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Synthesis and Antiprotozoal Activity of Pyridyl Analogues of Pentamidine

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A series of novel pyridyl analogues **1**−**18** of antiprotozoal drug 1,5-bis(4-amidinophenoxy)pentane (pentamidine) has been synthesized and tested for in vitro activities against *Trypanosoma brucei* rhodesiense, *Plasmodium falciparum*, and *Leishmania donovani*, and for cytotoxicity against mammalian cells. Antiprotozoal properties of compounds **1**−**18** depended on the placement of cationic moieties on the pyridine rings as well as the nature of substituents on the amidine groups. Diamidine **6** with cationic moieties adjacent to pyridine nitrogen atoms was the most promising compound in the series showing superior in vitro activities against *T. brucei* rhodesiense, *P. falciparum*, and *L. donovani* compared to pentamidine. An oral prodrug of diamidine **6**, diamidoxime **9**, administered at 25 mg/kg daily for 4 days, exhibited excellent antitrypanosomal efficacy in vivo curing all infected animals in the STIB900 acute mouse model of trypanosomiasis.

Introduction

In recent years, the emergence of drug resistant pathogens has led to treatment failures for a number of tropical diseases, including malaria,[1−6] human African trypanosomiasis (HAT[a] or sleeping sickness),[7−10] and leishmaniasis.[11−13] Reduced efficiency of the most commonly used medications requires the use of drug combinations or more expensive treatments,[1,3,4,6] which increases economic burden on affected nations. The need for safe and affordable antiprotozoal therapies capable of overcoming parasite resistance makes the identification of new drug candidates an urgent priority.

Since the discovery of its antiprotozoal potency in the early 1940s, 1,5-bis(4-amidinophenoxy)pentane (pentamidine)[14] has been used to treat early stage *Trypanosoma brucei* gambiense related HAT,[8,15,16] *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia,[17−19] and antimony-resistant leishmaniasis,[12,20,21] including several recent cases of cross-infections with HIV.[22,23] Pentamidine also displayed activity against several strains of malaria,[24−26] although it was never used to treat the disease. Because of protonation of its amidine groups at physiological pH, pentamidine has low oral activity.[8,27] For that reason, the drug requires parenteral administration, which makes the treatment less practical in rural areas. Pentamidine is well tolerated by most patients in spite of some reported adverse effects.[27−29]

The mechanism of antiprotozoal action of pentamidine is still not completely clear.[8,15,16,27,30] The ability of pentamidine to accumulate rapidly inside *Trypanosoma*[24,31] and *Plasmodium*[24,26] species inhibiting multiple targets[8,24,27,28] indicates the involvement of parasite specific uptake mechanisms. In *T. brucei*, the uptake of diamidines and melaminophenyl arsenicals is partially mediated by the P2 transporter.[24,30−37] The cross-resistance between these drugs, sharing recognition motifs for the carrier, was observed in laboratory strains of *Trypanosoma*[38−40] and was attributed to the loss of the P2 transporter.[15,31,35,36,41,42] However, pentamidine accumulates even in strains without significant P2 activity,[30] suggesting the presence of alternative routes of uptake.[24,35−37,41,42] *Plasmodium* species use different mode of accumulation for diamidines than trypanosomes. Pentamidine penetrates the infected erythrocyte membranes through a parasite-induced pore with properties of the new permeability pathway (NPP).[24,26]

The absence of widespread trypanosomal resistance to pentamidine in the field[8,24] makes it an attractive lead compound in the search for new antiprotozoal therapies.[9,24,43,44] The amidine groups in pentamidine are part of the recognition motif for the P2 transporter in *Trypanosoma* species,[30,33−35] and oxygen atoms in the aliphatic linker are essential for the activity of the drug against the parasite.[45] Earlier findings from this laboratory demonstrated that the replacement of the linker oxygens in pentamidine with nitrogen atoms improves antiprotozoal properties of resulting dications.[25,46−48] However, similar alternations on the phenyl rings in pentamidine have not been explored before. The reports of increased efficacies of pyridyl analogues of 2,5-bis(4-amidinophenoxy)-furan (furamidine)[49] have been recently published.[50,51] In our continuing search for new antiprotozoal drug candidates, we report the synthesis and antiprotozoal testing of a series of novel heterocyclic pentamidine analogues **1**−**18**. The phenyl rings in pentamidine were replaced with pyridyl fragments to improve uptake by the P2 carrier and possibly to increase the bioavailability of dications. The compounds **1**−**18** were screened in vitro against *T. brucei* rhodesiense (STIB900), chloroquine resistant *Plasmodium falciparum* (K1), axenic

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[a] Abbreviations: HAT, human African trypanosomiasis; HIV, human immunodeficiency virus; BBB, blood−brain barrier; MLSP, melarsoprol; CQ, chloroquine; ATMS, artemisinin; PPT, podophyllotoxin.

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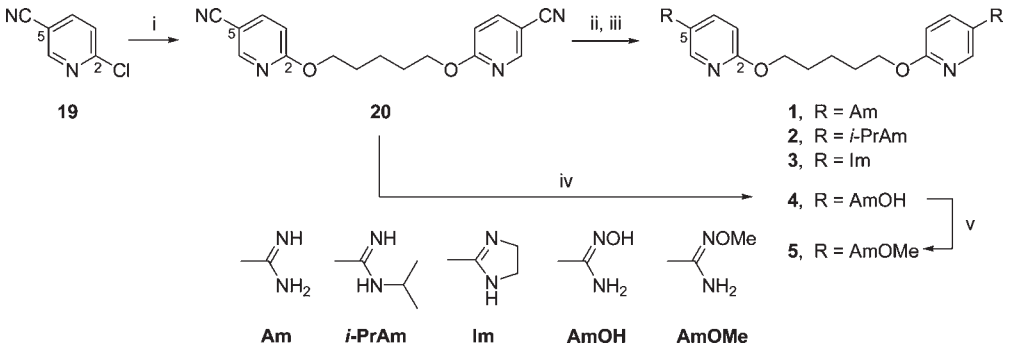
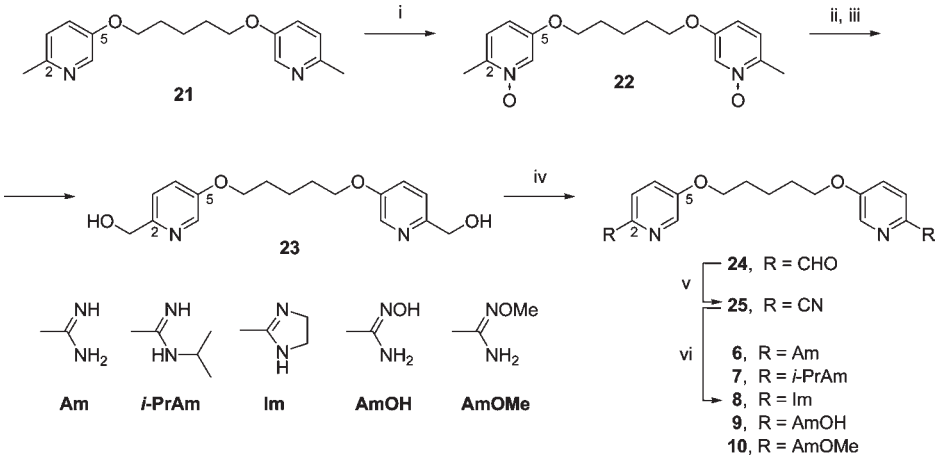
Scheme 1[a]



[a] Reagents and conditions: (i) NaH, 1,5-pentanediol, THF, 0 °C; (ii) HCl, EtOH, dioxane, 0 °C−ambient temperature; (iii) ammonia or amine, EtOH, ambient temperature; (iv) *t*-BuOK, $NH_2OH \cdot HCl$, DMSO; (v) 1 M NaOH, dimethyl sulfate, DMSO.

Scheme 2[a]



[a] Reagents and conditions: (i) MCPA, $CHCl_3$; (ii) Ac_2O, reflux; (iii) NEt_3, MeOH, reflux; (iv) MnO_2, $CHCl_3$; (v) $NH_2OH \cdot HCl$, $CHCl_3$, NEt_3, triphosgene; (vi) NaOMe, MeOH, then amine hydrochloride.

amastigotes of *L. donovani* (MHOM/SD/62/1S-$CL2_D$), and for cytotoxicity against rat myoblast cells (L6). Compounds exhibiting high antitrypanosomal activities in vitro underwent in vivo screening in the STIB900 acute mouse model of trypanosomiasis.

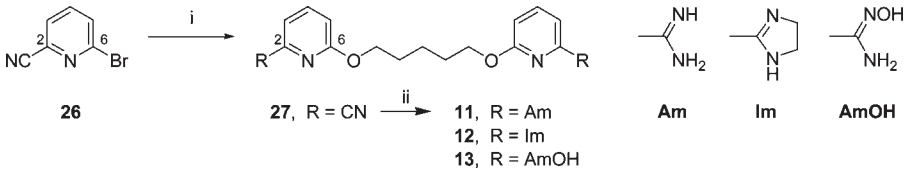
Chemistry

The synthesis of compounds **1**−**5** is depicted in Scheme 1. 6-Chloropyridine-3-carbonitrile (**19**) was prepared from commercially available 6-chloropyridine-3-carboxamide according to a literature procedure.[52] Interaction of **19** with 1,5-pentanediol in the presence of NaH at 0 °C resulted in 1,5-bis(3-cyanopyridine-6-oxy)pentane (**20**) in 83% yield. Compound **20** underwent Pinner reaction[53] to form a diimidate ester, which was reacted with ammonia or appropriate amines to yield dications **1**−**3**. Interaction of dinitrile **20** with $NH_2OH \cdot HCl$ in the presence of *t*-BuOK in DMSO led to the formation of 1,5-bis(3-*N*-hydroxyamidinopyridine-6-oxy)-pentane (**4**). Alkylation of compound **4** with dimethyl sulfate afforded 1,5-bis(3-*N*-methoxyamidinopyridine-6-oxy)pentane (**5**).

The synthesis of dications **6**−**10** is outlined in Scheme 2. 1,5-Bis(2-methylpyridine-5-oxy)pentane (**21**) was prepared by reaction of 6-methylpyridin-3-ol with 1,5-dibromopropane under reflux in 2-butanone in the presence of K_2CO_3 according to the published procedure.[54] Compound **21** was converted to 1,5-bis(2-pyridinaldehyde-5-oxy)pentane (**24**) in four steps following the previously described methodology.[55] Thus, oxidation of **21** with MCPA in chloroform afforded 1,5-bis(2-methylpyridine-5-oxy-1-oxide)pentane (**22**) in 65% yield, which underwent a rearrangement by treatment with acetic anhydride to afford 1,5-bis(2-acetoxymethylpyridin-5-oxy)pentane. Hydrolysis of the ester groups with triethylamine in methanol gave dialcohol **23**, which was oxidized with activated MnO_2 to form dialdehyde **24** in 39% yield over three steps. Compound **24** reacted with $NH_2OH \cdot HCl$ and was converted to dinitrile **25** in 32% yield using a one-pot procedure utilizing bis(trichloromethyl)carbonate as a dehydrating agent.[56] Because pyridine-2-carbonitriles do not form imidate esters under the conditions of the Pinner reaction, this transformation was carried out under basic conditions previously reported for the synthesis of 4-pyrine-carboxamidine hydrochloride.[57] Interaction of the resulting imidate ester with hydrochloride salts of appropriate amines yielded dications **6**−**10**.

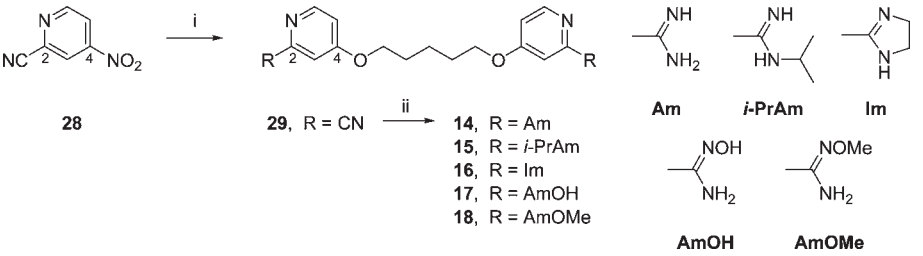
6-Bromopyridine-2-carbonitrile (**26**), a key intermediate for the synthesis of compounds **11**−**13** (Scheme 3), was prepared from commercially available 6-bromopyridine-2-carbaldehyde in 67% yield using the aforementioned one-pot procedure[56] employed for the synthesis of dinitrile **25**. Compound **26** was found to be labile at ambient temperature and therefore was used immediately after the preparation. Interaction

Scheme 3[a]



[a] Reagents and conditions: (i) NaH, 1,5-pentanediol, THF, 0 °C; (ii) NaOMe, MeOH, then amine hydrochloride.

Scheme 4[a]



[a] Reagents and conditions: (i) NaH, 1,5-pentanediol, THF, 0 °C; (ii) NaOMe, MeOH, then amine hydrochloride.

of **26** with 1,5-pentanediol in the presence of NaH at 0 °C formed dinitrile **27**, which reacted with methanol in the presence of catalytic amounts of sodium methoxide, followed by interaction with hydrochlorides of ammonia, ethylenediamine, or hydroxylamine, to form compounds **11**−**13**.

The synthesis of compounds **14**−**18** is shown in Scheme 4. Commercially available 4-nitropyridine-*N*-oxide was converted to 4-nitropyridine-2-carbonitrile (**28**) according to a literature procedure.[58] Compound **28** reacted with 1,5-pentanediol in the presence of NaH at 0 °C to give 1,5-bis(2-cyanopyridine-4-oxy)pentane (**29**) in 65% yield. Interaction of dinitrile **29** with catalytic amounts of sodium methoxide in methanol followed by interaction with the hydrochloride salts of appropriate amines afforded analogues **14**−**18**.

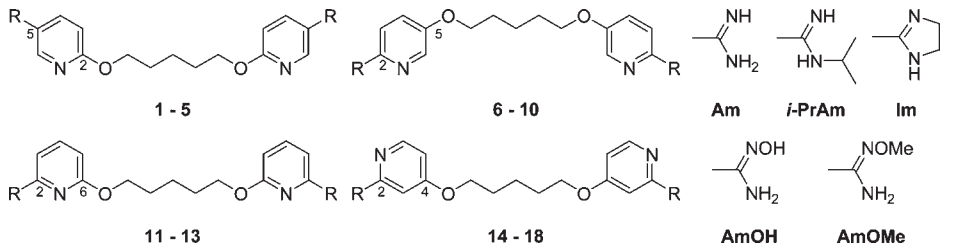
Results and Discussion

The effect of various structural variations of aromatic dications on their activities against *Trypanosoma*, *Plasmodium*, and *Leishmania* species have been previously investigated by others[14,59−67] and by us.[25,46,47,68−71] Earlier results from this laboratory demonstrated that aza-analogues prepared by the replacement of oxygen atoms in the carbon linker of pentamidine with nitrogens exhibited excellent antiprotozoal properties.[25,46−48] However, similar alternations on the aromatic rings of pentamidine have not been undertaken before. Here, we report a continued investigation of structure−activity relationship of pentamidine analogues in which phenyl rings have been replaced with pyridyl fragments. Structural modifications of dications used in this study included varying the position of the cationic moieties in the pyridine rings and the nature of the substituents on the amidine groups. Novel pentamidine analogues (**1**−**3**, **6**−**8**, **11**, **12**, **14**−**16**) and their amidoxime and *O*-methylamidoxime prodrugs (**4**, **5**, **9**, **10**, **13**, **17**, and **18**) were evaluated in vitro for activity against bloodstream form trypomastigotes of *T. brucei* rhodesiense (STIB900), chloroquine resistant *P. falciparum* (K1), axenic amastigotes of *L. donovani* (MHOM/SD/62/1S−CL2$_D$), and for cytotoxicity against rat myoblast cells (L6). The results of the screening are summarized in Table 1. For comparison to tested dications, we included the activities of pentamidine, melarsoprol (*T. brucei* rhodesiense), chloroquine and artemisinin (*P. falciparum*), and podophyllotoxin (L6). Finally, to demonstrate the degree of selectivity between parasite and rat myoblast cells (L6 line) three selectivity indexes[72] were calculated as follows: antitrypanosomal selectivity index SI$_T$, expressed as the ratio [IC$_{50}$ (L6-cells)/IC$_{50}$ (*T. brucei* rhodesiense)], antiplasmodial selectivity index SI$_P$, expressed as the ratio [IC$_{50}$ (L6-cells)/IC$_{50}$ (*P. falciparum*)], and antileishmanial selectivity index SI$_L$, expressed as the ratio [IC$_{50}$ (L6-cells)/IC$_{50}$ (*L. donovani*)]. Dications exhibiting high antitrypanosomal activities in vitro underwent in vivo screening in the STIB900 acute mouse model of trypanosomiasis (Table 2).

Overall, the tested compounds exhibited lower cytotoxicity compared to pentamidine regardless of the substitution on the amidine groups and the position of the nitrogen atoms in the aromatic rings, except for analogues **4**, **6**, **10**, and **12**. The arrangement of substituents on the aromatic rings affected relative cytotoxicities of dications **1**−**18**. For example, compounds **6**−**8** bearing the cationic groups in the 2-position were more cytotoxic than the corresponding 5-substituted isomers **1**−**3**. Concurrently, 2,6- and 2,4-disubstituted diamidines **11** and **14** exhibited higher cytotoxicity compared to diamidine **1**, while at the same time being less cytotoxic than analogue **6**. Among different types of dications, bis(*N*-isopropyl)amidines were usually less cytotoxic compared to diamidines and diimidazolines, which supports our previous findings.[48,71] Thus, bis(*N*-isopropyl)amidine **7** was 30 times less cytotoxic than diamidine **6** and nearly 3 times less cytotoxic relative to pentamidine.

In this study, the *N*-alkylation of cationic fragments reduced the activity of compounds **1**−**3**, **6**−**8**, **11**, **12**, and **14**−**16** against *T. brucei* rhodesiense and *L. donovani* compared to pentamidine, confirming previously published results.[25,60,63,64,66,71,73] Among the compounds **1**−**18**, only diamidines **1**, **6**, **11**, and **14** as well as the bis(*N*-isopropyl)amidine **7** exhibited antitrypanosomal IC$_{50}$ values less than 100 nM. Compound **6** was 3 times as active as pentamidine against *T. brucei* rhodesiense, while diamidine **1** was 23 times less potent against the pathogen than the drug. The 2,6- and 2,4-substituted dications **11**, **12**, and **14**−**16** displayed lower potencies against *T. brucei* rhodesiense than

Table 1. Cytotoxicity and in Vitro Antiprotozoal Activity of Dications **1**−**18**



		cytotoxicity[f]	*T. brucei* rhodesiense[g]		*P. falciparum*[i]		*L. donovani*[k]	
compd	R	IC_{50} (μM)	IC_{50} (μM)	SI_T[h]	IC_{50} (μM)	SI_P[j]	IC_{50} (μM)	SI_L[l]
1	Am	137	0.070	1957	0.329	416	39	4
2	*i*-PrAm	> 155	0.276	> 562	0.256	> 605	> 100	2
3	Im	111	1.42	78	1.86	60	> 100	1
4	AmOH	16.4	27.4	< 1	6.50	3	> 100	< 1
5	AmOMe	> 185	25.9	> 7	2.24	> 83	> 100	2
6	Am	4.90	0.001	4900	0.009	544	0.47	11
7	*i*-PrAm	148	0.034	4353	0.006	24667	6.0	25
8	Im	82.3	0.170	484	0.036	2286	36	2
9	AmOH	166	108	2	> 10.7	< 16	> 100	< 2
10	AmOMe	39.4	51.2	< 1	3.50	11	> 50	< 1
11	Am	114	0.019	6000	0.027	4222	4.1	28
12	Im	14.6	3.07	5	0.568	26	24	< 1
13	AmOH	52.2	19.0	3	10.5	5	> 50	1
14	Am	59.3	0.051	1163	0.016	3706	38	2
15	*i*-PrAm	> 155	1.70	> 91	1.33	117	> 100	2
16	Im	80.8	12.5	6	4.26	19	> 100	< 1
17	AmOH	159	12.0	13	> 2.10	76	45	4
18	AmOMe	138	18.2	8	> 2.00	69	> 100	1
PMD[a]		46.6	0.003	15533	0.058	803	1.8	25
MLSP[b]		7.78	0.004	1945				
CQ[c]		117			0.124	944		
ATMS[d]		450			0.006	75000		
PPT[e]		0.01						

[a] PMD, pentamidine. [b] MLSP, melarsoprol. [c] CQ, chloroquine. [d] ATMS, artemisinin. [e] PPT, podophyllotoxin. [f] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations. [g] *Trypanosoma brucei* rhodesiense (STIB900). Average of duplicate determinations. [h] Selectivity index for *T. brucei* rhodesiense (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. brucei* rhodesiense)]. [i] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations. [j] Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)]. [k] *Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of at least two independent determinations. [l] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].

Table 2. Activity of Select Pentamidine Analogues in the STIB900 Mouse Model of Trypanosomiasis

	in vitro	in vivo[b]			
compd	IC_{50} (nM)[a]	RA[c]	dose (mg/kg)	cures[d]	survival (days)[e]
melarsoprol	4	ip	4 × 2	4/4	> 60
		ip	4 × 1	2/4	> 51.5
pentamidine	7	ip	4 × 20	2/4	> 57.5
		ip	4 × 5	2/4	> 45
1	70	ip	4 × 5	0/4	17.75
4	27400	po	4 × 25	0/4	8.75
6	1	ip	4 × 5	1/4	> 36
7	34	ip	4 × 5	0/4	18.5
8	170	ip	4 × 5	0/4	12.75
9	108000	po	4 × 25	4/4	> 60
10	51200	po	4 × 25	0/4	19.5
11	19	ip	4 × 5	0/4	22.5
14	51	ip	4 × 5	0/4	14
17	12000	po	4 × 100	0/4	14.25
18	18200	po	4 × 100	0/4	11.75

[a] Average of duplicate determinations. [b] STIB900 acute mouse model. [c] RA, route of administration: intraperitoneal (ip) or oral (po). [d] Number of mice that survive and are parasite free for 60 days. [e] Average days of survival of all mice including the cured ones (60 days); untreated controls expired between day 7 and 10 post infection.

the corresponding 2,5-substituted isomers **6**−**8**. The diamidines **1**, **6**, **11**, and **14** were more selective for *T. brucei* rhodesiense than the corresponding *N*-substituted analogues. Compound **6** not only exhibited the highest antitrypanosomal activity in the series with an IC_{50} value of 1 nM but also demonstrated the second highest parasite selectivity among dications **1**−**18** (SI_T = 4900). Diamidine **11** was the most selective analogue in the group for *T. brucei* rhodesiense, showing SI_T = 6000.

Diamidine **6** was also the most active compound against *L. donovani*, being nearly 4 times more potent than pentamidine while at the same time showing significant selectivity for the pathogen. Compound **6** was the only analogue in the series exhibiting an antileishmanial IC_{50} value less than 1 μM. Antileishmanial properties of the 2,6- and 2,4-substituted dications **11**, **12**, and **14**−**16** diminished with respect to their 2,5-substituted counterparts **6**−**8** and to pentamidine as did the selectivity of the compounds **12** and **14**−**16** against the pathogen. Diamidine **11** was the most selective compound against *L. donovani* (SI_L = 28), showing superior selectivity for the pathogen compared to pentamidine. Its 2,4-substituted isomer **14**, however, was 9 times less active against the parasite

and exhibited antileishmanial selectivity that was 14 times lower relative to **11**.

Contrary to the antitrypanosomal and antileishmanial results, the *N*-alkyl substitution on the amidine groups improved antiplasmodial activities of bis(*N*-isopropyl)amidines **2** and **7** and diimidazoline **8** with respect to the corresponding diamidines **1** and **6**. The aza-analogues **6**−**8**, **11**, and **14** exhibited increased activity against *P. falciparum* compared to pentamidine (IC_{50} = 58 nM). Bis(*N*-isopropyl)amidine **7** was the most active compound in the series, showing antiplasmodial activity comparable to that of artemisinin (IC_{50} = 6 nM). Compounds **7**, **8**, **11**, and **14** exhibited antiplasmodial selectivity indexes (SI_P) that were 4- to nearly 30-fold greater than that of pentamidine, corroborating earlier findings.[71,73] While diamidine **6** displayed improved antiplasmodial properties compared to pentamidine, it demonstrated lower selectivity for *P. falciparum* than the drug. Dication **7**, which was significantly less cytotoxic than diamidine **6**, exhibited the highest antiplasmodial selectivity among all analogues (SI_P = 24667). It was 30 times more selective for *P. falciparum* than pentamidine. Dications **11**, **12**, **14**−**16** were less active against *P. falciparum* than the corresponding 2,5-substituted derivatives **6**−**8**, which correlates with comparative antiplasmodial activities of 3,3′- and 4,4′-substituted pentamidine isomers.[25] At the same time, diamidines **11** and **14** and diimidazoline **12** exhibited greater antimalarial potency compared to the 2,5-substituted analogues **1** and **3**. Diamidines **11** and **14** were more selective for *P. falciparum* relative to the 2,5-substituted analogues **1** and **6**. Among the 2,5-disubstituted dications **1**−**3** and **6**−**8**, bis(*N*-isopropyl)amidines were more selective for *P. falciparum* than diamidines and diimidazolines. Antiplasmodial selectivity of 2,4- and 2,6-disubstituted isomers **11**, **12**, and **14**−**16** decreased in the order Am > *i*-PrAm > Im.

As expected, diamidoximes **4**, **9**, **13**, and **17** and bis(*O*-methyl)amidoximes **5**, **10**, and **18** did not show significant in vitro activities against *T. brucei* rhodesiense, *P. falciparum*, and *L. donovani* due to the lack of metabolizing enzymes in these organisms.[50,74−76] While being significantly less active in vitro compared to corresponding amidines, aromatic diamidoximes and bis(*O*-methyl)amidoximes have been shown to rapidly convert to amidines in vivo.[77] Use of these derivatives as prodrugs for amidines creates an opportunity for developing orally bioavailable drug candidates.[50,78−80]

Select dications exhibiting promising in vitro activities against *T. brucei* rhodesiense were evaluated in vivo in the STIB900 mouse model of African trypanosomiasis (Table 2). The screening was conducted using intraperitoneal dosing at 5 mg/kg daily for 4 days for parent dications and oral administration at 25 mg/kg for 4 days for amidoximes and methamidoximes. The majority of analogues tested in the STIB900 acute mouse model of trypanosomiasis did not cure infected animals although several compounds increased the survival time compared to untreated controls. Despite high antitrypanosomal activity in vitro, diamidine **6** exhibited lower efficacy relative to pentamidine, curing only one out of four mice. This finding could possibly be explained by the poor pharmacodynamics of dication **6** because of the introduction of additional cationic centers in the molecule. Its amidoxime prodrug **9**, however, displayed excellent in vivo efficacy, providing 100% cures upon oral administration at 25 mg/kg. Despite several reports of superior in vivo efficacies of methamidoximes compared to the corresponding diamidoximes,[50,80] bis(*O*-methyl)amidoxime **10** was inactive in the acute mouse model. This lack of oral activity could be explained by the low rate of metabolic conversion of compound **10** to parent diamidine **6**. On the basis of promising in vivo results, diamidoxime **9** was assayed in the second-stage CNS mouse model (GVR35) of sleeping sickness. Unfortunately, compound **9** provided no cures in this model (data not shown), perhaps due to the lack of blood−brain barrier (BBB) permeability.

In conclusion, a series of pyridyl analogues of pentamidine was synthesized and tested in vitro against *T. brucei* rhodesiense, *P. falciparum*, and *L. donovani* as well as in vivo against *T. brucei* rhodesiense. We found that diamidine **6** possessing cationic substituents adjacent to nitrogen atoms in pyridine rings displayed superior activities against all tested parasites compared to pentamidine. While diamidine **6** was only moderately active in the STIB900 acute mouse model of trypanosomiasis, its prodrug diamidoxime **9** demonstrated excellent in vivo efficacy, curing four out of four animals upon oral administration. Although compound **9** did not provide cures in the CNS mouse model of infection, its BBB permeability could potentially be improved using a prodrug approach. Excellent in vitro activity of diamidine **6** and high in vivo efficacy of its prodrug diamidoxime **9** warrant further investigation of these dications as potential antiprotozoal drug candidates.

Experimental Section

Biology. Preparation of Compounds. Compounds were dissolved in 100% dimethylsulfoxide (DMSO) and finally diluted in culture medium prior to the assay. The DMSO concentration never exceeded 1% in the in vitro assays. For in vivo experiments, the compounds were dissolved in DMSO and further diluted with distilled H_2O to a final DMSO concentration of 10% prior to injection into the animals.

In Vitro Cytotoxicity Assay (L6 Rat Myoblast Cells). IC_{50} values were determined using the Alamar blue assay[81] and were carried out twice independently and in duplicate. Briefly, 4000 L6 cells were seeded in RPMI 1640 medium supplemented with L-glutamine 2 mM, HEPES 5.95 g/L, $NaHCO_3$ 2 g/L, and 10% fetal bovine serum in 96-well microtiter plates. The serial drug dilutions were incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin (Sigma) dissolved in 100 mL of phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2−3 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength 536 nm and an emission wavelength 588 nm. The IC_{50} values were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of *T. brucei* rhodesiense (STIB900). IC_{50} values were determined using the Alamar blue assay and were carried out twice independently and in duplicate. Briefly, the compounds were tested in Minimum Essential Medium with Earle's salts, supplemented as previously described[82] with the following modifications: 2-mercaptoethanol 0.2 mM, sodium pyruvate 1 mM, hypoxanthine 0.5 mM, and 15% heat-inactivated horse serum. Serial drug dilutions were prepared in 96-well microtiter plates and each well inoculated with 2000 bloodstream forms and incubated for 70 h at 37 °C under a humidified 5% CO_2 atmosphere. The viability marker Alamar blue (12.5 mg resazurin (Sigma) dissolved in 100 mL of phosphate buffered saline) (10 μL) was then added to each well and the plate was incubated for additional 2−6 h. The plates were read in a Spectramax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values

were calculated from the sigmoidal inhibition curves using the SoftmaxPro software.

In Vitro Growth Inhibition Assay of *P. falciparum* (K1). The determination of IC_{50} values against erythrocytic stages of *P. falciparum* was carried out twice independently and in duplicate using the [^{3}H]-hypoxanthine incorporation assay.[83,84] Briefly, the compounds were tested in RPMI 1640 medium 10.44 g/L, supplemented with Hepes 5.94 g/L, Albumax II 5 g/L, sodium bicarbonate 2.1 g/L, and neomycin 100 mg/L in 96-well microtiter plates. Infected human red blood cells in medium (hematocrit 1.25%, parasitemia 0.3%) were incubated with the drug dilutions in an atmosphere of 93% N_2, 4% CO_2, 3% O_2 at 37 °C. After 48 h, [^{3}H]-hypoxanthine (0.5 μCi/well) was added and the plates were incubated for additional 24 h under the same conditions. The wells were harvested with a Betaplate cell harvester and transferred on a glass fiber filter. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by a Betaplate liquid scintillation counter (Wallac, Zurich, Switzerland). The IC_{50} values were calculated from the sigmoidal inhibition curves using MS Excel.

Antileishmanial Assay. Axenic amastigotes of *L. donovani* (WHO designation MHOM/SD/62/1S-$CL2_D$) were adapted from promastigotes and grown in the amastigote medium described previously[85] at 37 °C. In a final volume of 60 μL, 6×10^4 parasites were added to each well of a 96-well plate except for negative control wells. Standard and test compounds were added as appropriate using 2-fold dilutions to allow a range of concentrations to be tested. Plates were then incubated at 37 °C for 72 h in a humidified environment containing 5% CO_2. The tetrazolium dye-based CellTiter reagent (Promega, Madison, WI) was used to assess parasite growth.[86] Several hours after adding 12 μL of the CellTiter reagent to each well of the plate, absorbance readings were taken at 490 nm using a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA). SoftMax Pro software (Amersham Biosciences, Piscataway, NJ) was used to calculate IC_{50} values by employing the dose−response equation $y=[(a-d)/(1+(x/c)^b)]+d$, where x = compound concentration, y = absorbance at 490 nm, a = upper asymptote, b = slope, c = IC_{50} value, and d = lower asymptote.

STIB900 Acute Mouse Model of Trypanosomiasis. Experiments were performed as previously reported[87] with minor modifications. Briefly, female NMRI mice were infected intraperitoneally (ip) with 2×10^4 STIB900 bloodstream forms. Experimental groups of four mice were treated ip with tested dications on 4 consecutive days from day 3 to day 6 postinfection. A control group was infected but remained untreated. The tail blood of all mice was checked for parasitemia until 60 days postinfection. Surviving and aparasitemic mice at day 60 were considered cured and then euthanized. Death of animals (including the aparasitemic mice, >60) was recorded to calculate the mean survival time in days.

Chemistry. General Experimental Information. All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Acros Organics and were used without further purification. Uncorrected melting points were measured on a Thomas−Hoover capillary melting point apparatus. All tested compounds are >95% pure by elemental analysis. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 spectrometer operating at 300 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane. Anhydrous ethanol was distilled over Mg/I_2 immediately prior to use. Reaction mixtures were monitored by TLC using Whatman silica gel 250 μm UV_{254} plates or by reverse phase HPLC. Organic layers of extraction mixtures were washed with saturated NaCl solution and dried over Na_2SO_4 or $MgSO_4$ before being evaporated under reduced pressure. Flash column chromatography was performed using Davisil grade 633, type 60A silica gel (200−425 mesh). Analytical HPLC chromatograms were recorded on an a Hewlett-Packard 1090 series II or Agilent 1200 chromatograph using an Agilent Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. The column temperature was maintained at 40 °C. Mobile phases consisted of mixtures of acetonitrile (0−75%) in water, both solvents containing formic acid (80 mM), ammonium formate (20 mM), and triethylamine (15 mM). Flow rates were maintained at 1.5 mL/min. In method A, the concentration of acetonitrile was increased linearly from 0 to 22.5% over 6 min, from 22.5 to 56.25% over 4 min, and maintained for 1 min prior to re-equilibration. In method B, the concentration of acetonitrile was increased linearly from 22.5 to 75% over 10 min and then maintained for 2 min prior to re-equilibration.

Preparative Reverse-Phase HPLC. Preparative reverse-phase HPLC was performed on a Varian ProStar chromatography workstation configured with two PS-215 pumps fitted with 50 mL pump heads, a Dynamax Microsorb C18 (60 Å) column (41.4 mm × 250 mm, 8 μm), PS-320 variable wavelength UV−vis detector, and a PS-701 fraction collector. Mobile phases consisted of mixtures of acetonitrile (0−75%) in water, both solvents containing formic acid (40 mM) and ammonium formate (10 mM). Flow rates were maintained at 40 mL/min. Detector wavelengths and mobile phase gradients were optimized for the individual compounds. Select fractions were analyzed for purity as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 2K or 6K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Flash Chromatography of Amidines on C_{18} Reversed Phase Silica Gel. The chromatographic column was half-filled with acetonitrile and packed with slurry of C_{18} Silica Gel (70 g) in acetonitrile (70−100 mL). The excess acetonitrile was drained out, and the top of the column was covered with a 2 cm pad of sand. The column was equilibrated with 150 mL of initial mobile phase consisting of water containing formic acid (40 mM) and ammonium formate (10 mM). A concentrated reaction mixture was dissolved in the initial mobile phase. In case of low solubility, heating of the mixture and/or addition of a small amount of methanol as a cosolvent were performed. After the reaction mixture was applied to the column, the elution began with initial mobile phase (150 mL) to remove the excess amine and then with a mobile phase consisting of a mixture of acetonitrile (0−75%) in water containing formic acid (40 mM) and ammonium formate (10 mM). Acetonitrile concentrations varied for each individual compound and contained 50−70% of the calculated amount of acetonitrile at the point of the retention time of the compound in analytical method A. After the purification was completed, the column was washed with acetonitrile (3 × 100 mL), ethanol (100 mL), deionized water (2 ×100 mL), and kept in acetonitrile or acetonitrile−water mixture. Select fractions were analyzed for purity as described above for analytical HPLC. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 2K lyophilizer. The lyophilized compounds were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Low resolution ESI mass spectra were recorded on an Agilent Technologies 1100 series LC/MSD trap spectrometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA, and were within ±0.4% of calculated values.

General Procedure for Syntheses of Dications 1−3. 1,5-Bis(3-amidinopyridine-6-oxy)pentane Dihydrochloride (1). A mixture of dry 1,4-dioxane (50 mL) and dry EtOH (20 mL) in a three-neck 250 mL flask equipped with a gas inlet tube, a thermometer, and a drying tube was saturated with gaseous HCl at 0 °C. 1,5-Bis(3-cyanopyridine-6-oxy)pentane (**20**) (2.50 g, 8.10 mmol) was added in one portion, the flask was sealed, and the mixture was stirred at ambient temperature until the starting material was no longer detectable by HPLC. The reaction mixture was diluted with dry ether, and the resulting precipitate was filtered

off under argon, washed with dry ether, and dried under high vacuum to yield a diimidate ester (3.00 g, 99%) that was reacted immediately with ammonia or appropriate amines.

The diimidate ester (0.50 g, 1.30 mmol) was suspended in dry EtOH (20 mL), and saturated ethanolic ammonia (10 mL) was added. The sealed reaction mixture was stirred at ambient temperature. The progress of the reaction was monitored by HPLC. After two days, the reaction mixture was diluted with dry ether and cooled in a freezer. A resulting precipitate was filtered off, purified by preparative HPLC, and recrystallized from 1 N HCl to yield compound **1** as a white powder (0.52 g, 93%); mp >125 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.50 (s, 4H), 9.24 (s, 4H), 8.71 (s, 2H), 8.16 (dd, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 2H), 7.02 (d, J = 8.2 Hz, 2H), 5.86 (br s, 2H), 4.38 (t, J = 6.0 Hz, 4H), 1.80 (m, 4H), 1.57 (m, 2H). HPLC (method A) t_R 4.73 min (100 area %). Anal. ($C_{17}H_{22}N_6O_2 \cdot 3.8HCl \cdot 1.9H_2O$) C, H, N, Cl.

1,5-Bis[3-(*N*-isopropylamidino)pyridine-6-oxy]pentane Dihydrochloride (2). Following the procedure described above for dication **1**, compound **2** was prepared from dinitrile **20**. A crude product was purified by preparative HPLC and recrystallized from 1 N HCl to yield **2** as a white powder (0.40 g, 32%); mp 163 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.69 (d, J = 7.7 Hz, 2H), 9.55 (s, 2H), 9.18 (s, 2H), 8.57 (d, J = 2.7 Hz, 2H), 8.07 (dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 6.67 (br s, 2H), 4.36 (t, J = 6.6 Hz, 4H), 4.07 (m, 2H), 1.79 (m, 4H), 1.55 (m, 2H), 1.27 (d, J = 6.6 Hz, 12H). HPLC (method A) t_R 5.68 min (100 area %). Anal. ($C_{23}H_{34}N_6O_2 \cdot 3.7HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis[3-(2-imidazolinyl)pyridine-6-oxy]pentane Dihydrochloride (3). Following the procedure described above for dication **1**, compound **3** was prepared from dinitrile **20**. A crude product was recrystallized several times from 1 N HCl to yield **3** as a white powder (0.30 g, 24%); mp 249−251 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.82 (s, 4H), 8.91 (d, J = 2.2 Hz, 2H), 8.33 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 4.39 (t, J = 6.6 Hz, 4H), 3.98 (s, 8H), 1.80 (m, 4H), 1.55 (m, 2H). HPLC (method A) t_R 5.69 min (100 area %). Anal. ($C_{19}H_{26}N_6O_2 \cdot 2.1HCl \cdot 0.6H_2O$) C, H, N, Cl.

1,5-Bis(3-*N*-hydroxyamidinopyridine-6-oxy)pentane Dihydrochloride (4). To a solution of $NH_2OH \cdot HCl$ (1.50 g, 22.0 mmol) in dry DMSO (30 mL) was added *t*-BuOK (2.25 g, 20.0 mmol) and the mixture was stirred at ambient temperature for 20 min. Dinitrile **20** (0.50 g, 1.60 mmol) was added in one portion, and the reaction mixture was stirred at ambient temperature for 2 days. The mixture was diluted with water and forming precipitate was filtered off. A crude product was purified by preparative HPLC to afford compound **4** as a free base; mp 197−200 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 2H), 8.42 (d, J = 2.2 Hz, 2H), 7.92 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 5.88 (s, 4H), 4.28 (t, J = 6.6 Hz, 4H), 1.78 (m, 4H), 1.55 (m, 2H). HPLC (method B) t_R 1.22 min (100 area %). Anal. ($C_{17}H_{22}N_6O_4 \cdot 0.3H_2O$) C, H, N.

A highly hygroscopic hydrochloride salt as a white powder was obtained by adding dry ether to a solution of compound **4** in EtOH saturated with gaseous HCl (0.11 g, 15%); mp 85 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.17 (br s, 2H), 9.15 (br s, 4H), 8.57 (d, J = 2.2 Hz, 2H), 8.06 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 5.80 (br s, 2H), 4.39 (t, J = 6.6 Hz, 4H), 1.80 (m, 4H), 1.55 (m, 2H). HPLC (method A) t_R 4.43 min (100 area %). Anal. ($C_{17}H_{22}N_6O_4 \cdot 3HCl \cdot 2H_2O \cdot 0.5EtOH$) C, H, N, Cl.

1,5-Bis(3-*N*-methoxyamidinopyridine-6-oxy)pentane Dihydrochloride (5). To a solution of a free base of **4** (0.60 g, 1.60 mmol) in dry DMSO (20 mL) at 0−5 °C was added dimethyl sulfate (1.0 mL, 10.1 mmol). The resulting mixture was stirred at ambient temperature for two days, diluted with water (100 mL), and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated to give a crude product, which was purified by preparative HPLC to give a free base of compound **5**; mp 102−105 °C. ^{1}H NMR (DMSO-d_6) δ 8.48 (d, J = 2.2 Hz, 2H), 7.96 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.40 (br s, 4H), 6.87 (d, J = 8.8 Hz, 2H), 4.32 (t, J = 6.6 Hz, 4H), 3.78 (s, 6H), 1.78 (m, 4H), 1.52 (m, 2H). Anal. ($C_{19}H_{26}N_6O_4$) C, H, N.

Compound **5** was prepared by recrystallization of its free base from 1 N HCl. White solid (0.28 g, 39%); mp 185−187 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.40 (d, J = 2.2 Hz, 2H), 7.90 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 6.13 (s, 4H), 4.28 (t, J = 6.6 Hz, 4H), 3.72 (s, 6H), 1.75 (m, 4H), 1.53 (m, 2H). HPLC (method B) t_R 4.63 min (100 area %). Anal. ($C_{19}H_{26}N_6O_4 \cdot 2.1HCl \cdot 0.4H_2O$) C, H, N, Cl.

General Procedure for Syntheses of Dications 6−18. 1,5-Bis(2-amidinopyridine-5-oxy)pentane Dihydrochloride (6). To a stirred suspension of 1,5-bis(3-cyanopyridine-6-oxy)pentane (**25**) (3.00 g, 9.70 mmol) in dry methanol (28 mL) was added 0.5 M solution of NaOMe (7.0 mL, 3.50 mmol). The reaction mixture was stirred at ambient temperature for three days to allow an imidate ester to form and was split in five equal portions. To a solution of the imidate ester (7 mL, 1.4 mmol) was added ammonium chloride (0.18 g, 3.00 mmol), and the resulting mixture was stirred at ambient temperature until the starting material was no longer detectable by HPLC. The solvent was evaporated, and a crude product was purified by column chromatography on C_{18} reversed phase silica gel and recrystallized from 1 N HCl to yield compound **6** as a white powder (0.27 g, 47%); mp 256−258 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 4H), 9.26 (s, 4H), 8.48 (d, J = 2.2 Hz, 2H), 8.37 (d, J = 8.8 Hz, 2H), 7.72 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 4.23 (t, J = 6.0 Hz, 4H), 1.86 (m, 4H), 1.62 (m, 2H). HPLC (method A) t_R 5.73 min (100 area %). Anal. ($C_{17}H_{22}N_6O_2 \cdot 2HCl \cdot 1.2H_2O$) C, H, N, Cl.

1,5-Bis[2-(*N*-isopropylamidino)pyridine-5-oxy]pentane Dihydrochloride (7). Following the procedure described above for dication **6**, compound **7** was prepared from dinitrile **25**. A crude product was purified by preparative HPLC and recrystallized from 1 N HCl to yield **7** as a yellowish powder (0.17 g, 24%); mp 254−255 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.70 (m, 4H), 9.51 (s, 2H), 8.55 (d, J = 8.8 Hz, 2H), 8.46 (d, J = 2.5 Hz, 2H), 7.72 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 2H), 4.30 (m, 2H), 4.24 (t, J = 6.1 Hz, 4H), 1.86 (m, 4H), 1.63 (m, 2H), 1.31 (d, J = 6.3 Hz, 12H). HPLC (method A) t_R 7.67 min (100 area %). Anal. ($C_{23}H_{34}N_6O_2 \cdot 2.6HCl \cdot 1.2H_2O$) C, H, N, Cl.

1,5-Bis[2-(2-imidazolinyl)pyridine-5-oxy]pentane Dihydrochloride (8). Following the procedure described above for dication **6**, compound **8** was prepared from dinitrile **25**. A crude product was purified by column chromatography on C_{18} reversed phase silica gel and recrystallized from 1 N HCl to afford **8** as a white powder (0.09 g, 14%); mp 211 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.82 (s, 4H), 8.51 (d, J = 2.7 Hz, 2H), 8.47 (d, J = 8.8 Hz, 2H), 7.74 (dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz, 2H), 4.34 (br s, 1H), 4.24 (t, J = 6.0 Hz, 4H), 3.99 (s, 8H), 1.85 (m, 4H), 1.62 (m, 2H). HPLC (method A) t_R 5.95 min (97.2 area %). Anal. ($C_{21}H_{26}N_6O_2 \cdot 3HCl \cdot 1.5H_2O$) C, H, N, Cl.

1,5-Bis(2-(*N*-hydroxyamidino)pyridine-5-oxy)pentane Dihydrochloride (9). Following the procedure described above for dication **6**, compound **9** was prepared from dinitrile **25**. A crude product was purified by column chromatography on C_{18} reversed phase silica gel and recrystallized from 1 N HCl to afford **9** as a white powder (0.26 g, 21%); mp 197 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.23 (br s, 2H), 9.05 (br s, 4H), 8.45 (d, J = 2.7 Hz, 2H), 8.22 (d, J = 8.8 Hz, 2H), 7.68 (dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz, 2H), 4.21 (t, J = 6.6 Hz, 4H), 4.10 (br s, 2H), 1.85 (m, 4H), 1.60 (m, 2H). HPLC (method A) t_R 7.49 min (100 area %). Anal. ($C_{17}H_{22}N_6O_4 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis(2-(*N*-methoxyamidino)pyridine-5-oxy)pentane Dihydrochloride (10). Following the procedure described above for dication **6**, compound **10** was prepared from dinitrile **25**. A crude product was purified by column chromatography on C_{18} reversed phase silica gel and recrystallized from 1 N HCl to afford **10** as a white powder (0.47 g, 36%); mp 65−67 °C. ^{1}H NMR (DMSO-d_6) δ 9.11 (br s, 4H), 8.40 (s, 2H), 8.17 (br s, 2H),

7.63 (d, J = 7.1 Hz, 2H), 4.19 (m, 4H), 3.84 (s, 6H), 1.82 (m, 4H), 1.60 (m, 2H). HPLC (method B) t_R 6.04 min (96.3 area %). Anal. ($C_{19}H_{26}N_6O_4 \cdot 3HCl \cdot 0.9H_2O$) C, H, N, Cl.

1,5-Bis(2-amidinopyridine-6-oxy)pentane Dihydrochloride (11). Following the procedure described above for dication **6**, compound **11** was prepared from 1,5-bis(2-cyanopyridine-6-oxy)pentane (**27**). A crude product was purified by preparative HPLC and recrystallized from 1 N HCl to yield **11** as a white powder (0.18 g, 67%); mp 217−220 °C. ^{1}H NMR (DMSO-d_6) δ 9.48 (s, 8H), 8.02 (t, J = 7.7 Hz, 2H), 7.94 (d, J = 7.1 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 4.46 (t, J = 6.0 Hz, 4H), 1.82 (m, 4H), 1.58 (m, 2H). HPLC (method A) t_R 5.79 min (100 area %). Anal. ($C_{17}H_{22}N_6O_2 \cdot 2HCl \cdot 2.1H_2O \cdot 0.2EtOH$) C, H, N, Cl.

1,5-Bis[2-(2-imidazolinyl)pyridine-6-oxy]pentane Dihydrochloride (12). Following the procedure described above for dication **6**, compound **12** was prepared from was prepared from dinitrile **27**. A crude product was recrystallized from 1 N HCl to yield **12** as a white powder (0.14 g, 47%); mp 244 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.73 (s, 4H), 8.03 (t, J = 7.7 Hz, 2H), 7.93 (d, J = 7.1 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 4.44 (t, J = 6.6 Hz, 4H), 4.03 (s, 8H), 1.80 (m, 4H), 1.57 (m, 2H). HPLC (method A) t_R 6.84 min (100 area %). Anal. ($C_{21}H_{26}N_6O_2 \cdot 2.2HCl \cdot 1.3H_2O$) C, H, N, Cl.

1,5-Bis[2-(*N*-hydroxyamidino)pyridine-6-oxy]pentane Dihydrochloride (13). Following the procedure described above for dication **6**, compound **13** was prepared from was prepared from dinitrile **27**. A crude product was purified by preparative HPLC and recrystallized from 1 N HCl to yield **13** as a white powder (0.10 g, 35%); mp 101−103 °C (dec). ^{1}H NMR (DMSO-d_6) δ 11.28 (br s, 2H), 8.94 (br s, 4H), 7.95 (t, J = 8.2 Hz, 2H), 7.72 (d, J = 7.1 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 4.44 (t, J = 6.0 Hz, 4H), 1.81 (m, 4H), 1.57 (m, 2H). HPLC (method A) t_R 7.89 min (100 area %). Anal. ($C_{17}H_{22}N_6O_4 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis(2-amidinopyridine-4-oxy)pentane Dihydrochloride (14). Following the procedure described above for dication **6**, compound **14** was prepared from 1,5-bis(3-cyanopyridine-6-oxy)pentane (**29**). A crude product was recrystallized from 1 N HCl to yield **14** as a white powder (0.58 g, 64%); mp 168−170 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.69 (s, 4H), 9.51 (s, 4H), 8.60 (d, J = 5.5 Hz, 2H), 8.10 (s, 2H), 7.34 (d, J = 5.5 Hz, 2H), 4.25 (t, J = 6.6 Hz, 4H), 1.87 (m, 4H), 1.61 (m, 2H). HPLC (method A) t_R 5.28 min (100 area %). Anal. ($C_{17}H_{22}N_6O_2 \cdot 2HCl \cdot 1H_2O$) C, H, N, Cl.

1,5-Bis[2-(*N*-isopropylamidino)pyridine-4-oxy]pentane Dihydrochloride (15). Following the procedure described above for dication **6**, compound **15** was prepared from dinitrile **29**. A crude product was purified by preparative HPLC, dissolved in 1 N HCl, and concentrated. An oily residue was slowly crystallized by adding dry ether to yield **15** as a white powder (0.32 g, 29%); mp 140 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.83 (s, 4H), 9.48 (s, 2H), 8.58 (d, J = 5.5 Hz, 2H), 8.05 (d, J = 2.2 Hz, 2H), 7.32 (dd, J_1 = 5.5 Hz, J_2 = 2.2 Hz, 2H), 4.26 (t, J = 6.0 Hz, 4H), 4.16 (m, 2H), 1.86 (m, 4H), 1.59 (m, 2H), 1.29 (d, J = 6.6 Hz, 12H). HPLC (method B) t_R 1.55 min (100 area %). Anal. ($C_{23}H_{34}N_6O_2 \cdot 3.5HCl \cdot 1.5H_2O$) C, H, N, Cl.

1,5-Bis[2-(2-imidazolinyl)pyridine-4-oxy]pentane Dihydrochloride (16). Following the procedure described above for dication **6**, compound **16** was prepared from dinitrile **29**. A crude product was recrystallized from 1 N HCl to yield **16** as a yellowish powder (0.18 g, 18%); mp 184−187 °C. ^{1}H NMR (DMSO-d_6) δ 10.98 (s, 4H), 8.62 (d, J = 5.5 Hz, 2H), 8.13 (d, J = 2.2 Hz, 2H), 7.35 (dd, J_1 = 5.5 Hz, J_2 = 2.2 Hz, 2H), 4.23 (t, J = 6.0 Hz, 4H), 4.02 (s, 8H), 1.86 (m, 4H), 1.59 (m, 2H). HPLC (method A) t_R 5.46 min (100 area %). Anal. ($C_{21}H_{26}N_6O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis(2-(*N*-hydroxyamidino)pyridine-4-oxy)pentane Dihydrochloride (17). Following the procedure described above for dication **6**, compound **17** was prepared from dinitrile **29**. A crude product was recrystallized from 1 N HCl to yield **17** as a white powder (0.47 g, 45%); mp 154−155 °C. ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 2H), 8.56 (br s, 6H), 7.86 (s, 2H), 7.31 (s, 2H), 4.27 (s, 4H), 1.87 (s, 4H), 1.59 (s, 2H). HPLC (method A) t_R 1.91 min (100 area %). Anal. ($C_{17}H_{22}N_6O_4 \cdot 2HCl \cdot 1.5H_2O$) C, H, N, Cl.

1,5-Bis(2-(*N*-methoxyamidino)pyridine-4-oxy)pentane Dihydrochloride (18). Following the procedure described above for dication **6**, compound **18** was prepared from dinitrile **29**. A crude product was recrystallized from 1 N HCl to yield **18** as a white powder (0.45 g, 40%); mp 109−111 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.53 (d, J = 6.6 Hz, 2H), 7.77 (d, J = 2.2 Hz, 2H), 7.37 (dd, J_1 = 6.6 Hz, J_2 = 2.2 Hz, 2H), 6.96 (br s, 4H), 4.32 (t, J = 5.5 Hz, 4H), 3.87 (s, 6H), 1.85 (m, 4H), 1.58 (m, 2H). HPLC (method A) t_R 5.76 min (100 area %). Anal. ($C_{19}H_{26}N_6O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

1,5-Bis(3-cyanopyridine-6-oxy)pentane (20). To a solution of 1,5-pentanediol (2.63 g, 25.0 mmol) in dry THF (50 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 2.63 g). The resulting mixture was stirred for 20 min and then was added to a solution of 6-chloro-3-cyanopyridine (**19**)[52] (7.00 g, 50.0 mmol) in dry THF (50 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for one day, diluted with chloroform, and a formed precipitate was filtered off. The filtrate was dried over $MgSO_4$ and concentrated to give a crude residue, which was recrystallized from chloroform to yield compound **20** as a white solid (6.50 g, 83%); mp 165−166 °C. ^{1}H NMR (DMSO-d_6) δ 8.67 (d, J = 2.2 Hz, 2H), 8.14 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 4.35 (t, J = 6.6 Hz, 4H), 1.79 (m, 4H), 1.54 (m, 2H). HPLC (method B) t_R 7.35 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 0.1H_2O$) C, H, N.

1,5-Bis(2-methylpyridine-5-oxy)pentane (21). A mixture of 3-hydroxy-6-methylpyridine (21.8 g, 200 mmol), 1,5-dibromopentane (23.0 g, 100 mmol), and potassium carbonate (27.6 g, 200 mmol) in water (50 mL) and DMF (100 mL) was stirred under reflux overnight. The reaction mixture was cooled in an ice bath, and a formed precipitate was collected, washed with water, and dried. A crude product was recrystallized from ethanol/water (1:1) to yield compound **21** as a white solid (9.50 g, 33%); mp 50−51 °C. ^{1}H NMR (DMSO-d_6) δ 8.13 (d, J = 2.7 Hz, 2H), 7.27 (dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz, 2H), 7.15 (d, J = 8.8 Hz, 2H), 4.02 (t, J = 6.6 Hz, 4H), 2.38 (s, 6H), 1.77 (m, 4H), 1.55 (m, 2H). HPLC (method B) t_R 1.71 min (100 area %). Anal. ($C_{17}H_{22}N_2O_2$) C, H, N.

1,5-Bis(2-methylpyridine-5-oxy-1-oxide)pentane (22). A solution of **21** (12.9 g, 45.0 mmol) and 3-chloroperoxybenzoic acid (77%, 22.5 g, 100 mmol) in chloroform (250 mL) was refluxed for 1 h. The reaction mixture was cooled, quenched with saturated solution K_2CO_3, stirred for 30 min, and filtered. An organic layer was separated, washed with brine (2 × 100 mL), dried over $MgSO_4$, filtered, and concentrated to give a crude product (9.25 g, 65%), which was used without further purification. An analytical sample was prepared by column chromatography eluting with chloroform and chloroform/ethanol (1:1) followed by recrystallization from ethyl acetate to yield compound **22** as a white solid; mp 123−125 °C. ^{1}H NMR (DMSO-d_6) δ 8.06 (d, J = 2.2 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 6.95 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 4.02 (t, J = 6.6 Hz, 4H), 2.26 (s, 6H), 1.75 (m, 4H), 1.54 (m, 2H). HPLC (method B) t_R 3.40 min (100 area %). Anal. ($C_{17}H_{22}N_2O_4$) C, H, N.

1,5-Bis(2-hydroxymethylpyridine-5-oxy)pentane (23). A solution of **22** (8.90 g, 28.0 mmol) in acetic anhydride (80 mL) was refluxed for 2 h, cooled, and concentrated. A residue was purified by column chromatography eluting initially with dichloromethane and then with ethyl acetate. The product was recrystallized from ethyl acetate/hexanes (1:1) to yield 1,5-bis(2-acetoxymethylpyridine-5-oxy)pentane (7.90 g, 70%); mp 92−94 °C. ^{1}H NMR (DMSO-d_6) δ 8.24 (d, J = 2.2 Hz, 2H), 7.40 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 5.05 (s, 4H), 4.07 (t, J = 6.0 Hz, 4H), 2.07 (s, 6H), 1.80 (m, 4H), 1.58 (m, 2H). HPLC (method B) t_R 5.66 min (100 area %). Anal. ($C_{21}H_{26}N_2O_6$) C, H, N.

To a solution of 1,5-bis(2-acetoxymethylpyridine-5-oxy)pentane (7.70 g, 19.0 mmol) in methanol (100 mL) was added

triethylamine (5.0 mL, 36.0 mmol), and the resulting mixture was refluxed for 40 h and then stirred at ambient temperature for two days. The mixture was concentrated, and a residue was purified by column chromatography eluting with chloroform and was recrystallized from ethyl acetate to yield compound **23** as a yellowish solid (5.00 g, 82%); mp 87−89 °C. ^{1}H NMR (DMSO-d_6) δ 8.17 (s, 2H), 7.36 (s, 4H), 5.25 (t, J = 6.0 Hz, 2H), 4.48 (d, J = 6.0 Hz, 4H), 4.05 (t, J = 6.6 Hz, 4H), 1.80 (m, 4H), 1.58 (m, 2H). HPLC (method B) t_R 1.56 min (100 area %). Anal. ($C_{17}H_{22}N_2O_4$) C, H, N.

1,5-Bis(2-pyridinaldehyd-5-oxy)pentane (24). To a stirred solution of 1,5-bis(2-hydroxymethylpyridine-5-oxy)pentane (4.60 g, 15.0 mmol) in chloroform (150 mL) was added activated manganese(IV) oxide (15.0 g, 173 mmol). The resulting mixture was stirred at ambient temperature for two2 days and another portion of manganese(IV) oxide (15.0 g, 173 mmol) was added. The reaction mixture was stirred for the next five days. Manganese(IV) oxide was filtered off and washed with chloroform (2 × 100 mL). A filtrate was concentrated to yield a crude product, which was recrystallized from ethyl acetate/hexanes (1:2) to yield compound **24** as a white solid (3.10 g, 68%); mp 103−104 °C. ^{1}H NMR (DMSO-d_6) δ 9.89 (s, 2H), 8.48 (d, J = 2.2 Hz, 2H), 7.93 (d, J = 8.8 Hz, 2H), 7.58 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 4.21 (t, J = 6.6 Hz, 4H), 1.85 (m, 4H), 1.62 (m, 2H). HPLC (method B) t_R 5.51 min (96.0 area %). Anal. ($C_{17}H_{18}N_2O_4$) C, H, N.

A One-Pot Conversion of Aldehydes to Nitriles. General Procedure. 1,5-Bis(2-cyanopyridine-5-oxy)pentane (25). To a solution of **24** (2.90 g, 9.23 mmol) and triethylamine (5.6 mL, 40 mmol) in dry chloroform (75 mL) at 0 °C was added hydroxylamine hydrochloride (1.40 g, 20.3 mmol) in small portions. The reaction mixture was stirred at ambient temperature for one day. After the starting material was no longer detectable by HPLC, triphosgene (5.64 g, 19.0 mmol) was added in small portions over a period of 15 min. The reaction mixture was stirred at room temperature for 2 h, quenched with water (10 mL), and was extracted with chloroform (3 × 30 mL). A chloroform solution was washed with brine, dried over Na_2SO_4, filtered, and concentrated. A crude product was purified by column chromatography eluting with chloroform followed by recrystallization from ethyl acetate/hexane to yield compound **25** as a white solid (0.90 g, 32%); mp 108−109 °C. ^{1}H NMR (DMSO-d_6) δ 8.44 (d, J = 2.7 Hz, 2H), 8.00 (d, J = 8.8 Hz, 2H), 7.59 (dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz, 2H), 4.18 (t, J = 6.6 Hz, 4H), 1.83 (m, 4H), 1.58 (m, 2H). HPLC (method B) t_R 6.28 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2 \cdot 0.3H_2O$) C, H, N.

1,5-Bis(2-cyanopyridine-6-oxy)pentane (27). 6-Bromopyridine-2-carbonitrile (**26**) was prepared from 6-bromopyridine-2-carbaldehyde according to the procedure described above for compound **25**. A crude product was purified by column chromatography eluting with chloroform to yield labile at room temperature nitrile **26** (3.30 g, 67%); mp 87−89 °C. ^{1}H NMR (DMSO-d_6) δ 8.14 (dd, J_1 = 6.3 Hz, J_2 = 2.2 Hz, 1H), 8.38 (m, 2H). HPLC (method B) t_R 3.29 min (95.6 area %).

To a solution of 1,5-pentanediol (1.51 g, 14.5 mmol) in dry THF (50 mL) was added sodium hydride (60% dispersion in mineral oil, 1.51 g) at 0 °C. The mixture was stirred for 20 min and was added to a solution of compound **26** (5.30 g, 29.0 mmol) in dry THF (50 mL). The resulting mixture was stirred for two days at ambient temperature, diluted with chloroform (100 mL), and filtered to separate a precipitate. A filtrate was washed with water, dried over $MgSO_4$, and concentrated. A crude product was purified by column chromatography eluting with dichloromethane to yield compound **27** as a white solid (1.10 g, 43%); mp 82−84 °C. ^{1}H NMR (DMSO-d_6) δ 7.91 (dd, J_1 = 8.2 Hz, J_2 = 7.1 Hz, 2H), 7.63 (d, J = 7.1 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 4.29 (t, J = 6.6 Hz, 4H), 1.79 (m, 4H), 1.52 (m, 2H). HPLC (method B) t_R 8.35 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2$) C, H, N.

1,5-Bis(2-cyanopyridine-4-oxy)pentane (29). To a solution of 1,5-pentanediol (0.68 g, 6.50 mmol) in dry THF (50 mL) was added sodium hydride (60% dispersion in mineral oil, 0.65 g) at 0 °C. The resulting mixture was stirred for 20 min and then was added to a solution of 4-nitropyridine-2-carbonitrile (**28**)[58] in dry THF (50 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for two days and a formed precipitate was filtered off. A filtrate was concentrated, and a residue was purified by column chromatography eluting with ethyl acetate/hexanes (1:1) followed by recrystallization from ethyl acetate/hexanes (1:3) to yield compound **29** as a yellowish solid (1.30 g, 65%); mp 109−110 °C. ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 5.5 Hz, 2H), 7.70 (s, 2H), 7.29 (d, J = 5.5 Hz, 2H), 4.15 (t, J = 6.6 Hz, 4H), 1.81 (m, 4H), 1.54 (m, 2H). HPLC (method B) t_R 5.92 min (100 area %). Anal. ($C_{17}H_{16}N_4O_2$) C, H, N.

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Supporting Information Available: Elemental analysis data for compounds **1**−**5**, **4**-base, **5**-base, **6**−**18**, **20**−**25**, **27**, **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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