Synthesis and in Vitro Antiprotozoal Activity of Bisbenzofuran Cations

Svetlana M. Bakunova,[†] Stanislav A. Bakunov,[†] Tanja Wenzler,[‡] Todd Barszcz,[§] Karl A. Werbovetz,[§] Reto Brun,[‡] James Edwin Hall,[†] and Richard R. Tidwell*[,†]

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599-7525, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, and Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, CH-4002 Basel, Switzerland

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Forty three cationic bisbenzofurans were synthesized either by interaction of *o*-hydroxyaldehydes with α-halogenated ketones followed by intramolecular ring closure or by a copper- or palladium-mediated heteroannulation of substituted *o*-iodophenols with terminal acetylenes. In vitro antiprotozoal activities of compounds **1**−**43** against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani* and cytotoxicity against mammalian cells were influenced by the position and the type of cationic substituents as well as the length of the carbon linker between aromatic moieties. One bisamidine displayed an antitrypanosomal efficacy comparable to that of pentamidine and melarsoprol. Twenty two compounds were more potent than pentamidine and seven dications were more effective than artemisinin against *P. falciparum*. Eight bisbenzofurans displayed activity against *L. donovani* superior to that of pentamidine. Overall, bisamidines connected by two-carbon linkers exhibited the highest efficacies against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*.

Introduction

Protozoan parasitic infections malaria, human African trypanosomiasis (HAT[a] or sleeping sickness), and leishmaniasis are re-emerging.[1−4] Malaria occurs in over 90 countries worldwide and is responsible for approximately 300 million acute cases each year, with 1.5 to 2.0 million fatalities. About 90% of those deaths occur in sub-Saharan African regions and are caused by *Plasmodium falciparum*, the species responsible for the most severe and life-threatening form of disease.[3,5] African sleeping sickness remains one of the most neglected diseases and is fatal if untreated.[6] There are two forms of HAT, depending on the parasite involved. *Trypanosoma brucei gambiense* causes a human chronic infection, endemic in western and central Africa; *T. b. rhodesiense* has a vast animal reservoir and causes acute illness in people in eastern and southern African countries.[2,7,8] Most episodes of HAT occur in the remote rural areas, where the surveillance is weak or nonexistent, with 50 to 70 thousand estimated cases.[9] *Leishmania* species cause a spectrum of disease ranging from self-healing cutaneous lesions to life-threatening visceral infections, with the clinical manifestations depending on the species of infecting parasite. Approximately 1.5−2.0 million new cases of leishmaniasis occur annually, with about 500 000 of them being of the potentially fatal visceral form. The coexistence of HIV and *Leishmania* species causing visceral disease[10] has in recent years resulted in hundreds of cases of dually infected individuals in Africa,[11] Latin America,[12] and Southern Europe.[13,14] In addition, approximately 700 parasitologically confirmed cases of leishmaniasis have been reported in United States and coalition forces participating in Operation Iraqi Freedom through mid-April 2004.[15]

The emergence of resistance to the cheapest and most commonly used drugs, such as chloroquine and sulfadoxine-pyrimethamine, represents a major obstacle in controlling malaria.[5,16] Many countries must now rely on therapies that are more expensive or use drug combinations (particularly with artemisinins) to try to slow the development of resistance.[17,18] Various antimalarial drugs in current usage are chemically related or have a similar mode of action, possibly sharing resistance mechanisms, thus increasing the risk of cross-resistance and clinical failure of newly introduced therapies.[3,19−21]

Of the four drugs clinically approved for treating HAT, three (suramin, pentamidine, and melarsoprol) were introduced more than half a century ago. Because suramin and pentamidine are ionized at physiological pH, they are unable to cross the blood−brain barrier in therapeutic concentrations and are thus used for the treatment of hemolymphatic early stage HAT, caused by *T. b. rhodesiense* and *T. b. gambiense* infections, respectively. The treatment of the second or neurological stage, when the parasites invade the central nervous system (CNS), relies on the organo-arsenical drug melarsoprol and the more recently registered eflornithine. The latter is ineffective against *T. b. rhodesiense* sleeping sickness and is used primarily to control CNS-involved HAT caused by *T. b. gambiense*. These antitrypanosomal therapies suffer from unacceptable toxicity, poor efficacy, difficulties of administration, and increasing treatment failures due to the development of parasite resistance.[1,2,7,8,22,23]

Pentavalent antimonial compounds have been the mainstay of antileishmanial chemotherapy since the 1940s (see refs 24, 25 for recent reviews of the chemotherapy of leishmaniasis), but these agents require a long course of parenteral administration, and antimonial unresponsiveness is becoming more prevalent, particularly in the Indian state of Bihar.[26] Amphotericin B has also been used as a second-line treatment, but it must also be administered parenterally, and the nephrotoxicity

* To whom correspondence should be addressed. Phone: (919) 966-4294. Fax: (919) 966-0704. E-mail: Tidwell@med.unc.edu
[†] The University of North Carolina
[‡] Swiss Tropical Institute
[§] The Ohio State University.

[a] Abbreviations: HAT, human African trypanosomiasis; CQ, chloroquine; CNS, central nervous system; PMD, pentamidine; MLSP, melarsoprol; ATMS, artemisinin; PPT, podophyllotoxin.

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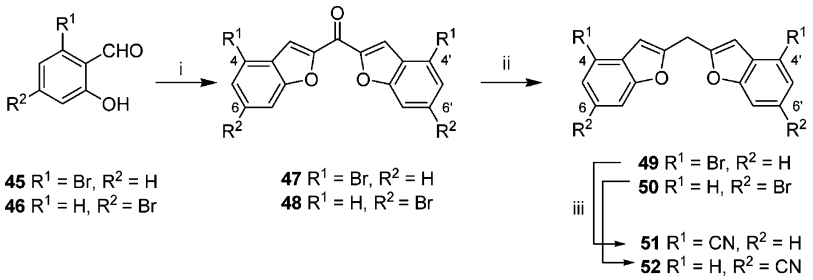
Scheme 1[a]



[a] Reagents and conditions: (i) 1,3-Dichloroacetone, K_2CO_3, 2-butanone, reflux, 4 h; (ii) $AlCl_3$, $LiAlH_4$, ether, 25 °C, 1 h, then 2 N HCl; (iii) CuCN, quinoline, reflux, 3 h.

caused by this agent is a serious limitation. Liposome-encapsulated amphotericin B, which is much less toxic than standard formulations, is too costly to be used for routine antileishmanial chemotherapy in the developing world. Miltefosine was recently registered in India as the first oral treatment for visceral leishmaniasis. Unfortunately, it cannot be used to treat pregnant women, has severe gastrointestinal side effects, and must also be given over a long course of treatment.[27]

Since safe, effective, and affordable orally active therapies capable of overcoming resistance are clearly needed, the identification of new antiprotozoal drug candidates should be an urgent priority. Aromatic diamidines demonstrate a wide range of antifungal and antiprotozoal activities,[28−33] although they have never been used for treatment of malaria because other effective therapies were available. The aromatic diamidine 1,5-bis(4-amidinophenoxy)pentane (pentamidine) is employed widely for treatment of early stage *T. b. gambiense* sleeping sickness, as well as antimony-resistant leishmaniasis and *Pneumocystis carinii* pneumonia. However, its poor oral activity[7] and the necessity of parenteral administration makes the treatment less practical, especially in remote areas, where most cases of HAT and leishmaniasis occur. Despite some adverse effects, such as hypotension, abdominal pain, vertigo, hypersalivation, hypoglycemia, nausea, and mild nephrotoxocity,[2,8,22,34] pentamidine is fairly well tolerated by patients.

The mechanism of antiprotozoal action of pentamidine is not completely understood.[7,23,34,35] It rapidly accumulates in millimolar concentrations inside African trypanosomes[36,37] and *Plasmodium*,[37,38] inhibiting multiple targets in the parasites.[7,22,23,37] Considering the low passive permeability of pentamidine due to its positive charges, such accumulation indicates the involvement of parasite specific transport mechanisms, which are important for selective activity of the drug.[35,36,38,39]

Phenyl oxygens in pentamidine are known to be a part of the structural recognition motif for the aminopurine transporter in *Trypanosoma* species and therefore are crucial for effective uptake by the P2 carrier.[35,39−41] Thus, replacement of these oxygen atoms in pentamidine with methylene groups resulted in a 20-fold loss in antitrypanosomal activity.[42] On the other hand, pentamidine analogues containing substituents in the ortho position to the phenyl oxygens demonstrate similar or even superior antiprotozoal properties as well as lower cytotoxicity compared to the parent compound.[30,31]

In our continuing search for novel dicationic compounds with enhanced efficacy and lowered toxicity as new antiprotozoal drug candidates, we synthesized a series of dicationic bisbenzofurans **1**−**43** as heterocyclic pentamidine analogues in which phenoxy fragments were included into a benzofuran structural motif. In the early 1970s, syntheses of six bisbenzofuran dications were reported[43−45] and the moderate antitrypanosomal properties of bis(5-amidinobenzofuran-2-yl)methane **4** and 1,2-bis(5-amidinobenzofuran-2-yl)ethane **8** were published,[44] but these compounds received very little attention since. Here we report the synthesis of **4** as well as the new approach to **8** and 41 analogues **1**−**3**, **5**−**7**, **9**−**43**. All dications were tested in vitro against *T. b. rhodesiense* (STIB900), *P. falciparum* (K1, resistant to chloroquine), and axenic amastigotes of *L. donovani* (MHOM/SD/62/1S-$CL2_D$) and evaluated for cytotoxicity against rat myoblast cells (L6).

Chemistry

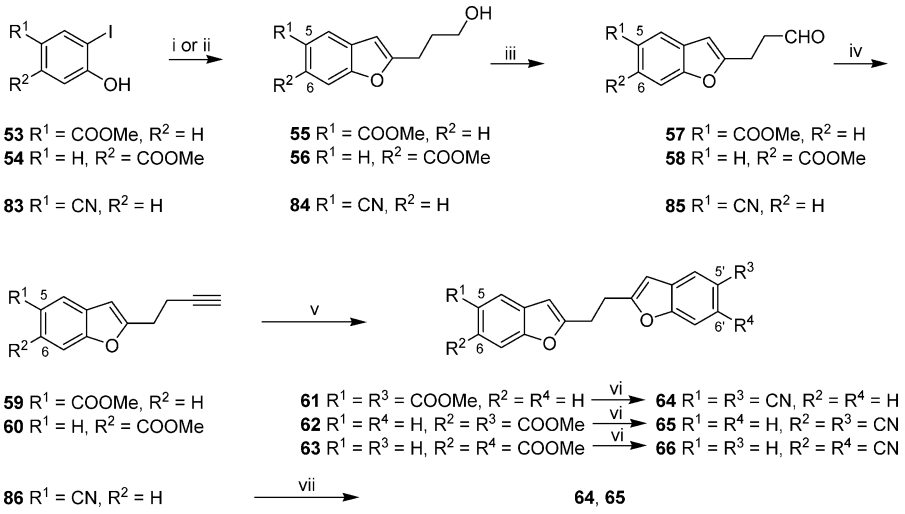
The benzofuran system was formed using two different approaches. The first one included interaction of *o*-hydroxyaldehydes with α-halogenated ketones[43,46] followed by intramolecular ring closure and was employed for synthesis of bisbenzofurans connected by a single carbon atom (Scheme 1). In the second method, used to prepare compounds with multiple methylene groups in the linker, the benzofuran ring was constructed by the reaction of *o*-iodophenols with terminal acetylenes. This approach was carried out under the conditions of both copper-catalyzed (modified[47,48] Castro reaction[49,50]) and palladium-catalyzed (Sonogashira-type reaction[51−57]) heteroannulation (Schemes 2, 3).

Bis(5-cyanobenzofuran-2-yl)methane **44** was prepared from the commercially available 5-bromosalicylaldehyde following the procedure described by Dann et al.[43,44]

Hydroxyaldehydes **45** and **46** were converted to dinitriles **51** and **52** in three steps in 39% and 42% yields, respectively (Scheme 1). Compounds **45** and **46**, prepared from 3-bromophenol by Reimer−Tiemann reaction,[58] reacted with 1,3-dichloroacetone in refluxing 2-butanone[46] providing ketones **47** and **48**. Reduction of compounds **47** and **48** with lithium aluminum hydride and aluminum chloride in dry ether afforded bis(benzofuran-2-yl)methanes **49** and **50**, which were converted to dinitriles **51** and **52** by means of CuCN in boiling quinoline, following the procedure outlined for the synthesis of **44**.[44]

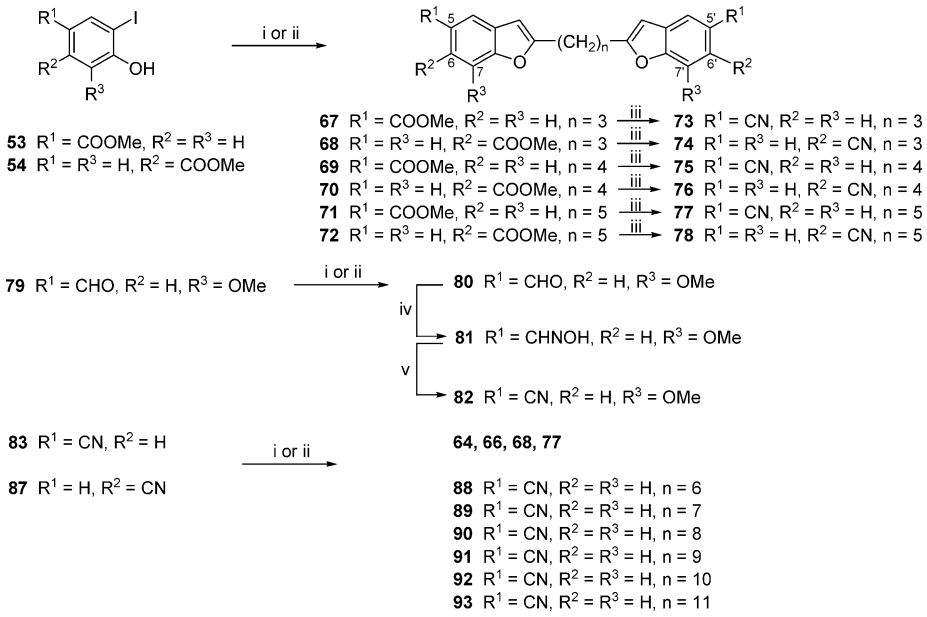
The synthesis of bisbenzofurans connected by the ethylene bridge is shown in Scheme 2. 4-Hydroxy-3-iodobenzoic acid[52,59] and 3-hydroxy-4-iodobenzoic acid[48,60,61] were prepared from 4- and 3-hydroxybenzoic acids, respectively,[60] following the iodination protocol of Edgar and Falling,[59] and were converted then into methyl benzoates **53** and **54** by acid-catalyzed esterification.[60] The high yields (80%) on the iodination step were obtained when 2 equiv of NaOH were used instead of one, as suggested in the original procedure.[59] Reaction of *o*-iodophenols **53** and **54** with 4-pentyn-1-ol in refluxing pyridine in the presence of cuprous oxide[47,48] afforded benzofuran alcohols **55**[52] and **56**, which were oxidized using the Swern method[62] to give aldehydes **57** and **58** in high yields. A one-pot procedure[63] for the conversion of aldehydes to alkynes was employed in the synthesis of benzofurans **59** and **60** containing

Scheme 2[a]



[a] Reagents and conditions: (i) 4-Pentyn-1-ol, Cu_2O, pyridine, 100 °C, 15 h; (ii) 4-pentyn-1-ol, $PdCl_2(PPh_3)_2$, CuI, 1,1,3,3-tetramethylguanidine or triethylamine, DMF, 25 °C or 80 °C, 3−24 h; (iii) 2 M oxalyl chloride in CH_2Cl_2, DMSO, CH_2Cl_2, −70 °C, 1 h, then NEt_3; (iv) dimethy1 1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH; (v) **53** or **54**, Cu_2O, pyridine, 100 °C, 15 h; (vi) Me_2AlNH_2, *o*-xylene, 80−100 °C, 3 h; (vii) **83** or **87**, Cu_2O, pyridine, 100 °C, 15 h.

Scheme 3[a]

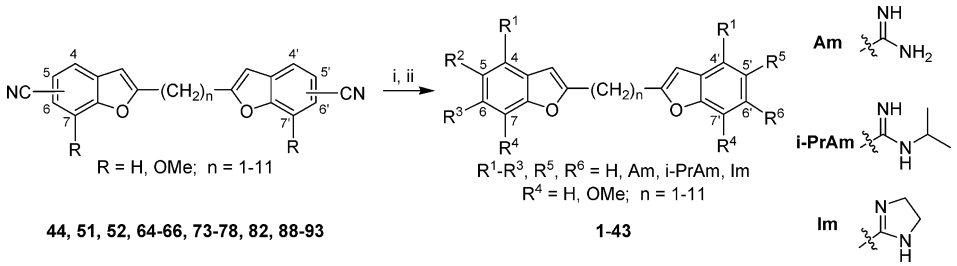


[a] Reagents and conditions: (i) Appropriate alkyldiyne, $PdCl_2(PPh_3)_2$, CuI, 1,1,3,3-tetramethylguanidine or triethylamine, DMF, 25 °C or 80 °C, 3−24 h; (ii) appropriate alkyldiyne, Cu_2O, pyridine, 100 °C, 15 h; (iii) Me_2AlNH_2, *o*-xylene, 80−100 °C, 3 h; (iv) $NH_2OH{\cdot}HCl$, EtOH, pyridine; (v) Ac_2O, reflux, 2 h.

acetylenic side chains. Thus, interaction of aldehydes **57** and **58** with dimethyl-1-diazo-2-oxopropylphosphonate[64,65] in the presence of K_2CO_3 in dry methanol afforded acetylenes **59** and **60**. Compounds **59** and **60** were reacted with methyl benzoates **53** and **54** under the conditions of modified Castro reaction[47] to provide bisbenzofurans **61**, **62**, and **63**, which were directly converted to dinitriles **64**, **65**, and **66** in moderate yields (40−50%) by treatment with dimethylaluminum amide.[66] Overall yield for the transformation of **53** to **64** was 9% over five steps and **54** was converted to **65** and to **66** in 19% and 21% yields, respectively. Although the Sonogashira reaction provided milder conditions for the construction of the heterocyclic ring and therefore represented a more attractive synthetic approach, the modified Castro method worked better for the syntheses of bisbenzofurans with a two-carbon linker, affording fewer impurities and higher yields of the desired compounds.

The same synthetic strategy, utilizing heteroannulation of *o*-iodophenol esters and terminal alkyldiynes, was employed for the synthesis of dinitriles containing three and more methylene groups in the linker (Scheme 3). Thus, interaction of 2 equiv of methyl benzoates **53** and **54** with commercially available 1,6-heptadiyne, 1,7-octadiyne, and 1,8-nonadiyne under the conditions of Castro or Sonogashira reactions led to bisbenzofurans **67**−**72** in one step, affording comparable yields with both methods. Treatment of esters **67**−**72** with dimethylaluminum amide provided dinitriles **73**, **74**, **75**,[45] **76**−**78** in moderate to high yields. Likewise, 7,7′-dimethoxy-substituted nitrile **82** was prepared from 5-iodovanillin **79** in three steps in 48% overall

Scheme 4[a]



[a] Reagents and conditions: (i) 1,4-Dioxane, EtOH, HCl, 1−3 days; (ii) appropriate amine, EtOH, 1−4 days.

yield. Heteroannulation of **79** and 1,8-nonadiyne, following the modified Castro protocol,[47] afforded dialdehyde **80**. Dioxime **81**, obtained by reaction of **80** with hydroxylamine, was dehydrated by treatment with acetic anhydride to give **82** (Scheme 3).

In general, introduction of cyano groups required additional synthetic steps and decreased the yields of the target compounds. Employment of cyano-substituted halogenophenols as starting materials allowed the synthetic pathway to be shortened and improved the reaction outcome by eliminating the low yielding transformations. 4-Hydroxy-3-iodobenzonitrile **83**[67] was prepared by iodination of 4-cyanophenol following the procedure of Edgar and Falling.[59] 3-Hydroxy-4-iodobenzonitrile **87** was synthesized as previously described.[61] The Sonogashira reaction of **83** with 4-pentyn-1-ol followed by the aforementioned sequence (Scheme 2) afforded acetylene **86**, which reacted both with **83** and **87** to give dinitriles **64** and **65** in four steps in 30% overall yield for both compounds. A one-step reaction of **83** and **87** with 1,5-hexadiyne and the reaction of **87** with 1,6-heptadiyne (Scheme 3) under the Castro conditions provided dinitriles **64**, **66**, and **68** in 24%, 22%, and 62% yields, respectively. The Sonogashira reaction of **83** with 1,8-nonadiyne afforded dinitrile **77** in 77% yield. Because yields of benzofurans were comparable in both the Castro and Sonogashira protocols as the length of the carbon linker increased but the latter occurred under milder conditions, the Sonogashira reaction was chosen to prepare dinitriles **88**−**93**. 1,9-Decadiyne was commercially available. 1,10-Undecadiyne, 1,11-dodecadiyne, 1,-12-tridecadiyne, 1,13-tetradecadiyne, and 1,14-pentadecadiyne were prepared according to the published procedure.[68] The aforementioned alkyldiynes reacted with **83** to give **88**−**93** in 19−71% yields.

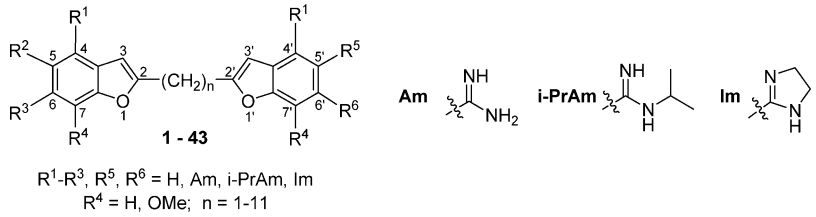
All dicationic bisbenzofuran amidines (**1**−**43**) were synthesized by the modified Pinner method[69] (Scheme 4). Dinitriles **44**, **51**, **52**, **64**,[44,45] **65**,[45] **66**,[45] **73**, **74**, **75**,[45] **76**−**78**, **82**, **88**,[45] **89**−**93** were converted to imidate esters, which reacted with ethanolic solutions of ammonia, isopropylamine, or ethylenediamine to give the bisamidines **1**, **4**,[44] **8**,[44] **11**,[45] **14**,[45] **17**, **20**, **23**,[45] **26**, **29**, **32**, **35**, **38**,[45] **39**−**43**, bis(*N*-isopropyl)amidines **2**, **5**, **9**, **12**, **15**, **18**, **21**, **24**, **27**, **30**, **33**, **36**, and bisimidazolines **3**, **6**, **7**, **10**, **13**, **16**, **19**, **22**, **25**, **28**, **31**, **34**, **37**, respectively (Table 1).

Results

A series of 43 dications bearing amidine (Am), *N*-isopropylamidine (i-PrAm), or imidazoline (Im) substituents in positions 4, 5, or 6 of the benzofuran rings and with the length of the alkyl linker between aromatic moieties ranging from one to eleven methylene groups was evaluated for antiprotozoal activity and cytotoxicity. The results of the in vitro testing against bloodstream form trypomastigotes of *T. b. rhodesiense* (STIB900), *P. falciparum* (K1, resistant to chloroquine), and axenic amastigotes of *L. donovani* (MHOM/SD/62/1S-CL2$_D$) as well as the assessment of cytotoxicity of the compounds **1**−**43** against rat myoblast cells (L6) are summarized in Table 2 and compared to that of pentamidine. Other controls employed were melarsoprol (*T. b. rhodesiense*), chloroquine and artemisinin (*P. falciparum*), and podophyllotoxin (L6). In addition to activities, four parameters included in Table 2 were calculated and analyzed: antitrypanosomal selectivity index SI$_T$, expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*T. b. rhodesiense*)]; antiplasmodial selectivity index SI$_P$, expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*P. falciparum*)]; selectivity (S$_{P/T}$), expressed as ratio [IC$_{50}$ (*T. b. rhodesiense*)/IC$_{50}$ (*P. falciparum*)]; and antileishmanial selectivity index SI$_L$, expressed as the ratio [IC$_{50}$ (L6)/IC$_{50}$ (*L. donovani*)]. Selectivity indexes SI$_T$, SI$_P$ and SI$_L$ reflect inhibition of the specific parasite relative to the L6 cells. Selectivity (S$_{P/T}$) reflects suppression of *P. falciparum* comparative to *T. b. rhodesiense*.

Cytotoxicity Study. The results from cytotoxicity studies clearly demonstrated that structural variations affected properties of bisbenzofurans **1**−**43**. In our survey 4-substituted dications were more cytotoxic than corresponding 5- or 6-substituted isomers. Compounds bearing cationic groups in the 5-position of the aromatic ring were less cytotoxic than their 6-substituted counterparts in all cases except derivatives **14** and **16**, which displayed lower cytotoxicity than that of 5,5′- or 5,6′-substituted isomers **8**, **11** and **10**, **13**. Compounds **11** and **12**, containing cationic groups in the 5- and 6′-positions were more cytotoxic than comparable 5,5′- and 6,6′-substituted derivatives **8**, **14** and **9**, **15**. Among different types of the tested cations, unsubstituted bisamidines were more cytotoxic. Substitution on the amidine moiety resulted in the reduction of cytotoxicity with *N*-isopropyl-substituted amidines typically being the least cytotoxic in the series. 5-Substituted bis(*N*-isopropyl)amidines **5**, **9**, **18**, **24**, and **30** displayed IC$_{50}$ values against L6 cells exceeding 100 μM. This group of compounds with lower cytotoxicity also included bisamidine **17** and bisimidazoline **6**. Nineteen 5-substituted derivatives displayed IC$_{50}$ values for cytotoxicity less than 100 μM. They included eleven bisamidines (**4**, **8**, **23**, **29**, **32, 38**−**43**), one bis(*N*-isopropyl)amidine (**33**), and five bisimidazolines (**10**, **19**, **25**, **31**, and **34**). In a series of 6-substituted cations only two bis(*N*-isopropyl)amidines (**15** and **21**) and two bisimidazolines (**7** and **16**) exhibited IC$_{50}$ values against L6 cells exceeding 100 μM. Those with the cytotoxicity values below this level consisted of four bisamidines (**14**, **20**, **26**, and **32**), two bis(*N*-isopropyl)amidines (**27** and **33**), and three bisimidazolines (**22**, **28**, and **34**). Among the 5,6′-substituted bisbenzofurans **11**−**13** only bis(*N*-isopropyl)amidine **12** revealed a cytotoxic IC$_{50}$ value exceeding 100 μM. Both bisamidine **11** and bisimidazoline **13** scored below that mark. Generally, elongation of the carbon bridge between benzofuran rings resulted in increased cytotoxicity against L6 cells. Thus, bisamidines **38**−**43** with the linker containing from

Table 1. Structures of Bisbenzofurans **1**−**43**



R^1-R^3, R^5, R^6 = H, Am, i-PrAm, Im
R^4 = H, OMe; n = 1-11

compd	*n*	R^1	R^2	R^3	R^4	R^5	R^6
1	1	Am	H	H	H	H	H
2	1	i-PrAm	H	H	H	H	H
3	1	Im	H	H	H	H	H
4	1	H	Am	H	H	Am	H
5	1	H	i-PrAm	H	H	i-PrAm	H
6	1	H	Im	H	H	Im	H
7	1	H	H	Im	H	H	Im
8	2	H	Am	H	H	Am	H
9	2	H	i-PrAm	H	H	i-PrAm	H
10	2	H	Im	H	H	Im	H
11	2	H	Am	H	H	H	Am
12	2	H	i-PrAm	H	H	H	i-PrAm
13	2	H	Im	H	H	H	Im
14	2	H	H	Am	H	H	Am
15	2	H	H	i-PrAm	H	H	i-PrAm
16	2	H	H	Im	H	H	Im
17	3	H	Am	H	H	Am	H
18	3	H	i-PrAm	H	H	i-PrAm	H
19	3	H	Im	H	H	Im	H
20	3	H	H	Am	H	H	Am
21	3	H	H	i-PrAm	H	H	i-PrAm
22	3	H	H	Im	H	H	Im
23	4	H	Am	H	H	Am	H
24	4	H	i-PrAm	H	H	i-PrAm	H
25	4	H	Im	H	H	Im	H
26	4	H	H	Am	H	H	Am
27	4	H	H	i-PrAm	H	H	i-PrAm
28	4	H	H	Im	H	H	Im
29	5	H	Am	H	H	Am	H
30	5	H	i-PrAm	H	H	i-PrAm	H
31	5	H	Im	H	H	Im	H
32	5	H	Am	H	OMe	Am	H
33	5	H	i-PrAm	H	OMe	i-PrAm	H
34	5	H	Im	H	OMe	Im	H
35	5	H	H	Am	H	H	Am
36	5	H	H	i-PrAm	H	H	i-PrAm
37	5	H	H	Im	H	H	Im
38	6	H	Am	H	H	Am	H
39	7	H	Am	H	H	Am	H
40	8	H	Am	H	H	Am	H
41	9	H	Am	H	H	Am	H
42	10	H	Am	H	H	Am	H
43	11	H	Am	H	H	Am	H

six to eleven methylene groups were more cytotoxic than pentamidine.

Antitrypanosomal Activity. All compounds **1**−**43** displayed antitrypanosomal activities in vitro, with IC_{50} values ranging from 8 nM to 18.7 μM. Sixteen bisbenzofurans (**4**, **8**, **11**−**15**, **19**, **20**, **23**−**27**, **35**, and **38**) displayed antitrypanosomal IC_{50} values less than 100 nM. Among those compounds were nine bisamidines (**4**, **8**, **11**, **14**, **20**, **23**, **26**, **35**, and **38**), four bis(*N*-isopropyl) amidines (**12**, **15**, **24**, and **27**) and three bisimidazolines (**13**, **19**, and **25**). Seven compounds (**4**, **8**, **19**, **23**−**25**, and **38**) possessed substituents in 5-positions, six isomers (**14**, **15**, **20**, **26**, **27**, and **35**) were 6-substituted, and three (**11**−**13**) were mixed derivatives containing their cationic groups both in 5- and 6′- positions. Eleven dications had a linker with an even number of carbon atoms, of which six compounds (**11**−**16**) were connected by an ethylene bridge and five compounds possessed a tetramethylene chain. Four compounds had one (**4**), three (**19** and **20**), or five (**35**) methylene groups in their linker.

Four bisamidines (**8**, **11**, **20** and **26**) demonstrated antitrypanosomal IC_{50} values less than 20 nM. The most potent compound, bisamidine **8** (IC_{50} = 8 nM), exhibited efficacy comparable to that of pentamidine and melarsoprol and was the only derivative in the series with the antitrypanosomal IC_{50} value less than 10 nM.

The position of substituents affected the trypanocidal properties of compounds **1**−**43**. Bisbenzofurans containing cationic substituents in the 5- or 6-positions were substantially more effective against *T. b. rhodesiense* than their 4-substituted counterparts. The relative antitrypanosomal activities of 5- and 6-substituted compounds were affected by the type of cationic group and the length of the carbon linker in the molecules. Thus, 6-substituted bisamidines and bis(*N*-isopropyl)amidines were generally more potent against *T. b. rhodesiense* than 5-substituted isomers, except bisamidine **8** and bis(*N*-isopropyl)amidines **12** and **30**, which displayed lower antitrypanosomal IC_{50} values. It is noteworthy that stepwise transition from the 6-substituted

Table 2. Cytotoxicity and in Vitro Antiprotozoal Activity of Bisbenzofurans **1**−**43**

	cytotoxicity[a]	*T. b. rhodesiense*[b]		*P. falciparum*[d]			*L. donovani*[g]	
compd	IC_{50} (μM)	IC_{50} (μM)	SI_T[c]	IC_{50} (μM)	SI_P[e]	$S_{P/T}$[f]	IC_{50} (μM)	SI_L[h]
1	22.5	0.201	112	0.068	331	3.0	20.8	1.1
2	158	14.0	11	0.918	172	15	>100	>1.6
3	74.6	18.7	4	2.12	35	8.8	>100	>0.75
4	94.2	0.072	1308	0.028	3364	2.6	3.80	25
5	>174	0.455	>382	0.102	>1706	4.5	11.3	>15
6	165	0.474	348	0.034	4853	14	13.8	12
7	124	4.64	27	0.022	5636	211	11.1	11
8	21.5	0.008	2688	0.003	7167	2.7	1.06	20
9	>179	0.148	>1209	0.003	>59667	49	4.14	>43
10	38.9	0.127	306	0.011	3536	12	5.31	7.3
11	19.0	0.015	1267	0.002	9500	7.5	1.54	12
12	123	0.040	3075	0.006	20500	6.7	1.97	62
13	58.1	0.090	646	0.046	1263	2.0	3.59	16
14	78.9	0.031	2545	0.004	19725	7.8	0.887	89
15	>171	0.060	>2850	0.005	>34200	12	2.46	>70
16	114	0.415	275	0.034	3353	12	3.88	29
17	104	0.145	717	0.041	2537	3.5	4.40	24
18	>168	0.133	>1263	0.009	>18667	15	31.1	>5.4
19	78.9	0.031	2545	0.004	19725	7.2	13.2	>6.0
20	38.5	0.019	2026	0.037	1041	0.51	2.38	16
21	155	0.122	1270	0.036	4306	3.4	5.68	27
22	81.5	0.355	230	0.353	231	1.0	4.65	18
23	34.6	0.051	678	0.032	1081	1.6	1.34	26
24	>163	0.099	>1646	0.010	>16300	9.9	12.7	>13
25	35.4	0.073	485	0.026	1362	2.8	1.97	18
26	12.0	0.011	1091	0.058	207	0.19	1.52	7.9
27	27.9	0.082	340	0.076	367	1.1	3.87	7.2
28	30.9	0.321	96	0.164	188	2.0	9.54	3.2
29	33.0	0.127	260	0.067	493	1.9	2.49	13
30	>161	0.101	>1594	0.020	>8050	5.1	6.85	>24
31	35.1	0.120	293	0.067	524	1.8	1.83	19
32	35.3	0.164	215	0.066	535	2.5	1.79	20
33	72.8	0.104	700	0.133	547	0.78	5.66	13
34	54.1	0.231	234	0.110	492	2.1	1.86	29
35	19.2	0.078	246	0.057	337	1.4	5.10	3.8
36	70.0	0.408	172	0.018	3889	23	8.10	8.6
37	13.2	0.531	25	0.133	99	4.0	3.76	3.5
38	2.78	0.083	33	0.364	8	0.23	4.30	0.65
39	2.74	0.158	17	0.279	10	0.57	2.79	0.98
40	2.18	0.220	10	0.694	3	0.32	12.2	0.18
41	2.16	0.119	18	0.296	7.3	0.40	3.16	0.68
42	2.32	0.178	13	0.287	8	0.62	2.31	1
43	1.85	0.210	9	0.219	8	0.96	2.97	0.62
PMD[i]	46.6	0.003	15533	0.058	803	0.05	1.83	25
MLSP[j]	7.78	0.004	1945					
CQ[k]	117			0.124	944			
ATMS[l]	450			0.006	75000			
PPT[m]	0.01							

[a] Cytotoxicity (L6 rat myoblast cells). Average of duplicate determinations.[85] [b] *Trypanosoma brucei rhodesiense* (STIB900). Average of duplicate determinations.[33,86,87] [c] Selectivity index for *T. b. rhodesiense* (SI_T), expressed as the ratio [IC_{50} (L6)/IC_{50} (*T. b. rhodesiense*)].[70] [d] *Plasmodium falciparum* (K1, resistant to chloroquine). Average of duplicate determinations.[88] [e] Selectivity index for *P. falciparum* (SI_P), expressed as the ratio [IC_{50} (L6)/IC_{50} (*P. falciparum*)].[70] [f] Selectivity ($S_{P/T}$), expressed as ratio [IC_{50} (*T. b. rhodesiense*)/IC_{50} (*P. falciparum*)]. [g] *Leishmania donovani* (MHOM/SD/62/1S-$CL2_D$) axenic amastigotes. Average of duplicate determinations.[89,90] [h] Selectivity index for *L. donovani* (SI_L), expressed as the ratio [IC_{50} (L6)/IC_{50} (*L. donovani*)].[70] [i] PMD, pentamidine. [j] MLSP, melarsoprol. [k] CQ, chloroquine. [l] ATMS, artemisinin. [m] PPT, podophyllotoxin.

bisamidine **14** to the 5,6′-substituted isomer **11** and the 5-substituted compound **8**, involving moving each of two amidine groups in the molecule into the 5 position, resulted in a 2-fold increase in antitrypanosomal activity. On the other hand, all 5,5′- and 5,6′-substituted bisimidazolines **6**, **10**, **13**, **19**, **25**, **31**, and **34**, connected by the linker containing from one to five methylene groups, demonstrated higher antitrypanosomal activity than the corresponding 6-substituted isomers **7**, **16**, **22**, **28**, and **37**.

Among bisbenzofurans with different types of cationic groups, unsubstituted amidines exhibited higher antitrypanosomal activity in vitro. Substitution on the amidine fragment usually reduced trypanocidal properties of the compounds **1**−**43**. Although for 5-substituted derivatives there was no apparent correlation between the type of the cationic substituents and their antitrypanosomal efficacies, in the case of 6-substituted bisbenzofurans, antitrypanosomal activity decreased in the order Am > *i*-PrAm > Im.

The length of a carbon linker between aromatic rings affected the trypanocidal properties of bisbenzofurans **1**−**43**. Antitrypanosomal activities of 5-substituted bis(*N*-isopropyl)amidines and bisimidazolines increased with carbon chain elongation. The lowest antitrypanosomal IC_{50} values in this group revealed compounds **24** and **25**, containing a tetramethylene linker. The same tendency was observed in the series of 6-substituted bisamidines, where compound **26** with a four-carbon bridge displayed the lowest IC_{50} value of 11 nM. Overall, among bisbenzofurans with identical cationic substituents in either 5- or 6- positions, derivatives with an even number of carbon atoms in the alkyl linker displayed higher antitrypanosomal activity.

All compounds **1**−**43** were selective against the *Trypanosoma* parasite (selectivity index[70] SI_T, defined as a ratio of cytotoxic IC_{50} to antitrypanosomal IC_{50} values, varied from 4 to more than 3000). Bisimidazolines were the least selective against *T. b. rhodesiense*, except compounds **19**, **31**, and **34**, in which cases corresponding bisamidines **17**, **29**, and **32** exhibited the lowest antitrypanosomal selectivity indexes. The most selective compound in the group against *T. b. rhodesiense* was bis(*N*-isopropyl)amidine **12** (SI_T = 3075), showing antitrypanosomal IC_{50} value of 40 nM. This molecule possessed an ethylene linker and contained substituents in 5- and 6′-positions. Bisamidine **8**, the most potent compound in the series, demonstrated highest antitrypanosomal selectivity index among bisamidines (SI_T = 2688). Overall, selectivity against *T. b. rhodesiense* decreased with elongation of the carbon chain, particularly in case of 6-substituted dications.

Antiplasmodial Activity. Dications **1**−**43** displayed antimalarial activities with IC_{50} values ranging from 2 nM to 2.1 μM (Table 2). Twenty four compounds (**4**, **6**−**21**, **23**−**26**, **30**, **35**, and **36**) displayed antiplasmodial activity comparable or exceeding that of pentamidine (IC_{50} = 58 nM). Among those compounds there were nine bisamidines (**4**, **8**, **11**, **14**, **17**, **20**, **23**, **26**, and **35**), eight bis(*N*-isopropyl)amidines (**9**, **12**, **15**, **18**, **21**, **24**, **30**, and **36**) and seven bisimidazolines (**6**, **7**, **10**, **13**, **16**, **19** and **25**). Twelve compounds (**8**−**12**, **14**, **15**, **18**, **19**, **24**, **30**, and **36**) demonstrated in vitro antiplasmodial IC_{50} values less than 20 nM. This group included three bisamidines (**8**, **11**, and **14**), seven bis(*N*-isopropyl)amidines (**9**, **12**, **15**, **18**, **24**, **30**, and **36**) and two bisimidazolines (**10** and **19**). Among them seven compounds (**8**−**10**, **18**, **19**, **24**, and **30**) had their cationic substituents in the 5 positions, three were 6-substituted isomers (**14**, **15**, and **36**), and two were 5,6′-substituted derivatives (**11** and **12**). Of the eight compounds with an even number of methylene units in the linker, seven (**8**−**12**, **14**, **15**) contained an ethylene bridge. Two compounds (**18** and **19**) had a propylene linker and two (**30** and **36**) were connected by five-carbon chains.

Seven of these dications (**8**, **9**, **11**, **12**, **14**, **15**, and **19**), all except bisimidazoline **19** connected by two-carbon linkers, exhibited antiplasmodial IC_{50} values lower than that of artemisinin (ATMS) (IC_{50} = 6 nM). Bisamidine **11**, bearing cationic substituents in the 5- and 6′-positions, was the most potent compound in the series, with an antiplasmodial IC_{50} value of 2 nM.

The position of the cationic substituents affected efficacies of bisbenzofurans **1**−**43** against *P. falciparum*. While 4-substituted isomers were significantly less potent, 5- and 6-substituted bisamidines displayed comparable antimalarial properties. 5-Substituted bis(*N*-isopropyl)amidines and bisimidazolines were more effective against *Plasmodium* than 6-substituted derivatives in all cases except for compounds **7** and **36**.

Among bisbenzofurans with different types of cationic substituents, bisamidines or bis(*N*-isopropyl)amidines were usually more potent against *P. falciparum* than bisimidazolines, except compound **19**, which revealed superior antiplasmodial efficacy compared to corresponding derivatives **17** and **18**. Bisamidines exhibited the best antiplasmodial activity when benzofuran moieties contained methylene or ethylene bridges. However, this tendency changed with elongation of the alkyl linkers. Thus, for both 5- and 6-substituted bisbenzofurans connected by three or more carbon atoms, bis(*N*-isopropyl)-amidines demonstrated better antiplasmodial efficacy.

All bisbenzofurans **1**−**43** were selective against the *P. falciparum* and displayed selectivity indexes[70] (SI_P, defined as a ratio of cytotoxic IC_{50} to antiplasmodial IC_{50} values), ranging from 3 to nearly 60000. Regardless of the position of the cationic substituents and the length of the alkyl chain between the aromatic moieties, bis(*N*-isopropyl)amidines displayed the highest antimalarial selectivity among the dications in all cases, except 4-substituted bisamidine **1** and 5-substituted bisimidazolines **6** and **19**, connected by the methylene (**1** and **6**) or the propylene linker (**19**). The most potent compound in the series, bisamidine **11**, possessed antiplasmodial selectivity index SI_P nearly twelve fold greater than that of pentamidine. Despite comparable potency of the most effective bisamidines and bis(*N*-isopropyl)amidines, the latter are attractive as potential antimalarial drug candidates due to their low cytotoxicity as well as high selectivity for the *Plasmodium* parasite. Thus, the most potent of the bis(*N*-isopropyl)amidines compound **9** not only demonstrated an antiplasmodial IC_{50} value of 3 nM but also was the most selective in the group against the *P. falciparum* (SI_P = 59667), showing antiplasmodial selectivity index 74-fold greater than that of pentamidine.

Dications **2**, **6**, **7**, **9**, **10**, **15**, **16**, **18**, **24**, and **36** displayed selectivity ($S_{P/T}$) (ratio of antitrypanosomal IC_{50} to antiplasmodial IC_{50} values) equal or greater than 10-fold. The most discriminatory of these compounds, bisimidazoline **7**, demonstrating 215-fold $S_{P/T}$ ratio, exhibited an antiplasmodial IC_{50} of 22 nM. Bisbenzofurans **9**, **15**, **18**, **24**, and **36**, with selectivity ($S_{P/T}$) between 10 and 46-fold, were the most effective of the ten dications as well as the least cytotoxic, with compound **9** providing the best combination of high antiplasmodial activity, high selectivity ($S_{P/T}$), and low cytotoxicity.

Antileishmanial Activity. All compounds except bis(*N*-isopropyl)amidine **2** and bisimidazoline **3**, both of which contained a mono-methylene linker, were active in the *L. donovani* axenic amastigote assay, demonstrating activity ranging from 0.89 to 31 μM. Ten bisbenzofurans **8**, **11**, **12**, **14**, **23**, **25**, **26**, **31**, **32**, and **34** displayed antileishmanial IC_{50} values less than 2 μM. Among those compounds there were six bisamidines (**8**, **11**, **14**, **23**, **26**, and **32**), one bis(*N*-isopropyl)-amidine (**12**), and three bisimidazolines (**25**, **31**, and **34**). Six compounds (**8**, **23**, **25**, **31**, **32**, and **34**) contained their substituents in the 5,5′ positions, two were 6,6′-substituted bisamidines (**14** and **26**), and two were mixed 5,6′-substituted derivatives (**11** and **12**). Seven bisbenzofurans possessed aliphatic linkers containing two (**8**, **11**, **12**, and **14**) and four (**23**, **25**, **26**) methylene groups, and three compounds (**31**, **32**, and **34**) contained a five-carbon chain. Five bisamidines (**8**, **11**, **14**, **23**, **26**, and **32**) as well as two bisimidazolines (**31** and **34**) exhibited antileishmanial IC_{50} values equal or superior to that of pentamidine (IC_{50} = 1.83 μM). Bisbenzofurans **8** and **14**, both connected by the ethylene bridge, were the most potent compounds among 5- and 6-substituted isomers, respectively. It is noteworthy that 5,6′-substituted bisamidine **11** displayed lower antileishmanial activity than both 5-substituted compound **8** and 6-substituted isomer **14**. Bisamidine **14** revealed the highest antileishmanial activity in the series, exhibiting an IC_{50} value of 887 nM.

Both 5- and 6-substituted isomers displayed comparable antileishmanial properties. However, placement of the cationic substituents in the 4-position of the benzofuran rings caused a significant loss of efficacy, especially for derivatives **2** and **3**.

Similar to *T. b. rhodesiense*, introduction of substituents in the cationic moiety usually afforded less potent compounds against *L. donovani* axenic amastigotes, although exceptions did occur. For both 5- and 6-substituted bisamidines and bis(*N*-

isopropyl)amidines, compounds connected by a linker containing two or four methylene groups displayed better activity. In case of bisimidazolines, however, the most effective compounds (**31** and **37**) contained a five-carbon chain.

All bisbenzofurans, except 4-substituted derivatives **1**−**3** and 5-substituted bisamidines **38**−**43**, containing six to eleven methylene groups in the alkyl linker, were selective against *L. donovani* and displayed selectivity indexes[70] (SI_L, defined as a ratio of cytotoxic IC_{50} to antileishmanial IC_{50} values), ranging from 3 to 89. Among bisbenzofurans with different types of cationic substituents, bisamidines or bis(*N*-isopropyl)amidines were generally more selective against *L. donovani* than bisimidazolines. The only exception was 7-methoxy-substituted bisimidazoline **34**, which exhibited a higher antileishmanial selectivity index SI_L than that of corresponding dications **32** and **33**. Although there was no clear correlation between the length of the carbon linker connecting benzofuran rings and the selectivity of 5-substituted derivatives, in the case of 6-substituted dications the selectivity against *L. donovani* decreased with elongation of the carbon chain. Among eleven compounds with antileishmanial selectivity indexes SI_L comparable or superior to that of pentamidine ($SI_L = 25$), five dications (**9**, **12**, **14**, **15**, and **16**) had ethylene linkers, two (**30** and **34**) were connected by five-carbon bridges, and two (**17** and **21**) contained three methylene groups in their alkyl chains. Two bisamidines (**4** and **23**) possessed a one- and four-carbon linker, respectively. Bisamidine **14** was not only the most potent compound in the series but also demonstrated the greatest selectivity against the *L. donovani* axenic amastigotes ($SI_L = 89$), which was more than 3-fold higher than that of pentamidine.

Discussion

The absence of widespread resistance to pentamidine in the field, despite its massive use as an HAT prophylactic,[7,37] makes the drug an attractive lead compound in a search for new antiprotozoal therapies.[6,17,37,71] The mechanism of action of pentamidine is not completely understood.[7,23,34,35] Its rapid accumulation inside *Trypanosoma* and *Plasmodium* species, resulting in the inhibition of multiple targets in the parasites,[22,23,36−38] suggests the involvement of parasite specific uptake mechanisms that are critical for the activity of pentamidine and absence of the prevalent resistance to it.[35,36,38,39]

In order to improve efficacy and selectivity against individual parasites, heterocyclic cationic pentamidine analogues containing benzofuran rings have been synthesized. This structural modification also intended to protect phenoxy groups from metabolic deactivation by including them in the aromatic rings. Such an alteration would allow bisbenzofurans to retain affinity for the aminopurine P2 transporter and therefore their antitrypanosomal activity.

In *T. brucei* species the P2 transporter partially mediates uptake of pentamidine and melaminophenyl arsenicals,[35−37,39,40,72−74] that share structural recognition motifs for the carrier.[35,39−41] Cross-resistance between pentamidine and arsenicals was observed in laboratory strains of *Trypanosoma* species[75−77] and was attributed to the loss of the P2 delivery system.[35,36,39,73,78] Pentamidine, however, accumulates even in strains without significant P2-transporter activity,[35] suggesting the presence of other routes of uptake. The existence of additional modes of accumulation[37,39,73,74,78] coupled with multiple modes of action might explain this lack of widespread resistance to pentamidine.

Data presented in Table 2 indicate that bisbenzofuran **8** displayed in vitro antitrypanosomal activity comparable to that of pentamidine and melarsoprol. Analysis of L6 cytotoxicity and antitrypanosomal selectivity indexes SI_T shows that six of the synthesized dications (**8**, **12**, **14**, **15**, **19**, and **20**) were less cytotoxic and more selective against *T. b. rhodesiense* than melarsoprol. None of the bisbenzofurans **1**−**43** reached the level of antitrypanosomal selectivity of pentamidine. The *P. falciparum* strain K1 (resistant to chloroquine) was in general very susceptible to the bisbenzofurans screened in this study. Twenty two dications (**4**, **6**−**21**, **23**−**25**, **30**, and **36**) displayed greater potency and were more selective against *P. falciparum* than pentamidine. Seven bisbenzofurans (**8**, **9**, **11**, **12**, **14**, **15**, and **19**) exhibited antiplasmodial IC_{50} values superior to that of artemisinin. In areas where the risk of cross-infection is high, it is important to possess therapies that are highly selective for *P. falciparum* over *T. b. rhodesiense* to treat patients infected with both organisms. Ten dications **2**, **6**, **7**, **9**, **10**, **15**, **16**, **18**, **24**, and **36** displayed selectivity ($S_{P/T}$) equal or greater than 10-fold. In this group of discriminatory compounds bisbenzofurans **9**, **15**, **18**, and **24**, revealing selectivity ($S_{P/T}$) between 10 and 46-fold, were the most effective against *P. falciparum* as well as the least cytotoxic. Ten bisbenzofurans (**8**, **11**, **12**, **14**, **23**, **25**, **26**, **31**, **32**, and **34**) were equally or more potent than pentamidine against *L. donovani* axenic amastigotes. Eleven bisbenzofurans (**4**, **9**, **12**, **14**−**17**, **21**, **23**, **30**, and **34**) displayed antileishmanial selectivity comparable or superior to that of pentamidine.

Three major structural variations were explored: the placement of substituents, the type of cationic groups, and the length of the linker between two benzofuran moieties. The evaluation of the effect of the position of cationic groups on the antiprotozoal efficacies of bisbenzofurans **1**−**43** demonstrated that 4-substituted derivatives **1**−**3** were significantly less active against all three parasites as well as more cytotoxic against L6 cells than their 5- and 6-substituted counterparts. These results revealed that the placement of cationic substituents affects the antiprotozoal properties of dications **1**−**43**. Both 5- and 6-substituted bisbenzofurans exhibited comparable activities against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. These findings correspond to the relative antimalarial activities of para- versus meta-substituted pentamidine congeners.[30]

Introduction of the substituents on the amidine groups usually decreased antitrypanosomal[28,79−81] and antileishmanial[32] activities of bisbenzofurans **1**−**43**. It is tempting to explain the reduced antitrypanosomal efficacy of bis(*N*-isopropyl)amidines and bisimidazolines by the higher affinity of unsubstituted bisamidines for the aminopurine transporter. However, as was previously shown, there is no direct correlation between the affinity for the P2 carrier and the antitrypanosomal efficacies of the compounds.[82,83] Antimalarial properties of bisbenzofurans **1**−**43** were affected by substitution on the cationic groups in a different way. Bis(*N*-isopropyl)amidines displayed antiplasmodial activities comparable to that of unsubstituted amidines. One possible explanation could be the different mechanism of uptake of diamidines in *P. falciparum*, where pentamidine penetrates the *Plasmodium*-infected erythrocyte membrane through a parasite-induced pore with properties of the new permeability pathway (NPP).[37,38] Because the uptake through the NPP is favorable for compounds with increased hydrophobicity,[84] one can speculate that substituted bisbenzofurans in which polar amidine groups are shielded by aliphatic substituents might exhibit higher activity compared to unsubstituted ones if antiplasmodial efficacy depended only on the parasite uptake. However, this assumption does not explain the lower antiplasmodial activities of bisimidazolines compared to that of both bisamidines and bis(*N*-isopropyl)amidines. Substitution on the

cationic groups also decreased the cytotoxicity of bisbenzofurans **1**−**43** against L6 cells.

The length of the linker between aromatic rings influenced the antiprotozoal properties of bisbenzofurans **1**−**43**. Bisamidines connected by two-carbon chains demonstrated the highest activity against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. The length of the alkyl bridge and therefore the distance between the two cationic moieties might affect not only the efficacies of bisbenzofurans but also the development of parasite resistance to this class of compounds. It was shown that *T. brucei* strains resistant to propamidine remain sensitive to pentamidine.[37,39] This flexibility of pentamidine plays an important role in the absence of the widespread resistance to the drug. Other diamidines with lesser flexibility are all prone to multidrug resistance.[37,39] Elongation of the carbon linker also decreased the selectivity of bisbenzofurans **1**−**43** against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. Clearly, the distance between two cationic groups in bisbenzofurans influences the efficacy and cytotoxicity of the compounds and might as well affect the development of parasite resistance.

Currently, we cannot offer any reasonable explanation of how and why the substitution on the amidine groups affects the antiprotozoal properties of bisbenzofurans **1**−**43**. To better understand the mode of action and thus improve the efficacy of aromatic diamidines, further studies of the influence of the type of cationic substituents and the distance between aromatic moieties on uptake and intracellular distribution of dicationic bisbenzofurans are needed.

Conclusions

We have reported the synthesis and the results of the in vitro testing of bisbenzofurans **1**−**43** against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* as well as the cytotoxicity of these compounds against L6 mammalian cells. For the synthesis of bisbenzofurans connected by a single carbon atom, *o*-hydroxyaldehydes reacted with α-halogenated ketones followed by intramolecular ring closure. Compounds with multiple methylene groups in the linker were synthesized by means of copper- or palladium-mediated heteroannulation of substituted *o*-iodophenols with terminal acetylenes. We found that the position and the type of the cationic substituents in the molecule and the length of the linker between the aromatic moieties affected cytotoxicity and in vitro antiprotozoal activity of the compounds **1**−**43**. 5-Substituted bisbenzofurans were generally less cytotoxic than compounds bearing their substituents in the 4- or 6-positions. Cytotoxicity also decreased with substitution on the cationic groups, with bis(*N*-isopropyl)amidines usually being the least cytotoxic in the series, and increased with the elongation of the carbon linker. Consequently, the selectivity of bisbenzofurans **1**−**43** against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* decreased as number of methylene groups in the alkyl bridge increased. 4-Substituted bisbenzofurans were significantly less active against all parasites than corresponding 5- and 6-substituted isomers. Substitution on the cationic moieties of bisbenzofurans resulted in compounds with inferior antitrypanosomal and antileishmanial activity. Bisamidines and bis(*N*-isopropyl)amidines were more efficient against *P. falciparum* than bisimidazolines. Bisamidines connected by two-carbon chains demonstrated the highest activity against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani*. Bisamidine **8** displayed an antitrypanosomal IC_{50} value of 8 nM comparable to that of pentamidine and melarsoprol. Bisbenzofurans **4**, **6**−**21**, **23**−**25**, **30**, and **36** exhibited antiplasmodial activity exceeding that of pentamidine and were up to 74-fold more selective against *P. falciparum*. Seven dications (**8**, **9**, **11**, **12**, **14**, **15**, and **19)** revealed higher antiplasmodial efficacies than that of artemisinin. Bisbenzofurans **2**, **6**, **7**, **9**, **10**, **15, 16**, **18**, **24**, and **36** were discriminatory for *P. falciparum* over *T. b. rhodesiense*, showing parasite selectivity ratio ($S_{P/T}$) equal or greater than 10-fold. Bis(*N*-isopropyl)amidine **9** provided the best combination of low cytotoxicity, high antiplasmodial activity ($IC_{50} = 3$ nM), and high selectivity ($S_{P/T} = 46$). Compounds **8**, **11**, **14**, **23**, **26**, **31**, **32**, and **34** displayed activity against *L. donovani* axenic amastigotes superior to that of pentamidine ($IC_{50} = 1.83$ μM). Bisamidine **14**, exhibiting an antileishmanial IC_{50} value of 887 nM, was the most potent compound in the series against *L. donovani*. It was twice as active and more than three times as selective against the parasite as pentamidine. Promising in vitro efficacies of selected bisbenzofuran cations against *T. b. rhodesiense*, *P. falciparum*, and *L. donovani* as well as their reduced cytotoxicity compared to pentamidine warrant further investigation of the antiprotozoal properties of these compounds in vivo and the evaluation of oral activities of their prodrugs.

Experimental Section

General Experimental. In vitro antitrypanosomal, antiplasmodial, and antileishmanial activities and cytotoxicities were determined following established protocols.[33,85−90]

Uncorrected melting points were measured on a Thomas-Hoover Capillary melting point apparatus. ^{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. Gravity and flash column chromatography were performed using Davisil grade 633 type 60A silica gel (200−425 mesh). Analytical HPLC chromatograms were recorded on a Hewlett-Packard 1090 Series II 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 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 and then from 22.5 to 56.25% over 4 min and finally maintained for 1 min. In method B, the concentration of acetonitrile was increased linearly from 22.5 to 75% over 10 min and then maintained for 2 min. In method C, the concentration of acetonitrile was increased linearly from 45 to 75% over 10 min and then maintained for 2 min. In method D, the concentration of acetonitrile was increased linearly from 45 to 75% over 7 min and then maintained for 6 min. Chromatographic data were recorded and analyzed using HP ChemStation software.

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 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 using an Agilent Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and the latter mobile phases on an Agilent Technologies 1100 chromatograph. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop

2K lyophilizer. Acquired bisbenzofurans, as free bases, were dissolved in ethanol and converted into HCl salts with aqueous HCl.

Flash Chromatography of Amidines on C_{18} Reversed Phase Silica Gel. Chromatographic column was half-filled with acetonitrile and packed with a slurry of C_{18} silica gel (70 g) in acetonitrile (70−100 mL). Excess of acetonitrile was drained out, and the top of the column was covered with 2 cm pad of sand. The column was balanced with 150 mL of initial mobile phase that consisted 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 excess of amine and then with a mobile phase consisting of 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 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), and deionized water (2 × 100 mL) and kept in acetonitrile or acetonitrile−water mixture. Select fractions were analyzed for purity using an Agilent Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) and described mobile phases on an Agilent Technologies 1100 chromatograph. Residues of evaporated pooled purified fractions were reconstituted in water and lyophilized on a VirTis BenchTop 2K lyophilizer. Acquired bisbenzofurans, as free bases, 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.

Materials. All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Acros Organics and were used without further purification.

General Procedure for Syntheses of Diamidines (1−43). Bis-(4-amidinobenzofuran-2-yl)methane Dihydrochloride (1). A mixture of dry 1,4-dioxane (40 mL) and dry EtOH (10 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. Bis(4-cyanobenzofuran-2-yl)methane (**41**) (1.42 g, 4.8 mmol) was added in one portion, the flask was sealed, and the mixture was stirred at room temperature until the starting material was no longer detectable by HPLC (1 day). After dilution of the reaction mixture with dry ether, the diimidate (2.11 g, 96%), was filtered off under argon, washed with diethyl ether, dried under high vacuum, and reacted immediately with appropriate amines.

The diimidate (0.70 g, 1.5 mmol) was suspended in dry EtOH saturated with gaseous ammonia (19 mL). The sealed reaction mixture was stirred at room temperature. The progress of the reaction was monitored by HPLC. After 4 days, the reaction mixture was diluted with diethyl ether (50 mL) and placed in a freezer overnight. The resulting precipitate was collected by filtration, washed with diethyl ether, and dried under vacuum to give 0.45 g (89%) of crude material, which was recrystallized from 1.5 M HCl to afford **1**, as a brown solid (0.14 g, 21%): mp 310−311 °C. ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 4H), 9.36 (s, 4H), 7.91 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 7.7 Hz, 2H), 7.50 (dd, J = 8.2 and 7.7 Hz, 2H), 7.00 (s, 2H), 4.64 (s, 2H). HPLC (method A) t_R 4.67 min (100 area %). Anal. ($C_{19}H_{16}N_4O_2$·2HCl·1H_2O) C, H, N, Cl.

Bis(4-(*N*-isopropyl)amidinobenzofuran-2-yl)methane Dihydrochloride (2). Brown solid (0.06 g, 8%): mp 210 °C (dec) (diethyl ether/EtOH/1 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.77 (d, J = 8.4 Hz, 2H), 9.55 (s, 2H), 9.41 (s, 2H), 7.87 (d, J = 7.7 Hz, 2H), 7.55 (d, J = 7.1 Hz, 2H), 7.48 (dd, J = 7.7 and 7.1 Hz, 2H), 6.96 (s, 2H), 4.66 (s, 2H), 4.18 (m, 2H), 1.31 (d, J = 6.0 Hz, 12H). *M/Z* 417.2 (MH^+ of free base). HPLC (method A) t_R 6.29 min (100 area %). Anal. ($C_{25}H_{28}N_4O_2$·2HCl·1.5H_2O·0.5C_2H_5OH) C, H, N, Cl.

Bis(4-(2-imidazolinyl)benzofuran-2-yl)methane Dihydrochloride (3). Light yellow solid (0.48 g, 70%): mp 333−335 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.70 (s, 4H), 8.00 (d, J = 8.2 Hz, 2H), 7.79 (d, J = 7.7 Hz, 2H), 7.56 (dd, J = 8.2 and 7.7 Hz, 2H), 7.26 (s, 2H), 4.68 (s, 2H), 4.04 (s, 8H). HPLC (method A) t_R 5.41 min (98.2 area %). Anal. ($C_{23}H_{20}N_4O_2$·2HCl·0.5H_2O) C, H, N, Cl.

Bis(5-amidinobenzofuran-2-yl)methane Dihydrochloride (4).[44] Yellow solid (0.18 g, 19%): mp 212−214 °C (lit.[44] 201−206 °C). ^{1}H NMR (DMSO-d_6) δ 9.40 (s, 4H), 9.16 (s, 4H), 8.15 (d, J = 1.6 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.73 (dd, J = 8.8 and 1.6 Hz, 2H), 7.02 (s, 2H), 4.59 (s, 2H). Anal. ($C_{19}H_{16}N_4O_2$·2HCl·0.6H_2O) C, H, N, Cl.

Bis(5-(*N*-isopropyl)amidinobenzofuran-2-yl)methane Dihydrochloride (5). White solid (0.75 g, 65%): mp 238−240 °C. ^{1}H NMR (DMSO-d_6) δ 9.58 (d, J = 7.8 Hz, 2H), 9.44 (s, 2H), 9.09 (s, 2H), 8.03 (d, J = 1.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.63 (dd, J = 8.8 and 1.6 Hz, 2H), 7.00 (s, 2H), 4.59 (s, 2H), 4.08 (m, 2H), 1.29 (d, J = 6.0 Hz, 12H). HPLC (method B) t_R 1.49 min (100 area %). Anal. ($C_{25}H_{28}N_4O_2$·2HCl·1.5H_2O) C, H, N, Cl.

Bis(5-(2-imidazolinyl)benzofuran-2-yl)methane Dihydrochloride (6). Light yellow solid (0.27 g, 25%): mp 233−235 °C. ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 4H), 8.38 (s, 2H), 7.96 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.06 (s, 2H), 4.60 (s, 2H), 4.01 (s, 8H). HPLC (method A) t_R 5.83 min (99.6 area %). Anal. ($C_{23}H_{20}N_4O_2$·2HCl·1.1H_2O) C, H, N, Cl.

Bis(6-(2-imidazolinyl)benzofuran-2-yl)methane Dihydrochloride (7). Light brown solid (0.13 g, 50%): mp 341−343 °C. ^{1}H NMR (DMSO-d_6) δ 10.82 (s, 4H), 8.40 (s, 2H), 7.96 (d, J = 8.2 Hz, 2H), 7.88 (d, J = 8.2 Hz, 2H), 7.03 (s, 2H), 4.66 (s, 2H), 4.01 (s, 8H). HPLC (method A) t_R 5.65 min (100 area %). Anal. ($C_{23}H_{20}N_4O_2$·2HCl·2H_2O) C, H, N, Cl.

1,2-Bis(5-amidinobenzofuran-2-yl)ethane Dihydrochloride (8)[44] was prepared from dinitrile **59** (0.40 g, 1.28 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a yellow solid (0.17 g, 32%): mp 356 °C (dec) (lit.[44] 293−295 °C (dec)). ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 4H), 9.10 (s, 4H), 8.06 (s, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 6.87 (s, 2H), 3.34 (s, 4H). HPLC (method A) t_R 5.89 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2$·2HCl·1.3H_2O) C, H, N, Cl.

1,2-Bis(5-(*N*-isopropyl)amidinobenzofuran-2-yl)ethane Dihydrochloride (9) was prepared from dinitrile **59** (0.40 g, 1.28 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a white solid (0.18 g, 29%): mp 350 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.54 (d, J = 8.0 Hz, 2H), 9.41 (s, 2H), 9.06 (s, 2H), 7.95 (d, J = 1.6 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.59 (dd, J = 8.8 and 1.6 Hz, 2H), 6.85 (s, 2H), 4.07 (m, 2H), 3.34 (s, 4H), 1.27 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R 5.41 min (100 area %). Anal. ($C_{26}H_{30}N_4O_2$·2HCl) C, H, N, Cl.

1,2-Bis(5-(2-imidazolinyl)benzofuran-2-yl)ethane Dihydrochloride (10) was prepared from dinitrile **59** (0.40 g, 1.28 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a beige solid (0.25 g, 42%): mp 366 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.68 (s, 4H), 8.29 (s, 2H), 7.90 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.8 Hz, 2H), 6.94 (s, 2H), 4.00 (s, 8H), 3.34 (s, 4H). HPLC (method A) t_R 4.69 min (100 area %). Anal. ($C_{24}H_{22}N_4O_2$·2HCl·1.7H_2O) C, H, N, Cl.

1-(5-Amidinobenzofuranyl-2)-2-(6-amidinobenzofuranyl-2)-ethane Dihydrochloride (11)[45] was prepared from dinitrile **65**. The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **11** (0.24 g, 46%): mp >300 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.38 (s, 2H), 9.34 (s, 2H), 9.14 (s, 4H), 8.09 (s, 1H), 8.08 (s, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.70 (m, 2H), 6.87 (s, 2H), 3.33 (d, J = 7.5 Hz, 4H). HPLC (method A) t_R 5.83 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2$·2HCl·2H_2O) C, H, N, Cl.

1-(5-*N*-Isopropylamidinobenzofuranyl-2)-2-(6-*N*-isopropylamidinobenzofuranyl-2)ethane Dihydrochloride (12) was prepared

from dinitrile **65**. The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **12** (0.20 g, 32%): mp 220 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.57 (m, 2H), 9.48 (s, 1H), 9.43 (s, 1H), 9.12 (d, J = 7.2 Hz, 2H), 7.97 (d, J = 8.2 Hz, 2H), 7.74 (d, J = 8.2 Hz, 2H), 7.59 (m, 2H), 6.85 (s, 2H), 4.10 (m, 2H), 3.35 (d, J = 7.5 Hz, 4H), 1.29 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R 7.68 min (100 area %). Anal. ($C_{26}H_{30}N_4O_2$·2HCl·2.5H_2O) C, H, N, Cl.

1-(5-(2-Imidazolinyl)benzofuranyl-2)-2-(6-(2-imidazolinyl)-benzofuranyl-2)ethane Dihydrochloride (13) was prepared from dinitrile **65**. The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **13** (0.15 g, 25%): mp 225 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.79 (s, 2H), 10.72 (s, 2H), 8.35 (s, 1H), 8.30 (s, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 8.8 Hz, 1H), 6.90 (s, 1H), 6.88 (s, 1H), 3.98 (s, 8H), 3.34 (s, 4H). HPLC (method A) t_R 6.61 min (100 area %). Anal. ($C_{24}H_{22}N_4O_2$·2HCl·2.5H_2O) C, H, N, Cl.

1,2-Bis(6-amidinobenzofuran-2-yl)ethane Dihydrochloride (14)[45] was prepared from dinitrile **60** (0.13 g, 0.42 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a light yellow solid (0.12 g, 60%): mp 362 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 4H), 9.15 (s, 4H), 8.10 (d, J = 1.1 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H), 7.69 (dd, J = 8.2 and 1.1 Hz, 2H), 6.88 (s, 2H), 3.34 (s, 4H). HPLC (method A) t_R 5.53 min (100 area %). Anal. ($C_{20}H_{18}N_4O_2$·2HCl·1H_2O) C, H, N, Cl.

1,2-Bis(6-(*N*-isopropyl)amidino benzofuran-2-yl)ethane Dihydrochloride (15) was prepared from dinitrile **60** (0.13 g, 0.42 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a light yellow solid (0.13 g, 54%): mp 346 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.58 (d, J = 8.2 Hz, 2H), 9.47 (s, 2H), 9.11 (s, 2H), 7.98 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 6.86 (s, 2H), 4.10 (m, 2H), 3.34 (s, 4H), 1.29 (d, J = 6.6 Hz, 12H). HPLC (method A) t_R 7.51 min (100 area %). Anal. ($C_{26}H_{30}N_4O_2$·2HCl·1.2H_2O) C, H, N, Cl.

1,2-Bis(6-(2-imidazolinyl)benzofuran-2-yl)ethane Dihydrochloride (16). Yellow solid (0.21 g, 84%) (EtOH/DMSO/3 M HCl): mp >360 °C. ^{1}H NMR (DMSO-d_6/D_2O) δ 10.60 (br s, 4H), 8.14 (d, J = 1.1 Hz, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.75 (dd, J = 8.2 and 1.1 Hz, 2H), 6.91 (s, 2H), 4.02 (s, 8H), 3.38 (s, 4H). HPLC (method A) t_R 6.27 min (100 area %). Anal. ($C_{24}H_{22}N_4O_2$·1.9HCl·1H_2O) C, H, N, Cl.

1,3-Bis(5-amidinobenzofuran-2-yl)propane Dihydrochloride (17). White solid (0.47 g, 71%): mp 168−170 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 4H), 9.06 (s, 4H), 8.08 (d, J = 1.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.69 (dd, J = 8.8 and 1.6 Hz, 2H), 6.88 (s, 2H), 2.95 (t, J = 7.1 Hz, 4H), 2.17 (p, J = 7.1 Hz, 2H). HPLC (method B) t_R 1.31 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2$·2HCl·1H_2O) C, H, N, Cl.

1,3-Bis(5-(*N*-isopropyl)amidinobenzofuran-2-yl)propane Dihydrochloride (18). White solid (0.42 g, 53%): mp 195−197 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.58 (d, J = 7.7 Hz, 2H), 9.45 (s, 2H), 9.13 (s, 2H), 7.98 (s, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 6.86 (s, 2H), 4.10 (m, 2H), 2.95 (t, J = 7.1 Hz, 4H), 2.17 (p, J = 7.1 Hz, 2H), 1.29 (d, J = 6.6 Hz, 12H). HPLC (method B) t_R 2.52 min (100 area %). Anal. ($C_{27}H_{32}N_4O_2$·2HCl·1H_2O) C, H, N, Cl.

1,3-Bis(5-(2-imidazolinyl)benzofuran-2-yl)propane Dihydrochloride (19). White solid (0.72 g, 97%): mp 203−205 °C. ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 4H), 8.32 (d, J = 1.6 Hz, 2H), 7.94 (dd, J = 8.8 and 1.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 6.88 (s, 2H), 4.01 (s, 8H), 2.96 (t, J = 7.1 Hz, 4H), 2.19 (p, J = 7.1 Hz, 2H). HPLC (method A) t_R 7.25 min (100 area %). Anal. ($C_{25}H_{24}N_4O_2$·2HCl·1.2H_2O) C, H, N, Cl.

1,3-Bis(6-amidinobenzofuran-2-yl)propane Dihydrochloride (20). Light yellow solid (0.56 g, 53%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.42 (s, 4H), 9.21 (s, 4H), 8.11 (s, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 8.2 Hz, 2H), 6.85 (s, 2H), 2.97 (t, J = 7.1 Hz, 4H), 2.18 (p, J = 7.1 Hz, 2H). HPLC (method B) t_R 1.14 min (100 area %). Anal. ($C_{21}H_{20}N_4O_2$·2HCl·0.3H_2O) C, H, N, Cl.

1,3-Bis(6-(*N*-isopropyl)amidinobenzofuran-2-yl)propane Dihydrochloride (21). White solid (0.52 g, 41%): mp 289−291 °C (dec). ^{1}H NMR (DMSO-d_6) δ 9.54 (d, J = 6.6 Hz, 2H), 9.42 (s, 2H), 9.06 (s, 2H), 7.97 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 6.85 (s, 2H), 4.06 (m, 2H), 2.96 (t, J = 7.1 Hz, 4H), 2.20 (p, J = 7.1 Hz, 2H), 1.29 (d, J = 6.0 Hz, 12H). HPLC (method B) t_R 2.33 min (100 area %). Anal. ($C_{27}H_{32}N_4O_2$·2HCl·1H_2O) C, H, N, Cl.

1,3-Bis(6-(2-imidazolinyl)benzofuran-2-yl)propane Dihydrochloride (22). White solid (0.69 g, 59%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.75 (s, 4H), 8.31 (s, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 8.2 Hz, 2H), 6.86 (s, 2H), 4.02 (s, 8H), 2.98 (t, J = 6.6 Hz, 4H), 2.20 (p, J = 6.6 Hz, 2H). HPLC (method B) t_R 1.45 min (100 area %). Anal. ($C_{25}H_{24}N_4O_2$·2HCl·2H_2O) C, H, N, Cl.

1,4-Bis(5-amidinobenzofuran-2-yl)butane Dihydrochloride (23).[45] White solid (0.13 g, 38%): mp 325−327 °C (dec) (1.5 M HCl) (lit.[45] 335 °C (dec)). ^{1}H NMR (DMSO-d_6) δ 9.35 (s, 4H), 9.13 (s, 2H), 9.08 (s, 2H), 8.08 (d, J = 1.6 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.69 (dd, J = 8.2 and 1.6 Hz, 2H), 6.82 (s, 2H), 2.91 (br s, 4H), 1.81 (br s, 4H). HPLC (method B) t_R 1.75 min (100 area %). Anal. ($C_{22}H_{22}N_4O_2$·2HCl·0.5H_2O) C, H, N, Cl.

1,4-Bis(5-(*N*-isopropyl)amidinobenzofuran-2-yl)butane Dihydrochloride (24). White solid (0.17 g, 41%): mp 283−285 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.56 (d, J = 8.2 Hz, 2H), 9.42 (s, 2H), 9.09 (s, 2H), 7.96 (d, J = 1.6 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.59 (dd, J = 8.2 and 1.6 Hz, 2H), 6.80 (s, 2H), 4.08 (m, 2H), 2.91 (br s, 4H), 1.81 (br s, 4H), 1.29 (d, J = 6.0 Hz, 12H). Anal. ($C_{28}H_{34}N_4O_2$·2HCl·1.2H_2O) C, H, N, Cl.

1,4-Bis(5-(2-imidazolinyl)benzofuran-2-yl)butane Dihydrochloride (25). White solid (0.25 g, 63%): mp 323−324 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.78 (s, 4H), 8.33 (d, J = 1.6 Hz, 2H), 7.94 (dd, J = 8.8 and 1.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 6.84 (s, 2H), 4.01 (s, 8H), 2.91 (br s, 4H), 1.82 (br s, 4H). HPLC (method A) t_R 7.82 min (100 area %). Anal. ($C_{26}H_{26}N_4O_2$·2HCl·2.5H_2O) C, H, N, Cl.

1,4-Bis(6-amidinobenzofuran-2-yl)butane Dihydrochloride (26). White solid (0.45 g, 73%): mp 328−330 °C (dec) (diethyl ether/EtOH/1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.38 (s, 4H), 9.14 (s, 4H), 8.09 (s, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 6.81 (s, 2H), 2.93 (br s, 4H), 1.82 (br s, 4H). HPLC (method A) t_R 7.15 min (100 area %). Anal. ($C_{22}H_{22}N_4O_2$·2HCl·1.5H_2O) C, H, N, Cl.

1,4-Bis(6-(*N*-isopropyl)amidinobenzofuran-2-yl)butane Dihydrochloride (27). White solid (0.37 g, 51%): mp 282−283 °C (dec) (diethyl ether/EtOH/1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.55 (d, J = 8.2 Hz, 2H), 9.45 (s, 2H), 9.09 (s, 2H), 7.97 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.2 Hz, 2H), 6.81 (s, 2H), 4.08 (m, 2H), 2.93 (br s, 4H), 1.81 (br s, 4H), 1.29 (d, J = 6.0 Hz, 12H). HPLC (method B) t_R 2.92 min (100 area %). Anal. ($C_{28}H_{34}N_4O_2$·2HCl·0.5H_2O) C, H, N, Cl.

1,4-Bis(6-(2-imidazolinyl)benzofuran-2-yl)butane Dihydrochloride (28). White solid (0.37 g, 54%): mp 343−345 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.66 (s, 4H), 8.28 (s, 2H), 7.84 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 6.84 (s, 2H), 4.02 (s, 8H), 2.94 (br s, 4H), 1.83 (br s, 4H). HPLC (method B) t_R 1.94 min (100 area %). Anal. ($C_{26}H_{26}N_4O_2$·2HCl·0.7H_2O) C, H, N, Cl.

1,5-Bis(5-amidinobenzofuran-2-yl)pentane Dihydrochloride (29). White solid (0.40 g, 67%): mp 156−157 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 4H), 9.14 (br s, 4H), 8.07 (s, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.68 (dd, J = 8.8 and 1.6 Hz, 2H), 6.80 (s, 2H), 2.84 (t, J = 7.7 Hz, 4H), 1.78 (m, 4H), 1.45 (m, 2H). HPLC (method B) t_R 2.29 min (100 area %). Anal. ($C_{23}H_{24}N_4O_2$·2HCl·1.6H_2O) C, H, N, Cl.

1,5-Bis(5-(*N*-isopropyl)amidinobenzofuran-2-yl)pentane Dihydrochloride (30) was prepared from dinitrile **72** (0.48 g, 1.36 mmol) to give, after preparative HPLC and recrystallization from aqueous HCl, a white solid (0.20 g, 32%): mp 193−195 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.58 (d, J = 7.0 Hz, 2H), 9.46 (s, 2H), 9.16 (s, 2H), 7.97 (s, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 4.11 (m, 2H), 2.85 (m, 4H), 1.77

(m, 4H), 1.45 (m, 2H) 1.29 (d, J = 6.0 Hz, 12H). HPLC (method A) t_R 8.57 min (98.0 area %). Anal. ($C_{29}H_{36}N_4O_2$·2HCl·0.7H_2O) C, H, N, Cl.

1,5-Bis(5-(2-imidazolinyl)benzofuran-2-yl)pentane Dihydrochloride (31). White solid (0.22 g, 37%): mp 155−157 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.69 (s, 4H), 8.28 (d, J = 1.6 Hz, 2H), 7.89 (dd, J = 8.8 and 1.6 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 6.82 (s, 2H), 4.01 (s, 8H), 2.85 (t, J = 7.1 Hz, 4H), 1.78 (m, 4H), 1.46 (m, 2H). HPLC (method A) t_R 8.25 min (100 area %). Anal. ($C_{27}H_{28}N_4O_2$·2HCl·2H_2O) C, H, N, Cl.

1,5-Bis(5-amidino-7-methoxybenzofuranyl-2)pentane Dihydrochloride (32). White solid (0.31 g, 60%): mp 187−189 °C (dec) (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 4H), 9.20 (s, 4H), 7.70 (s, 2H), 7.36 (s, 2H), 6.77 (s, 2H), 4.02 (s, 6H), 2.83 (t, J = 7.1 Hz, 4H), 1.76 (m, 4H), 1.43 (m, 2H). HPLC (method A) t_R 8.25 min (100 area %). Anal. ($C_{25}H_{28}N_4O_4$·2HCl) C, H, N, Cl.

1,5-Bis(5-*N*-isopropylamidino-7-methoxybenzofuranyl-2)pentane Dihydrochloride (33). The crude product was purified by preparative HPLC to yield a white solid (0.13 g, 22%): mp 165−170 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.58 (d, J = 7.7 Hz, 2H), 9.48 (s, 2H), 9.13 (s, 2H), 7.56 (s, 2H), 7.23 (s, 2H), 6.76 (s, 2H), 4.09 (m, 2H), 4.02 (s, 6H), 2.83 (t, J = 7.1 Hz, 4H), 1.76 (m, 4H), 1.43 (m, 2H), 1.29 (d, J = 6.6 Hz, 12H). HPLC (method A) t_R 9.29 min (100 area %). Anal. ($C_{31}H_{40}N_4O_4$·2.1HCl·2.4H_2O) C, H, N, Cl.

1,5-Bis(5-(2-imidazolinyl)-7-methoxybenzofuranyl-2)pentane Dihydrochloride (34). The crude product was purified by preparative HPLC to yield a white solid (0.17 g, 30%): mp 197−200 °C (1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 4H), 7.90 (s, 2H), 7.65 (s, 2H), 6.79 (s, 2H), 4.01 (s, 14H), 2.83 (t, J = 7.7 Hz, 4H), 1.76 (m, 4H), 1.43 (m, 2H). HPLC (method A) t_R 8.36 min (100 area %). Anal. ($C_{29}H_{32}N_4O_4$·2HCl·2.5H_2O) C, H, N, Cl.

1,5-Bis(6-amidinobenzofuran-2-yl)pentane Dihydrochloride (35). White solid (0.25 g, 26%): mp 243−245 °C (dec) (EtOH/1 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.42 (s, 4H), 9.22 (s, 4H), 8.10 (s, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 2.86 (t, J = 7.1 Hz, 4H), 1.78 (m, 4H), 1.44 (m, 2H). Anal. ($C_{23}H_{24}N_4O_2$·2HCl·0.7H_2O) C, H, N, Cl.

1,5-Bis(6-(*N*-isopropyl)amidinobenzofuran-2-yl)pentane Dihydrochloride (36). White solid (0.48 g, 40%): mp 193−195 °C (dec) (*i*-PrOH/1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 9.55 (d, J = 7.0 Hz, 2H), 9.45 (s, 2H), 9.10 (s, 2H), 7.96 (s, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 4.10 (m, 2H), 2.86 (m, 4H), 1.78 (m, 4H), 1.46 (m, 2H), 1.29 (d, J = 6.0 Hz, 12H). HPLC (method B) t_R 3.47 min (100 area %). Anal. ($C_{29}H_{36}N_4O_2$·2HCl·1.3H_2O) C, H, N, Cl.

1,5-Bis(6-(2-imidazolinyl)benzofuran-2-yl)pentane Dihydrochloride (37). White solid (0.62 g, 49%): mp 308−310 °C (dec) (*i*-PrOH/1.5 M HCl). ^{1}H NMR (DMSO-d_6) δ 10.74 (s, 4H), 8.31 (s, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.2 Hz, 2H), 6.81 (s, 2H), 4.02 (s, 8H), 2.87 (t, J = 7.1 Hz, 4H), 1.79 (m, 4H), 1.45 (m, 2H). Anal. ($C_{27}H_{28}N_4O_2$·2HCl·1.5H_2O·0.3*i*-PrOH) C, H, N, Cl.

1,6-Bis(5-amidinobenzofuranyl-2)hexane Dihydrochloride (38).[45] The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **38** (0.54 g, 48%): mp 307−309 °C (lit.[45] 305 °C (dec)). ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 4H), 9.19 (s, 4H), 8.08 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 2.83 (t, J = 6.6 Hz, 4H), 1.73 (br s, 4H), 1.42 (br s, 4H). HPLC (method A) t_R 8.19 min (100 area %). Anal. ($C_{24}H_{26}N_4O_2$·2HCl·0.2H_2O) C, H, N, Cl.

1,7-Bis(5-amidinobenzofuranyl-2)heptane Dihydrochloride (39). The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **39** (0.69 g, 74%): mp 130−132 °C. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 4H), 9.12 (s, 4H), 8.07 (s, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 6.79 (s, 2H), 2.83 (t, J = 7.1 Hz, 4H), 1.71 (br s, 4H), 1.37 (s, 6H). HPLC (method A) t_R 8.47 min (99.0 area %). Anal. ($C_{25}H_{28}N_4O_2$·2HCl·2H_2O) C, H, N, Cl.

1,8-Bis(5-amidinobenzofuranyl-2)octane Dihydrochloride (40). The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **40** (0.76 g, 82%): mp 302−303 °C. ^{1}H NMR (DMSO-d_6) δ 9.41 (s, 4H), 9.23 (s, 4H), 8.09 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 2.82 (t, J = 6.6 Hz, 4H), 1.71 (br s, 4H), 1.33 (s, 8H). HPLC (method A) t_R 8.82 min (100 area %). Anal. ($C_{26}H_{30}N_4O_2$·2.3HCl) C, H, N, Cl.

1,9-Bis(5-amidinobenzofuranyl-2)nonane Dihydrochloride (41). The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **41** (0.63 g, 55%): mp 167−170 °C. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 4H), 9.12 (s, 4H), 8.07 (s, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.69 (d, J = 8.7 Hz, 2H), 6.79 (s, 2H), 2.81 (t, J = 7.1 Hz, 4H), 1.68 (br s, 4H), 1.31 (br s, 10H). HPLC (method B) t_R 4.36 min (98.5 area %). Anal. ($C_{27}H_{32}N_4O_2$·2HCl·1.5H_2O) C, H, N, Cl.

1,10-Bis(5-amidinobenzofuranyl-2)decane Dihydrochloride (42). The crude product was purified by flash chromatography on C_{18} reversed phase silica gel to afford **42** (0.75 g, 55%): mp 281−283 °C. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 4H), 9.13 (s, 4H), 8.07 (s, 2H), 7.76 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 2.81 (t, J = 7.1 Hz, 4H), 1.69 (br s, 4H), 1.29 (m, 12H). HPLC (method B) t_R 4.81 min (98.4 area %). Anal. ($C_{28}H_{34}N_4O_2$·2HCl·0.2H_2O) C, H, N, Cl.

1,11-Bis(5-amidinobenzofuranyl-2)undecane Dihydrochloride (43). The crude product was purified by flash chromatography on reversed phase silica gel to afford **43** (0.43 g, 48%): mp 230−233 °C. ^{1}H NMR (DMSO-d_6) δ 9.36 (s, 4H), 9.13 (s, 4H), 8.07 (s, 2H), 7.76 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 6.79 (s, 2H), 2.81 (t, J = 7.1 Hz, 4H), 1.68 (m, 4H), 1.27 (m, 14H). HPLC (method B) t_R 5.27 min (100 area %). Anal. ($C_{29}H_{36}N_4O_2$·2.1HCl·1.3H_2O) C, H, N, Cl.

General Procedure for Synthesis of Bisbenzofuranmethanones (47, 48). Bis(4-bromobenzofuran-2-yl)methanone (47). Dried K_2CO_3 (7.00 g, 50.1 mmol) was added to a solution of 2-bromo-6-hydroxybenzaldehyde (**45**) (6.57 g, 32.3 mmol) in dry 2-butanone (35 mL). The suspension was refluxed for 30 min and allowed to cool. A solution of 1,3-dichloroacetone (3.00 g, 24.2 mmol) in dry 2-butanone (5 mL) was added. The reaction mixture was refluxed for 4 h, cooled, filtered through a pad of Celite (2 cm), and concentrated. A dark residue was recrystallized from $CHCl_3$ to give **47** as a brown solid (5.05 g, 76%): mp 223−225 °C. ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 2H), 7.92 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 7.7 Hz, 2H), 7.57 (dd, J = 8.2 and 7.7 Hz, 2H). HPLC (method B) t_R 9.83 min (100 area %). Anal. ($C_{17}H_8Br_2O_3$) C, H, Br.

Bis(6-bromobenzofuran-2-yl)methanone (48). Yellow solid (2.16 g, 76%): mp 176−177 °C (CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 8.26 (s, 2H), 8.17 (br s, 2H), 7.87 (d, J = 8.8 Hz, 2H), 7.61 (dd, J = 8.7 and 1.6 Hz, 2H). HPLC (method B) t_R 9.62 min (100 area %). Anal. ($C_{17}H_8Br_2O_3$) C, H, Br.

General Procedure for Synthesis of Bisbenzofuranmethanes (49, 50). Bis(4-bromobenzofuran-2-yl)methane (49). A solution of aluminum chloride (4.80 g, 11.4 mmol) in dry diethyl ether (70 mL) was added dropwise under Ar to a stirred suspension of lithium aluminum hydride (0.98 g, 25.8 mmol) in dry diethyl ether (100 mL). Vacuum-dried **47** (4.80 g, 11.4 mmol) was added in small portions. The reaction mixture was stirred at room temperature for 1 h followed by careful addition of 2 M HCl (100 mL) and extraction with diethyl ether (2 × 100 mL). The ether solution was dried over $CaCl_2$ and concentrated to provide **49** as a white solid (3.84 g, 83%): mp 142−143 °C (diethyl ether) ^{1}H NMR (DMSO-d_6) δ 7.60 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 7.7 Hz, 2H), 7.24 (dd, J = 8.2 and 7.7 Hz, 2H), 6.80 (s, 2H), 4.52 (s, 2H). HPLC (method B) t_R 10.67 min (100 area %). Anal. ($C_{17}H_{10}Br_2O_3$) C, H, Br.

Bis(6-bromobenzofuran-2-yl)methane (50). White solid (1.43 g, 99%): mp 146−147 °C (diethyl ether). ^{1}H NMR (DMSO-d_6) δ 7.85 (br s, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.39 (dd, J = 8.2 and 1.6 Hz, 2H), 6.82 (s, 2H), 4.44 (s, 2H). HPLC (method B) t_R 10.43 min (100 area %). Anal. ($C_{17}H_{10}Br_2O_3$) C, H, Br.

General Procedure for Synthesis of Bisbenzofuranmethane Carbodinitriles (51, 52). Bis(4-cyanobenzofuran-2-yl)methane (51). A mixture of bis(4-bromobenzofuran-2-yl)methanone (**49**) (3.50 g, 8.62 mmol) and CuCN (6.12 g, 69.0 mmol) was heated

under reflux in dry quinoline (10 mL) for 2 h. The hot reaction mixture was poured into 2 M HCl and stirred at 50−70 °C for 1 h. A precipitate formed was separated, dried under vacuum, and suspended in chlorobenzene (300 mL). The mixture was stirred for 1 h at ambient temperature and filtered. The filtrate was concentrated, and a dark solid residue (2.00 g, 78%) was purified by column chromatography eluting with $CHCl_3$ to produce **51** as a light yellow solid (1.62 g, 63%): mp 173−174 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 7.96 (d, J = 8.2 Hz, 2H), 7.77 (d, J = 7.7 Hz, 2H), 7.46 (dd, J = 8.2 and 7.7 Hz, 2H), 7.09 (s, 2H), 4.64 (s, 2H). HPLC (method B) t_R 7.92 min (100 area %). Anal. ($C_{19}H_{10}N_2O_2$) C, H, N.

Bis(6-cyanobenzofuran-2-yl)methane (52). Yellow solid (0.55 g, 56%): mp 207−209 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.19 (s, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.00 (s, 2H), 4.61 (s, 2H). HPLC (method B) t_R 7.92 min (96.6 area %). Anal. ($C_{19}H_{10}N_2O_2 \cdot 0.3H_2O$) C, H, N.

General Procedure for the Copper-Mediated Heteroannulation. 5-Methoxycarbonyl-2-(3-hydroxypropyl-1)benzofuran (55).[52] A mixture of methyl 4-hydroxy-3-iodobenzoate (**53**) (35.0 g, 126 mmol), 4-pentyn-1-ol (11.0 g, 131 mmol), and copper(I) oxide (12.6 g, 88.2 mmol) in dry pyridine (150 mL) was stirred at 100−120 °C overnight. The mixture was allowed to cool to ambient temperature, diluted with EtOAc (200 mL), filtered through a Celite pad (5 cm), and concentrated. The residue was dissolved in EtOAc (300 mL), washed with 2 M HCl (50 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography, eluting with (1:1) hexanes/EtOAc, followed by recrystallization from (2:1) hexanes/diethyl ether to produce **55** as a light yellow solid (14.4 g, 49%). ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 6.74 (s, 1H), 4.59 (br s, 1H), 3.86 (s, 3H), 3.49 (br s, 2H), 2.84 (t, J = 7.1 Hz, 2H), 1.85 (m, 2H). HPLC (method B) t_R 4.20 min (100 area %).

6-Methoxycarbonyl-2-(3-hydroxypropyl-1)benzofuran (56). Following the procedure described above for **55**, **56** was prepared from **54** and 4-pentyn-1-ol as a light yellow solid (8.90 g, 78%): mp 63−65 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.04 (s, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.65 (d, J = 8.2 Hz, 1H), 6.74 (s, 1H), 4.59 (br s, 1H), 3.87 (s, 3H), 3.49 (t, J = 6.0 Hz, 2H), 2.86 (t, J = 7.7 Hz, 2H), 1.85 (m, 2H). HPLC (method B) t_R 4.17 min (96.5 area %). Anal. ($C_{13}H_{14}O_4 \cdot 0.1H_2O$) C, H.

5-Methoxycarbonyl-2-(2-formylethyl-1)benzofuran (57). Oxalyl chloride (2 M solution in CH_2Cl_2, 40 mL, 80 mmol) was placed under Ar into a reaction flask, equipped with addition funnel, inlet thermometer, and drying tube charged with Drierite and cooled to −70 °C. A mixture of DMSO (12.2 mL, 171 mmol) and dry CH_2Cl_2 (30 mL) was added dropwise. After 30 min a solution of **55** (14.3 g, 61.0 mmol) in CH_2Cl_2 was slowly added and the mixture was stirred at −70 °C for 2 h. After that time, triethylamine (45.2 mL, 405 mmol) was added dropwise at −60 °C. The mixture was allowed to warm to ambient temperature, water (100 mL) was added carefully, and the stirring was continued for 10 min. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 75 mL). Combined organic layers were washed with 2 M HCl (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography, eluting with (5:1) hexanes/EtOAc, followed by recrystallization from (3:1) hexanes/EtOAc to produce **52** as a light yellow solid (12.9 g, 91%): mp. 48−49 °C. ^{1}H NMR (DMSO-d_6) δ 9.70 (s, 1H), 8.20 (s, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 6.75 (s, 1H), 3.87 (s, 3H), 3.07 (m, 2H), 2.96 (m, 2H). Anal. ($C_{13}H_{12}O_4 \cdot 0.1H_2O$) C, H.

6-Methoxycarbonyl-2-(2-formylethyl-1)benzofuran (58). Following the procedure described above for **57**, **58** was prepared from **56** as a light yellow solid (2.78 g, 85%): mp 57−58 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 9.76 (s, 1H), 8.04 (s, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 6.75 (s, 1H), 3.87 (s, 3H), 3.09 (m, 2H), 2.95 (m, 2H). Anal. ($C_{13}H_{12}O_4$) C, H.

5-Methoxycarbonyl-2-(3-butynyl-1)benzofuran (59). A solution of dimethyl 1-diazo-2-oxopropylphosphonate[64] (8.90 g, 46.4 mmol) in dry methanol (50 mL) was added to a stirring mixture of **57** (8.50 g, 36.6 mmol) and K_2CO_3 (10.2 g, 74.0 mmol) in dry methanol (500 mL). The mixture was stirred at ambient temperature until complete conversion was attained according to HPLC (2 h) and concentrated. The residue was diluted with diethyl ether (500 mL), washed with water (2 × 100 mL), and dried over $CaCl_2$. The solvent was removed and the residue was recrystallized from (9:1) hexanes/EtOAc to produce **59** as a white solid (7.66 g, 92%): mp 57−58 °C. ^{1}H NMR (DMSO-d_6) δ 8.23 (d, J = 1.6 Hz, 1H), 7.88 (dd, J = 8.8 and 1.6 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 6.82 (s, 1H), 3.86 (s, 3H), 3.00 (t, J = 7.1 Hz, 2H), 2.84 (t, J = 2.8 Hz, 1H), 2.63 (td, J = 7.1 and 2.8 Hz, 2H). HPLC (method B) t_R 6.73 min (100 area %). Anal. ($C_{14}H_{12}O_3 \cdot 0.2H_2O$) C, H.

6-Methoxycarbonyl-2-(3-butynyl-1)benzofuran (60). Following the procedure described above for **59**, **60** was prepared from **58** as a white solid (0.40 g, 98%): mp 68−69 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.05 (d, J = 1.1 Hz, 1H), 7.84 (dd, J = 8.2 and 1.1 Hz, 1H), 7.68 (d, J = 8.2 Hz, 1H), 6.83 (s, 1H), 3.87 (s, 3H), 3.02 (t, J = 7.1 Hz, 2H), 2.86 (t, J = 2.8 Hz, 1H), 2.63 (td, J = 7.1 and 2.8 Hz, 2H). HPLC (method B) t_R 6.71 min (97.6 area %). Anal. ($C_{14}H_{12}O_3 \cdot 0.1H_2O$) C, H.

1,2-Bis(5-methoxycarbonylbenzofuran-2-yl)ethane (61). Following the procedure described above for **55**, **61** was prepared from **53** and **59** as a white solid (5.24 g, 43%): mp 165−167 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 2H), 7.87 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 6.82 (s, 2H), 3.86 (s, 6H), 3.33 (s, 2H), 3.29 (s, 2H). Anal. ($C_{22}H_{18}O_6 \cdot 0.8H_2O$) C, H.

1-(5-Methoxycarbonylbenzofuran-2-yl)-2-(6-methoxycarbonylbenzofuran-2-yl)ethane (62). Following the procedure described above for **55**, **62** was prepared from **53** and **54** as a white solid (1.70 g, 69%): mp 108−109 °C (CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 1H), 8.05 (s, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H), 6.83 (s, 2H), 3.87 (s, 3H), 3.86 (s, 3H), 3.30 (s, 4H). HPLC (method B) t_R 7.86 min (100 area %). Anal. ($C_{22}H_{18}O_6$) C, H.

1,2-Bis(6-methoxycarbonylbenzofuran-2-yl)ethane (63). Following the procedure described above for **55**, **63** was prepared from **54** and **60** as a white solid (2.00 g, 48%): mp 175−177 °C (CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 8.04 (br s, 2H), 7.83 (dd, J = 8.2 and 1.1 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 6.83 (s, 2H), 3.87 (s, 6H), 3.33 (s, 4H). HPLC (method B) t_R 9.16 min (100 area %). Anal. ($C_{22}H_{18}O_6$) C, H.

1,2-Bis(5-cyanobenzofuran-2-yl)ethane (64).[44] **Method 1. Conversion of Bisbenzofuran Esters to Dinitriles with Dimethylaluminum Amide.** Anhydrous NH_3 was bubbled through dry *o*-xylene (100 mL) for 20 min at 0 °C. A 2.0 M solution of $AlMe_3$ in toluene (27 mL, 54.0 mmol) was added, the NH_3 was passed through the mixture for 20 min, and then the solution was stirred at room temperature for 1 h. 1,2-Bis(5-methoxycarbonylbenzofuran-2-yl)ethane (**61**) (5.00 g 13.2 mmol) was added in one portion. The mixture was stirred at 100−110 °C for 3 h, allowed to cool to ambient temperature, and diluted with $CHCl_3$ (250 mL). Water (100 mL) was added dropwise with vigorous stirring, and a precipitate formed was filtered off. The filtrate was concentrated to give crude **64** (4.00 g, 97%), which was purified by column chromatography, eluting with CH_2Cl_2, to give the desired product as a light yellow solid (1.96 g, 48%): mp 243−244 °C (CH_2Cl_2) (lit.[44] 248−250 °C). ^{1}H NMR (DMSO-d_6) δ 8.10 (br s, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.68 (dd, J = 8.2 and 1.6 Hz, 2H), 6.82 (s, 2H), 3.31 (s, 4H). HPLC (method B) t_R 8.15 min (100 area %). Anal. ($C_{20}H_{12}N_2O_2 \cdot 0.6H_2O$) C, H, N.

Method 2. Copper-Mediated Heteroannulation. A mixture of 4-hydroxy-3-iodobenzonitrile (**83**) (10.0 g, 40.0 mmol), Cu_2O (3.15 g, 22.0 mmol), and 1,5-hexadiene (50% solution in pentane, 4.00 g, 25.0 mmol) in pyridine (100 mL) was stirred at room temperature for 5 h and then refluxed overnight. The mixture was concentrated, diluted with chloroform, filtered through a pad of Celite (2 cm), and concentrated. An oily residue was treated with acetonitrile (50 mL) to form a precipitate, which was recrystallized from CH_2Cl_2 to give pure **64** (1.90 g, 24%).

Method 3. Palladium-Catalyzed Heteroannulation. 4-Hydroxy-3-iodobenzonitrile (**83**) (2.52 g, 10.3 mmol) was added to a

stirred suspension of DMF (8 mL), triethylamine (6 mL), $PdCl_2(PPh_3)_2$ (0.28 g, 2 mol %), and CuI (0.28 g). The resulting mixture was stirred at room temperature for 15 min, and 5-cyano-2-(3-butynyl-1)benzofuran (**86**) (2.00 g, 10.2 mmol) was added at once. The resulting mixture was heated at 80 °C for 15 min, slowly cooled to ambient temperature, and stirred for 2 days. A precipitate formed was filtered off, washed with ethyl acetate, and dried under vacuum to yield **64** (2.00 g, 63%).

1-(5-Cyanobenzofuranyl-2)-2-(6-cyanobenzofuranyl-2)-ethane (65) was prepared from **61** following the procedure described above for **64** in Method 1. Light yellow solid (0.65 g, 46%): mp 235−237 °C (CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1H), 8.10 (s, 1H), 7.74 (d, J = 8.2 Hz, 2H), 7.67 (dd, J = 8.2 and 1.6 Hz, 1H), 7.61 (dd, J = 8.2 and 1.6 Hz, 1H), 6.86 (s, 1H), 6.82 (s, 1H), 3.31 (s, 4H). HPLC (method B) t_R 6.43 min (100 area %). Anal. ($C_{20}H_{12}N_2O_2 \cdot 0.2H_2O$) C, H, N.

Following the procedure described above for **64** in Method 3, **65** was prepared from 5-cyano-2-(3-butynyl-1)benzofuran (**86**) and 3-hydroxy-4-iodobenzonitrile (**87**) as a light yellow solid (2.22 g, 63%).

1,2-Bis(6-cyanobenzofuran-2-yl)ethane (66).[45] Following the procedure described above for **64** in Method 1, **66** was prepared from **63** as a light yellow solid (0.56 g, 27%): mp 266−267 °C (CH_2Cl_2) (lit.[45] 272−274 °C). ^{1}H NMR (DMSO-d_6) δ 8.16 (s, 2H), 7.75 (d, J = 8.2 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 6.88 (s, 2H), 3.35 (s, 4H). HPLC (method B) t_R 8.26 min (100 area %). Anal. ($C_{20}H_{12}N_2O_2 \cdot 0.1H_2O$) C, H, N.

Following the procedure described above for **64** in Method 2, **66** was prepared from 3-hydroxy-4-iodobenzonitrile (**87**) and 1,5-hexadiyne as a light yellow solid (1.77 g, 22%)

1,3-Bis(5-methoxycarbonylbenzofuran-2-yl)propane (67). Following the procedure described above for **64** in Method 2, **67** was prepared from **53** and 1,6-heptadiyne as a white solid (6.37 g, 75%): mp 87−88 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 1.6 Hz, 2H), 7.86 (dd, J = 8.8 and 1.6 Hz, 2H), 7.61 (d, J = 8.8 Hz, 2H), 6.79 (s, 2H), 3.86 (s, 6H), 2.92 (t, J = 7.1 Hz, 4H), 2.15 (m, 2H). HPLC (method B) t_R 9.67 min (100 area %). Anal. ($C_{23}H_{20}O_6 \cdot 0.5H_2O$) C, H.

1,3-Bis(6-methoxycarbonylbenzofuran-2-yl)propane (68). Following the procedure described above for **64** in Method 2, **68** was prepared from **54** and 1,6-heptadiyne as a white solid (6.10 g, 44%): mp 100−101 °C (hexanes/benzene). ^{1}H NMR (DMSO-d_6) δ 8.01 (s, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 6.79 (s, 2H), 3.87 (s, 6H), 2.94 (t, J = 7.1, 4H), 2.17 (m, 2H). HPLC (method B) t_R 9.63 min (93.0 area %). Anal. ($C_{23}H_{20}O_6$) C, H.

Following the procedure described above for **64** in Method 3, **68** was prepared from **54** and 1,6-heptadiyne. The reaction mixture was stirred at 80 °C for 3 days, cooled to ambient temperature, diluted with water (100 mL), and extracted with ethyl acetate (3 × 150 mL). The extracts were dried over $MgSO_4$ and concentrated to give a crude that was purified by column chromatography on silica gel eluting with (1:1) hexanes/chloroform to yield **68** as a white solid (8.50 g, 66%).

1,4-Bis(5-methoxycarbonylbenzofuran-2-yl)butane (69). Following the procedure described above for **64** in Method 2, **69** was prepared from **53** and 1,7-octadiyne as a white solid (6.0 g, 79%): mp 130−131 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.19 (d, J = 1.1 Hz, 2H), 7.86 (dd, J = 8.8 and 1.1 Hz, 2H), 7.61 (d, J = 8.8 Hz, 2H), 6.75 (s, 2H), 3.86 (s, 6H), 2.88 (br s, 4H), 1.80 (br s, 4H). Anal. ($C_{24}H_{22}O_6 \cdot 0.6H_2O$) C, H.

1,4-Bis(6-methoxycarbonylbenzofuran-2-yl)butane (70). Following the procedure described above for **64** in Method 2, **70** was prepared from **54** and 1,7-octadiyne as a white solid (4.96 g, 70%): mp 130−131 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.01 (br s, 2H), 7.83 (dd, J = 8.2 and 1.1 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 6.75 (s, 2H), 3.86 (s, 6H), 2.90 (m, 4H), 1.80 (m, 4H). HPLC (method B) t_R 10.19 min (100 area %). Anal. ($C_{24}H_{22}O_6$) C, H.

Following the procedure described above for **64** in Method 3, **70** was prepared from **54** and 1,7-octadiyne as a white solid (5.62 g, 45%).

1,5-Bis(5-methoxycarbonylbenzofuran-2-yl)pentane (71). Following the procedure described above for **64** in Method 2, **71** was prepared from **53** and 1,8-nonadiyne as a yellow solid (6.15 g, 72%): mp 83−85 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 8.16 (d, J = 1.6 Hz, 2H), 7.84 (dd, J = 8.8 and 1.6 Hz, 2H), 7.59 (d, J = 8.8 Hz, 2H), 6.71 (s, 2H), 3.86 (s, 6H), 2.81 (t, J = 7.1 Hz, 4H), 1.76 (m, 4H), 1.43 (m, 2H). HPLC (method B) t_R 10.79 min (100 area %). Anal. ($C_{25}H_{24}O_6$) C, H.

Following the procedure described above for **64** in Method 3, **71** was prepared from **53** and 1,8-nonadiyne as a yellow solid (0.71 g, 75%)

1,5-Bis(6-methoxycarbonylbenzofuran-2-yl)pentane (72). Following the procedure described above for **64** in Method 2, **72** was prepared from **54** and 1,8-nonadiyne as a yellow solid (8.19 g, 84%): mp 83−85 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 8.01 (s, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 6.73 (s, 2H), 3.87 (s, 6H), 2.84 (t, J = 7.1 Hz, 4H), 1.77 (m, 4H), 1.45 (m, 2H). HPLC (method B) t_R 10.63 min (100 area %). Anal. ($C_{25}H_{24}O_6$) C, H.

Following the procedure described above for **64** in Method 3, **71** was prepared from **54** and 1,8-nonadiyne as a yellow solid (8.00 g, 99%)

1,3-Bis(5-cyanobenzofuran-2-yl)propane (73). Following the procedure described above for **64** in Method 1, **73** was prepared from **67** as a white solid (2.47 g, 49%): mp 153−154 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.07 (d, J = 1.6 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 7.65 (dd, J = 8.8 and 1.6 Hz, 2H), 6.79 (s, 2H), 2.93 (t, J = 7.1 Hz, 4H), 2.15 (p, J = 7.1 Hz, 2H). HPLC (method B) t_R 8.69 min (96.1 area %). Anal. ($C_{21}H_{14}N_2O_2 \cdot 0.2$EtOAc) C, H, N.

1,3-Bis(6-cyanobenzofuran-2-yl)propane (74). Following the procedure described above for **64** in Method 1, **74** was prepared from **68** as a white solid (2.92 g, 58%): mp 199−201 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 2H), 7.71 (d, J = 7.7 Hz, 2H), 7.60 (d, J = 7.7 Hz, 2H), 6.83 (s, 2H), 2.95 (t, J = 7.7 Hz, 4H), 2.17 (p, J = 7.7 Hz, 2H). HPLC (method B) t_R 8.64 min (100 area %). Anal. ($C_{21}H_{14}N_2O_2$) C, H, N.

Following the procedure described above for **64** in Method 2, **74** was prepared from 3-hydroxy-4-iodobenzonitrile (**87**) and 1,6-heptadiyne as a white solid (5.38 g, 62%).

Following the procedure described above for **64** in Method 3, **74** was prepared from 3-hydroxy-4-iodobenzonitrile (**87**) and 1,6-heptadiyne as a white solid (0.78 g, 47%).

1,4-Bis(5-cyanobenzofuran-2-yl)butane (75).[45] Following the procedure described above for **64** in Method 1, **75** was prepared from **69** as a white solid (2.02 g, 40%): mp 187−188 °C (hexanes/EtOAc) (lit.[45] 190−192 °C). ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 6.76 (s, 2H), 2.90 (m, 4H), 1.80 (m, 4H). HPLC (method B) t_R 9.40 min (100 area %). Anal. ($C_{22}H_{16}N_2O_2 \cdot 0.3H_2O$) C, H, N.

1,4-Bis(6-cyanobenzofuran-2-yl)butane (76). Following the procedure described above for **64** in Method 1, **76** was prepared from **70** as a white solid (1.57 g, 38%): mp 215−217 °C ($CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 6.80 (s, 2H), 2.91 (br s, 4H), 1.80 (br s, 4H). HPLC (method B) t_R 9.32 min (100 area %). Anal. ($C_{22}H_{16}N_2O_2 \cdot 0.4H_2O$) C, H, N.

1,5-Bis(5-cyanobenzofuran-2-yl)pentane (77). Following the procedure described above for **64** in Method 1, **77** was prepared from **71** as a white solid (1.71 g, 42%): mp 133−134 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.06 (s, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 6.71 (s, 2H), 2.83 (m, 4H), 1.77 (m, 4H), 1.44 (m, 2H). HPLC (method B) t_R 9.90 min (100 area %). Anal. ($C_{23}H_{18}N_2O_2 \cdot 0.1H_2O$) C, H, N.

Following the procedure described above for **64** in Method 3, **77** was prepared from 3-hydroxy-4-iodobenzonitrile (**83**) and 1,8-nonadiyne as a light yellow solid (13.7 g, 77%).

1,5-Bis(6-cyanobenzofuran-2-yl)pentane (78). Following the procedure described above for **64** in Method 1, **78** was prepared from **72** as a white solid (3.30 g, 50%): mp 106−107 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.11 (br s, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.62 (dd, J = 8.2 and 1.0 Hz, 2H), 6.79 (s, 2H), 2.87 (t, J = 7.1 Hz, 4H), 1.78 (m, 4H), 1.44 (m, 2H). HPLC (method B) t_R 9.89 min (95.2 area %). Anal. ($C_{23}H_{18}N_2O_2$) C, H, N.

1,5-Bis(5-formyl-7-methoxybenzofuran-2-yl)pentane (80). Following the procedure described above for **64** in Method 2, **80** was prepared from 5-iodovaniline **79** as a white solid (6.01 g, 78%): mp 110−112 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 9.98 (s, 2H), 7.75 (s, 2H), 7.33 (s, 2H), 6.76 (s, 2H), 3.99 (s, 6H), 2.82 (t, J = 7.1 Hz, 4H), 1.76 (m, 4H), 1.42 (m, 2H). Anal. ($C_{25}H_{24}O_6 \cdot 1H_2O$) C, H.

1,5-Bis(5-formyl-7-methoxybenzofuran-2-yl)pentane Bisoxime (81). Bisaldehyde **80** (5.50 g, 13.0 mmol) and hydroxylamine hydrochloride (2.10 g, 30.0 mmol) were dissolved in a mixture of dry pyridine (20 mL) and dry ethanol (20 mL). The reaction mixture was stirred under Ar at ambient temperature for 2 h. The mixture was concentrated to half of its volume and diluted with water to form an oily residue. The liquid was decanted, and the residue was dissolved in ethanol (30 mL), filtered through a paper filter, and diluted with water (100 mL). The ethanol was evaporated. During the concentration, a precipitation occurred. The precipitate was filtered off and recrystallized from aqueous EtOH to yield **81** as a white solid (5.00 g, 85%): mp 125−127 °C. ^{1}H NMR (DMSO-d_6) δ 11.06 (s, 2H), 8.16 (s, 2H), 7.27 (d, J = 1.6 Hz, 2H), 7.13 (d, J = 1.6 Hz, 2H), 6.59 (s, 2H), 3.92 (s, 6H), 2.77 (t, J = 7.1 Hz, 4H), 1.73 (m, 4H), 1.42 (m, 2H). Anal. ($C_{25}H_{26}N_2O_6 \cdot 0.3H_2O$) C, H, N.

1,5-Bis(5-cyano-7-methoxybenzofuran-2-yl)pentane (82). A mixture of **81** (3.00 g, 6.71 mmol) in acetic anhydride (10 mL) was refluxed for 2 h. The reaction mixture was cooled down, diluted with water (100 mL) and stirred for 2 h at ambient temperature. A precipitate formed was separated and recrystallized from a mixture of hexanes and EtOAc to yield **82** as a white solid (2.30 g, 83%): mp 136−137 °C. ^{1}H NMR (DMSO-d_6) δ 7.66 (s, 2H), 7.31 (s, 2H), 6.69 (s, 2H), 3.97 (s, 6H), 2.80 (t, J = 7.1 Hz, 4H), 1.71 (m, 4H), 1.43 (m, 2H). Anal. ($C_{25}H_{22}N_2O_4 \cdot 0.2AcOH$) C, H, N.

5-Cyano-2-(3-hydroxypropyl-1)benzofuran (84). Following the procedure described above for **64** in Method 3, **84** was prepared from 4-hydroxy-3-iodobenzonitrile **83** and 4-pentyn-1-ol as a white solid (8.91 g, 74%): mp 65−66 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (d, J = 1.0 Hz, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.66 (dd, J = 8.2 and 1.0 Hz, 1H), 6.73 (s, 1H), 4.58 (t, J = 4.9 Hz, 1H), 3.48 (q, J = 6.0 Hz, 2H), 2.86 (t, J = 7.1 Hz, 2H), 1.85 (m, 2H). HPLC (method B) t_R 3.72 min (100 area %). Anal. ($C_{12}H_{11}NO_2$) C, H, N.

5-Cyano-2-(2-formylethyl-1)benzofuran (85). Following the procedure described above for **57**, **85** was prepared from **84** as a light yellow solid (6.51 g, 84%): mp 52−53 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 8.10 (br s, 1H), 7.73 (d, J = 8.2 Hz, 1H), 7.67 (dd, J = 8.2 and 1.6 Hz, 1H), 6.75 (s, 1H), 3.09 (t, J = 7.1 Hz, 2H), 2.95 (t, J = 7.1 Hz, 2H). Anal. ($C_{12}H_9NO_2$) C, H, N.

5-Cyano-2-(3-butynyl-1)benzofuran (86). Following the procedure described above for **59**, **86** was prepared from **85** as a white solid (4.70 g, 77%): mp 110−112 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.13 (br s, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.68 (dd, J = 8.2 and 1.6 Hz, 1H), 6.82 (s, 1H), 3.02 (t, J = 7.1 Hz, 2H), 2.84 (t, J = 2.2 Hz, 1H), 2.63 (m, 2H). HPLC (method B) t_R 6.31 min (100 area %). Anal. ($C_{13}H_9NO$) C, H, N.

1,6-Bis(5-cyanobenzofuran-2-yl)hexane (88). Following the procedure described above for **64** in Method 3, **88** was prepared from **83** and 1,9-decadiyne[68] as a light yellow solid (4.31 g, 71%): mp 184−185 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 6.73 (s, 2H), 2.81 (t, J = 7.1 Hz, 4H), 1.71 (br s, 4H), 1.40 (br s, 4H). HPLC (method B) t_R 10.54 min (100 area %). Anal. ($C_{24}H_{20}N_2O_2 \cdot 0.2EtOAc$) C, H, N.

1,7-Bis(5-cyanobenzofuran-2-yl)heptane (89). Following the procedure described above for **64** in Method 3, **89** was prepared from **83** and 1,10-undecadiyne[68] as a light yellow solid (2.01 g, 43%): mp 133 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 6.72 (s, 2H), 2.80 (t, J = 7.1 Hz, 4H), 1.68 (br s, 4H), 1.35 (m, 6H). HPLC (method B) t_R 10.98 min (100 area %). Anal. ($C_{25}H_{22}N_2O_2 \cdot 0.2hexane$) C, H, N.

1,8-Bis(5-cyanobenzofuranyl-2)octane (90). Following the procedure described above for **64** in Method 3, **90** was prepared from **83** and 1,11-dodecadiyne[68] as a light yellow solid (1.50 g, 23%): mp 167−168 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 6.72 (s, 2H), 2.80 (t, J = 7.1 Hz, 4H), 1.68 (m, 4H), 1.32 (br s, 8H). HPLC (method B) t_R 11.91 min (100 area %). Anal. ($C_{26}H_{24}N_2O_2 \cdot 0.3EtOAc$) C, H, N.

1,9-Bis(5-cyanobenzofuranyl-2)nonane (91). Following the procedure described above for **64** in Method 3, **91** was prepared from **83** and 1,12-tridecadiyne[68] as a light yellow solid (1.20 g, 24%): mp 95−97 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 2H), 7.72 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 6.72 (s, 2H), 2.79 (t, J = 7.1 Hz, 4H), 1.67 (m, 4H), 1.29 (br s, 10H). HPLC (method C) t_R 11.40 min (100 area %). Anal. ($C_{27}H_{26}N_2O_2$) C, H, N.

1,10-Bis(5-cyanobenzofuranyl-2)decane (92). Following the procedure described in Method 3 for **64**, **92** was prepared from **83** and 1,13-tetradecadiyne[68] as a light yellow solid (1.91 g, 36%): mp 141−143 °C (EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 6.72 (s, 2H), 2.80 (t, J = 7.1 Hz, 4H), 1.67 (m, 4H), 1.25 (m, 12H). HPLC (method D) t_R 9.93 min (100 area %). Anal. ($C_{28}H_{28}N_2O_2 \cdot 0.1EtOAc$) C, H, N.

1,11-bis(5-cyanobenzofuranyl-2)undecane (93). Following the procedure described above for **64** in Method 3, **93** was prepared from **83** and 1,14-pentadecadiyne[68] as a white solid (1.01 g, 19%): mp 87−89 °C (hexanes/EtOAc). ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 2H), 7.70 (d, J = 8.2 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 6.72 (s, 2H), 2.79 (t, J = 7.1 Hz, 4H), 1.68 (m, 4H), 1.25 (m, 14H). HPLC (method D) t_R 10.12 min (97.9 area %). Anal. ($C_{29}H_{30}N_2O_2 \cdot 0.3hexane$) C, H, N.

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Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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