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**Original article**

# Synthesis and anti-*Pneumocystis carinii* pneumonia activity of novel dicationic dibenzothiophenes and orally active prodrugs

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**Abstract** – Dicationic carbazoles have been found to be highly active against a rat model of *Pneumocystis carinii* pneumonia (PCP). Unfortunately, amidoxime derivatives, designed as prodrugs, were inactive against PCP even though the corresponding amidines were highly active. In the present work, a series of 2,8- and 3,7-bis cationic dibenzothiophenes was synthesized and assayed for anti-PCP activity. Three of the compounds proved to be more potent and less toxic than a standard anti-PCP drug (pentamidine) when given intravenously. Unlike the carbazoles, a dibenzothiophene amidoxime prodrug given orally reduced the parasite load by more than 99%. While no quantitative correlation was seen between anti-PCP activity and DNA binding, a strong level of DNA binding was found to be necessary for antimicrobial activity.


***Pneumocystis carinii* pneumonia / dibenzothiophenes / dications / DNA / amidines**

## 1. Introduction

This work is a continuation of studies designed to develop novel dicationic molecules (pentamidine related) for the treatment of *Pneumocystis carinii* pneumonia (PCP), a significant AIDS related opportunistic infection. A number of novel dicationic molecules, including pentamidine analogues [1, 2] and molecules having various spacers between the cationic groups [3–10] have been prepared and assayed against a rat model of PCP. Dicationically substituted carbazoles are among the most active against PCP and least toxic in the rat model of the disease [9]. However, the potential clinical use of aromatic dications is severely limited by their lack of oral bioavailability. Research examining the metabolic interconversion of pentamidine and its amidoxime derivatives [11–16] led to the hypothesis that amidoximes are effective, orally bioavailable prodrugs of pharmacologically active amidines [17]. However, the carbazole diamidoximes lacked significant anti-PCP activity upon oral or intravenous dosing, despite the high activity of the corresponding diamidines. These diamidoximes appear to be reduced to the amidine and diamidine species in the presence of rat liver homogenates but not intact BRL 3A hepatocytes [6 and unpublished data]. Structural features which may account for this inactivity in vivo include the presence of a nitrogen atom and the absence of an ether linkage in the spacer between the two amidoxime groups [6, 7, 18]. The replacement of the carbazole nitrogen atom by isosteric oxygen and sulfur to form dibenzofurans and dibenzothiophenes, respectively, is a logical step toward optimizing the activity of the carbazole diamidoximes. While several of the dibenzofuran diamidines had intravenous anti-PCP activity comparable to pentamidine, the diamidoximes lacked significant

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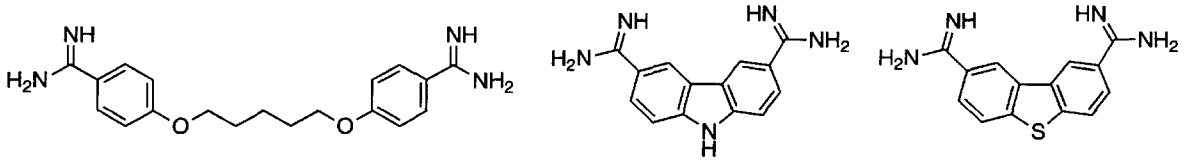


**Figure 1.** Structures of (from left) pentamidine, 3,6-diamidinocarbazole, and 2,8-diamidinodibenzothiophene.

activity when given orally [10]. The current study investigates the synthesis and anti-PCP activity of novel dicationic dibenzothiophenes *(figure 1)*.

## 2. Chemistry

The 2,8-bis cationic dibenzothiophenes were prepared from dibenzothiophene *(figure 2)*. Dibenzothiophene was brominated following a known procedure [19], and the 2,8-dibromo intermediate underwent cyanodebromination to give dinitrile **9** [20]. Compounds **1–3** were prepared by standard Pinner syntheses [2, 9] from **9**. The Pinner synthesis was chosen for amidoxime **3** because of previous experience with carbazole [9] and dibenzofuran analogues [10], which could not be prepared by direct reaction of the nitrile precursors with hydroxylamine [14]. The imidazoline **4** was conveniently prepared by the neat fusion of dinitrile **9** with a mixture of ethylenediamine and ethylenediamine dihydrochloride at 300 °C [9].

It was anticipated that sulfone analogues of the dibenzothiophene dications would be more water-soluble than the corresponding dibenzothiophenes, hence the representative sulfone **5** was prepared. The most expedient method of preparing **5** proved to be the oxidation of **4**. Compound **9** [20] and its dibromo precursor [19] were successfully oxidized to the corresponding sulfones (in 77 and 90% yields, respectively) using 30% hydrogen peroxide in acetic acid [21, 22]. However, these intermediates proved to be extremely insoluble in organic solvents. An attempted Pinner synthesis beginning with the sulfone analogue of **9** failed to give the desired amidine. The hydrogen peroxide oxidation of **4** in acetic acid was sluggish, presumably due to the low solubility of the starting material in acetic acid. The oxidation of **4** to **5** proceeded with no problems when trifluroacetic acid was used in place of acetic acid. The resultant ditrifluoroacetate salt was converted to the free base using sodium hydroxide, and then to the dihydrochloride salt using ethanolic HCl.

The preparation of the 3,7-dications **6–8** was less straightforward. The literature provided little insight for ring closure reactions yielding dibenzothiophenes with desired substituents in place. A different approach would involve electrophilic substitution (at positions 3 and 7) of the sulfone or sulfoxide derivative of dibenzothiophene. The dibromo intermediate **12** has been prepared by the dibromination of dibenzothiophene sulfone [22, 23], followed by the lithium aluminium hydride reduction of the sulfone moiety [24]. Both of these reported procedures proved to be difficult to reproduce. The bromination step, which requires refluxing in neat bromine, resulted in a mixture of mono and dibromo products, the separation of which was hindered by their poor solubility. The hydride reduction resulted in a mixture of deoxygenation and debromination products. Therefore the sulfone approach was abandoned in favour of the sulfoxide approach, as sulfoxides are known to undergo substitution and reduction under milder conditions [25]. The attempted oxidation of dibenzothiophene by a known chlorination-hydrolysis method [26] resulted in 2,8-dichlorodibenzothiophene. Dibenzothiophene sulfoxide was prepared successfully by the ceric ammonium nitrate oxidization of dibenzothiophene [27]. There appears to be no report in the literature of the direct bromination of dibenzothiophene sulfoxide. Attempted bromination resulted in no reaction using various solvents, or in mixtures of products using neat bromine. The sulfoxide was nitrated to intermediate **10** by a known procedure [25]. The same authors reported the stannous chloride reduction of **10** to **11** (isolated as the free base). This reaction proved to be difficult to reproduce. A more expedient method was the palladium-catalysed hydrogenation of **10** in ethanol, with the isolation of **11** as the dihydrochloride salt. The best method to prepare **13** from **11** was via intermediate **12**. Dihydrochloride salt **11** readily underwent bis-diazotization with sodium nitrate in aqueous HCl, and the diazonium salt was reacted with a solution of copper(I) bromide in 48% HBr to give dibromo intermediate **12**. Reaction of **12** with copper(I) cyanide in 1-methyl-2-pyrrolidinone [20] gave dinitrile **13**. The dintirile was subjected to standard Pinner conditions to give the 3,7-diamidines **6–8**.

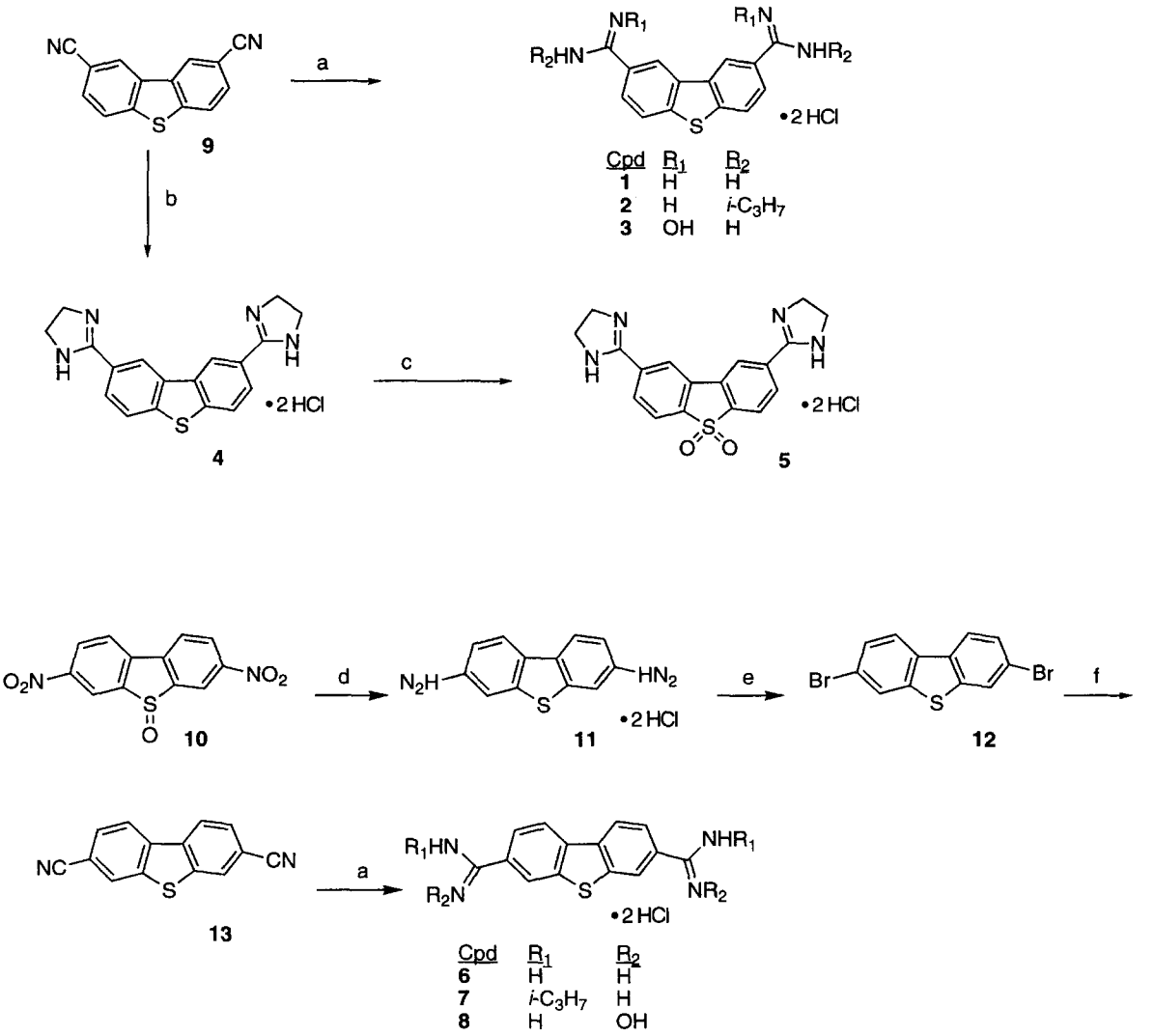


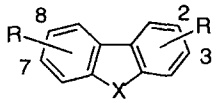

**Figure 2.** Synthesis of dicationic dibenzothiophenes[a]
[a]Key: (a) EtOH, HCl, 1,4-dioxane –5–25 °C, then appropriate amine, EtOH; (b) $H_2N(CH_2)_2NH_2 \cdot 2HCl$, $H_2N(CH_2)_2NH_2$, 300 °C, 30 min; (c) 30% $H_2O_2$, TFA, 16 h; (d) $H_2$, 10% Pd/C, EtOH, 2 h, then EtOH/HCl; (e) $NaNO_2$, aq. HCl, 0 °C, 45 min, then CuBr, 48% HBr, Δ, 2 h; (f) CuCN, 1-methyl-2-pyrrolidinone, 3 h.

## 3. Results and discussion

### *3.1. Activity against P. carinii pneumonia*

The activity of the compounds against PCP in the rat model of disease is shown in *table I.* The activity is expressed as the percent of cysts counted in treated groups compared to saline treated controls. All of the compounds in the initial screen were given by tail vein injection at a dose of 10 μmol/kg/d for 14 d. The test compounds were compared for efficacy with pentamidine at a dose of 22 μmol/kg/d. Three of the compounds tested (**1**, **2** and **7**) were found to be more potent than pentamidine, reducing the parasite load by 99.9% or more when given intravenously at less than half the dose of pentamidine. Furthermore, none of the compounds exhibited significant toxicity in the rat model at the 10 μmol/kg

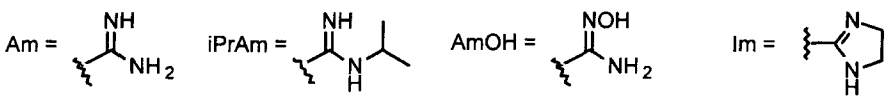
**Table I.** Activity against *Pneumocystis carinii* pneumonia (PCP) and DNA binding by novel dicationic dibenzothiophenes.




| Compound[a] | Position of R | R[b] | X | Anti-*Pneumocystis carinii* activity | | | DNA binding *ΔTm* (°C)[f] |
|---|---|---|---|---|---|---|---|
| | | | | Dose (μmol/kg)[c] | Toxicity[d] | % Saline control ± std. error[e] | |
| Saline control | – | – | – | – | 0 | 100.00 ± 14.86 | – |
| Pentamidine | – | – | – | 22.0 | ++ | 0.74 ± 0.12 | 10.7 |
| **1** | 2,8 | Am | S | 10.0 | 0 | 0.10 ± 0.03 | 12.4 |
| **2** | 2,8 | iPrAm | S | 10.0 | + | 0.01 ± 0.01 | 6.4 |
| | | | | 5.0 | 0 | 0.04 ± 0.01 | |
| | | | | 2.5 | 0 | 0.19 ± 0.10 | |
| | | | | 1.0 | 0 | 12.76 ± 4.02 | |
| | | | | 0.1 | 0 | 94.78 ± 28.30 | |
| **3** | 2,8 | AmOH | S | 10.0 | + | 0.82 ± 0.45 | – |
| | | | | 33.0 p.o. | 0 | 0.85 ± 0.59 | |
| **4** | 2,8 | Im | S | 10.0 | 0 | 28.03 ± 12.72 | 19.0 |
| **5** | 2,8 | Im | $SO_2$ | 10.0 | 0 | 93.48 ± 9.58 | 2.3[g] |
| **6** | 3,7 | Am | S | 10.0 | 0 | 35.95 ± 9.59 | 15.0 |
| **7** | 3,7 | iPrAm | S | 10.0 | + | 0.04 ± 0.02 | 14.5 |
| **8** | 3,7 | AmOH | S | 10.0 | + | 111.64 ± 76.53 | – |
| | | | | 33.0 p.o. | 0 | 45.56 ± 26.12 | |

[a] All compounds were isolated as the dihydrochloride salt.

[b]




[c] Expressed as μmol/kg/d, given via tail vein injection to groups of 8 rats for 14 d, except for compounds **3** and **8**, which were given to groups of 4 rats by both i.v. injection and oral gavage (p.o.). [d]Toxicity scores are subjective evaluations of overt toxicity in dexamethasone immunosuppressed rats. A score of '0' indicates no observable deleterious effects from dosing. A score of '+' indicates slight hypoactivity with compounds **2** and **7**, and tail inflammation with compounds **3** and **8**. A score of '++' indicates hypoactivity, dyspnea, and ataxia with pentamidine. [e]Cysts/g lung counts were $69.18 \times 10^6$ for the saline control group and $0.51 \times 10^6$ for the pentamidine group. These scores were pooled across experiments involving compounds **1**–**8**. Saline: $n = 55$; pentamidine: $n = 52$. [f]Change in thermal melting determined on calf thymus DNA. [g]Tested as the ditrifluoroacetate salt.

screening dose. In contrast, compounds **4** and **6** showed diminished activities, and **5** was inactive. Comparison of the activities of **4** and **5** demonstrates the decreased activity resulting from the oxidation of the sulfur atom from the sulfide to the sulfone state. The inactivity of **5** is due, at least in part, to its weak binding to DNA. One may also speculate whether the sulfone oxygen atoms may impair the ability of the molecule to cross cell membranes.

Notable structure-activity observations arise upon comparing the anti-PCP activities of the dibenzothiophenes to those of the dibenzofurans [10] and carbazoles [9]. The carbazole 3,6- and 2,7-diamidines, diimidazolines, and di-*N*-isopropylamidines all exhibited excellent anti-PCP activity. Among the 2,8-substituted dibenzothiophenes (analogues of the 3,6-substituted carbazoles), the diamidine **1** and the di-*N*-isopropylamidine **2** were highly active, as were the analogous carbazoles. However, the diimidazoline **4** was only weakly active, in contrast to the analogous carbazole. A similar trend was observed among the 2,8-dibenzofurans, although the latter compounds were less active than either the carbazoles or the dibenzothiophenes. The dibenzothiophene 3,7-di-*N*-isopropylamidine **7** was highly active, as was the analogous carbazole. On the other hand, diamidine **6** was only weakly active, unlike the highly active analogous carbazole. Again, a similar trend was observed among the 3,7-substituted dibenzofurans, with the dibenzofurans being less active than the dibenzothiophenes. 3,7-Diimidazolinodibenzothiophene (the 3,7 regioisomer

of **4**) was not prepared because it was presumed to be inactive, in light of the diminished activities of **4** and both dibenzofuran diimidazolines [10].

A dose response study was performed upon compound **2**, the most potent compound in the series. Only the highest dose (10 μmol/kg) produced mild side effects which were manifested as very slight and transient hypoactivity. Excellent anti-PCP activity (more than 99% reduction of the parasite load) was retained at the 5 μmol/kg dose, and even at the 2.5 μmol/kg dose (one-tenth the dose of pentamidine). The anti-PCP activity was lost when the dose was reduced to 1 μmol/kg/d or less.

The most significant finding of this study was the activity of amidoxime **3** (designed as a prodrug of **1**) when given orally. When given intravenously at 10 μmol/kg/d, **3** had somewhat diminished activity relative to **1** at the same dose, but similar activity to pentamidine at half the dose of pentamidine. Comparable anti-PCP activity (99% reduction of the parasite load) was maintained when **3** was given orally at 33 μmol/kg/d. In addition, the oral activity of **3** was significantly greater than that of pentamidoxime at the same dose [6]. In contrast to the carbazole diamidoximes, dibenzothiophene diamidoxime **3** acts as prodrug of the active diamidine **1**. The inactivity of the carbazole diamidoximes appears to result from the inability of the compounds to enter hepatocytes, where the amidoximes are reduced to the active amidines. The carbazole nitrogen atom has been implicated in this inactivity, although for reasons that are not well understood [6]. The replacement of the carbazole NH group with isosteric sulfur resulted in greatly enhanced oral activity of the diamidoximes. In contrast, diamidoxime **8** was inactive against PCP when given intravenously or orally. This result was probably due to the diminished activity of the corresponding diamidine **6**.

### *3.2. DNA binding*

Previous studies have shown an important link between the ability of dicationic molecules to bind to the minor groove of DNA and exert antimicrobial activity. A minimal $\Delta T_m$ value of 5.0 °C has been shown to be necessary for antimicrobial activity [9, 28–31] The past work clearly indicated that the DNA binding and antimicrobial potency and specificity were dependent on the nature and size of the spacer between the cationic moieties.

All of the dibenzothiophenes which were active against PCP, except for the amidoxime prodrugs, were bound to calf thymus DNA with a change in thermal melting of at least 5 °C. Compounds **1**, **2**, and **7**, which were highly active against PCP, exhibited $\Delta T_m$ values of 12.4, 6.4, and 14.5 °C, respectively. Among these three compounds, **2** was the most potent against PCP but was the least strongly bound to DNA. The sulfone **5**, which was inactive against PCP, exhibited a $\Delta T_m$ value of only 2.3 °C. On the other hand, compounds **4** and **6**, which were the most tightly bound to DNA ($\Delta T_m$ values of 19.0 and 15.0 °C, respectively) were only weakly active against PCP. An interesting observation is the sharply contrasting $\Delta T_m$ values of **4** and its sulfone analogue **5**. Not only do the sulfone oxygen atoms cause decreased anti-PCP activity, but also decreased DNA binding. The oxygen atoms, projecting in opposite directions away from the plane of the dibenzothiophene system, may sterically prevent **5** from fitting into the DNA minor groove. In at least one dicationic carbazole, the carbazole NH atom serves as a hydrogen bonding donor with DNA base pairs [32]. The mode of DNA binding of dicationic dibenzothiophenes, and more specifically, the role of the sulfur atom as a hydrogen bond donor, have yet to be investigated.

In conclusion, while a minimal level of binding to DNA was required for anti-PCP activity, no quantitative relationship was observed between anti-PCP activity and the strength of DNA binding. These results are consistent with previous findings in our laboratories.

## 4. Experimental protocols

### *4.1. Biological studies*

The anti-PCP activity of the compounds was determined using a standard procedure [4], as was the binding of the molecules to DNA as determined by thermal melting of calf thymus DNA [33].

### *4.2. Chemistry*

Uncorrected melting points were measured on a Thomas Hoover capillary melting point apparatus or a Mel-Temp II apparatus. IR spectra were recorded in Nujol mulls or KBr pellets on a Perkin-Elmer 1320 spectrophotometer. $^1$H NMR spectra were recorded on Varian XL 400 and Bruker AMX-500 MHz spectrometers. Anhydrous ethanol was distilled over Mg immediately prior to use. Isopropyl amine was distilled over KOH prior to use. Ethanolic solutions of hydroxylamine were prepared by treating solutions of hydroxylamine hydrochloride with equimolar amounts of sodium ethoxide (21% solution in denatured alcohol), and filtering off the resultant sodium chloride. Reaction products were dried over $P_2O_5$ at 77 or 110 °C at 0.1 mm Hg. Unless stated otherwise, reactions

were monitored by TLC on silica or by reverse phase HPLC. HPLC chromatograms were recorded as previously described [9] with the following modifications. A Dupont Zorbax Rx C8 column (3.5 μ 3.0 mm × 15 cm) was used. Mobile phases consisted of mixtures of acetonitrile (3.75–67.5% v/v) in water containing tetramethylammonium chloride, sodium heptanesulfonate, and phosphate buffer pH 2.5 (10 mM each). In method A the concentration of acetonitrile was maintained at 3.75% for 0.5 min, increased to 45% following a linear gradient over 12.5 min, increased immediately to 67.5% following a linear gradient over 3 min, then maintained at 67.5% for 4.5 min. Method B was identical to method A, except that the acetonitrile concentrations were 5, 47, 68, and 68%, at the respective time points. FAB mass spectra were recorded on a VG 70-SEQ Hybrid spectrometer (caesium ion gun, 30 kV). Microanalyses were performed by Atlantic Microlab, Norcross, GA., and were within ± 0.4% of calculated values. Intermediates **9** and **10** were each prepared in two steps from dibenzothiophene using known procedures [19, 20, 25, 27]. Dibenzothiophene was purchased from Aldrich Chemical Co., Milwaukee, WI.

*4.2.1. General procedure for Pinner syntheses of compounds* **1–3**

Method A. A stirred suspension of the dinitrile and anhydrous ethanol in 1,4-dioxane in a 3-neck flask equipped with a hydrogen chloride inlet tube, a thermometer, and a drying tube was cooled in an ice-salt bath. Hydrogen chloride was introduced into the system at such a rate that the temperature of the reaction mixture did not exceed 5 °C, until the system was saturated with HCl. The reaction mixture was then tightly stoppered and stirred at room temperature until the nitrile was no longer detectable by IR or HPLC. The reaction mixture was diluted with ether. The crude imidate was filtered off under $N_2$, then reacted immediately with the appropriate amine.

Method B was similar to method A, except that the dioxane was first quickly saturated with hydrogen chloride without regard to temperature. The solution was cooled to 0 °C before the nitrile and the ethanol were added. Hydrogen chloride was passed slowly through the system for 15–30 min to ensure complete saturation.

*4.2.1.1. 2,8-Diamidinodibenzothiophene dihydrochloride* (**1**)

The imidate was prepared from 2,8-dicyanodibenzothiophene (**9**, 2.35 g, 10.0 mmol), ethanol (2.98 g, 64.7 mmol) and 1,4-dioxane (150 mL) by method A. After 19 d the crude diimidate (3.66 g, 91.4%) was filtered off under nitrogen and was added to a solution of ammonia (12.48 g, 7.33 mmol) in anhydrous ethanol (100 mL) in a stoppered flask. The mixture was stirred overnight at 40–55 °C. The reaction mixture was concentrated, and the crude product was filtered off and washed with ether. The crude product was nearly completely dissolved in hot water (150 mL) and filtered through a layer of Norit-A (4–5 cm thick) over a pad of Celite 545. The filtrate was concentrated to ca. 25 mL. The resultant solid was triturated with ethanol and filtered off. Several crystallizations from acetone-water gave a white powder (0.75 g, 22%): m.p. > 300 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.50 (br s, 8 H), 9.14 (s, 2 H), 8.39 (d, $J$ = 8.4 Hz, 2 H), 8.04 (d, $J$ = 8.4 Hz, 2 H); FAB-MS *m/z* 269 ($MH^+$ of free base); HPLC (method A) $t_R$ 10.48 min (96.2 area %). Anal. ($C_{14}H_{13}N_4S{\cdot}2HCl{\cdot}H_2O$) C, H, N.

*4.2.1.2. 2,8-Bis(N-isopropylamidino)dibenzothiophene dihydrochloride* (**2**)

The imidate was prepared from 2,8-dicyanodibenzothiophene (**9**, 2.36 g, 10.1 mmol), anhydrous ethanol (5.0 mL, 86 mmol), and 1,4-dioxane (200 mL) by method B. The crude imidate (4.30 g, 107%) was filtered off after 27 d. A portion of the crude imidate (2.06 g) was stirred overnight at room temperature in a solution of isopropyl amine (10 mL, 235 mmol) under nitrogen. The resultant precipitate was filtered off to give a white solid (1.10 g, 53.7%): m.p. > 300 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.8 (v br s, 6 H), 9.05 (s, 2 H), 8.36 (d $J$ = 8.4 Hz, 2 H), 7.94 (d $J$ = 8.4 Hz, 2 H), 4.19 (m, 2 H), 1.34 (d, $J$ = 6.3 Hz, 12 H); FAB-MS *m/z* 353 ($MH^+$ of free base); HPLC (method A) $t_R$ 11.80 min (98.6 area %). Anal. ($C_{20}H_{24}N_4S{\cdot}2HCl{\cdot}H_2O$) C, H, N, S, Cl.

*4.2.1.3. 2,8-Bis(N-hydroxyamidino)dibenzothiophene dihydrochloride* (**3**)

The imidate was prepared from 2,8-dicyanodibenzothiophene (**9**, 2.00 g, 8.54 mmol), ethanol (3.0 mL, 51 mmol) and 1,4-dioxane (120 mL) by method B. After 20 d the crude imidate was filtered off and was stirred at reflux for 6 h in a solution of hydroxylamine prepared from hydroxylamine hydrochloride (6.09 g, 87.3 mmol), and sodium ethoxide (32 mL of a 21% solution, 85 mmol) and ethanol (85 mL). The crude product was filtered off and washed with ether, then converted to the dihydrochloride salt by treatment with ethanol and ethanolic HCl. The crude dihydrochloride salt was recrystallized from ethanol-ether to give a white solid (1.68 g, 52%): m.p. > 300 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.10 (br s, 2 H), 8.81 (d, $J$ = 1.4 Hz, 2 H), 8.21 (d, $J$ = 8.5 Hz, 2 H), 7.90 (dd, $J$ = 8.5 and 1.4 Hz, 2 H), 7.62 (br s, 3 H), 6.85 (br s, 4 H); FAB-MS *m/z* 301 ($MH^+$ of free

base); HPLC (method B) $t_R$ 9.93 min (95.4 area %). Anal. ($C_{14}H_{12}N_4O_2S \cdot 2HCl \cdot 2H_2O$) C, H, N, S, Cl.

*4.2.1.4. 2,8-Bis(2-imidazolinyl)dibenzothiophene dihydrochloride* (**4**)

A slurry of finely pulverized 2,8-dicyanodibenzothiophene (**9**, 1.78 g, 5.02 mmol), and ethylenediamine dihydrochloride (8.86 g, 66.6 mmol) in ethylenediamine (10 mL, 150 mmol) in a 50 mL beaker was heated at 310 °C in a sand bath for 30 min with occasional manual stirring. The reaction mixture was nearly completely dissolved in hot water (ca. 100 mL) and filtered through Celite 545. The filtrate was cooled in an ice bath, then alkalinized with 2 N NaOH solution (75 mL). The free base was filtered off, partially dried, and suspended in hot ethanol (50 mL). The mixture was treated with ethanolic HCl (10 mL), and the undissolved solid was filtered off to give (as the dihydrochloride salt) beige micro crystals (1.41 g, 81%): m.p. > 330 °C; $^1$H NMR (500 MHz, TFA-*d*) δ 8.96 (s, 2 H), 8.11 (d, *J* = 8.4 Hz, 2 H), 7.90 (d, *J* = 8.4 Hz, 2 H), 4.27 (s, 8 H); $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.08 (s, 4 H), 9.23 (d *J* = 21.6 Hz, 2 H), 8.42 (d, *J* = 8.5 Hz, 2 H), 8.18 (dd, *J* = 8.4 and 1.6 Hz, 2 H), 4.07 (s, 8 H); *m/z* 321 ($MH^+$ of free base); HPLC (method A) $t_R$ 11.023 min (95.5 area%). Anal. ($C_{18}H_{16}N_4S \cdot 2HCl \cdot H_2O$) C, H, N, S, Cl.

*4.2.1.5. 2,8-Bis(2-imidazolinyl)-5,5-dioxodibenzothiophene dihydrochloride* (**5**)

Hydrogen peroxide (5 mL of a 30% solution) was added to a solution of 2,8-bis(2-imidazolinyl) dibenzothiophene dihydrochloride (**4**, 0.67 g, 1.7 mmol) in trifluoroacetic acid (10 mL). An exothermic reaction resulted. Another portion (5 mL) of the peroxide solution was added, and the mixture was stirred overnight at room temperature. HPLC analysis showed a mixture of the desired product and the purported sulfoxide intermediate. Another portion of the peroxide solution (10 mL) was added and the reaction was allowed to proceed for a total of 40 h. The reaction mixture was basified with 2 N NaOH solution. The resultant free base (0.53 g, 89% recovery) was filtered off, washed with water, and dried. The solid was nearly completely dissolved in hot ethanol (80 mL) and filtered through Celite 545. The filtrate was concentrated to ca. 40 mL and treated with ethanolic HCl (10 mL) to give ivory crystals (0.51 g, 71%): m.p. > 300 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.30 (s, 4 H), 9.05 (s, 2 H), 8.47 (d, *J* = 8.1 Hz, 2 H), 8.31 (dd, *J* = 8.1 and 1.4 Hz, 2 H), 4.09 (s, 8 H); *m/z* 353 ($MH^+$ of free base); HPLC (method A) $t_R$ 10.09 min (98.3 area%). Anal. ($C_{18}H_{16}N_4O_2S \cdot 2HCl \cdot 0.5H_2O$) C, H, N, Cl.

*4.2.2. General procedure for Pinner syntheses of compounds* **6–8**

These compounds were prepared analogously to **1–3** using method B described above.

*4.2.2.1. 3,7-Diamidinodibenzothiophene dihydrochloride* (**6**)

The imidate intermediate was prepared from 3,7-dicyanodibenzothiophene (**13**, 3.52 g, 15.0 mmol), ethanol (10 mL, 170 mmol), and 1,4-dioxane (225 mL). After 9 d the crude imidate (5.70 g, 96% recovery) was collected. An aliquot (2.87 g) of the crude imidate was added to anhydrous ethanol (63 mL) saturated with ammonia. The solid dissolved and a new precipitate formed. The mixture was stirred overnight at room temperature in a stoppered flask. The crude product was filtered off, suspended in ethanol and treated with ethanolic HCl. Recrystallization from water-isopropyl alcohol gave a white solid (0.63 g, 26%): m.p. 385–388 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.65 (s, 4 H), 9.43 (2, 4 H), 8.78 (d, *J* = 8.2 Hz, 2 H), 8.69 (2, 2 H), 8.01 (d, *J* = 8.2 Hz, 2 H); *m/z* 269 ($MH^+$ of free base); HPLC (method B) $t_R$ 9.16 min (98.1 area%). Anal. ($C_{14}H_{12}N_4S \cdot 2HCl \cdot 0.25 H_2O$) C, H, N, S, Cl.

*4.2.2.2. 3,7-Bis(N-isopropylamidino)dibenzothiophene dihydrochloride* (**7**)

An aliquot (2.87 g) of the crude imidate described above was suspended in ethanol (30 mL) and diluted with isopropyl amine (20 mL, 230 mmol). Solids went into solution and a precipitate had formed after the mixture had stirred at room temperature overnight. The excess amine was distilled off. The cooled reaction mixture was diluted with ether, and the crude product was filtered off. Recrystallization from water-isopropyl alcohol gave white crystals (1.51 g, 49%); m.p. 337–338 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.86 (d, *J* = 8.3 Hz, 2 H), 9.70 (s, 2 H), 9.37 (s, 2 H), 8.75 (d, *J* = 8.4 Hz, 2 H), 8.59 (s, 2 H), 7.92 (d, *J* = 8.4 Hz, 2 H), 4.16 (m, 2 H), 1.32 (d, *J* = 6.2 Hz, 12 H); *m/z* 353 ($MH^+$ of free base); HPLC (method B) $t_R$ 11.12 min (95.6 area%). Anal. ($C_{20}H_{24}N_4S \cdot 2HCl$) C, H, N, S, Cl.

*4.2.2.3. 3,7-Bis(N-hydroxyamidino)dibenzothiophene dihydrochloride* (**8**)

The imidate intermediate was prepared from 3,7-dicyanodibenzothiophene (**13**, 1.00 g, 4.26 mmol), ethanol (5.5 mL, 94 mmol), and 1,4-dioxane (75 mL). After 5 d the crude imidate (1.66 g, 97% recovery) was collected and suspended in anhydrous ethanol (15 mL). The suspension was diluted with a solution of hydroxylamine prepared from hydroxylamine (3.5 g, 50 mmol), sodium ethoxide (21% solution in denatured alcohol,

18 mL, 48 mmol), and ethanol (60 mL). Solids went into solution and a new precipitate formed. After 2.5 h the mixture was diluted with ether, and the crude product was filtered off. A suspension of the crude product in water (10 mL) was diluted with 2 N HCl (2–3 mL) until the solid dissolved. The turbid solution was filtered, and the filtrate was diluted with conc. HCl (20 mL). The resultant precipitate was filtered off to give a white solid (0.58 g, 36%): m.p. > 300 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.44 (br s, 2 H), 9.20 (br s, 3 H), 8.74 (d, $J$ = 8.4 Hz, 2 H), 8.59 (d, $J$ = 1.5 Hz, 2 H), 7.91 (dd, $J$ = 8.4 and 1.5 Hz, 2 H); high resolution FAB MS $m/z$ 301.0730 (calcd $m/z$ 301.0759 for $C_{14}H_{13}N_4O_2S$); HPLC (method B) $t_R$ 8.87 min (96.4 area%). Anal. ($C_{14}H_{12}N_4O_2S$·2HCl·0.85$H_2O$) C, H, N, S, Cl.

*4.2.3. 3,7-Diaminodibenzothiophene dihydrochloride* (**11**)

3,7-Dinitro-5-oxo-dibenzothiophene (**10**, 9.60 g, 33.1 mmol) was hydrogenated in four batches at 45 psi over 10% palladium on carbon in ethanol. In each batch, 300 mL of ethanol and 0.4–0.5 g of catalyst were used, and the reaction time was 2 h. After each hydrogenation, the catalyst was filtered off, and the filtrate was immediately treated with ethanolic HCl (10–15 mL) to give a white precipitate. The combined precipitates were collected to give a white solid (8.68 g, 91.5%): m.p. 280 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.36 (d, $J$ = 8.4 Hz, 2 H), 7.92 (s, 2 H), 7.42 (dd, $J$ = 8.4 and 1.6 Hz, 2H); HPLC (method B) $t_R$ 9.58 min (96.2 area%).

*4.2.4. 3,7-Dibromodibenzothiophene* (**12**)

A suspension of 3,7-diaminodibenzothiophene dihydrochloride (**11**, 7.32 g, 25.5 mmol) in water (50 mL) and conc. HCl (13 mL) was cooled in an ice-salt bath to 5 °C. A solution of sodium nitrite (5.27 g 76.4 mmol) in water (15 mL) was added dropwise at such a rate that the temperature of the reaction mixture did not exceed 10 °C. After 45 min the reaction mixture was poured into a solution of copper(I) bromide in 48% HBr (90 mL). The mixture was heated at reflux for 2 h, cooled, and poured into ice-water. (total volume ca. 1 500 mL). The resultant yellow precipitate was filtered off, dried, and sublimed (168 °C, 0.2 mm Hg) to give a pale yellow solid (6.84 g, 78.6%): m.p. 171–173 °C (lit. [24] 180 °C); $^1$H NMR (400 MHz, CDCl3) δ 7.98 (d, $J$ = 1.7 Hz, 2 H), 7.96 (d, $J$ = 8.6 Hz, 2 H), 7.58 (dd, $J$ = 8.6 and 1.7 Hz, 2 H).

*4.2.5. 3,7-Dicyanodibenzothiophene* (**13**)

A refluxing solution of 3,7-dibromodibenzothiophene (**12**, 1.56 g, 4.56 mmol) and copper(I) cyanide (1.27 g, 14.2 mmol) in 1-methyl-2-pyrrolidinone (10 mL) was stirred under nitrogen for 3 h. The cooled reaction mixture was treated with a solution of $FeCl_3$ (3.29 g) in conc. HCl (10 mL). After the initial exotherm, the mixture was stirred with heat for 1 h. The mixture was poured over ice. The resultant grey precipitate was filtered off, dried, and sublimed (280 °C, 0.3 mm Hg) to give white powder (0.89 g, 84%): m.p. > 330 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.76 (s, 2 H), 8.71 (d, $J$ = 8.2 Hz, 2 H), 8.01 (dd, $J$ = 8.2 and 1.4 Hz, 2 H); HPLC (method B) $t_R$ 16.84 min (93.5 area%). Anal. ($C_{14}H_6N_2S$·0.25$H_2O$) C, H, N.

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