Chemical and Biological Oxidation of Thiophene: Preparation and Complete Characterization of Thiophene *S*-Oxide Dimers and Evidence for Thiophene *S*-Oxide as an Intermediate in Thiophene Metabolism *in Vivo* and *in Vitro*

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Abstract: Direct evidence for the involvement of thiophene *S*-oxide as a key primary reactive intermediate in the metabolism of thiophene (**1**) in rats was obtained from the isolation of two diastereoisomeric thiophene *S*-oxide dimers, **4a** and **4b**, both *in vitro* (oxidation of thiophene with rat liver microsomes) and *in vivo* (isolation of **4a** from rat urine). The structure of these dimers was established after an original preparation of identical samples by oxidation of thiophene with H_2O_2 and CF_3COOH. In fact, the H_2O_2/CF_3COOH system appeared to be the best oxidizing agent for the selective transformation of thiophene to its *S*-oxide. The complete determination of the structures of **4a** and **4b** was carried out for the first time by X-ray diffraction for the former and by a sequence of chemical reactions for the latter. The reported results indicate two fates for thiophene *S*-oxide *in vivo*: (i) its dimerization *via* a Diels−Alder reaction and (ii) its reaction with nucleophiles such as glutathione leading eventually to mercapturates. These results together with recent literature data on thiophene derivatives suggest that thiophene *S*-oxides, a class of reactive intermediates whose chemistry is still not well-known, could play a central role in the metabolism and toxic effects of thiophenes in mammals. This situation would be different from that observed in the metabolism of other aromatic compounds, such as benzene or furan, in which arene oxides are predominant intermediates.

Introduction

Aromatic and heteroaromatic compounds are ubiquitous components not only of food and drugs but also of environmental pollutants. Living organisms have developed efficient systems for their elimination after oxidative metabolism. Unfortunately, this metabolic oxidation may lead to reactive intermediates able to covalently bind to DNA and proteins leading to toxic effects.[1] It is now well established that arene oxides derived from cytochrome P450-dependent oxidation of aromatic compounds play a central role not only in the oxidative metabolism but also in the appearance of the toxic (e.g., carcinogenic or cytotoxic) effects of these compounds.[2] These highly reactive intermediates either are deactivated by epoxide hydrolases or glutathione and glutathione transferases or are covalently bound to cell macromolecules.[3,4] While the metabolism of furans[5] has also been shown to proceed *via* arene oxide intermediates, the mechanism of metabolism of thiophene derivatives is much less well understood.[6] The intermediate formation of thiophene 2,3-epoxide has been postulated to explain the formation of a mercapturate urinary metabolite in rats treated with thiophene.[7] More recent results about the oxidative metabolism of thiophene itself[8] and of a 3-aroylthiophene[9] were more in favor of thiophene *S*-oxides than arene oxides as primary metabolites of thiophene compounds. These results showed the formation of adducts which should result from a Michael-type addition of a thiol-containing trapping agent to a thiophene *S*-oxide intermediate (Figure 1). Such unstable thiophene *S*-oxides would constitute a novel class of reactive metabolites.

In fact, the chemistry of thiophene *S*-oxides is still not well-known.[10] Only two bearing bulky substituents at positions 2

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Table 1. Comparison of ^1H and ^{13}C NMR Data of Thiophene *S*-Oxide Dimers **4a** and **4b,** Sesquioxide **5**, and Monosulfoxide **6**[a]

	^1H and ^{13}C NMR chemical shifts[b]							
compd	2	3	3a	4	5	6	7	7a
4a	6.50 (137.2)	6.37 (137.7)	4.34 (54.0)	4.02 (67.7)	5.93 (125.4)	6.27 (130.2)	4.05 (64.5)	4.70 (61.5)
4b	6.49 (137.5)	6.62 (143.0)	4.84 (56.4)	3.97 (64.3)	6.07 (130.8)	6.19 (128.3)	4.39 (63.2)	4.07 (73.6)
5	6.59 (135.6)	6.69 (139.4)	4.49 (49.1)	4.09 (65.0)	6.12 (127.5)	6.29 (129.7)	4.26 (63.8)	4.30 (62.0)
6	5.95 (128.2)	5.44 (120.8)	4.25 (54.9)	4.08 (65.5)	6.04 (128.4)	6.06 (128.5)	4.03 (65.5)	4.63 (49.7)

[a] All spectra were recorded at 300 K; solvent resonances were used for internal calibration, the spectra of the thiophene *S*-oxide dimers **4a** and **4b**, sesquioxide **5**, and monoxide **6** were run in CD_3CN on a 500 MHz instrument; ^1H and ^{13}C chemical shifts are indicated; ^{13}C shifts are in parentheses. (b) Labeling of carbon atoms is as in Figure 4. Assigment of the ^1H and ^{13}C NMR signals were made after the following experiments: (i) NOESY experiments showed only one clear NOE between two protons separated by more than two carbons; those protons are H_3 and H_4, since the H_3H_4 distance is the shortest one between two protons separated by more than two carbons, as shown in the X-ray structure of **4a** (3.1 Å; all the other distances are longer than 3.5 Å). (ii) COSY 45 experiments allowed one to assign all other ^1H signals by correlation with those of H_3 and H_4, and these assigments were in agreement with those derived from decoupling experiments and short distance NOEs. (iii) Reverse COSY $^{13}C-^1H$ correlation experiments led to ^{13}C NMR signal assignment.

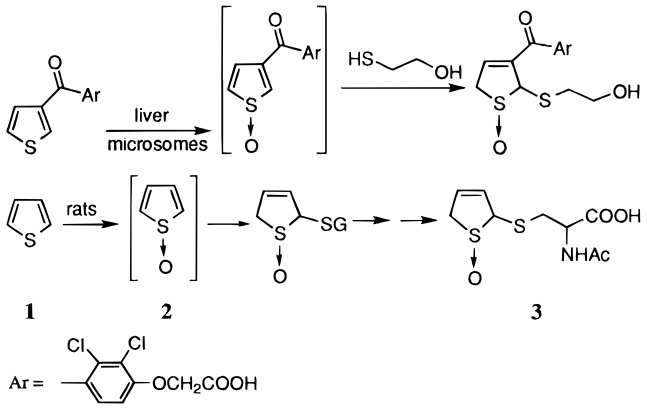

Figure 1.

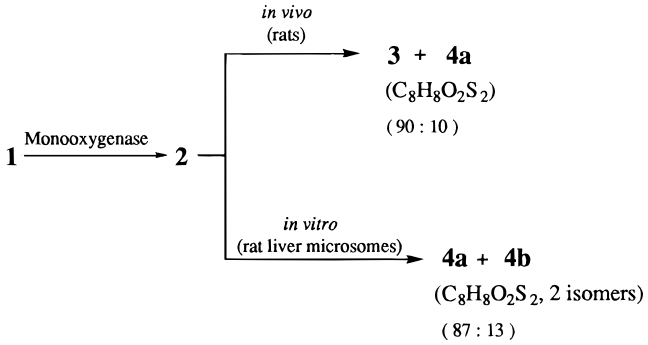

Figure 2.

and 5 have been prepared in low yields (5%) by chemical oxidation of the corresponding thiophenes and have been characterized in solution by UV and NMR spectroscopy.[11] Other thiophene *S*-oxides have been produced as transient species[12] and only characterized at the level of their Diels−Alder products[13] or metal complexes.[14] Quite recently, the first complete X-ray structure of a thiophene *S*-oxide, 2,5-diphenylthiophene *S*-oxide, has been published.[15] Apart from the Diels−Alder reactivity of thiophene *S*-oxide itself[13a,16] and some of its alkyl-substituted derivatives,[17] limited data are available on the chemical reactivity of thiophene *S*-oxides. Thiophene *S*-oxide dimers resulting from Diels−Alder dimerization of thiophene *S*-oxide have never been obtained by direct oxidation of thiophene.[16] Here we describe a new method for the transient formation of thiophene *S*-oxide which is based on a controlled oxidation of thiophene with H_2O_2 and CF_3COOH and leads to thiophene *S*-oxide dimers in good yields. The structure of these thiophene *S*-oxide dimers was completely established for the first time by chemical as well as spectroscopic techniques including X-ray crystallography of one dimer. We also report the formation of thiophene *S*-oxide dimers in the metabolism of thiophene *in vivo* in rats and *in vitro* by rat liver microsomes. These results provide strong evidence for the key role of thiophene *S*-oxide in thiophene bioactivation and show the two main fates of such poorly known reactive intermediates: (i) their dimerization by a Diels−Alder reaction and (ii) their reaction with nucleophiles such as glutathione, which could be responsible for the toxic effects reported for some thiophene derivatives.[18]

Results

***In Vivo* and *in Vitro* Metabolism of Thiophene.** Metabolism of radioactively labeled thiophene (**1**) *in vivo* in rats afforded two main urinary metabolites which were separated by HPLC. The main product, which represented about 90% of the overall radioactivity detected in the urine within 33 h after administration and ca. 20% of the overall administered dose, was characterized previously[19] and found to be mercapturate **3** (Figures 1 and 2). More recently, the minor metabolite **4a**, representing ca. 10% of the urinary radioactivity and 2% of the administered dose, could be isolated by HPLC. Its mass spectrum (chemical ionization; NH_3) indicated a molecular mass of 200 Da, and its ^1H and ^{13}C NMR spectra revealed the existence of eight inequivalent CH units, four in the aliphatic and four in the olefinic region of the spectrum (Table 1). Finally IR spectroscopy showed strong absorptions at 1030, 1040, and 1075 cm^{-1}, which are characteristic of the presence of sulfoxide

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functions. All of these data were in accordance with a chemical formula of $C_8H_8O_2S_2$ for the minor metabolite **4a** and therefore indicated the latter to be a dimer of thiophene *S*-oxide (**2**).

In vitro metabolism of labeled thiophene (**1**) with liver microsomes of rats pretreated with dexamethasone in the presence of NADPH and dioxygen[20] led to two major metabolites, **4a** and **4b** (Figure 2) in a relative ratio of 87:13, representing ca. 90% of the radioactivity detected in the aqueous phase and 12% of the overall starting dose. Metabolites **4a** and **4b** were separated by preparative HPLC. The major metabolite **4a** exhibited an HPLC retention time and ^{1}H NMR, IR, and mass spectra identical to those of the minor metabolite isolated from the urine of rats treated with thiophene. The minor metabolite **4b** obtained from microsomal oxidation of thiophene exhibited spectral properties very similar to those of **4a**: (i) a molecular mass *m*/*z* of 200 in mass spectrometry, (ii) eight inequivalent hydrogens from ^{1}H NMR (Table 1), and (iii) the presence of strong bands around 1050 cm^{-1} indicative for SO functions in its IR spectrum. On the basis of these data, **4b** appeared to be an isomer of **4a**.

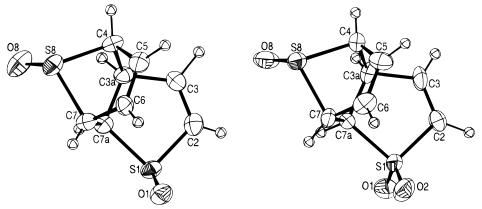
Chemical Oxidation of Thiophene. At that stage, it was not possible to determine the detailed stereochemistry of metabolites **4a** and **4b** from the available spectroscopic data obtained from the limited amounts of *in vivo* and *in vitro* metabolites (0.5−3 mg). Therefore, we have undertaken a study in order to develop a chemical system able to reproduce the metabolic oxidation of thiophene which could lead to thiophene *S*-oxide dimers in sufficient amounts for complete structural characterization. In fact, there was only one report in the literature on thiophene *S*-oxide dimers.[12] It was concerned with a tentative synthesis of thiophene *S*-oxide (**2**) by a double elimination starting from *trans*-3,4-(dimesyloxy)tetrahydrothiophene sulfoxide. This reaction has been reported to lead to a compound whose mass spectrum and elemental analysis are in agreement with a thiophene *S*-oxide dimer but whose stereochemistry was not determined. Interestingly, thiophene *S*-oxide dimers have never been obtained by direct thiophene oxidation. The only literature reports on chemical thiophene oxidation were concerned with reactions with peracids which provided the *syn*,*endo*-3a,4,7,7a-tetrahydro-4,7-epithiobenzo[*b*]-thiophene 1,1,8-trioxide (**5**) (see Figure 4), the so-called sesquioxide.[16] Its formation was explained by a Diels−Alder reaction of thiophene *S*-oxide with the corresponding thiophene *S*,*S*-dioxide.[21] This prompted us to reinvestigate the chemical oxidation of thiophene.

As shown in Table 2, oxidation of thiophene with $NaIO_4$, a classical and mild sulfoxidation reagent,[22] even in large excess, failed to give compounds such as **4** or **5**. Other classical oxidants such as dimethyldioxirane (entry 2) or *meta*-chloroperbenzoic acid (*m*-CPBA, entry 3) led to the major formation of **5** but not to thiophene *S*-oxide dimers **4**, even when using an oxidant/thiophene ratio of 0.2. On the contrary, oxidation of thiophene with H_2O_2 and CF_3COOH, a system recently reported to selectively oxidize 2,5-disubstituted thiophenes to the corresponding *S*-oxides,[15] led to compounds **4a** and **4b** (78:22 relative yield) in a 60−70% overall yield (see path A, Figure 4). When an oxidant/thiophene ratio smaller than 0.35 was used, **4a** and **4b** were the major reaction products and formation of **5** was almost negligible (entries 5 and 6). Upon increasing the oxidant/thiophene ratio from 0.35 to 1.0, the **4**/**5** ratio increased from 98:2 to 64:36 (entries 7 and 8). The selectivity of the H_2O_2/CF_3COOH system for sulfoxidation (and not sulfonation) of thiophenes has been attributed to a possible protonation of the thiophene *S*-oxide by CF_3COOH,[23] rendering its further oxidation more difficult. In agreement with this proposition, thiophene oxidation with *m*-CPBA led to **5**; whereas, in the presence of CF_3COOH, the thiophene *S*-oxide dimers **4a** and **4b** were obtained as major products (entry 4).

Table 2. Oxidation of Thiophene with Various Oxidizing Agents[a]

entry	oxidant[b]	presence of CF_3COOH	time (h)[c]	**4** (%)[d,e]	**5** (%)[d]	**4**:**5**
1	$NaIO_4$ (1.0−10)	no	96	nd[f]	nd	
2	dioxirane (0.20)	no	0.5	nd	26[g]	
3	*m*-CPBA (0.20)	no	192	nd	76	
4	*m*-CPBA (0.20)	yes	15	77	nd	
5	30% H_2O_2 (0.17)	yes	11	57	traces	99:1
6	30% H_2O_2 (0.35)	yes	14	62	1	98:2
7	30% H_2O_2 (0.70)	yes	20	33	4	89:11
8	30% H_2O_2 (1.00)	yes	29	21	12	64:36

[a] In methylene chloride at 20 °C, except for entry 1 where oxidation was performed in an acetone/water mixture either at 20 °C or at reflux. [b] Oxidant/thiophene ratio in parentheses, CF_3COOH/thiophene ratio ca. 3:1. [c] Time until complete consumption of the oxidant was indicated by using KI/starch. [d] Yields (based on the oxidant) were determined by analytical HPLC (for conditions, see the Experimental Section) directly from the water extracts of the crude mixture, error ca. 5% of given values. [e] Includes both isomers, **4a** and **4b**. [f] Not detected (nd). [g] Complex mixture with **5** as the main product.



4a **5**

Figure 3.

Compounds **4a** and **4b**, as well as sesquioxide **5**, were thus obtained upon oxidation of thiophene either with the H_2O_2/CF_3-COOH system or with *m*-CPBA, respectively. They were separated and purified with silica gel chromatography followed by preparative HPLC for the separation of dimers **4a** and **4b**. It is noteworthy that HPLC-monitoring at an early stage of the thiophene oxidation reaction indicated an initial **4a**/**4b** ratio of 85:15, slightly different from the final one of 78:22. This was rationalized in terms of an acid-catalyzed isomerization,[23] **4a** and **4b** being interconvertible in the presence of an excess of CF_3COOH. Accordingly, treatment of pure **4a** and **4b** with 50 equiv of CF_3COOH in acetonitrile at ambient temperature led to identical **4a**/**4b** mixtures (20:80 relative ratio) after several days, indicating that **4b** is the thermodynamically favored isomer.

Structure Determination of Compounds 4 and 5. The ORTEP drawing of the X-ray crystal structure of sesquioxide **5**[24] is shown in Figure 3. It definitively establishes the *endo* configuration of the two rings and the *syn* configuration of the bridging sulfoxide,[25] which were previously proposed from NMR studies using lanthanide shift reagents.[26]

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(24) Sesquioxide **5** was crystallized from acetonitrile. Compound **5** is *monoclinic* and possesses the following structural parameters: $P2_1/n$; a = 943.5(4) pm; b = 1242.4(5) pm; c = 724.1(3) pm; β = 94.19(4)°; V = 846.5(6) $\times 10^6$ pm^3; Z = 4; d_{calcd} = 1.697 g/cm^{-3}; 1379 independent reflections; R = 0.0720. Further details of the crystal structure of sesquioxide **5** are available on request from the Cambridge Crystallographic Data Centre by quoting the names of the authors and the journal citation.

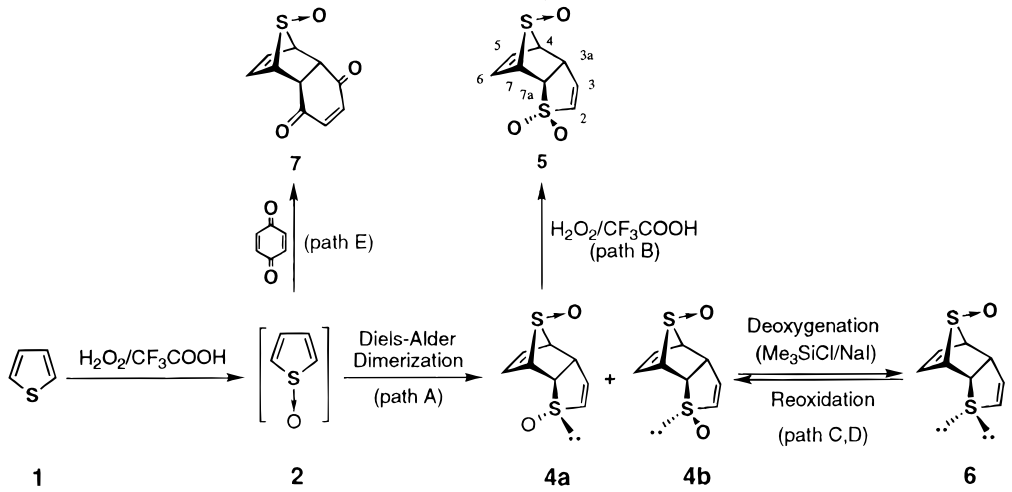
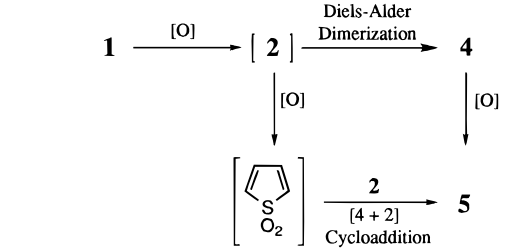


Figure 4.

The X-ray crystal structure of thiophene *S*-oxide dimer **4a**[27] (Figure 3) clearly established the configurations of the ring junction and bridging sulfoxide to be identical to those of sesquioxide **5**. Moreover, it demonstrated a *syn* configuration of the nonbridging sulfoxide group. This is in complete agreement with chemical experiments showing that oxidation of **4a** either with 1.1 equiv of H_2O_2 in the presence of CF_3-COOH or with *m*-CPBA exclusively led to the formation of sesquioxide **5** (path B, Figure 4).

As far as the structure of isomer **4b** is concerned, the following experiments have been performed to deduce its configuration from those of dimer **4a** and sesquioxide **5**. Selective deoxygenation of the nonbridging sulfoxide group of **4a** with trimethylsilyl chloride and sodium iodide in dry acetonitrile[28] afforded monosulfoxide **6** (path C, Figure 4). Compound **6** was detected in about 90% yield in the ^{1}H NMR spectrum of the crude reaction mixture but was only obtained in a 23% yield after silica column chromatography. Actual deoxygenation of the vinylic sulfoxide group was indicated by a significant upfield shift of the two protons in positions 2 and 3 (δ 6.50 and 6.37 for dimer **4a** and δ 5.95 and 5.44 for **6** respectively, Table 1). The structure shown for compound **6** in Figure 4 is in complete agreement with its ^{1}H and ^{13}C NMR spectra, mass spectra, and elemental analysis. Reoxidation of **6** with 1.0 equiv of either $NaIO_4$ or *m*-CPBA yielded **4a** and **4b** as the only products (path D, Figure 4) with a 8:92 and 16:84 relative ratio, respectively. Since $NaIO_4$ and *m*-CPBA are known to oxidize cyclic thioethers preferentially at the sterically less hindered site,[29] the minor formation of **4a** was expected



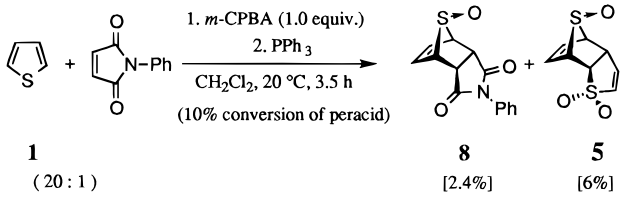


Figure 5.

and the major formation of **4b** from **6** indicates an *anti* configuration of the nonbridging sulfoxide for **4b**. This structure of **4b** is further supported by experiments showing that it is neither oxidized with *m*-CPBA nor with H_2O_2/CF_3COOH under conditions leading to a complete oxidation of its isomer **4a** to **5** (1.1 equiv of the oxidant). This should be due to the much lower accessibility of the sulfur lone pair of its nonbridging sulfoxide group. These results clearly established the detailed structure of the two main products, **4a** and **4b**, obtained by controlled oxidation of **1** with H_2O_2 and CF_3COOH. They should derive from the *S*-oxidation of **1** followed by a Diels−Alder dimerization of thiophene *S*-oxide (**2**). Intermediate formation of this *S*-oxide was also suggested by the formation of adduct **7** as the only product (32% yield) detected upon oxidation of **1** by H_2O_2 and CF_3COOH in the presence of 1 equiv of 1,4-benzoquinone as a Diels−Alder trapping reagent (path E, Figure 4).[13a]

Two mechanisms may apply for the formation of sesquioxide **5** in the oxidation of thiophene with H_2O_2/CF_3COOH or *m*-CPBA. The first one involves a Diels−Alder dimerization of thiophene *S*-oxide (**2**) followed by a further oxidation of the nonbridging vinylic sulfoxide in dimers **4a** and **4b**. The second one implies an oxidation of the thiophene *S*-oxide monomer (**2**) to the corresponding thiophene *S*,*S*-dioxide intermediate, which may react with thiophene *S*-oxide in a Diels−Alder reaction to directly give **5** (Figure 5). In order to distinguish between these two mechanisms, oxidation of thiophene with *m*-CPBA was performed in the presence of a small amount of

(25) Since the nomenclature for the stereochemical prefixes of unsymmetrically substituted brigde atoms is far from being unique, we prefer, with regard to earlier communications concerning Diels−Alder reactions of thiophene *S*-oxides, to name the configuration of the sulfoxide group *syn* when the oxygen atom directs toward the second ring and *anti* when it directs away from it. For a more detailed discussion, see: Macauley, J. B.; Fallis, A. G. *J. Am. Chem. Soc.* **1990**, *112*, 1136−1144 and references cited therein.

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(27) Crystals of thiophene *S*-oxide dimer **4a** were obtained from acetonitrile. Dimer **4a** is *orthorhombic* with the following structural parameters: Pb_{ca}; a = 2027.9(8) pm; b = 1013.7(4) pm; c = 784.3(3) pm; β = 90°; V = 1612.3(1) × 10^6 pm^3; Z = 8; d_{calcd} = 1.650 g/cm^{-3}; 1291 independent reflections; R = 0.0433. Further details of the crystal structure of dimer **4a** are available on request from the Cambridge Crystallographic Data Centre by quoting the names of the authors and the journal citation.

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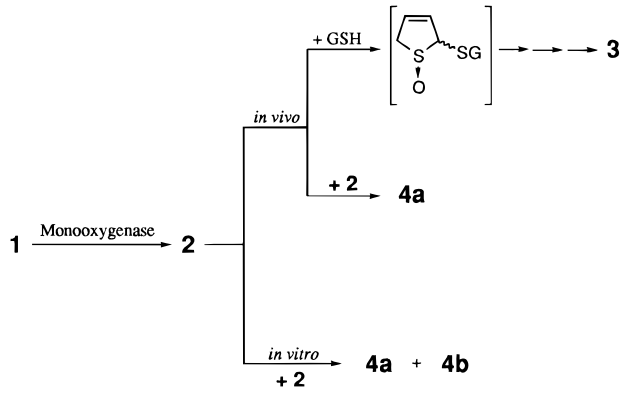

Figure 6.

N-phenylmaleimide as a competing Diels−Alder trapping agent (thiophene/*m*-CPBA/*N*-phenylmaleimide molar ratio = 20:20:1). Neither thiophene *S*-oxide dimers **4a** and **4b** nor sesquioxide **5** were observed until after complete consumption of *N*-phenylmaleimide (1.5 h). The oxidation was then allowed to continue for a further period of 2 h and was then quenched by addition of an excess of triphenylphosphine (total peracid consumption ca. 10%). This procedure yielded **5** as a major product (6% yield) and adduct **8** (2.4% yield) resulting from the competing Diels−Alder reaction of thiophene *S*-oxide (**2**) with *N*-phenylmaleimide. However, one failed to detect the formation of **4a** and **4b** and of products resulting from Diels−Alder reaction of thiophene *S*,*S*-dioxide with itself[30] or with *N*-phenylmaleimide. This lack of formation of products derived from Diels−Alder reactions of thiophene *S*,*S*-dioxide favors of a mechanism of formation of **5** involving a further oxidation of **4a** and **4b**.

As far as the characterization of adduct **8** is concerned, it was performed by ^{1}H and ^{13}C NMR, IR, mass spectrometry, and elemental analysis. Compound **8** was obtained in 32% yield from oxidation of thiophene with 0.2 equiv of H_2O_2 and CF_3-COOH in the presence of 1 equiv of *N*-phenylmaleimide.

Discussion

The aforementioned results provide strong evidence for the formation of thiophene *S*-oxide as a major reactive intermediate in the oxidative metabolism of thiophene *in vitro* and *in vivo* in rats (Figure 6). Interestingly, the metabolic patterns obtained from *in vitro* oxidation (liver microsomes) of **1** and from its chemical oxidation (H_2O_2/CF_3COOH) are very similar (major formation of **4a** and minor formation of **4b**), indicating that **4a** and **4b** are formed in both cases by a Diels−Alder dimerization of the intermediate thiophene *S*-oxide. The development of a chemical system (H_2O_2/CF_3COOH) able to reproduce the microsomal oxidation of thiophene was crucial for the synthesis of compounds **4a** and **4b** and the nonambiguous determination of their structure.

Oxidation of thiophene derivatives with H_2O_2 and CF_3COOH thus appears to represent the method of choice for the preparation of thiophene *S*-oxides with minimum formation of *S*,*S*-dioxides. This method has been successfully applied to the synthesis of some relatively stable 2,5-disubstituted thiophene *S*-oxides[15] and benzothiophene *S*-oxides.[9a] Here, it was applied to the *in situ* formation of reactive thiophene *S*-oxide itself. The success of this system for selective sulfoxidation of thiophenes could reside in the presence of a strong acid and partial protonation of thiophene *S*-oxides which would prevent them from further oxidation.[23] Regardless, the H_2O_2/CF_3COOH system could be useful to study the mechanisms of microsomal oxidation of thiophene derivatives, as demonstrated here for thiophene itself.

Detection of **4a** in the urine of rats treated with **1** suggests that formation of thiophene *S*-oxide also occurs *in vivo* and is a more direct evidence for such an intermediate than formation of **3** that has been reported previously.[8] *In vivo*, the major metabolite **3** does not derive from Diels−Alder dimerization of thiophene *S*-oxide. It could result from a Michael-type addition of glutathione at position 2 of thiophene *S*-oxide followed by the usual transformation of glutathione adducts to the corresponding mercapturates.[31] In the hepatocytes which are greatly involved in the metabolism of xenobiotics, the glutathione concentration is high (1−5 mM), and the formation of its adducts with electrophilic metabolites is generally catalyzed by glutathione *S*-transferases.[31] Our results indicate that the trapping of thiophene *S*-oxide by glutathione is very efficient *in vivo*. The lack of formation of an equivalent of **3** *in vitro*, after oxidation of **1** with rat liver microsomes in the presence of NADPH, dioxygen, and glutathione (5 mM) as a trapping agent (data not shown) could be due to the absence of the appropiate glutathione *S*-transferase in the liver microsomes used.

Formation of a thiophene *S*-oxide as a key reactive intermediate in the metabolism of a 3-aroylthiophene has been deduced from the isolation of its adduct with mercaptoethanol, resulting from Michael-type addition of this thiol at position 2 of the corresponding *S*-oxide[9a] (see Figure 1). Moreover, the formation of 5-hydroxy-2-aroylthiophenes upon metabolic oxidation of 2-aroylthiophenes and the suicide inactivation of P450 2C9 during oxidation of one of these 2-aroylthiophenes[32] have been explained *via* the intermediate formation of such highly reactive *S*-oxides and their fast reaction with nucleophiles. All of these data suggest that, for the oxidative metabolism of thiophene derivatives in mammals, thiophene *S*-oxides must be considered, in addition to arene oxides, as crucial, primary reactive intermediates which are able to dimerize or to react with nucleophiles in the cell. The pharmacological and toxicological consequences of the formation of these reactive species, whose chemistry is hardly known, remain to be determined.

Experimental Section

General Aspects. Melting points were taken on a Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 and AMX 500 spectrometers (250 or 500 MHz and 63 MHz, respectively),and the resonance of the solvent was used as internal standard. Infrared spectra were recorded on a Perkin-Elmer 783 ratio-recording infrared spectrophotometer. Mass spectra were taken on a Nermag R 1010 spectrometer. Elemental analyses were carried out by the Service Régional de Microanalyse de l' Université Paris VI. Column chromatography was done by using Merck Kieselgel 60 (70−230 mesh), the adsorbant/substrate ratio being ca. 100:1. Thin layer chromatography (TLC) was accomplished by using Polygram SIL G/UV_{254} (40 × 80 mm) from Macherey-Nagel. Products were visualized by using iodine vapor or by means of a 5% ethanolic solution of molybdophosphoric acid. Hydrogen peroxide (30%, Prolabo), thiophene (Janssen), trifluoroacetic acid (Janssen), *N*-phenylmaleimide (Aldrich), and 1,4-benzoquinone (Aldrich) were of highest commercially available quality and used without further purification. Tritiation of thiophene at positions 2 and 5 was achieved according to a previously described method.[33]

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***In Vivo* Metabolism of Radioactively Labeled Thiophene (1) in Rats.** The experimental details of the *in vivo* administration of labeled thiophene and the collection of the rat urine were previously described.[8] Mercapturate **3** and thiophene *S*-oxide dimer **4a** were separated by analytical HPLC (Hypersil MOS column [5 μm, 250 × 4.6 mm], flow 1.0 mL/min, gradient A (0.1% CF_3COOH/H_2O) and B (90:10 CH_3CN/H_2O); 0% B for 5 min, changing to 60% B within 12 min, plateauing at 60% for 5 min, and then changing to 100% B within 2 min). Fractions of 0.5 mL were collected, and the radioactivity was measured after addition of 2.0 mL of Pico-Fluor 40 to each sample.

1-*syn*,8-*syn*,*endo*-3a,4,7,7a-Tetrahydro-4,7-epithiobenzo[*b*]thiophene 1,8-Dioxide (4a): mp 160 °C (dec.); TLC (10:1 methylene chloride/methanol) 0.60; ^{1}H NMR (CD_3CN, 500 MHz, 27 °C) δ 4.02 (m, 1 H), 4.05 (m, 1 H), 4.34 (m, 1 H), 4.70 (dd, $J_1 = 7.0$ Hz, $J_2 = 3.8$ Hz, 1 H), 5.93 (m, 1 H), 6.27 (m, 1 H), 6.37 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.3$ Hz, 1 H), 6.50 (dd, $J_1 = 6.0$ Hz, $J_2 = 1.6$ Hz, 1 H); ^{13}C NMR (CD_3CN, 63 MHz, 27 °C) δ 54.0 (d), 61.5 (d), 64.5 (d), 67.7 (d), 125.4 (d), 130.2 (d), 137.2 (d), 137.7 (d); IR (KBr) ν 3085, 3060, 1600, 1350, 1310, 1295, 1230, 1170, 1140, 1075, 1040, 1030, 1000 cm^{-1}; MS (chemical ionization, NH_3) *m*/*z* 218 (M + NH_4, 49), 201 (M + H, 100), 185 ([M − O] + H, 14), 170 ([M − SO] + NH_4, 6), 153 ([M − SO] + H, 10).

***In Vitro* Metabolism of Radioactively Labeled Thiophene (1) with Rat Liver Microsomes.**[20] In a total volume of 1.0 mL of a 0.1 M phosphate buffer (pH 7.4) containing a suspension of liver microsomes from rats pretreated with dexamethasone (22 mg of protein/mL, 1.8 nmol of cytochrome P450/mg protein) and 2.5 μmol of tritiated thiophene (specific activity 0.16 mCi/mmol), the reaction was started by addition of 1.0 μmol of NADP, 10 μmol of glucose-6-phosphate, and 2 units of glucose-6-phosphate dehydrogenase. After incubation for 30 min at 37 °C, the reaction was quenched by adding 1.0 mL of isooctane (2,4,4-trimethylpentane) to extract the excess of unreacted thiophene (**1**). The phases were separated, and the aqueous phase was analyzed. The thiophene *S*-oxide dimers **4a** and **4b** were separated by analytical HPLC (Hypersil MOS column [5 μm, 250 × 4.6 mm], flow 1.0 mL/min, gradient 95:5 A (0.1% CF_3COOH/H_2O)/B (50:50 CH_3CN/H_2O) for 6 min, and then changing to 100% B within 5 min). Fractions of 0.5 mL were collected, and the radioactivity was determined after addition of 2 mL of Pico-Fluor 40 to each sample.

1-*anti*,8-*syn*,*endo*-3a,4,7,7a-Tetrahydro-4,7-epithiobenzo[*b*]thiophene 1,8-Dioxide (4b): mp 161−162 °C (dec.); TLC (10:1 methylene chloride/methanol) 0.57; ^{1}H NMR (CD_3CN, 500 MHz, 27 °C) δ 3.97 (m, 1 H), 4.07, (m, 1 H), 4.39 (m, 1 H), 4.84 (m, 1 H), 6.07 (m, 1 H), 6.19 (m, 1 H), 6.49 (dd, $J_1 = 6.0$ Hz, $J_2 = 1.9$ Hz, 1 H), 6.62 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.4$ Hz, 1 H); ^{13}C NMR (CD_3CN, 63 MHz, 27 °C) δ 56.4 (d), 63.2 (d), 64.3 (d), 73.6 (d), 128.3 (d), 130.8 (d), 137.5 (d), 143.0 (d); IR (KBr) ν 3080, 3040, 2990, 1600, 1340, 1325, 1270, 1230, 1180, 1170, 1070, 1040, 1025 cm^{-1}; MS (chemical ionization, NH_3) *m*/*z* 218 (M + NH_4, 100), 201 (M + H, 55), 185 ([M − O] + H, 4), 170 ([M − SO] + NH_4, 13), 153 ([M − SO] + H, 7). Anal. Calcd. for $C_8H_8O_2S_2$ (200.28): C, 47.97; H, 4.03. Found: C, 48.07; H, 3.94.

Attempted Oxidation of Thiophene (1) with $NaIO_4$. Thiophene (533 mg, 6.63 mmol, 0.5 mL) in 5 mL of acetone was allowed to react with 1.35 g (6.33 mmol) of $NaIO_4$ in 10 mL of water at room temperature for 24 h followed by a further 24 h at reflux. No product formation could be detected by using TLC and NMR techniques. The result was unchanged after an additional 9 equiv of $NaIO_4$ and heating of the reaction mixture under reflux for several days.

Oxidation of Thiophene (1) with Dimethyldioxirane. Thiophene (53.3 mg, 0.633 mmol, 51 μL) was dissolved in 1.0 mL of methylene chloride and 0.2 equiv of dimethyldioxirane (as ca. 0.1 M acetone solution) was added. Stirring at ambient temperature was continued for 30 min until no more peroxide could be detected by KI/starch. The solvent was removed at 20 °C (15 Torr), and the crude product was analyzed by TLC and ^{1}H NMR spectroscopy using 1,2-dichloroethane as an internal standard. The reaction yielded sesquioxide **5** as the main product (26%) together with a complex mixture of unidentified products. No thiophene *S*-oxide dimers (**4a** and **4b**) could be detected neither by ^{1}H NMR spectroscopy nor by HLPC on the aqueous extract of the crude reaction mixture. Sesquioxide **5** was identified by comparison of its ^{1}H and ^{13}C NMR characteristics with literature data.[26]

Preparation of Thiophene *S*-Oxide Dimers 4a and 4b by Oxidation of Thiophene (1) with 30% H_2O_2/CF_3COOH. Thiophene (2.13 g, 25.3 mmol, 2.0 mL) dissolved in 30 mL of methylene chloride together with 8.91 g (78.1 mmol, 6.0 mL) of CF_3COOH and 0.5 mL (4.40 mmol, 0.18 equiv) of 30% H_2O_2. The reaction mixture was stirred for 11 h until no more peroxide could be detected with KI/starch and then extracted three times with 10 mL of water. The aqueous phase was evaporated at 25 °C (0.1 Torr), the residue was taken up into 5 mL of water, and the solution was neutralized to pH 6 with saturated $NaHCO_3$ solution. The mixture was then evaporated to dryness at 25 °C (0.1 Torr), and the mixture of diastereoisomers **4a** and **4b** was isolated by silica gel column chromatography. After the residue was eluted with 10:1 methylene chloride/methanol, 254 mg (58%) of the **4a** and **4b** mixture (78:22 rel ratio) was obtained as a colorless solid. The isomers were separated by semipreparative isocratic HPLC (Hypersil MOS column [5 μm, 250 × 7.8 mm], flow 2.3 mL/min, eluent 98:2 H_2O/CH_3CN).

General Procedure for the Determination of Product Ratios after Peracid Oxidations of Thiophene (1). Thiophene (533 mg, 6.63 mmol, 0.5 mL) was dissolved in a mixture containing 2.23 g (19.5 mmol, 1.5 mL) of CF_3COOH and the appropiate amount of the oxidant in 10 mL of methylene chloride. The solution was stirred at ambient temperature until no more peroxide could be detected with KI/starch. The reaction mixture was extracted three times with 10 mL of water, and the aqueous phase was evaporated to dryness at 25 °C (0.1 Torr). The residue was taken up into 5 mL of water, neutralized to pH 6 with saturated $NaHCO_3$ solution, and again evaporated to dryness at 25 °C (0.1 Torr). Product quantification was performed directly on the crude reaction mixture after isocratic HPLC (Hypersil MOS column [5 μm, 250 × 4.6 mm], flow 1.0 mL/min, eluent 98:2 H_2O/CH_3CN) and UV detection ($\lambda = 220$ nm). The yields of dimers **4a** and **4b** and of sesquioxide **5** are given in Table 1.

HPLC Monitoring of the Oxidation of Thiophene (1) by 30% H_2O_2/CF_3COOH. The thiophene (**1**) oxidation was repeated as described above. At specific time intervals, small samples of about 100 μL were taken out of the crude reaction mixture and 0.5 mL of water was added to these samples. The phases were separated, and the **4a**/**4b** ratio was determined after isocratic HPLC (conditions see above) and UV detection ($\lambda = 220$ nm) to be 85:15 after 1 h and 75:25 after 14 h (complete conversion of H_2O_2).

Trifluoroacetic Acid-Catalyzed Isomerization of the Dimers 4a and 4b. The thiophene *S*-oxide dimer **4a** (or **4b**) (2.00 mg, 10.0 μmol) was dissolved in 1.0 mL of acetonitrile and 56.9 mg (500 μmol, 50 equivalents) of trifluoroacetic acid were added. The isomerization was followed by isocratic HPLC (conditions see above) for several days. It led to **4a** and **4b** mixtures with a similar **4a**:**4b** ratio (ca. 20:80).

Oxidation of Dimer 4a with H_2O_2 and CF_3COOH. The thiophene *S*-oxide dimer **4a** (10.0 mg, 49.9 μmol) was dissolved in 1.0 mL of water, and a mixture of 6.2 μL (54.8 μmol, 10% excess) of 30% H_2O_2 and 27.1 mg (238 μmol) of trifluoroacetic acid was added. Stirring at ambient temperature was continued for 4 h until no more dimer **4a** could be detected by TLC (10:1 methylene chloride/methanol). TLC and ^{1}H NMR with 1,2-dichloroethane as an internal standard revealed the formation of sesquioxide **5** as the only detectable product (94% yield).

Attempted Oxidation of Dimer 4b with H_2O_2 and CF_3COOH. The above procedure was repeated with 10.0 mg (49.9 μmol) of the thiophene *S*-oxide dimer **4b**. After 4 h of stirring at ambient temperature, no transformation of **4b** could be observed by TLC (10:1 methylene chloride/methanol) or HPLC.

Deoxygenation of Dimer 4a with Me_3SiCl/NaI. Compound **4a** (208 mg, 1.04 mmol) and 308 mg (2.08 mmol) of sodium iodide were dissolved in 10 mL of dry acetonitrile under a nitrogen gas atmosphere. To the stirred solution was added 112 mg (1.04 mmol, 131 μL) of trimethylsilyl chloride, and the deeply brownish reaction mixture was stirred for 1.5 h at room temperature. The reaction mixture was treated with 1.5 mL of a saturated aqueous solution of sodium thiosulfate and then with 10 mL of water and 25 mL of methylene chloride. The phases were separated, and the aqueous phase was extracted two times with 10 mL of methylene chloride. After drying over $MgSO_4$, the solvent was removed at 20 °C (15 Torr) to obtain 143 mg of a yellow crude product. Purification by silica gel column chromatography by eluting

with 20:1 methylene chloride/methanol afforded 45 mg (23%) of **6** as a colorless solid.

8-*syn*,*endo*-3a,4,7,7a-Tetrahydro-4,7-epithiobenzo[*b*]thiophene 8-Oxide (6): mp 123−124 °C; TLC (20:1 methylene chloride/methanol) 0.36; ^{1}H NMR ($CDCl_3$, 250 MHz, 27 °C) δ 4.03 (m, 1 H), 4.10 (m, 1 H), 4.42 (m, 1 H), 4.80 (dd, $J_1 = 10.1$ Hz, $J_2 = 3.8$ Hz, 1 H), 5.46 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.4$ Hz, 1 H), 5.97 (dd, $J_1 = 6.0$ Hz, $J_2 = 2.0$ Hz, 1 H), 6.09 (m, 2 H); ^{13}C NMR ($CDCl_3$, 63 MHz, 27 °C) δ 49.0 (d), 54.4 (d), 65.1 (d), 67.0 (d), 119.5 (d), 128.0 (d), 128.5 (d), 128.9 (d); IR (KBr) ν 3050, 2920, 2850, 1590, 1340, 1315, 1260, 1235, 1200, 1165, 1140, 1070, 1030, 1005 cm^{-1}; MS (chemical ionization, NH_3) *m*/*z* 202 (M + NH_4, 17%), 185 (M + H, 100%), 137 ([M − SO] + H, 24%). Anal. Calcd. for $C_8H_8OS_2$ (184.28): C, 52.14; H, 4.38. Found: C, 52.40; H, 4.41.

Oxidation of Monosulfoxide 6 with *m*-CPBA. Monosulfoxide **6** (3.10 mg, 16.8 μmol) was dissolved in 1.0 mL of methylene chloride, and the solution was cooled to 0 °C. Then, 2.90 mg (16.8 μmol) of *m*-CPBA, dissolved in 0.1 mL of methylene chloride, was added, and the mixture was stirred for 20 min until no peroxide could be detected by using KI/starch. As shown by TLC (20:1 methylene chloride/methanol), the starting material had been completely consumed. The solvent was removed at 20 °C (15 Torr), and the crude product was taken up in 0.8 mL CD_3CN with 1,2-dichloroethane present as an internal standard. According to NMR spectroscopy, the only products were the thiophene *S*-oxide dimers **4a** and **4b** in a 14:86 ratio.

Oxidation of Monosulfoxide 6 with $NaIO_4$. Monosulfoxide **6** (2.40 mg, 13.0 μmol) was dissolved in 0.5 mL of acetone, and 2.79 mg (13.0 μmol) of sodium periodate in 0.3 mL of water was added. Stirring at ambient temperature was continued for 4 h until no starting material could be detected by TLC (20:1 methylene chloride/methanol). The **4a**/**4b** product ratio was determined directly from the reaction mixture by isocratic HPLC (Hypersil MOS column [5 μm, 250 × 4.6 mm], flow 1.0 mL/min, 98:2 H_2O/CH_3CN) to be 8:92.

Oxidation of Thiophene (1) with *m*-CPBA in the Presence of 0.05 Equiv of *N*-Phenylmaleimide. *m*-CPBA (399 mg, 2.31 mmol) and 20.0 mg (115 μmol, 0.05 equiv) of *N*-phenylmaleimide were dissolved in 30 mL of methylene chloride, and 194 mg (2.31 mmol, 182 μL) of thiophene was added. After 1.5 h of stirring at ambient temperature, *N*-phenylmaleimide was completely consumed (TLC, CH_2Cl_2). The mixture was left reacting for a further 2 h, and the oxidation was then quenched by addition of 606 mg (2.31 mmol) of triphenylphosphine. The solvent was removed at 20 °C (15 Torr), and the crude product submitted to silica gel chromatography. When the residue was eluted with 20:1 methylene chloride/methanol, 15.1 mg (2.4%) of Diels−Alder adduct **8** were isolated as a first fraction followed by 14.0 mg (6%) of sesquioxide **5**.

Preparation of Diels−Alder Adduct 8 by Oxidation of Thiophene (1) with H_2O_2 and CF_3COOH. Thiophene (1.88 g, 22.3 mmol, 2.0 mL) and 1.53 g (8.81 mmol) of *N*-phenylmaleimide were dissolved in a mixture of 8.91 g (78.1 mmol, 6.0 mL) of trifluoroacetic acid and 20 mL of methylene chloride. After 0.5 mL (4.41 mmol, 0.20 equiv) of 30% H_2O_2 was added, the yellow reaction mixture was stirred at ambient temperature for 12 h until no more peroxide could be detected by KI/starch. The solution was then washed two times with 20 mL of water and one time with 20 mL of a saturated solution of $NaHCO_3$, the organic phase was dried over $MgSO_4$, and the solvent was evaporated at 20 °C (15 Torr). The crude product was suspended in 15 mL of methylene chloride, and the unsoluble product was filtered, washed several times with 5 mL of hot methylene chloride, and dried. The Diels−Alder adduct **8** was thus obtained (382 mg, 32%) as a slightly brown, analytically pure solid.

***N*-Phenyl-8-*syn*,*endo*-2a,3,6,6a-tetrahydro-3,6-epithiobenzo[*c*]pyrrole-2,7-dione 8-Oxide (8):** mp 193−194 °C (dec.); TLC (20:1 methylene chloride/methanol) 0.47; ^{1}H NMR (DMSO-d_6, 250 MHz, 27 °C) δ 4.08 (s, br, 2 H), 4.41 (s, br, 2 H), 6.42 (s, br, 2 H), 7.16 (d, $J = 7.2$ Hz, 2 H), 7.47 (d, $J = 7.6$ Hz, 3 H); ^{13}C NMR (DMSO-d_6, 63 MHz, 27 °C) δ 44.6 (d), 63.4 (d), 126.7 (d), 128.2 (d), 128.4 (d), 128.6 (d), 131.9 (s), 174.2 (s); IR (KBr) ν 1735, 1520, 1415, 1250, 1230, 1220, 1185, 1155, 1110 cm^{-1}; MS (EI) *m*/*z* 273 (M, 48), 225 (M − SO, 100). Anal. Calcd. for $C_{14}H_{11}NO_3S$ (273.32): C, 61.52; H, 4.06; N, 5.13. Found: C, 61.54; H, 4.01; N, 5.11.

Trapping of *in Situ* Generated Thiophene *S*-Oxide with 1,4-Benzoquinone. Thiophene (21.3 mg, 253 μmol) was dissolved in 0.8 mL of $CDCl_3$ and 89.1 mg (0.781 mmol) of trifluoroacetic acid, and 3.00 mg (88.2 μmol) of 30% H_2O_2 and 28.6 mg (0.265 mmol) of 1,4-benzoquinone were added. The reaction mixture was stirred for 15 h at room temperature until complete consumption of H_2O_2 (detected by KI/starch). In addition to unreacted 1,4-benzoquinone, only the previously reported Diels−Alder adduct **7**[13a] was detected in the ^{1}H NMR spectrum of the crude product mixture in 32% yield.

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Supporting Information Available: Crystal data and structural information for **4a** and **5** (8 pages). See any current masthead page for ordering and Internet access instructions.

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