in H_2O (50 mL). The mixture was stirred at 60 °C for 5 h, diluted with H_2O (100 mL), and acidified to pH 2.2 (1 M HCl). The resulting precipitate was collected by filtration and dried to give **29** (2.23 g, 92%).

1-Methyl-4-{1-methyl-4-[1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}-2-pyrrolecarboxylic Acid (30). A mixture of the isoquinoline **28** (7.17 g, 41.4 mmol) and HBTU (14.9 g, 39.3 mmol) in NMP (50 mL) and DIEA (10 mL) was stirred for 2 h at 25 °C, treated with a solution of trimer **26** (15.00 g, 34.5 mmol) in NMP (50 mL) and DIEA (10 mL), stirred for 19 h at 25 °C, added dropwise to ice−water (1 L). The resulting precipitate was collected by filtration, suspended in EtOH (150 mL),

and treated with a solution of KOH (10 g) in H_2O (300 mL). The mixture was stirred at 70 °C for 6 h, diluted with H_2O (600 mL), and washed with AcOEt (1 × 100 mL). The aqueous layer was acidified to pH 2.4 (6 M HCl) and the resulting precipitate collected by filtration and dried to give **30** (8.00 g, 43%).

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4,5-dichloro-isothiazole-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (31). Prepared according to general procedure (A) from 4,5-dichloroisothiazole-2-carboxylic acid[11] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-([1,2,3]-thiadiazole-4-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (32). Prepared according to general procedure (A) from 1,2,3-thiadiazole-4-carboxylic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-methyl-[1,2,3]thiadiazole-5-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (33). Prepared according to general procedure (A) from 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(thiazole-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (34). Prepared according to general procedure (A) from thiazole-2-carboxylic acid[23] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (35). Prepared according to general procedure (A) from 3-chlorothiophene-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(1-methyl-pyrrole-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (36). Prepared according to general procedure (A) from 1-methyl-pyrrole-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(furan-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (37). Prepared according to general procedure (A) from 2-furoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(furan-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (38). Prepared according to general procedure (A) from 3-furoic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-5-phenyl-thiophene-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (39). Prepared according to general procedure (A) from acid **9** and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-pyridine-3-yl-thiazole-4-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (40). Prepared according to general procedure (A) from 2-(pyrid-3-yl)-thiazole-4-carboxylic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorobenzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (41). Prepared according to general procedure (A) from 3-chlorobenzo[*b*]thiophene-2-carboxylic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3,5-dichloro-thieno[3,2-b]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (42). Prepared according to general procedure (B) from 3,5-dichlorothieno[3,2-b]thiophene-2-carbonyl chloride[24] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[*d*]isothiazole-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (43). Prepared according to general procedure (A) from 1,2-benzoisothiazole-3-carboxylic acid (Dalton Chemical Labs Inc.) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[*b*]furan-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (44). Prepared according to general procedure (A) from benzo[*b*]furan-2-carboxylic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-methyl-benzo[*b*]furan-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (45). Prepared according to general procedure (A) from 3-methyl-benzo[*b*]furan-2-carboxylic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(indole-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (46). Prepared according to general procedure (A) from 1H-indole-2-carboxylic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(1-methyl-indole-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (47). Prepared according to general procedure (A) from 1-methylindole-2-carboxylic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(imidazo[2,1-b]thiazole-6-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (48). Prepared according to general procedure (A) from the acid **16** and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(imidazo[1,2-a]pyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (49). A mixture of the acid **19** (23.4 mg, 0.14 mmol) and oxalyl chloride (1 mL) was stirred at 25 °C for 1 h and evaporated. The residue was dissolved in DMF (1 mL) and DIEA (0.25 mL), treated with the amine **7**, stirred for 16 h at 25 °C, and diluted with 50% aqueous AcOH (14 mL). Purification according to general protocol A gave **49**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(imidazo[1,2-a]pyrimidine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (50). Prepared according to general procedure (A) from imidazo[1,2-a]pyrimidine-2-carboxylic acid[25] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(4-benzamido-1-methyl-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (51). Pre-

pared according to general procedure (A) from benzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-chlorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (52). Prepared according to general procedure (A) from 4-chlorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[1,3]dioxole-5-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (53). Prepared according to general procedure (B) from piperonyloyl chloride (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(pyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (54). Prepared according to general procedure (A) from picolinic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-nicotinamido-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (55). Prepared according to general procedure (A) from nicotinic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(pyridine-4-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (56). Prepared according to general procedure (A) from isonicotinic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-methyl-pyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (57). Prepared according to general procedure (A) from 3-methylpicolinic acid (TCI) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(5-butyl-pyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (58). Prepared according to general procedure (A) from fusaric acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(5-chloro-6-hydroxy-nicotinamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (59). Prepared according to general procedure (A) from 5-chloro-6-hydroxy-nicotinic acid (Fluka) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(5-methyl-pyrazine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (60). Prepared according to general procedure (A) from 5-methyl-pyrazine-2-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzimidazole-5-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (61). Prepared according to general procedure (A) from benzimidazole-5-carboxylic acid (Avocado) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[1,2,5]thiadiazole-5-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (62). Prepared according to general procedure (A) from benzo[1,2,5]thiadiazole-5-carboxylic acid (obtained from the corresponding methyl ester (Avocado) by saponification under standard conditions) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[1,2,5]oxadiazole-5-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (63). Prepared according to general procedure (A) from benzo[1,2,5]oxadiazole-5-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(naphthalene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (64). Prepared according to general procedure (A) from 2-naphthoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-oxo-2H-chromene-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (65). Prepared according to general procedure (A) from coumarin-3-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (66). Prepared according to general procedure (A) from isoquinoline-3-carboxylic acid (TCI) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-methyl-[1,6]naphthyridine-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (67). Prepared according to general procedure (A) from 2-methyl-1,6-naphthyridine-3-carboxylic acid (Peakdale Molecular Ltd.) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(quinoxaline-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (68). Prepared according to general procedure (A) from quinoxaline-2-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-([1,8]-naphthyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (69). Prepared according to general procedure (A) from 1,8-naphthyridine-2-carboxylic acid (Peakdale Molecular Ltd.) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-([1,6]-naphthyridine-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (70). Prepared according to general procedure (A) from 1,6-naphthyridine-2-carboxylic acid (Peakdale Molecular Ltd.) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (71). Prepared according to general procedure (A) from thiophene-2-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-fluoro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (72). Prepared according to general procedure (A) from 3-fluoro-thiophene-2-carboxylic acid[26] (modified procedure: the Schiemann reaction was performed in the solid-state instead of using xylene as a solvent) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-bromo-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-

2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (73). Prepared according to general procedure (A) from 3-bromo-thiophene-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-methyl-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (74). Prepared according to general procedure (A) from 3-methyl-thiophene-2-carboxylic acid (Acros) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-cyano-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (75). Prepared according to general procedure (A) from the acid **12** and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-methoxy-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (76). Prepared according to general procedure (A) from 3-methoxy-thiophene-2-carboxylic acid (obtained from the corresponding methyl ester (Avocado) by saponification under standard conditions) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-hydroxy-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (77). A solution of **76** (20 mg, 0.031 mmol) in CH_2Cl_2 (1.5 mL) and $CHCl_3$ (0.5 mL) was treated with $AlCl_3$ (50 mg, 0.37 mmol) and stirred for 30 h at 25 °C and for 24 h at 60 °C. The mixture was treated with 50% aqueous AcOH (8 mL). The layers were separated and the aqueous phase purified by HPLC.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-methylsulfanyl-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (78). Prepared according to general procedure (A) from 3-(methylthio)-thiophene-2-carboxylic acid[27] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(5-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (79). Prepared according to general procedure (A) from 5-chlorothiophene-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3,4,5-trichloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (80). Prepared according to general procedure (A) from 3,4,5-trichlorothiophene-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4,5-dichloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (81). Prepared according to general procedure (A) from 4,5-dichlorothiophene-2-carboxylic acid[28] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-4-(propane-2-sulfonyl)-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (82). Prepared according to general procedure (A) from 3-chloro-4-(propane-2-sulfonyl)-thiophene-2-carboxylic acid (Maybridge) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (83). Prepared according to general procedure (A) from benzo[*b*]thiophene-2-carboxylic acid (Lancaster) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(7-chloro-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (84). Prepared according to general procedure (A) from 7-chloro-benzo[*b*]thiophene-2-carboxylic acid[29] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3,6-dichloro-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (85). Prepared according to general procedure (A) from 3,6-dichloro-benzo[*b*]thiophene-2-carboxylic acid (Asinex) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-6-methyl-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (86). Prepared according to general procedure (B) from 3-chloro-6-methyl-benzo[*b*]thiophene-2-carbonyl chloride[30] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-6-fluoro-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (87). Prepared according to general procedure (A) from 3-chloro-6-fluorobenzo[*b*]thiophene-2-carboxylic acid (Asinex) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-6-trifluoromethyl-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (88). Prepared according to general procedure (B) from 3-chloro-6-trifluoromethyl-benzo[*b*]thiophene-2-carbonyl chloride[30] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-5,6-difluoro-benzo[*b*]thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (89). Prepared according to general procedure (B) from the acid chloride **21** and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(7-chloro-1,3-dioxa-5-thia-s-indacene-6-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (90). Prepared according to general procedure (B) from 7-chloro-1,3-dioxa-5-thia-s-indacene-6-carbonyl chloride[19] and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(1,3-dioxa-5-thia-s-indacene-6-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (91). Prepared according to general procedure (A) from the acid **25** and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-chlorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (92). Prepared according to general procedure (A) from 2-chlorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpho-

line (93). Prepared according to general procedure (A) from 3-chlorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-fluorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (94). Prepared according to general procedure (A) from 2-fluorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-fluorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (95). Prepared according to general procedure (A) from 3-fluorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-fluorobenzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (96). Prepared according to general procedure (A) from 4-fluorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2,4-difluoro-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (97). Prepared according to general procedure (B) from 2,4-difluorobenzoyl chloride (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-chloro-2-fluoro-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (98). Prepared according to general procedure (A) from 4-chloro-2-fluorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(2-chloro-4-fluoro-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (99). Prepared according to general procedure (A) from 2-chloro-4-fluorobenzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-methoxy-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}morpholine (100). Prepared according to general procedure (B) from 4-anisoyl chloride (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-difluoromethoxy-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (101). Prepared according to general procedure (A) from 4-(difluoromethoxy) benzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-trifluoromethoxy-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}morpholine (102). Prepared according to general procedure (B) from 4-(trifluoromethoxy)-benzoyl chloride (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(4-chloro-2-methoxy-benzamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (103). Prepared according to general procedure (A) from 4-chloro-2-methoxy-benzoic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(quinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (104). Prepared according to general procedure (A) from quinoline-3-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(quinoline-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (105). Prepared according to general procedure (A) from quinaldic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-1-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-morpholine (106). Prepared according to general procedure (A) from isoquinoline-1-carboxylic acid (Aldrich) and **7**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}thiomorpholine (107). Prepared according to general procedure (C) from 4-(2-aminoethyl)thiomorpholine (Oakwood) and **29**.

1-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}piperidine (108). Prepared according to general procedure (C) from 1-(2-aminoethyl)piperidine (Aldrich) and **29**.

4-Fluoro-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (109). Prepared according to general procedure (C) from 1-(2-amino-ethyl)-4-fluoro-piperidine (Oakwood) and **29**.

4,4-Difluoro-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (110). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4,4-difluoro-piperidine (Oakwood) and **29**.

4-Hydroxy-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (111). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4-hydroxy-piperidine (Oakwood) and **29**.

4-Methyl-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(3-chloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperazine (112). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4-methyl-piperazine (Oakwood) and **29**.

1-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}pyrrolidine (113). Prepared according to general procedure (C) from 1-(2-aminoethyl)pyrrolidine (Aldrich) and **29**.

4-{3-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-propyl}morpholine (114). Prepared according to general procedure (C) from 4-(3-aminopropyl)morpholine (Aldrich) and **29**.

1-{3-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-propyl}piperidine (115). Prepared according to general

procedure (C) from 1-(3-aminopropyl)piperidine (Aldrich) and **29**.

***N,N*-Dimethyl-3-{1-methyl-4-[1-methyl-4-(1-methyl-4-(3-chloro-thiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (116).** Prepared according to general procedure (C) from *N*,*N*-dimethylaminopropylamine (Aldrich) and **29**.

2-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-ethyl}pyridine (117). Prepared according to general procedure (C) from 2-(2-aminoethyl)pyridine (Lancaster) and **29**.

2-{[1-Methyl-4-(1-methyl-4-(1-methyl-4-(3-chlorothiophene-2-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-methyl}pyridine (118). Prepared according to general procedure (C) from 2-(aminomethyl)pyridine (TCI) and **29**.

4-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-thiomorpholine (119). Prepared according to general procedure (C) from 4-(2-aminoethyl)thiomorpholine (Oakwood) and **30**.

1-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-piperidine (120). Prepared according to general procedure (C) from 1-(2-aminoethyl)piperidine (Aldrich) and **30**.

4-Fluoro-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (121). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4-fluoropiperidine (Oakwood) and **30**.

4,4-Difluoro-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (122). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4,4-difluoro-piperidine (Oakwood) and **30**.

4-Hydroxy-1-{2-[1-methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}piperidine (123). Prepared according to general procedure (C) from 1-(2-aminoethyl)-4-hydroxypiperidine (Oakwood) and **30**.

1-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-piperazine (124). Prepared according to general procedure (C) from 1-(2-aminoethyl)piperazine (Aldrich) and **30**.

1-{2-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]ethyl}-pyrrolidine (125). Prepared according to general procedure (C) from 1-(2-aminoethyl)pyrrolidine (Aldrich) and **30**.

4-{3-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]propyl}morpholine (126). Prepared according to general procedure (C) from 4-(3-aminopropyl)morpholine (Aldrich) and **30**.

1-{3-[1-Methyl-4-(1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]propyl}-piperidine (127). Prepared according to general procedure (C) from 1-(3-aminopropyl)piperidine (Aldrich) and **30**.

***N,N*-Dimethylamino-3-{1-methyl-4-[1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (128).** Prepared according to general procedure (C) from *N*,*N*-dimethylaminopropylamine (Aldrich) and **30**.

***N,N*-Dimethylamino-2,2-dimethyl-3-{1-methyl-4-[1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (129).** Prepared according to general procedure (C) from *N*,*N*,2,2-tetramethyl-1,3-propane-diamine (TCI) and **30**.

4-Methyl-piperazin-1-yl-{1-methyl-4-[1-methyl-4-(1-methyl-4-(isoquinoline-3-carboxamido)-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-pyrrole-2-methanone} (130). Prepared according to general procedure (C) from 1-methyl-piperazine (Aldrich) and **30**.

DNA Binding Properties. DNase I footprint titration experiments were performed as described by Trauger and Dervan.[31] The plasmid pTrc99a (from Amersham Pharmacia Biotech, Inc) was used as a DNA-binding probe for all DNase I footprint titration experiments. The 3′-^{32}P end-labeled 352-bp DNA fragment was prepared by digesting pTrc99a with EcoRI and PvuII with simultaneous fill-in using Sequenase v. 2.0, [α-^{32}P]-ATP, and [α-^{32}P]-TTP.

Microbiology. Protocol as described.[32,33] All isolates tested were ATCC strains.

Acute Tolerability. Groups of ≥ 4 ICR female mice (ca. 25 g) received a single IV bolus in the lateral tail vein. Mice were monitored for 48−72 h for clinical observations. Mice were then sacrificed by CO_2 inhalation and a gross necropsy including body and organ weights was performed. Blood was collected for clinical chemistry analysis at the time of euthanasia and analyzed within several hours of collection.

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Supporting Information Available: ^{1}H NMR spectra, mass spectra, and purity analysis data (HPLC−UV). This material is available free of charge via the Internet at http://pubs.acs.org.

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