Covalent Modification of Cyclooxygenase-2 (COX-2) by 2-Acetoxyphenyl Alkyl Sulfides, a New Class of Selective COX-2 Inactivators†

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All of the selective COX-2 inhibitors described to date inhibit the isoform by binding tightly but noncovalently at the substrate binding site. Recently, we reported the first account of selective covalent modification of COX-2 by a novel inactivator, 2-acetoxyphenyl hept-2-ynyl sulfide (**70**) (*Science* **1998**, *280*, 1268−1270). Compound **70** selectively inactivates COX-2 by acetylating the same serine residue that aspirin acetylates. This paper describes the extensive structure−activity relationship (SAR) studies on the initial lead compound 2-acetoxyphenyl methyl sulfide (**36**) that led to the discovery of **70**. Extension of the *S*-alkyl chain in **36** with higher alkyl homologues led to significant increases in inhibitory potency. The heptyl chain in 2-acetoxyphenyl heptyl sulfide (**46**) was optimum for COX-2 inhibitory potency, and introduction of a triple bond in the heptyl chain (compound **70**) led to further increments in potency and selectivity. The alkynyl analogues were more potent and selective COX-2 inhibitors than the corresponding alkyl homologues. Sulfides were more potent and selective COX-2 inhibitors than the corresponding sulfoxides or sulfones or other heteroatom-containing compounds. In addition to inhibiting purified COX-2, **36**, **46**, and **70** also inhibited COX-2 activity in murine macrophages. Analogue **36** which displayed moderate potency and selectivity against purified human COX-2 was a potent inhibitor of COX-2 activity in the mouse macrophages. Tryptic digestion and peptide mapping of COX-2 reacted with [*1-*^{14}*C-acetyl*]-**36** indicated that selective COX-2 inhibition by **36** also resulted in the acetylation of Ser516. That COX-2 inhibition by aspirin resulted from the acetylation of Ser516 was confirmed by tryptic digestion and peptide mapping of COX-2 labeled with [*1-*^{14}*C-acetyl*]salicyclic acid. The efficacy of the sulfides in inhibiting COX-2 activity in inflammatory cells, our recent results on the selectivity of **70** in attenuating growth of COX-2-expressing colon cancer cells, and its selectivity for inhibition of COX-2 over COX-1 in vivo indicate that this novel class of covalent modifiers may serve as potential therapeutic agents in inflammatory and proliferative disorders.

Introduction

The committed step in prostaglandin and thromboxane biosynthesis involves the conversion of arachidonic acid to PGH_2, which is catalyzed by the sequential action of the cyclooxygenase (COX) and peroxidase (PER) activities of prostaglandin endoperoxide synthase (PGHS or COX; EC 1.14.99.1) (Scheme 1).[1] COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2.[2−4] COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal (GI) tract and thromboxane which triggers platelet aggregation in blood platelets.[5] COX-2 is inducible and short-lived; its expression is stimulated in response to endotoxins, cytokines, and mitogens.[6−8] Importantly, COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages) and in the central nervous system.[9−12] Overall, these observations suggest that COX-1 and COX-2 serve different physiological and pathophysiological functions. The differential tissue distribution of COX-1 and COX-2 provides a basis for the development of selective COX-2 inhibitors as antiinflammatory and analgesic agents without the GI and hematologic liabilities that plague all currently marketed nonsteroidal antiinflammatory drugs (NSAIDs), most of which inhibit both COX-1 and COX-2.[13]

Two general structural classes of selective COX-2 inhibitors are commonly reported in the literature. These include the diarylheterocycles (e.g., Celecoxib) and the acidic sulfonamide analogues (e.g., CGP 28238) (Figure 1).[14−20] Structure−activity relationship (SAR) studies, particularly on the diarylheterocycles, have indicated that the oxidation state of the sulfur is a key determinant of selectivity: sulfones and sulfonamides are selective for COX-2, whereas sulfoxides and sulfides are not. For example, reduction of the sulfone moiety in the selective COX-2 inhibitor SC 8092 to the corresponding sulfide generates SC 8076, a selective COX-1 inhibitor (see Figure 1).[21] All of the selective COX-2 inhibitors described to date inhibit the isoform by

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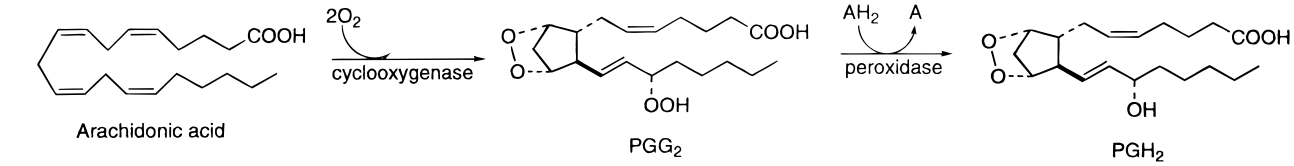
Scheme 1



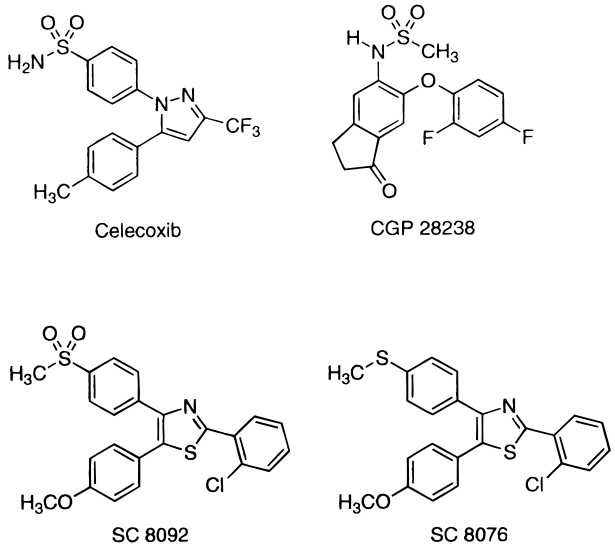


Figure 1. Selective COX-2 inhibitors.

binding tightly but noncovalently at the cyclooxygenase active site.[22]

Aspirin is the only known NSAID that covalently modifies both COX-1 and COX-2 by acetylating an active site serine residue (Ser530 in COX-1 and Ser516 in COX-2) (Scheme 2).[23−25] Aspirin is significantly more potent against COX-1 than COX-2.[13] In our search for selective COX-2 inactivators, we elected to structurally modify aspirin to a selective COX-2 inactivator. Our initial approach involved the replacement of the carboxylate moiety in aspirin with the methyl sulfone functionality. There were two reasons for this substitution. First, the interaction between the carboxylate of aspirin and the arginine residue adjacent to the active site serine residue of COX-1 and COX-2 is a strong ionic interaction that may overwhelm more subtle interactions necessary to establish selectivity for one isoform. Second, the methyl sulfone moiety is responsible for selective COX-2 inhibition in the diarylheterocycle series. Unfortunately, 2-acetoxyphenyl methyl sulfone (**80**) was devoid of inhibitory potency against either isoform. However, 2-acetoxyphenyl methyl sulfide (**36**) (see Scheme 2) was identified as a lead compound that demonstrated moderate inhibitory potency and selectivity for COX-2.[26] This inhibition profile is opposite to that observed with the diarylheterocycles. Systematic structural modification on **36** led to the development of 2-acetoxyphenyl hept-2-ynyl sulfide (**70**) as the most potent and selective inhibitor in the series. Compound **70** was 60 times more reactive against COX-2 than aspirin and 100 times more selective for its inhibition. Furthermore, selective inhibition of COX-2 by **70** resulted in the acetylation of the same serine residue that aspirin acetylates. This report summarizes the detailed investigation on the SAR requirements for 2-acetoxyphenyl alkyl sulfides as selective COX-2 inhibitors.

Results and Discussion

Chemistry. The preparation of 2-acetoxyphenyl alkyl sulfides **36**−**76**, 2-acetoxyphenyl heptyl sulfoxide (**77**), and 2-acetoxyphenyl alkyl sulfones **80** and **81** is shown in Scheme 3. Commercially available 2-hydroxythiophenol (**1**) was alkylated with the appropriate alkyl halide in the presence of $KHCO_3$ to generate the corresponding 2-hydroxyphenyl alkyl sulfide analogues **2**−**35**, which upon acetylation with Ac_2O gave the desired acetates **36**−**76** (Tables 1, 3, and 4). Synthesis of 2-acetoxyphenyl alkoxy (compounds **63** and **64**), 2-acetoxyphenyl alkenyl (**65**), and some 2-acetoxyphenyl alkynyl sulfides (compounds **68**−**71**, and **73**) (Tables 3 and 4) also required the synthesis of the starting alkoxy, alkenyl, and alkynyl bromides, respectively, from the reaction of the corresponding alcohols with carbon tetrabromide and triphenylphosphine. Controlled oxidation of 2-acetoxyphenyl heptyl sulfide (**46**) in the presence of Oxone[27] gave the sulfoxide **77** in near quantitative yields. The 2-acetoxyphenyl methyl and 2-acetoxyphenyl heptyl sulfone derivatives **80** and **81** were prepared by the oxidation of the 2-hydroxyphenyl alkyl sulfides in the presence of H_2O_2 to generate the corresponding 2-hydroxyphenyl alkyl sulfones **78** and **79**, which were then acetylated to afford **80** and **81**.

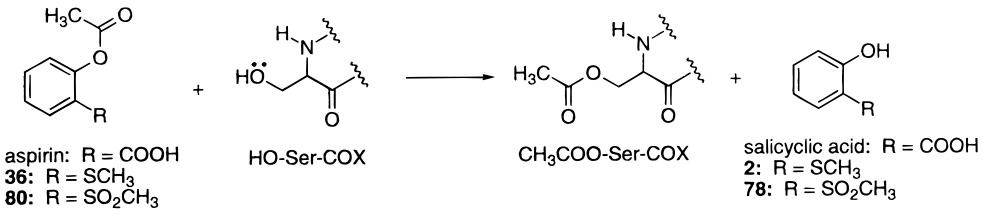
The synthesis of 2-acetoxyphenyl pentyl-5-acetate sulfide (**61**) and 2-acetoxyphenyl pentan-5-ol sulfide (**62**) analogues is shown in Scheme 4. Alkylation of **1** with 5-bromopentyl acetate afforded phenol **23** which was acetylated to afford **61**. Base-catalyzed hydrolysis of **23** generated 2-hydroxyphenyl pentan-5-ol sulfide (**24**). Selective acetylation of the phenolic moiety in **24** in the presence of 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine[28] gave **62**.

The synthesis of 2-(±)-[(2-acetoxyphenyl)thio]oct-3-yne analogue (±)-**72** was achieved in four steps (Scheme 5). Reaction of 1-hexyne (**82**) in the presence of nBuLi and acetaldehyde gave the alkynol (±)-**83**, which was converted to the corresponding alkynyl bromide (±)-**84** in the presence of CBr_4 and triphenylphosphine. S-Alkylation of **1** with (±)-**84** gave the phenol (±)-**34** which upon acetylation furnished the desired acetate (±)-**72**.

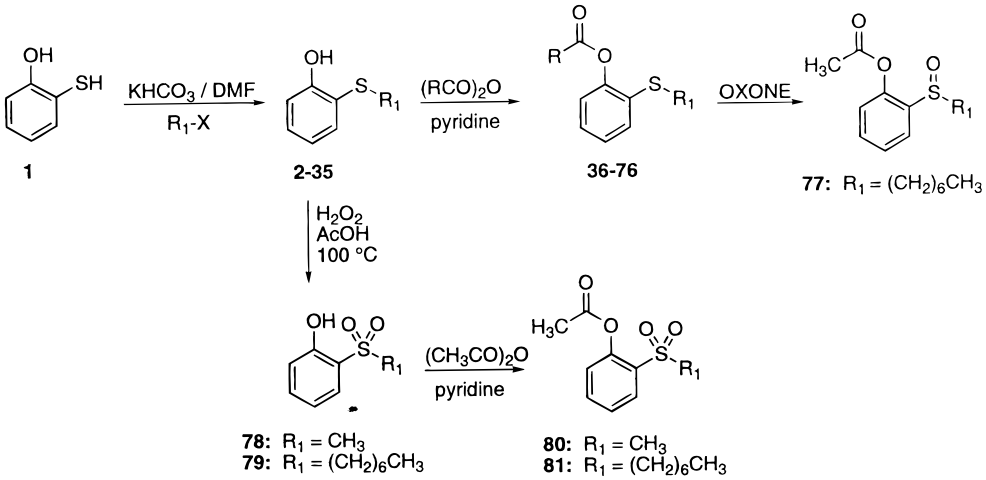
Acylation of 2-hydroxyphenyl hept-2-ynyl sulfide (**32**) with functionalities other than acetyl was carried out as outlined in Scheme 6. Acylation of **32** with propionic anhydride gave **74**, whereas reaction with bromoacetyl bromide gave the corresponding bromoacetyl analogue **75**. The carbamoyloxy derivative **76** was obtained by the reaction of **32** with chlorosulfonyl isocyanate[29] and the methanesulfonyloxy derivative **85** was prepared by reacting **32** with CH_3SO_2Cl.

The synthesis of 2-acetoxy-3-(methylthio)benzoic acid (**90**) was achieved in five steps (Scheme 7); the key step included the introduction of the carboxylate moiety in **2** by a directed ortho metalation strategy.[30] The first step in the synthesis involved conversion of the phenolic

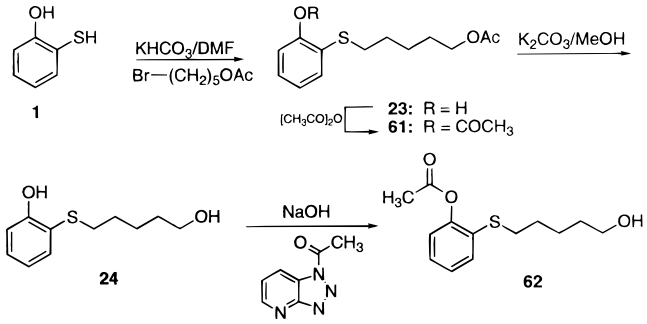
Scheme 2



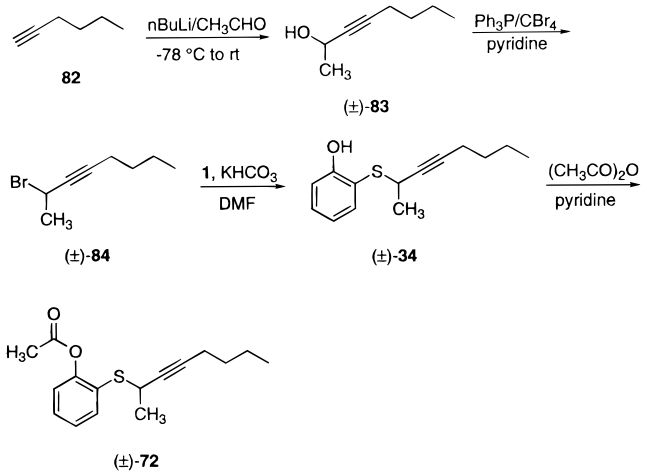
Scheme 3



Scheme 4



Scheme 5

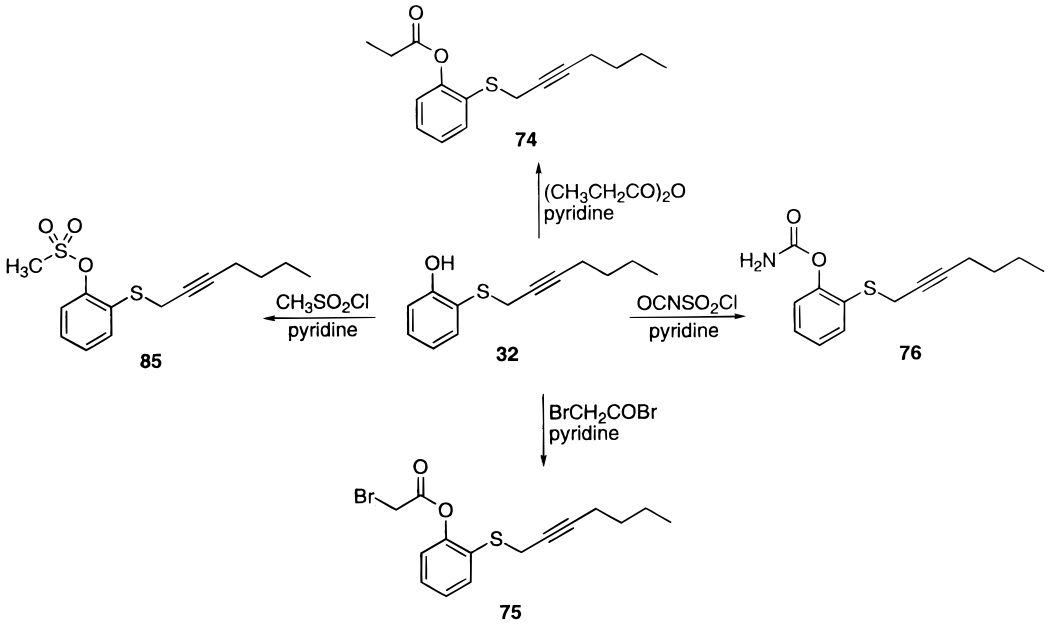


OH group in **2** to the corresponding methoxymethyl (MOM) derivative **86** in the presence of MOM-Cl and KF on activated alumina as the base. The MOM group was chosen because of its ability to direct aromatic lithiation ortho to itself, even in the presence of other ortho directing groups, a consequence of its superior lithium complexation.[31,32] Reaction of **86** with nBuLi followed by treatment with methyl chloroformate gave the MOM-protected methyl salicylate analogue **87**. Acid-catalyzed hydrolysis of the MOM group afforded the methyl salicylate derivative **88**. Base-catalyzed hydrolysis of the methyl ester generated the salicylic acid **89**, which upon acetylation furnished the aspirin derivative **90**. That the lithiation had occurred ortho to the MOM group in **86** to generate **87** and not ortho to the methylthio group to generate **91** was confirmed by NOE assignments on the corresponding methyl salicylate analogue **88**.[33]

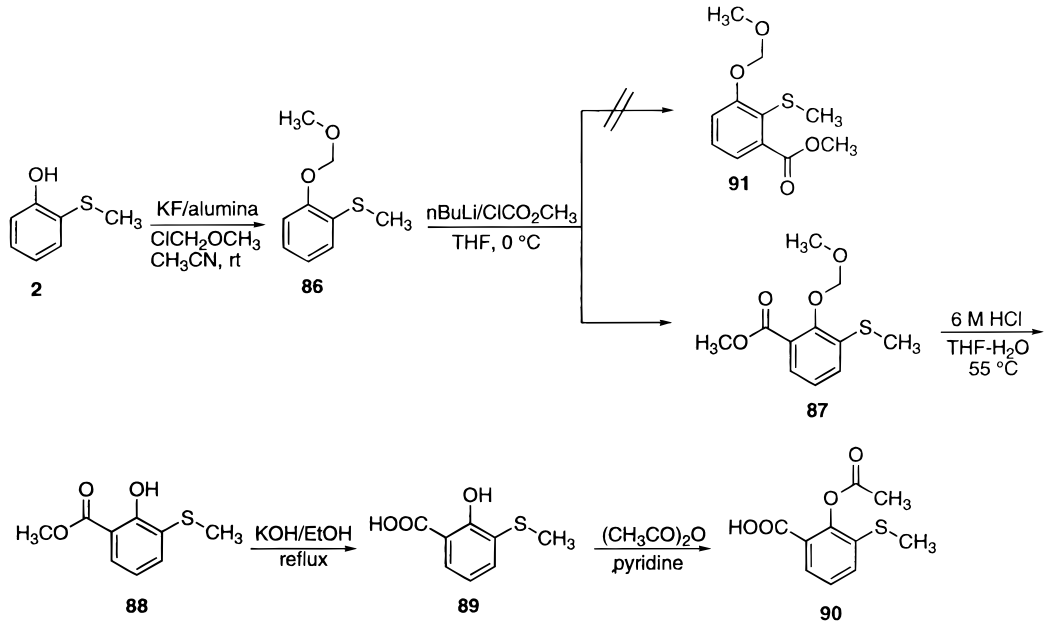
The synthesis of 2-acetoxy-3-fluorophenyl methyl sulfide (**96**) and 2-acetoxy-3,5-difluorophenyl methyl sulfide (**103**) analogues was also approached by the directed ortho metalation strategy and is depicted in Schemes 8 and 9, respectively. In the synthesis of **96**, 2-fluorophenol (**92**) was converted to the MOM derivative **93**. Since fluorine is also an efficient ortho directing group,[34] we chose the MOM functionality as the phenolic OH protecting group. Lithiation of **93** with nBuLi followed by treatment of the resultant yellow aryllithium intermediate with methyl disulfide afforded **94**. Hydrolysis of **94** in the presence of acid gave the phenol **95** which was converted to the desired acetate **96** following reaction with Ac_2O (Scheme 8).

During the synthesis of **103**, reaction of the MOM-protected difluorophenol **98** with nBuLi did not result in the incorporation of lithium ortho to the MOM group; instead lithiation occurred ortho to the two fluorines (see Scheme 9). The resultant pink aryllithium species was reacted with TMS-Cl to generate the aryltrimethylsilyl derivative **99**. A second lithiation of **99** with nBuLi followed by reaction with methyl disulfide gave **100**. Fluoride-mediated desilylation of **100** generated **101**

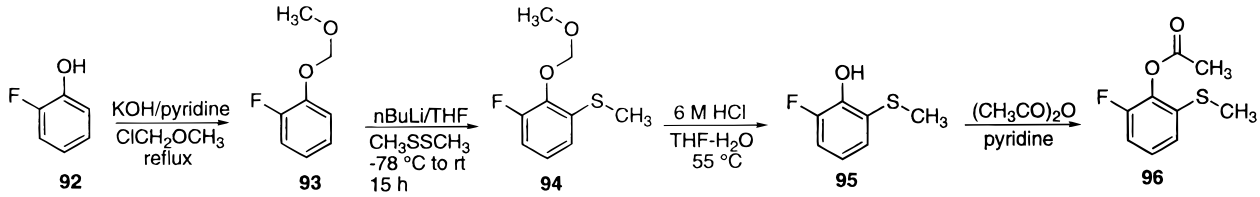
Scheme 6



Scheme 7



Scheme 8

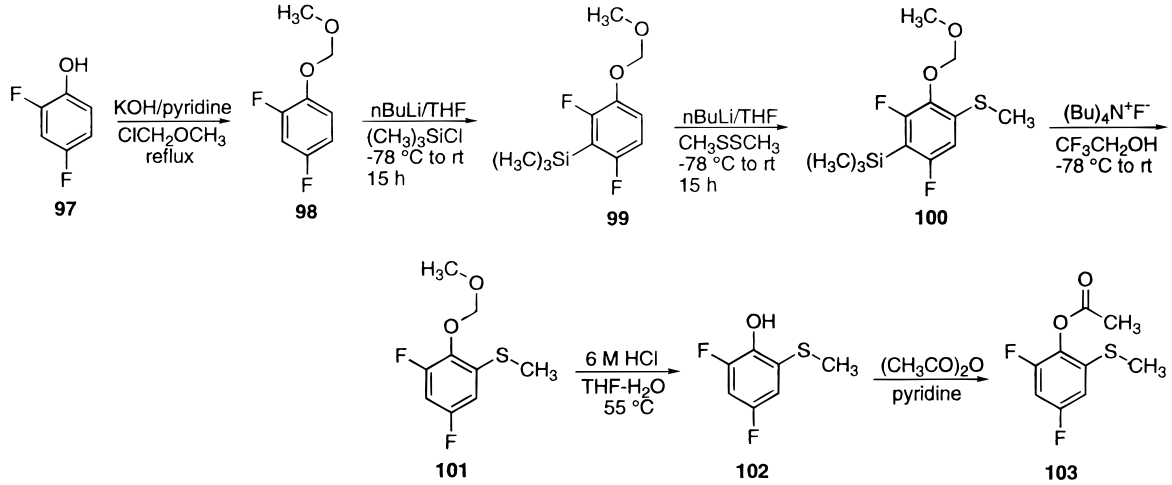


which was hydrolyzed in the presence of acid to afford phenol **102** which was subsequently acetylated to obtain the desired acetate **103**.

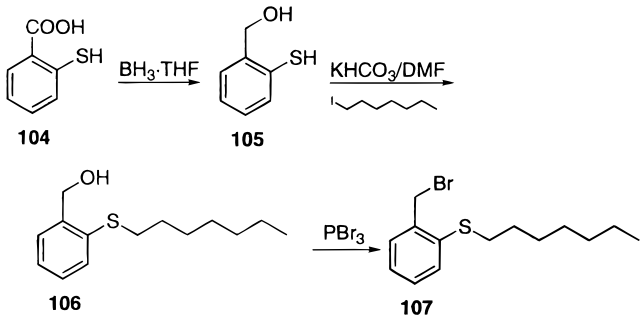
The preparation of the 2-(bromomethyl)phenyl heptyl sulfide (**107**) analogue which possesses a reactive alkyl halide moiety instead of the acetoxy group is shown in Scheme 10. Thiosalicylic acid (**104**) was reduced with borane−THF to afford the corresponding benzyl alcohol analogue **105** which was alkylated with 1-iodoheptane in the presence of $KHCO_3$ to generate 2-(hydroxymethyl)phenyl heptyl sulfide (**106**) which upon reaction with PBr_3 led to the formation of the desired benzyl bromide derivative **107**.

During the course of our SAR studies, we substituted the S atom in 2-acetoxyphenyl heptyl sulfide (**46**) with bioisoteric heteroatoms including Se, NCH_3, and O or replaced it with a methylene group. The synthesis of 2-acetoxyphenyl heptyl selenide derivative **111** is out-

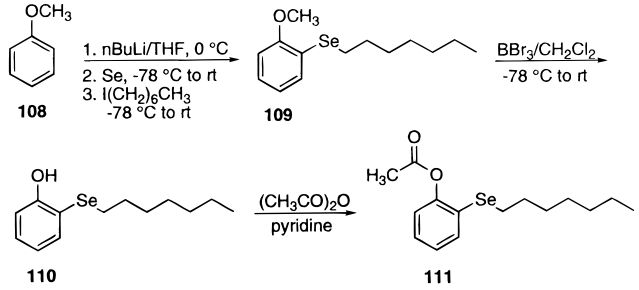
Scheme 9



Scheme 10



Scheme 11



lined in Scheme 11. The synthesis was adapted from a previously reported methodology for generating aryl alkyl sulfides and selenides.[35,36] Briefly, aromatic lithiation of anisole (**108**) with nBuLi followed by reaction of the aryllithium intermediate with elemental selenium generated the aryl selenide lithium species in situ, which was then treated with 1-iodoheptane to afford **109**. Demethylation of **109** with BBr_3 afforded the phenol **110** which was acetylated to furnish **111**.

The carbon homologue **116** was also generated in a similar manner as depicted in Scheme 12. Lithiation of MOM-protected phenol **113** with nBuLi followed by treatment with 1-iodooctane gave **114**. Acid-catalyzed hydrolysis of **114** gave 2-octylphenol (**115**) which was acetylated to yield **116**.

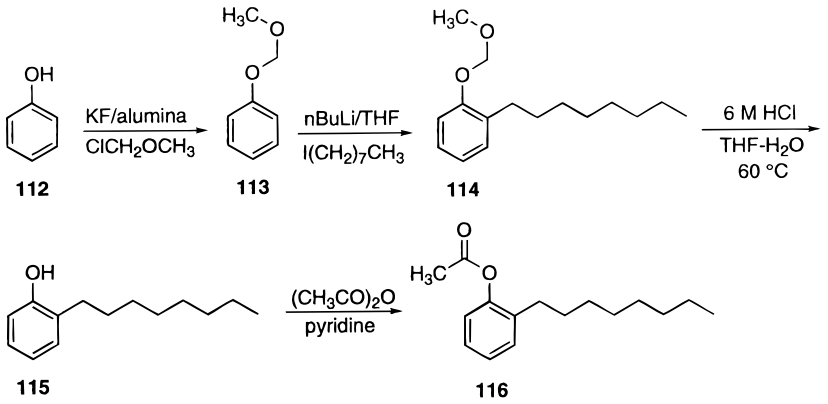
The synthesis of 2-(acetoxyphenyl)-*N*-heptyl-*N*-methylamine (**121**) and the 2-acetoxyphenyl heptyl and 2-acetoxyphenyl hept-2-ynyl ether analogues **125** and **126** is shown in Scheme 13. Methylation of 2-aminophenol (**117**) with CH_3I in the presence of $KHCO_3$ gave a mixture of the monomethyl- and dimethylamine derivatives **118** and **119** which were readily separable by chromatography. Alkylation of **118** with 1-iodoheptane gave the tertiary amine **120** which was converted to the desired acetate **121** following treatment with Ac_2O. The ether derivatives **125** and **126** were obtained in a similar fashion by alkylation of catechol (**122**) with 1-iodoheptane or 1-bromohept-2-yne to generate **123** and **124** and then derivatization with Ac_2O to afford the acetates.

The synthesis of the isomeric 2-acetoxy-3-(heptylthio)-naphthalene (**132**) and the 2-acetoxy-1-(heptylthio)-naphthalene (**136**) analogues is outlined in Schemes 14 and 15. The first step in the synthesis of **132** involved the methylation of 2-naphthol (**127**) followed by aromatic lithiation of the methyl naphthyl ether **128** with nBuLi. The naphthyllithium intermediate was treated in situ with elemental sulfur followed by treatment with 1-iodoheptane to afford predominantly **129** along with small amounts (~5%) of the isomer **130** as contaminant.[37] Following chromatography and recrystallization, pure **129** was demethylated with BBr_3 to give 3-(heptylthio)naphth-2-ol which upon acetylation gave **132**.

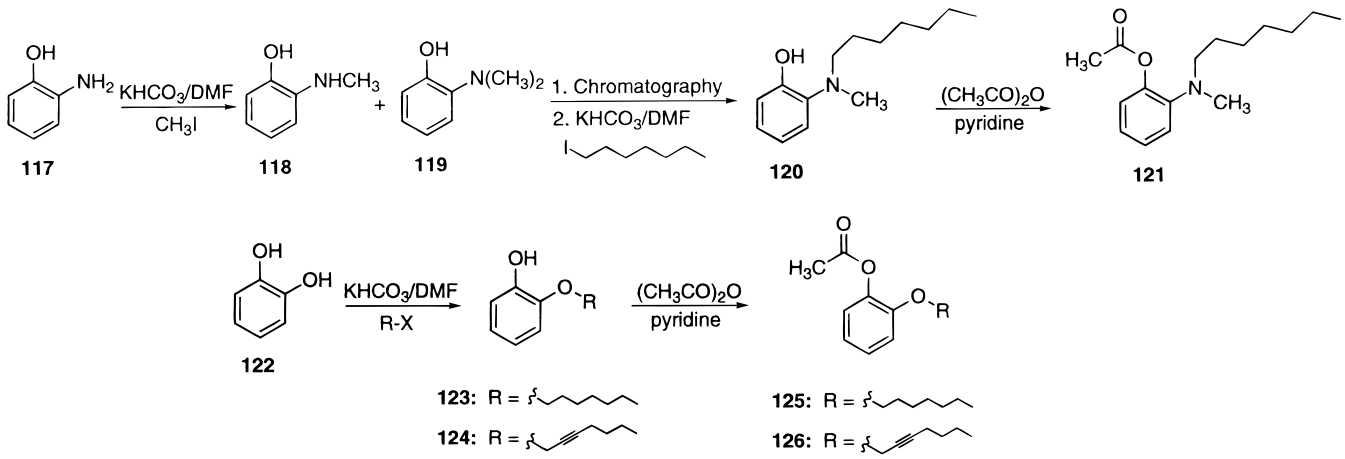
Regiospecific introduction of the heptylthio moiety at the 1 position in the naphthalene ring to generate **130** was achieved by dehalometalation of 1-bromonaphthyl methyl ether (**134**) in the presence of nBuLi, elemental sulfur, and 1-iodoheptane. *O*-Demethylation of **130** with BBr_3 followed by treatment of the naphthol derivative **135** with Ac_2O gave the desired acetate **136**.

Enzymology. 1. Selective Covalent Modification of COX-2 by 2-Acetoxyphenyl Methyl Sulfide (36). Incubation of purified human COX-2 or ovine COX-1 with a 1000-fold excess of **36** led to a time-dependent loss of the cyclooxygenase activity of COX-2 as monitored by the oxygen uptake assay. The cyclooxygenase activity of COX-1, however, remained unaffected. 2-Hydroxyphenyl methyl sulfide (**2**), the hydrolysis product of **36**, and 2-acetoxyphenyl methyl sulfone (**80**) did not inhibit either isozyme activity. Furthermore, **36** had no inhibitory effect on the peroxidase activity of COX-2 suggesting that selective COX-2 inhibition arises from an interaction(s) of **36** at the cyclooxygenase active site. Tryptic digestion and peptide mapping of COX-2 labeled with [*1-*^{14}*C-acetyl*]-**36** revealed that the radioactivity was incorporated into a

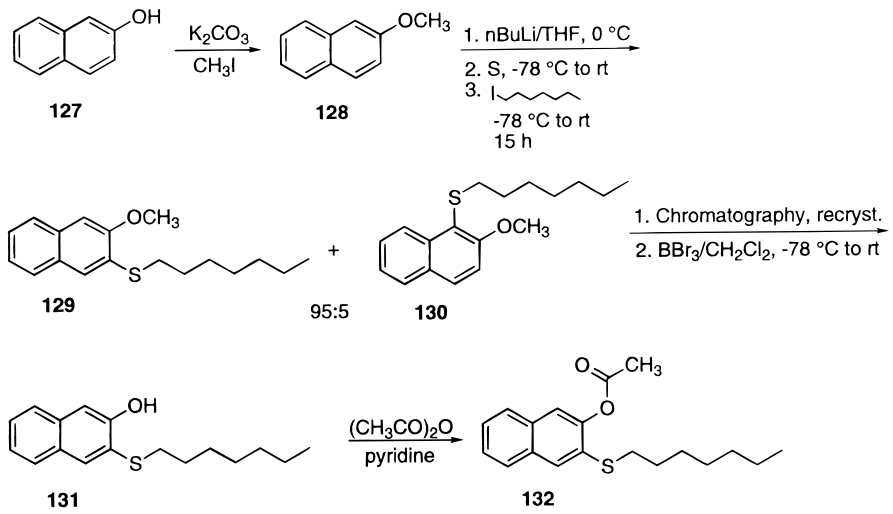
Scheme 12



Scheme 13



Scheme 14



single peptide. The same peptide was obtained following labeling of COX-2 with [*1-*^{14}*C-acetyl*]aspirin (see Figure 2). Electrospray mass spectroscopy revealed the structure of the peptide as acetylated tripeptide S-L-K (data not shown). This peptide is present in the COX-2 sequence at positions 516−518 and contains the complementary serine residue acetylated by aspirin in COX-1.[24] These results not only establish that inhibition of COX-2 by **36** is due to a selective covalent modification of the isozyme but also confirm that COX-2 inhibition by aspirin results in the acetylation of Ser516.

IC_{50} values for the inhibition of purified human COX-2 and ovine COX-1 by **36** were determined by the thin-layer chromatography (TLC) assay (Table 1). HoloCOX-2 (88 nM) or holoCOX-1 (22 nM) in 100 mM Tris-HCl, pH 8.0, containing 500 μM phenol was treated with several concentrations of **36** at 25 °C for 2 h. Since the recombinant COX-2 had a lower specific activity than ovine COX-1, the concentrations of protein were adjusted such that the precentage of total products obtained following catalysis of arachidonic acid by the two isoforms was comparable. The cyclooxygenase

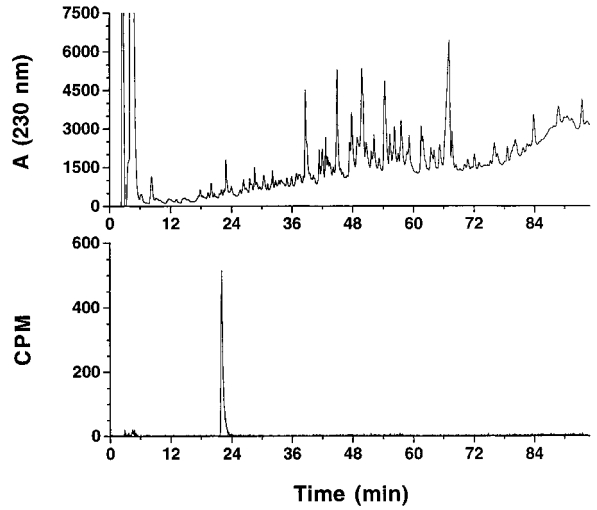
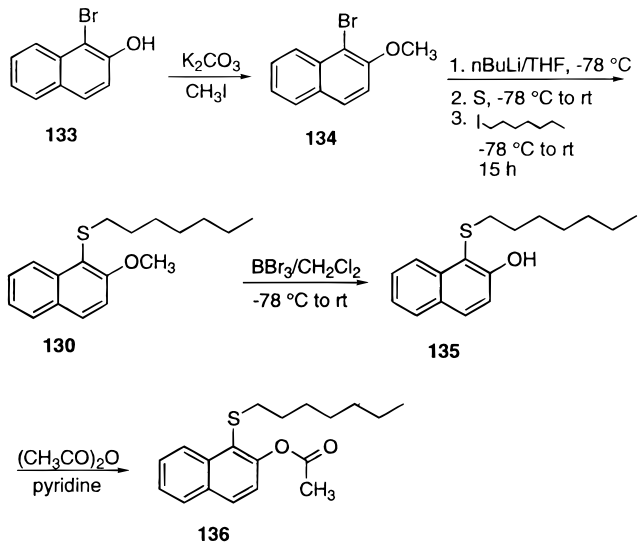


Figure 2. Peptide map of COX-2 acetylated by [*1-^{14}C-acetyl*]-salicylic acid. Hematin-reconstituted hCOX-2 (14 μM) was treated with 30 equiv of [*1-^{14}C-acetyl*]salicylic acid for 1.5 h at 25 °C. After dialysis, the protein was digested with 44:1 TPCK−trypsin for 24 h at 37 °C. The peptides were injected on a Beckman ODS (C18) reversed-phase column as described in the Experimental Section. The fragments were monitored with a UV detector (top trace) and a radioactive detector (bottom trace).

Scheme 15

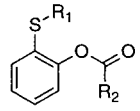


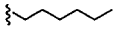
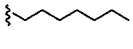
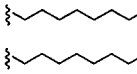
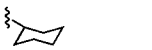
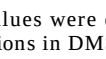
reaction was initiated by the addition of [*1-^{14}C*]arachidonic acid (50 μM) at 37 °C for 30 s. Control experiments in the absence of inhibitor indicated ~25−30% conversion of fatty acid substrate to products which was sufficient for assessing the inhibitory properties of all the test compounds described in this study. Under these conditions, **36** displayed selective time- and dose-dependent inhibition of COX-2 [IC_{50}(COX-2) ~ 250 μM; IC_{50}(COX-1) > 5000 μM] whereas aspirin preferentially inhibited COX-1 [IC_{50}(COX-2) ~ 62.5 μM; IC_{50}(COX-1) ~ 12.5 μM].

2. Strategies To Improve the Inhibitory Potency of 36 as a Selective COX-2 Inhibitor: (A) Increasing the Acetate Reactivity. Acetylation of the weakly nucleophilic hydroxyl group of serine by aspirin is thought to result from the initial binding of its *o*-carboxylate to an arginine residue which juxtaposes the acetyl moiety to the serine hydroxyl group.[38] Therefore, we decided to incorporate a carboxylate functionality ortho to the acetate in **36** as an attempt to mimic the interactions of aspirin as a cyclooxygenase inhibitor. However, aspirin analogue **90** was devoid of any inhibitory properties toward either isozyme [IC_{50}(COX-2 and COX-1) > 2000 μM].

Attempts to increase the acetyl reactivity by inductive destabilization of the carbonyl moiety in **36** by incorporation of electron-withdrawing substituents was also tried. However, the trifluoromethyl (**38**), the α-chloroacetyl (**39**), and the α-bromoacetyl (**40**) analogues displayed nonselective inhibition of both isozymes. Furthermore, the mono- and difluoro analogues **96** and **103** were inactive against both isoforms. Replacement of the acetate linkage with a propionyloxy moiety (compound **37**) resulted in an inactive analogue (see Table 1).

Table 1

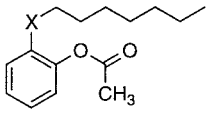


Compound	R_1	R_2	IC_{50}, (μM)[a]	
			hCOX-2	oCOX-1
aspirin	---	---	62.5	12.5
36[b]	CH_3	CH_3	250	> 5000
37	CH_3	C_2H_5	> 1000	> 1000
38	CH_3	CF_3	260	260
39	CH_3	CH_2Cl	360	390
40	CH_3	CH_2Br	510	320
41	C_2H_5	CH_3	200	375
42		CH_3	66	66
43		CH_3	34	40
44		CH_3	5.0	5.0
45		CH_3	3.5	8.0
46[b]		CH_3	2.0	6.0
47		CH_3	> 40	> 40
48		CH_3	> 40	> 40
49		CH_3	> 40	> 40
50		CH_3	> 40	> 40

[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with purified hCOX-2 or oCOX-1 for 2 h at rt; assays were run in duplicate. IC_{50} values are the average of duplicate determinations for each test compound. [b] The corresponding phenols were inactive.

(B) Alkyl Chain Length Extensions and Heteroatom Changes. Chain length extension of the *S*-methyl group in **36** to higher alkyl homologues revealed significant increases in COX-2 inhibitory potencies, albeit with some loss of selectivity (see Table 1). For example, replacement of the *S*-methyl group in **36** with a *S*-heptyl chain afforded 2-acetoxyphenyl heptyl sulfide (**46**) which was 125 times more potent than **36** as a COX-2 inhibitor [compound **36:** IC_{50}(COX-2) ~ 250 μM; compound **46:** IC_{50}(COX-2) ~ 2 μM].

Table 2



Compound	X	IC_{50}, (μM)[a]	
		hCOX-2	oCOX-1
46	S	2.0	6.0
77	SO	> 40	> 40
81	SO_2	> 40	> 40
111	Se	12	12
116	CH_2	> 40	> 40
121	NCH_3	> 40	> 40
125	O	> 40	> 40

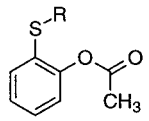
[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with purified hCOX-2 or oCOX-1 for 2 h at rt; assays were run in duplicate. IC_{50} values are the average of duplicate determinations for each test compound.

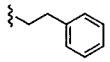
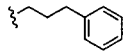
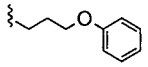
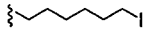
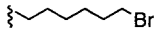
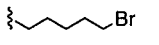
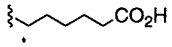
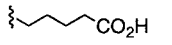
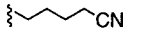
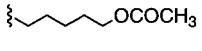
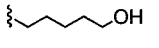
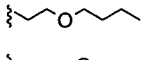
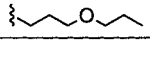
However, **46** was only 3 times selective for COX-2 [IC_{50}(COX-2) ~ 2 μM; IC_{50}(COX-1) ~ 6 μM]. Further chain length extensions or cyclizations led to the octyl or nonyl analogues (compounds **47** and **48**) and the cycloxhexyl or cycloheptyl derivatives **49** and **50**, respectively, which were inferior in inhibitory potency. Replacement of the sulfur in the heptyl analogue **46** with Se, NCH_3, or O and CH_2 or oxidation of the sulfide in **46** to the corresponding sulfoxide **77** or the sulfone **81** indicate that compounds with S as the heteroatom are the most potent (Table 2).

3. SAR Studies on 2-Acetoxyphenyl Heptyl Sulfide (46). Initial structural changes to improve COX-2 potency and selectivity of **46** included modifications on the heptyl chain of **46**. Introduction of a terminal hydrophobic iodo moiety led to compound **55** which was more selective as a COX-2 inhibitor than **46**, albeit with somewhat lower inhibitory potency against COX-2 (Table 3). Introduction of polar terminal substitutents on the heptyl chain such as carboxylate, cyano, acetoxy, or hydroxy generated compounds **58**−**62** which were much inferior to **46** as COX-2 inhibitors. Inclusion of arylalkyl moieties (compounds **51**−**54**) generated derivatives which displayed similar COX-2 selectivity ratios as **46**, but they were less potent as inhibitors [compound **51:** IC_{50}(COX-2) ~ 250 μM; IC_{50}(COX-1) > 400 μM]. It is noteworthy to point out that COX-2 inhibition was quite sensitive toward the inclusion of substituents on the heptyl chain. For example, introduction of an oxygen in the 3-position of the heptyl side chain furnished analogue **63** which shared similar COX-2 inhibitory potency and selectivity as **46**, whereas an ether linkage at the 4-position of the heptyl chain gave **64**, which did not reveal any significant inhibition of either isozyme even at high concentrations [compound **63:** IC_{50}(COX-2) ~ 7 μM; IC_{50}(COX-1) ~ 22 μM; compound **64:** IC_{50}(COX-2 or COX-1) > 50 μM].

Other structural modifications on **46** included replacement of the acetoxy moiety with a bromomethyl group to afford **107** (see Scheme 10) as a potential alkylating agent for the hydroxyl group of Ser516;

Table 3



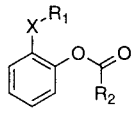
Compound	R	IC_{50}, (μM)	
		hCOX-2	oCOX-1
51		250	> 400
52		100	> 150
53		> 1000	> 1000
54		> 1000	> 1000
55		10	70
56		12	25
57		9.5	34
58		50	50
59		> 50	> 50
60		> 50	> 50
61		50	50
62		> 50	> 50
63		7.0	22
64		> 50	> 50

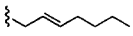
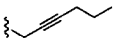
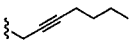
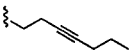
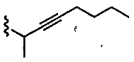
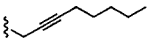
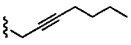
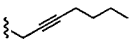
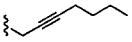
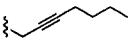
[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with purified hCOX-2 or oCOX-1 for 1 h at rt; assays were run in duplicate. IC_{50} values are the average of duplicate determinations for each test compound.

however, **107** was devoid of any inhibitory properties toward both COX-1 and COX-2. Since previous studies have shown that the fatty acid binding site in COX-2 is larger than that in COX-1,[39] we decided to synthesize the bulkier acetoxynaphthyl heptyl sulfide isomers **132** and **136** (see Schemes 14 and 15) as selective COX-2 inhibitors, but these analogues were also devoid of any inhibitory potency toward COX-2 or COX-1 (IC_{50}'s > 100 μM).

Introduction of a trans-double bond in the 2-position of the heptyl chain led to **65** which displayed similar COX-2 potency and selectivity as **46**, whereas incorporation of a triple bond in the 2-position gave 2-acetoxyphenyl hept-2-ynyl sulfide (**70**), which displayed the most potent and selective COX-2 inhibition in the series [IC_{50}(COX-2) ~ 0.8 μM; IC_{50}(COX-1) ~ 17 μM] (Table 4). Compound **70** was ~ 20-fold selective as a COX-2 inhibitor. Introduction of a triple bond in the alkyl derivatives **42**−**47** gave the alkynyl analogues **66**−**73** which displayed more potent and selective COX-2 inhibition than the corresponding saturated compounds

Table 4



Compound	R_1	R_2	X	IC_{50}, (μM)[a]	
				hCOX-2	oCOX-1
65		CH_3	S	11	22
66		CH_3	S	25	40
67		CH_3	S	20	35
68		CH_3	S	5.0	20
69		CH_3	S	3.0	14
70[b]		CH_3	S	0.8	17
71		CH_3	S	6.5	18
72		CH_3	S	7.0	15
73		CH_3	S	7.0	33
74		C_2H_5	S	> 40	> 40
75		CH_2Br	S	26	20
76		CH_2NH_2	S	> 40	> 40
126		CH_3	O	> 40	> 40

[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with purified hCOX-2 or oCOX-1 for 1 h at rt; assays were run in duplicate. IC_{50} values are the average of duplicate determinations for each test compound. [b] The corresponding phenol **32** was inactive.

(see Table 4). Interestingly, the oct-2-ynyl analogue **73** was capable of potent and selective COX-2 inhibition, whereas the corresponding octyl analogue **47** did not reveal significant inhibition of either isozyme at similar concentration ranges [compound **73**: IC_{50}(COX-2) ~ 7 μM; IC_{50}(COX-1) ~ 33 μM; compound **47:** IC_{50}(COX-1 and COX-2) > 40 μM]. Branching of the hept-2-ynyl chain at the 1-position with a methyl group [compound (±)-**72**] or movement of the triple bond from the 2- to the 3-position (compound **71**) led to some losses in selectivity. As observed with **36**, replacement of the acetyl group in **70** with other acylating moieties such as propionyloxy, α-bromoacetoxy, carbamoyloxy, or methylsulfonyloxy moieties (compounds **74**−**76** and **85**) afforded less potent inhibitors.

The kinetic constants for the time- and concentration-dependent inhibition of COX-2 by **70** were compared to those obtained with aspirin. Reconstituted COX-2 (5 μM) was preincubated with 2, 4, 8, 20, and 40 equiv of **70** or 20, 40, 80, and 200 equiv of aspirin, respectively. Periodic measurements of COX-2 activity were conducted by diluting aliquots from the reaction mixture in 100 mM Tris-HCl, pH 8.0, containing 500 μM phenol and [*1-*^{14}*C*]arachidonic acid (50 μM). The percentage of total products observed at different inhibitor concentrations was divided by the percentage of total products observed for protein samples preincubated for the same time with DMSO. Semilogarithmic plots of the percent

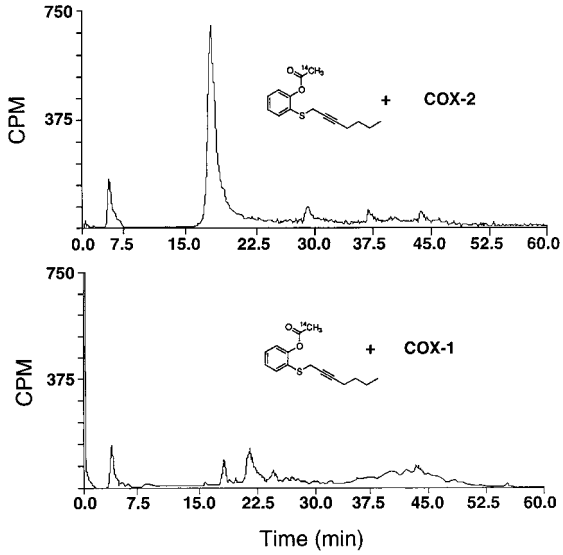


Figure 3. Selective covalent modification of COX-2 by **70**. Hematin-reconstituted hCOX-2 (14 μM) or oCOX-1 (14 μM) in 100 mM Tris-HCl, pH 8.0, containing 500 μM phenol was treated with 25 equiv of [*1-*^{14}*C-acetyl*]-**70** for 2 h at 25 °C. The isozymes were dialyzed overnight and injected on a reversed-phase Vydac C4 column as described in the Experimental Section. Only the radioactive traces corresponding to the intact proteins are presented. Labeled COX-2 (top trace); labeled COX-1 (bottom trace).

initial enzyme activity remaining versus time were constructed, and the apparent rates of pseudo-first-order inactivation (k_{obs}) at each inhibitor concentration were obtained. The reciprocal values of k_{obs} were plotted against the reciprocal of inhibitor concentration to generate k_{inact} and K_i values. Under these conditions, the second-order rate constants for the time- and dose-dependent inhibition of COX-2 by **70** and aspirin were k_{inact}/K_i ~ 0.18 and 0.003 $min^{-1}\cdot\mu M^{-1}$, respectively. These results indicated that **70** was 60 times more potent than aspirin as a COX-2 inhibitor. The corresponding hydrolysis product of **70**, i.e., **32**, was inactive. Like aspirin, COX-2 inhibited by **70** produced no prostaglandin products but did generate 15-hydroxyeicosatetraenoic acid (15-HETE).[40] When [*1-*^{14}*C-acetyl*]-**70** was reacted with the isoforms, the degree of incorporation of the [^{14}C]acetyl moiety into COX-2 and COX-1 correlated well with the relative inhibitory potency against the two isozymes (ratio of ^{14}C incorporated into COX-2 vs COX-1 = 15.4) (Figure 3). As with **36** and aspirin, tryptic digestion, peptide mapping, and subsequent mass spectrometric analysis of COX-2 acetylated with [*1-*^{14}*C-acetyl*]-**70** led to the identification of the acetylated residue as Ser516.[26]

4. Inhibition of COX-2 Activity in Intact Cells. The ability of 2-acetoxyphenyl alkyl sulfides to inhibit COX-2 in intact cells was assayed in RAW264.7 macrophages in which COX-2 activity was induced by pathologic stimuli. The macrophages were treated with lipopolysaccharide (500 ng/mL) and γ-interferon (10 U/mL) for 7 h to induce COX-2 and then treated with several concentrations of 2-acetoxythioanisole (**36**), 2-acetoxyphenyl heptyl sulfide (**46**), 2-acetoxyphenyl hept-2-ynyl sulfide (**70**), or aspirin for 30 min at 37 °C. The IC_{50} values for inhibition of prostaglandin D_2 (PGD_2) by **36**, **46**, **70**, and aspirin were 6, 0.85, 0.5, and 36 μM, respectively (Figure 4). The results from these studies

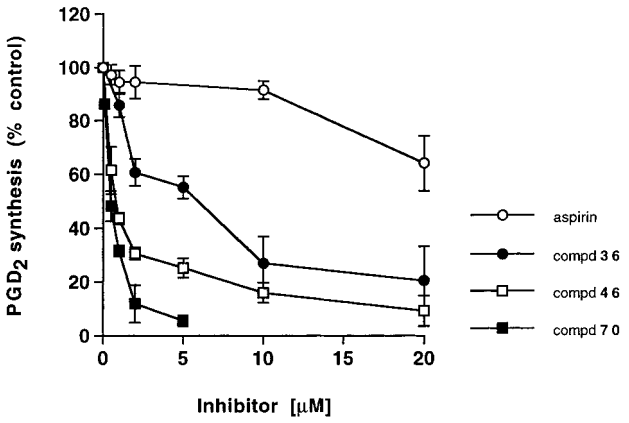


Figure 4. Inhibition of COX-2 activity in RAW264.7 murine macrophages. Cells were activated for 7 h at 37 °C in serum-free DMEM with LPS (500 ng/mL) and γ-interferon (10 U/mL). Vehicle (DMSO) or aspirin, **36**, **46**, or **70** in DMSO was added at the indicated concentrations for 30 min at 37 °C. The cyclooxygenase reaction was initiated by adding 20 μM [*1-*^{14}*C*]-arachidonic acid for 15 min at 37 °C. The medium was collected into cold termination solution (Et_2O/MeOH/1 M citrate, 30:4:1), and prostanoid products were quantified as described in the Experimental Section.

indicate that 2-acetoxyphenyl alkyl sulfides are superior to aspirin in inhibiting COX-2 activity in intact inflammatory cells.

In addition to inhibiting COX-2 activity in macrophages, the hept-2-ynyl derivative **70** also inhibited growth of HCA-7 colon cancer cells, which express high levels of COX-2. HCT-15 colon cancer cells, which do not express COX-2, were resistant to inhibition by **70**.[26] The IC_{50} value for inhibition of the growth of HCA-7 cells by **70** ($IC_{50} \sim 2$ μM) is lower than the published IC_{50} value for the COX-2 selective inhibitor SC-58125.[41]

Compound **70** was evaluated in vivo in a rat air pouch model.[26] A dose of 5 mg/kg **70** lowered prostaglandin E_2 (PGE_2) levels in the pouch exudate by 95 ± 1% but did not affect serum thromboxane B_2 (TxB_2) levels. Increasing the dose to 50 mg/kg completely inhibited PGE_2 levels but only decreased TxB_2 levels by 11%. In contrast, indomethacin (2 mg/kg) completely inhibited PGE_2 by 100% and TxB_2 by 90%. Thus, **70** is a potent and selective COX-2 inhibitor in vivo as well.

Summary

The results of this investigation have led to the discovery of the first selective covalent inactivators of COX-2. SAR studies indicate that the sulfides are more potent than other heteroatom-containing compounds as cyclooxygenase inhibitors. Furthermore, oxidation of the sulfides to the corresponding sulfoxides or sulfones is detrimental toward inhibitory potency. The lack of inhibition by the sulfones is opposite to that observed with diarylheterocycles. Systematic variation of the acyl group, alkyl group, aryl substitution pattern, and heteroatom of the initial lead compound **36** led to the identification of **70** as the most selective and potent COX-2 inhibitor in the series. As in previous studies from our laboratory on *N*-(substituted)maleimides,[42] the heptyl side chain in 2-acetoxyphenyl alkyl sulfides was optimum for inhibitory potency. Introduction of a triple bond in the heptyl side chain of **46** led to enhanced potency and selectivity for COX-2. Unlike aspirin, 2-acetoxyphenyl alkyl sulfides do not contain a carboxylate moiety and are incapable of binding to the positively charged arginine residue (Arg106) in COX-2, yet these compounds acetylate the same serine residue (Ser516) as aspirin. Furthermore, they preferentially acetylate COX-2, whereas aspirin preferentially acetylates COX-1. The structural basis for COX-2 selectivity by **70** has been probed by site-directed mutagenesis.[26] The results of those experiments reveal that 2-acetoxyphenyl alkyl sulfides selectively inhibit COX-2 by binding at previously uncharacterized regions in the COX-2 active site. For example, the Arg106Gln and the Tyr341Ala mutants which are resistant to inhibition by the carboxylate-containing NSAIDs including aspirin[43] are effectively inhibited by **70**. The side pocket triple mutant Val509Ile:Arg499His:Val420Ile which incorporates the major amino acid changes between COX-2 and COX-1[44] and which accounts for the selectivity of the diarylheterocycles[45] is also potently inhibited by **70**. In contrast, this mutant is resistant to inhibition by diarylheterocycles such as DuP 697.

The results of this investigation have established that potent, covalent inactivators of COX-2 can be designed. In light of COX-2's role in inflammation and COX-1's role in gastric protection, it is likely that 2-acetoxyphenyl alkyl sulfides can serve as therapeutic equivalents of aspirin in inflammatory and proliferative diseases without the deleterious ulcerogenic side effects which limit aspirin's use, particularly in long-term therapy. The efficacy of **70** as a selective COX-2 inhibitor in vivo, coupled to its ability to attenuate growth of COX-2-expressing colon cancer cells, indicates that these compounds may serve not only as novel, nonulcerogenic antiinflammatory agents but also as potential cancer chemopreventive agents.

Experimental Section

Chemistry. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. Acetonitrile was distilled over calcium hydride. Where other anhydrous solvents were required, Aldrich anhydrous solvents were used. All other solvents were HPLC grade. Reagents which were obtained commercially (Aldrich, Milwaukee, WI, or Lancaster, PA) were used without further purification. Reactions requiring anhydrous conditions were conducted in flame-dried glassware under argon. Analytical TLC (Analtech uniplates) was used to follow the course of reactions. Silica gel (Fisher, 60−100 mesh) was used for column chromatography. Chemical yields are unoptimized specific examples of one preparation. ^{1}H NMR and ^{13}C NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on a Bruker WP-360 or AM-400 spectrometer; chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are given in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HRMS) were obtained on a Kratos Concept II HH four-sector mass spectrometer.

General Procedure for the Synthesis of the 2-Acetoxyphenyl Alkynyl Sulfides. Step 1. Synthesis of the Bromoalkyne Analogues: 1-Bromohept-2-yne. A reaction mixture containing hept-2-yn-1-ol (1.1 g, 10 mmol) in 25 mL of dry THF was treated with Ph_3P (5.24 g, 20 mmol), dry pyridine (0.8 mL, 10 mmol), and CBr_4 (3.31 g, 10 mmol). After stirring for 4 h at room temperature, the reaction mixture was diluted with water and the aqueous solution was extracted

with Et_2O (3 × 15 mL). The combined organic solution was washed with 1 M HCl and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting oil was triturated with hexanes (2 × 5 mL), and the combined washings were concentrated in vacuo. Chromatography on silica gel (hexanes) afforded 1-bromohept-2-yne (1.2 g, 71%). Synthesis of the 2-hydroxyphenyl alkyl or 2-hydroxyphenyl aryl sulfides did not require the preparation of the alkyl bromides, as they were commercially available.

Step 2. Alkylation of 2-Hydroxythiophenol: 2-Hydroxyphenyl Hept-2-ynyl Sulfide (32). A reaction mixture containing 2-hydroxythiophenol (**1**; 0.5 g, 3.96 mmol) in 4 mL of dry DMF was treated with $KHCO_3$ (0.45 g, 4.52 mmol) and 1-bromohept-2-yne (0.69 g, 3.96 mmol) and allowed to stir at room temperature overnight. The mixture was diluted with water and extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 3:97) gave **32** as a pale-yellow oil (0.62 g, 71%): ^{1}H NMR ($CDCl_3$) δ 7.51−7.54 (dd, 1 H, J = 7.7 and 1.7 Hz, ArH), 7.27−7.31 (t, 1 H, ArH), 6.98−7.01 (dd, 1 H, J = 8.1 and 0.6 Hz, ArH), 6.86−6.90 (t, 1 H, J = 7.6 Hz, ArH), 6.78 (s, 1 H, OH), 3.39−3.40 (t, 2 H, J = 2.3 Hz, CH_2), 2.11−2.16 (m, 2 H, CH_2), 1.26−1.46 (m, 4 H, CH_2), 0.86−0.97 (t, 3 H, J = 7.1 Hz, CH_3).

Step 3. Acetylation of 2-Hydroxyphenyl Alkyl Sulfides: 2-Acetoxyphenyl Hept-2-ynyl Sulfide (70). A reaction mixture containing **32** (0.66 g, 3 mmol), dry pyridine (0.26 mL, 3.2 mmol), and Ac_2O (0.3 mL, 3.2 mmol) in 5 mL of dry CH_2Cl_2 was stirred at room temperature for 6 h. Water was added to the reaction mixture, and the aqueous solution was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was washed with water, dried ($MgSO_4$), and filtered, and the solvent was concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave the desired acetate as a pale-yellow oil (0.72 g, 92%): ^{1}H NMR ($CDCl_3$) δ 7.53−7.56 (dd, 1 H, J = 7.4 and 1.9 Hz, ArH), 7.22−7.27 (m, 2 H, ArH), 7.08−7.09 (dd, 1 H, J = 7.6 and 1.8 Hz, ArH), 3.57−3.58 (t, 2 H, J = 2.3 Hz, CH_2), 2.34 (s, 3 H, CH_3), 2.13−2.17 (m, 2 H, CH_2), 1.32−1.45 (m, 4 H, CH_2), 0.85−0.89 (t, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.03, 149.51, 131.25, 128.65, 127.82, 126.81, 122.52, 84.46, 74.96, 30.59, 22.36, 21.79, 20.80, 18.42, 13.54; FAB-MS 263 (MH^+), 262 (M^+, 45), 220 (100), 95 (30), 79 (90); HRMS (CI) calcd for $C_{15}H_{19}O_2S$ (MH^+) 263.11058, found 263.11040.

General Procedure for the Synthesis of 2-Acetoxyphenyl Haloalkyl Sulfides. Step 1. 2-Hydroxyphenyl 6-Iodohexyl Sulfide (17). To a solution of **1** (0.25 g, 2 mmol) in 4 mL of dry DMF was added $KHCO_3$ (0.2 g, 2 mmol) and 1,6-diiodohexane (0.67 g, 2 mmol). After stirring overnight at room temperature, the solution was diluted with water and extracted with Et_2O (3 × 5 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 2:98) gave **17** as a yellow oil (0.29 g, 44%): ^{1}H NMR ($CDCl_3$) δ 7.44−7.47 (dd, 1 H, J = 7.7 and 1.6 Hz, ArH), 7.23−7.30 (m, 1 H, ArH), 6.97−7.01 (dd, 1 H, J = 8.2 and 1.3 Hz, ArH), 6.85−6.90 (m, 1 H, ArH), 6.74 (s, 1 H, OH), 3.14−3.19 (t, 2 H, J = 6.9 Hz, CH_2), 2.66−2.71 (t, 2 H, J = 7.4 Hz, CH_2), 1.77−1.84 (m, 2 H, CH_2), 1.54−1.59 (m, 2 H, CH_2), 1.36−1.47 (m, 4 H, CH_2).

Step 2. 2-Acetoxyphenyl 6-iodohexyl sulfide (55) was prepared by the acetylation of **17** with Ac_2O. Title compound was obtained as a colorless oil (0.18 g, 95%) upon chromatography on silica gel (EtOAc/hexanes, 2:98): ^{1}H NMR ($CDCl_3$) δ 7.36−7.39 (m, 1 H, ArH), 7.20−7.26 (m, 2 H, ArH), 7.03−7.07 (m, 1 H, ArH), 3.16−3.20 (t, 2 H, J = 7.0 Hz, CH_2), 2.85−2.90 (t, 2 H, J = 7.3 Hz, CH_2), 2.35 (s, 3 H, CH_3), 1.77−1.86 (m, 2 H, CH_2), 1.60−1.68 (m, 2 H, CH_2), 1.42−1.45 (m, 4 H, CH_2); HRMS (CI) calcd for $C_{14}H_{20}IO_2S$ (MH^+) 379.01880, found 379.01895.

General Procedure for the Synthesis of 2-Acetoxyphenyl Alkanol Sulfides. Step 1. 2-Hydroxyphenyl Pentyl Acetate Sulfide (23). To a solution of **1** (0.5 g, 4 mmol) in 10 mL of dry DMF were added $KHCO_3$ (0.48 g, 4.8 mmol) and 5-bromopentyl acetate (1.26 g, 6 mmol). After stirring overnight at room temperature, the solution was diluted with water and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 10:90) gave **23** as a colorless oil (0.79 g, 78%): ^{1}H NMR ($CDCl_3$) δ 7.44−7.47 (dd, 1 H, J = 7.7 and 1.6 Hz, ArH), 7.25−7.26 (m, 1 H, ArH), 6.98−7.00 (dd, 1 H, J = 8.2 and 1.2 Hz, ArH), 6.85−6.89 (m, 1 H, ArH), 6.75 (s, 1 H, OH), 4.01−4.04 (t, 2 H, J = 6.5 Hz, CH_2), 2.67−2.71 (t, 2 H, J = 7.3 Hz, CH_2), 2.04 (s, 3 H, CH_3), 1.43−1.62 (m, 6 H, CH_2).

Step 2. 2-Hydroxyphenyl Pentan-5-ol Sulfide (24). To a solution of **23** (0.66 g, 2.59 mmol) in 5 mL of MeOH/water (3:1) was added K_2CO_3 (0.54 g, 3.89 mmol), and this solution was stirred for 8 h at room temperature. The mixture was extracted with EtOAc (3 × 5 mL), and the combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 40:60) gave **24** as a colorless oil (0.51 g, 92%): ^{1}H NMR ($CDCl_3$) δ 7.44−7.48 (dd, 1 H, J = 7.7 and 1.7 Hz, ArH), 7.24−7.29 (m, 1 H, ArH), 6.97−7.00 (dd, 1 H, J = 8.2 and 1.2 Hz, ArH), 6.84−6.91 (m, 1 H, ArH), 6.75 (s, 1 H, OH), 3.61−3.65 (t, 2 H, J = 6.3 Hz, CH_2), 2.70−2.76 (t, 2 H, J = 7.2 Hz, CH_2), 1.24−1.64 (m, 6 H, CH_2).

Step 3. 2-Acetoxyphenyl Pentan-5-ol Sulfide (62). To a solution of **24** (0.295 g, 1.39 mmol) in 1.4 mL of 1 N NaOH at room temperature was added 1-acetyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (0.25 g, 1.53 mmol) in THF (5.5 mL). The reaction mixture was stirred for 1 h and neutralized with 2 N HCl. The aqueous solution was extracted with Et_2O (3 × 10 mL), and the combined ether extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 20:80) gave the desired product **62** as a colorless oil (0.207 g, 59%): ^{1}H NMR ($CDCl_3$) δ 7.36−7.38 (m, 1 H, ArH), 7.20−7.23 (m, 2 H, ArH), 7.04−7.06 (m, 1 H, ArH), 3.62−3.65 (t, 2 H, J = 6.3 Hz, CH_2), 2.87−2.91 (t, 2 H, J = 7.3 Hz, CH_2), 2.35 (s, 3 H, CH_3), 1.51−1.66 (m, 6 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 169.18, 149.29, 129.97, 129.79, 126.93, 126.53, 122.60, 62.56, 32.90, 32.10, 28.65, 24.84, 20.80; HRMS (CI) calcd for $C_{13}H_{19}O_3S$ (MH^+) 255.10549, found 255.10558.

General Procedure for the Synthesis of 2-Acetoxyphenyl Carboxyalkyl Sulfides. Step 1. 2-Hydroxyphenyl 5-Carboxypentyl Sulfide (20). To a solution of **1** (1.5 g, 12 mmol) in 5 mL of dry DMF were added $KHCO_3$ (1.4 g, 14 mmol) and 6-bromohexanoic acid (2.34 g, 12 mmol). Upon stirring at room temperature for 10 h, the mixture was diluted with water and extracted with Et_2O (3 × 20 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 30:70) gave **20** as a semisolid (2.1 g, 75%): ^{1}H NMR ($CDCl_3$) δ 7.43−7.46 (dd, 1 H, J = 7.7 and 1.6 Hz, ArH), 7.23−7.39 (m, 1 H, ArH), 6.97−7.00 (dd, 1 H, J = 7.0 and 1.0 Hz, ArH), 6.84−6.89 (dt, 1 H, J = 7.6 and 1.4 Hz, ArH), 2.34−2.71 (t, 2 H, J = 7.1 Hz, CH_2), 2.04−2.36 (t, 2 H, J = 7.3 Hz, CH_2), 1.53−1.67 (m, 4 H, CH_2), 1.38−1.48 (m, 2 H, CH_2).

2-Hydroxyphenyl 4-cyanobutyl sulfide (22) was prepared as described above. Title compound was obtained as a crystalline white solid upon recrystallization of the crude product with hexanes (0.61 g, 93%): mp = 77−79 °C; ^{1}H NMR ($CDCl_3$) δ 7.44−7.47 (dd, 1 H, J = 7.7 and 1.5 Hz, ArH), 7.28−7.31 (m, 1 H, ArH), 6.98−7.01 (dd, 1 H, J = 7.0 and 1.1 Hz, ArH), 6.86−6.91 (t, 1 H, J = 7.5 and 1.1 Hz, ArH), 6.66 (s, 1 H, OH), 2.70−2.74 (t, 2 H, J = 6.9 Hz, CH_2), 2.32−2.37 (t, 2 H, J = 6.6 Hz, CH_2), 1.67−1.80 (m, 4 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 135.92, 131.33, 121.35, 120.87, 114.92, 35.50, 28.44, 24.17, 16.81.

Step 2. 2-Acetoxyphenyl 5-carboxypentyl sulfide (58) was prepared by the acetylation of **20** with Ac_2O. The title compound was obtained as a white solid (0.82 g, 87%) upon purification by chromatography on silica gel (EtOAc/hexanes,

20:80 then 40:60): ^{1}H NMR ($CDCl_3$) δ 7.35−7.38 (m, 1 H, ArH), 7.19−7.23 (m, 2 H, ArH), 7.03−7.06 (m, 1 H, ArH), 2.85−2.89 (t, 2 H, J = 7.1 Hz, CH_2), 2.33−2.38 (t merged with a s, 5 H, J = 7.2 Hz, CH_2 and CH_3), 1.60−1.70 (m, 4 H, CH_2), 1.44−1.52 (m, 2 H, CH_2); HRMS (CI) calcd for $C_{14}H_{19}O_4S$ (MH^+) 283.10040, found 283.10019.

2-Acetoxyphenyl 4-cyanobutyl sulfide (60) was prepared by the acetylation of **22** with Ac_2O. The title compound was obtained as a colorless oil (0.39 g, 87%) upon purification by chromatography on silica gel (EtOAc/hexanes, 5:95): ^{1}H NMR ($CDCl_3$) δ 7.38−7.40 (m, 1 H, ArH), 7.22−7.28 (m, 2 H, ArH), 7.05−7.08 (m, 1 H, ArH), 2.89−2.93 (t, 2 H, J = 6.6 Hz, CH_2), 2.35−2.38 (t merged with a s, 5 H, J = 6.9 Hz, CH_2 and CH_3), 1.74−1.82 (m, 4 H, CH_2); ^{13}C NMR ($CDCl_3$) δ 169.09, 149.75, 130.80, 128.82, 127.62, 126.66, 122.78, 119.30, 32.37, 27.79, 24.17, 20.80, 16.73; HRMS (CI) calcd for $C_{13}H_{16}NO_2S$ (MH^+) 250.09018, found 250.09029.

2-[((±)-2-Acetoxyphenyl)thio]oct-3-yne (72). Step 1. (±)-Oct-3-yn-2-ol (83). To a solution of 1-hexyne (**82**; 2 g, 24.3 mmol) in 30 mL of freshly distilled THF at −78 °C was added nBuLi (2.5 M solution in hexanes, 11 mL, 27 mmol) under argon. The resultant yellow solution was stirred at −78 °C for 30 min and at 0 °C for 15 min. Acetaldehyde (∼2 mL) was added to this solution at −78 °C, and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 30 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 1:99 then 10:90) gave **83** as a colorless oil (2.1 g, 73%): ^{1}H NMR ($CDCl_3$) δ 4.48−4.52 (q, 1 H, J = 6.0 Hz, CH), 2.17−2.22 (d of t, 2 H, J = 6.7 and 1.5 Hz, CH_2), 1.37−1.65 (complex m, 7 H, 4 CH_2 and 1 CH_3), 0.88−0.93 (t, 3 H, J = 7.2 Hz, CH_3).

Step 2. 2-Bromooct-3-yne (84) was prepared according to the procedure described for 1-bromohept-2-yne. Title compound was obtained as a colorless oil (0.9 g, 65%) upon purification on silica gel (EtOAc/hexanes, 1:99): ^{1}H NMR ($CDCl_3$) δ 4.64−4.67 (m, 1 H, CH), 2.21−2.26 (m, 2 H, CH_2), 1.88−1.9 (d, 3 H, J = 6.8 Hz, CH_3), 1.36−1.54 (m, 4 H, CH_2), 0.88−0.93 (t, 3 H, J = 6.9 Hz, CH_3).

Step 3. 2-[((±)-2-Hydroxyphenyl)thio]oct-3-yne (34) was prepared according to the procedure described for **29**. Title compound was obtained as a colorless oil (0.1 g, 55%) upon purification on silica gel (EtOAc/hexanes, 5:95): ^{1}H NMR ($CDCl_3$) δ 7.49−7.52 (dd, 1 H, J = 7.7 and 1.4 Hz, ArH), 7.26−7.33 (t, 1 H, J = 8.5 and 1.4 Hz, ArH), 6.98−7.01 (dd, 1 H, J = 8.1 Hz, 1.4 Hz, ArH), 6.85−6.90 (m and s, 2 H, ArH and OH), 3.69−3.73 (m, 1 H, CH), 2.12−2.21 (m, 2 H, CH_2), 1.19−1.49 (d and m, 7 H, CH_3 and 2 CH_2), 0.86−0.97 (t, 3 H, J = 7.2 Hz, CH_3).

Step 4. 2-[((±)-2-Acetoxyphenyl)thio]oct-3-yne (72) was prepared according to the procedure described for **70**. Title compound was obtained as a colorless oil (91 mg, 78%) upon purification on silica gel (EtOAc/hexanes, 5:95): ^{1}H NMR ($CDCl_3$) δ 7.60−7.65 (dd, 1 H, J = 7.6 and 1.6 Hz, ArH), 7.17−7.35 (m, 2 H, ArH), 7.06−7.10 (dd, 1 H, J = 7.8 and 1.4 Hz, ArH), 3.90−3.96 (m, 1 H, CH), 2.34 (s, 3 H, CH_3), 2.11−2.19 (m, 2 H, CH_2), 1.24−1.49 (m merged with d, 7 H, 2 CH_2 and CH_3), 0.84−0.91 (t, 3 H, J = 7.1 Hz, CH_3); HRMS (CI) calcd for $C_{16}H_{21}O_2S$ (MH^+) 277.12623, found 277.12635.

2-(α-Bromoacetoxy)phenyl Hept-2-ynyl Sulfide (75). To a solution of **32** (0.2 g, 0.9 mmol) in 2 mL of anhydrous CH_2Cl_2 at 0 °C were added anhydrous pyridine (71 mg, 0.9 mmol) and α-bromoacetyl bromide (0.18 g, 0.9 mmol) under argon. The solution was stirred at room temperature for 6 h. The reaction was carefully quenched with water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 2:98) gave **75** as a colorless oil (0.26 g, 84%): ^{1}H NMR ($CDCl_3$) δ 7.56−7.59 (dd, 1 H, J = 7.2 and 1.9 Hz, ArH), 7.23−7.33 (m, 2 H, ArH), 7.10−7.13 (dd, 1 H, J = 7.5 and 2.0 Hz, ArH), 4.12 (s, 2 H, CH_2), 3.57−3.59 (t, 2 H, J = 2.2 Hz, CH_2), 2.12−2.18 (m, 2 H, CH_2), 1.27−1.45 (m, 4 H, CH_2), 0.85−0.90 (t, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.28, 149.35, 131.92, 128.40, 128.11, 126.90, 122.05, 84.61, 74.84, 30.55, 25.51, 22.68, 21.77, 18.39, 13.51; FAB-MS 341 (MH^+, 65), 221 (50), 95 (70), 79 (100); HRMS (CI) calcd for $C_{15}H_{18}BrO_2S$ (MH^+) 341.02109, found 341.02081.

2-(Carbamoyloxy)phenyl Hept-2-ynyl Sulfide (76). To a solution of **32** (1 g, 4.54 mmol) in 10 mL of anhydrous CH_2Cl_2 at 0 °C was added chlorosulfonyl isocyanate (1.9 g, 13.5 mmol) under argon. The solution was stirred at room temperature for 3 h. The reaction was quenched with water and extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 10:90, then 50:50) gave the desired carbamate **76** as a pale-yellow solid (0.5 g, 42%): mp = 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 7.15−7.30 (m, 4 H, ArH), 4.81−5.10 (bs, 2 H, NH_2), 3.60−3.62 (t, 2 H, J = 2.2 Hz, CH_2), 2.13−2.18 (m, 2 H, CH_2), 1.30−1.48 (m, 4 H, CH_2), 0.85−0.90 (t, 3 H, J = 7.1 Hz, CH_3); HRMS (CI) calcd for $C_{14}H_{18}NO_2S$ (MH^+) 264.10582, found 264.10576.

2-Acetoxyphenyl Heptyl Sulfoxide (77). To a solution containing **46** (0.15 g, 0.53 mmol) in acetone/water (2:1, 6 mL) was added Oxone (0.36 g, 0.56 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 45 min and then at room temperature for 15 min. Water was added to the mixture followed by extraction with EtOAc (2 × 10 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 30:70) gave **77** as a colorless oil (0.137 g, 87%): ^{1}H NMR ($CDCl_3$) δ 7.88−7.91 (dd, 1 H, J = 7.2 and 1.6 Hz, ArH), 7.46−7.54 (m, 2 H, ArH), 7.16−7.19 (dd, 1 H, J = 7.8 and 1.6 Hz, ArH), 2.85−2.93 (m, 1 H, CH_2), 2.75−2.82 (m, 1 H, CH_2), 2.32 (s, 3 H, CH_3), 1.61−1.81 (m, 1 H, CH_2), 1.42−1.47 (m, 1 H, CH_2), 1.23−1.39 (m, 8 H, CH_2), 0.85−0.89 (t, 3 H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 168.14, 146.14, 153.82, 131.70, 126.86, 125.38, 122.57, 55.36, 31.38, 28.64, 28.39, 22.37, 22.02, 20.57, 13.88; HRMS (CI) calcd for $C_{15}H_{23}O_3S$ (MH^+) 283.13679, found 283.13666.

General Procedure for the Synthesis of 2-Acetoxyphenyl Alkyl Sulfones. Step 1. Oxidation of 2-Hydroxyphenyl Alkyl Sulfides: 2-Hydroxyphenyl Methyl Sulfone (78). To a solution containing 2-hydroxythioanisole (**2**; 1 g, 7.13 mmol) in 20 mL of glacial AcOH was added 30% H_2O_2 (14 mL) dropwise at 0 °C. After the addition was complete, the reaction was stirred at 100 °C for 4 h and then allowed to stir overnight at room temperature. The solution was concentrated in vacuo, and the residue was purified by chromatography on silica gel (EtOAc/hexanes, 10:90) to afford **78** as a white solid (0.8 g, 65%) which was further recrystallized from EtOH/H_2O: mp = 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1 H, OH), 7.67−7.71 (dd, 1 H, J = 8.2 and 1.6 Hz, ArH), 7.51−7.56 (t, 1 H, J = 8.7 and 1.6 Hz, ArH), 7.02−7.06 (m, 2 H, ArH), 3.13 (s, 3 H, CH_3); FAB-MS 173 (MH^+, 55), 157 (55), 93 (30), 79 (100).

2-Hydroxyphenyl heptyl sulfone (79) was similarly prepared by the oxidation of **8** with H_2O_2. The sulfone **79** was obtained as a colorless oil (0.212 g, 62%) upon purification on silica gel (EtOAc/hexanes, 10:90): ^{1}H NMR ($CDCl_3$) δ 7.62−7.64 (dd, 1 H, J = 6.9 Hz, 1.2 Hz, ArH), 7.51−7.55 (t, 1 H, J = 7.1 Hz, ArH), 7.01−7.05 (m, 2 H, ArH), 3.11−3.15 (t, 2 H, J = 8.0 Hz, CH_2), 1.70−1.78 (m, 2 H, CH_2), 1.24−1.36 (m, 8 H, CH_2), 0.84−0.87 (t, 3 H, J = 7.2 Hz, CH_3).

Step 2. 2-Acetoxyphenyl methyl sulfone (80) was prepared by the acetylation of **78** with Ac_2O. Title compound was recrystallized from EtOH/H_2O to afford a crystalline white solid (1.12 g, 91%): mp = 107−109 °C; ^{1}H NMR ($CDCl_3$) δ 8.01−8.04 (dd, 1 H, J = 7.9 and 1.6 Hz, ArH), 7.65−7.68 (t, 1 H, J = 7.7 and 1.5 Hz, ArH), 7.41−7.46 (t, 1 H, J = 7.7 and 1.0 Hz, ArH), 7.25−7.28 (d, 1 H, J = 8.0 Hz, ArH), 3.12 (s, 3 H, CH_3), 2.30 (s, 3 H, CH_3).

2-Acetoxyphenyl heptyl sulfone (81) was prepared by the acetylation of **79** with Ac_2O. Title compound was obtained as a colorless oil (0.148 g, 96%) upon purification on silica gel (EtOAc/hexanes, 5:95): ^{1}H NMR ($CDCl_3$) δ 7.98−8.00 (dd, 1 H, J = 7.9 and 1.6 Hz, ArH), 7.65−7.69 (t, 1 H, J = 7.9 and

1.6 Hz, ArH), 7.41−7.45 (t, 1 H, J = 7.7 Hz, ArH), 7.24−7.26 (d, 1 H, ArH), 3.21−3.25 (t, 2 H, J = 7.9 Hz, CH_2), 2.37 (s, 3 H, CH_3), 1.62−1.72 (m, 2 H, CH_2), 1.24−1.38 (m, 8 H, CH_2), 0.84−0.87 (t, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 168.42, 148.62, 134.96, 131.08, 130.72, 126.51, 125.03, 55.37, 31.33, 28.58, 28.16, 22.43, 22.36, 20.94, 13.95; FAB-MS 299 (MH^+, 40), 257 (100), 79 (24); HRMS (CI) calcd for $C_{15}H_{23}O_4S$ (MH^+) 299.13170, found 299.13154.

2-(Methylsulfonyloxy)phenyl Hept-2-ynyl Sulfide (85). To a solution of **32** (0.2 g, 0.9 mmol) in 2 mL of anhydrous CH_2Cl_2 at 0 °C were added anhydrous pyridine (71 mg, 0.9 mmol) and methanesulfonyl chloride (0.1 g, 0.9 mmol) under argon. The solution was stirred at room temperature for 6 h. The reaction was quenched with water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave the desired methylsulfonyloxy derivative **85** as a colorless oil (0.22 g, 84%): ^{1}H NMR ($CDCl_3$) δ 7.51−7.53 (dd, 1 H, J = 7.6 and 2.6 Hz, ArH), 7.40−7.42 (dd, 1 H, J = 7.9 and 2.8 Hz, ArH), 7.26−7.30 (m, 2 H, ArH), 3.66 (t, 2 H, J = 1.3 Hz CH_2), 3.24 (s, 3 H, CH_3), 2.11−2.13 (m, 2 H, CH_2), 1.26−1.41 (m, 4 H, CH_2), 0.84−0.87 (t, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 147.63, 130.77, 129.18, 127.82, 127.59, 123.20, 84.74, 74.54, 38.50, 30.52, 21.97, 21.77, 18.37, 13.53.

2-Acetoxy-3-(methylthio)benzoic Acid (90). Step 1. 2-(Methoxymethyleneoxy)thioanisole (86). A solution of 2-hydroxythioanisole (**2**; 1 g, 7.14 mmol) in 30 mL of freshly distilled CH_3CN was treated with potassium fluoride-activated alumina powder (8 g) and methoxymethyl chloride (0.72 g, 9.0 mmol), and this mixture was stirred at room temperature for 12 h under argon. The solution was filtered over Celite, and the filtrate was concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) afforded **86** as a colorless oil (1.1 g, 85%): ^{1}H NMR ($CDCl_3$) δ 7.01−7.17 (m, 4 H, ArH), 5.25 (s, 2 H, CH_2), 3.52 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3); FAB-MS 184 ($MH^+ - 1$, 20), 167 (25), 149 9 (100).

Step 2. Methyl 3-(Methylthio)-2-(methoxymethyleneoxy)benzoate (87). To a solution of **86** (1.06 g, 5.7 mmol) in 25 mL of freshly distilled THF at 0 °C was added nBuLi (2.5 M solution in hexanes, 2.6 mL, 6.27 mmol) under argon. The resultant yellow solution was stirred at 0 °C for 1 h. Following the addition of methyl chloroformate (1.1 g, 11.7 mmol) at −78 °C, the solution was stirred at room temperature for 24 h. The reaction was quenched with saturated NH_4Cl and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic solution was washed with brine and water, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford an oil. Chromatography on silica gel (EtOAc/hexanes, 4:96 then 10:90) gave **87** as a colorless oil (0.61 g, 44%): ^{1}H NMR ($CDCl_3$) δ 7.55−7.58 (dd, 1 H, J = 7.7 and 1.6 Hz, ArH), 7.28−7.31 (dd, 1 H, J = 7.8 and 1.6 Hz, ArH), 7.13−7.18 (t, 1 H, J = 7.8 Hz, ArH), 5.10 (s, 2 H, CH_2), 3.90 (s, 3 H, CH_3), 3.63 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3); FAB-MS 243 ($MH^+ - 1$, 30), 211 (100), 166 (20).

Step 3. Methyl 3-(Methylthio)salicylate (88). A reaction mixture of **87** (0.6 g, 2.47 mmol) in THF (230 μL), water (2 mL), and 6 M HCl (5 mL) was heated at 60 °C for 6 h. The solution was poured into an equal volume of brine and extracted with Et_2O (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **88** as a crystalline white solid (0.3 g, 61%): ^{1}H NMR ($CDCl_3$) δ 11.38 (s, 1 H, OH), 7.65−7.69 (dd, 1 H, J = 8.0 and 1.5 Hz, ArH), 7.35−7.38 (dd, 1 H, J = 7.6 and 1.3 Hz, ArH), 6.86−6.91 (t, 1 H, J = 7.8 Hz, ArH), 3.96 (s, 3 H, CH_3), 2.46 (s, 3 H, CH_3); FAB-MS 199 (MH^+, 70), 198 (M^+, 95), 167 (100), 153 (8), 138 (10).

Step 4. 3-(Methylthio)salicylic Acid (89). A reaction mixture containing **88** (50 mg, 0.25 mmol) and powdered KOH (56 mg, 1 mmol) in EtOH/H_2O (3.6 mL:0.36 mL) was heated under reflux for 3.5 h. The solution was cooled to room temperature and acidified with 1 M HCl. The aqueous solution was extracted with EtOAc (3 × 10 mL). The combined organic solution was washed with brine and water, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford **89** as a crystalline white solid (38 mg, 82%): ^{1}H NMR (DMSO-d_6) δ 7.57−7.60 (d, 1 H, J = 7.8 Hz, ArH), 7.39−7.41 (d, 1 H, J = 7.5 Hz, ArH), 6.92−6.97 (t, 1 H, J = 7.7 Hz, ArH), 2.41 (s, 3 H, CH_3); FAB-MS 185 (MH^+, 20), 184 (M^+, 20), 167 (75), 102 (70), 79 (100).

Step 5. 2-Acetoxy-3-(methylthio)benzoic Acid (90). A reaction mixture containing **89** (46 mg, 0.25 mmol), dry pyridine (50 μL, 0.61 mmol), and acetyl chloride (43 μL, 0.61 mmol) in 2 mL of dry CH_2Cl_2 was stirred at room temperature for 12 h. The solution was concentrated in vacuo, and the residue was partitioned between water and EtOAc. The organic solution was washed with 1 M HCl and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 70:30 then 50:50) gave the desired acetate **90** as a white solid (22 mg, 40%): ^{1}H NMR ($CDCl_3$) δ 7.86−7.89 (d, 1 H, J = 7.7 Hz, ArH), 7.46−7.44 (d, 1 H, J = 7.6 Hz, ArH), 7.32−7.36 (t, 1 H, J = 7.8 Hz, ArH), 2.45 (s, 3 H, CH_3), 2.38 (s, 3 H, CH_3); FAB-MS 227 (MH^+, 15), 226 (M^+, 10), 167 (50), 157 (30), 102 (100); HRMS (CI) calcd for $C_{10}H_{11}O_4S$ (MH^+) 227.03780, found 227.03773.

2-Acetoxy-3-fluorophenyl Methyl Sulfide (96). Step 1. 2-Fluoro-1-(methoxymethyl)phenol (93). To a solution of 2-fluorophenol (**92**; 2 g, 17.84 mmol) in 30 mL of dry pyridine was added powdered KOH (1 g, 17.71 mmol). The resulting solution was treated with methoxymethyl chloride (1.8 g, 22.49 mmol), heated to reflux for 3.5 h, cooled, and partitioned between 1 M NaOH and Et_2O. The organic solution was washed with 1 M HCl (2 × 30 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **93** as a yellow oil (2 g, 74%): ^{1}H NMR ($CDCl_3$) δ 6.95−7.22 (m, 4 H, ArH), 5.21 (s, 2 H, CH_2), 3.52 (s, 3 H, CH_3).

Step 2. 3-Fluoro-2-(methoxymethyleneoxy)phenyl Methyl Sulfide (94). To a solution of **93** (1 g, 6.4 mmol) in 25 mL of freshly distilled THF at −78 °C was added nBuLi (2.5 M solution in hexanes, 3 mL, 7.25 mmol) under argon. The resultant yellow solution was stirred at −78 °C for 2.5 h, and then dimethyl disulfide (0.68 g, 7.25 mmol) was added to the solution which was stirred at room temperature for 24 h. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 30 mL). The combined organic solution was washed with brine and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **94** as a pale-yellow oil (0.81 g, 79%): ^{1}H NMR ($CDCl_3$) δ 7.00−7.04 (m, 1 H, ArH), 6.89−6.92 (m, 2 H, ArH), 5.18 (s, 2 H, CH_2), 3.64 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.92, 153.64, 124.54, 124.43, 120.70, 120.66, 113.22, 113.96, 98.58, 98.50, 57.60, 14.74; FAB-MS 203 (MH^+, 5), 202 (M^+, 10), 185 (15), 171 (20), 157 (35), 137 (20), 93 (34), 79 (100).

Step 3. 3-Fluoro-2-hydroxyphenyl Methyl Sulfide (95). A reaction mixture of **94** (0.57 g, 2.8 mmol) in THF (230 μL), water (2 mL), and 6 M HCl (5 mL) was heated at 60 °C for 6 h. The solution was poured into an equal volume of brine and extracted with Et_2O (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **95** as a pale-yellow oil (0.19 g, 46%): ^{1}H NMR ($CDCl_3$) δ 7.17−7.21 (dd, 1 H, J = 9.0 and 1.2 Hz, ArH), 7.00−7.06 (t, 1 H, J = 8.3 and 1.3 Hz, ArH), 6.79−6.86 (m, 1 H, ArH), 6.23−6.24 (d, 1 H, J = 1.9 Hz, OH), 2.38 (s, 3 H, CH_3).

Step 4. 2-Acetoxy-3-fluorophenyl Methyl Sulfide (96). A reaction mixture containing **95** (0.14 g, 0.9 mmol), dry pyridine (74 μL, 0.92 mmol), and Ac_2O (74 μL, 0.92 mmol) in 2 mL of dry CH_2Cl_2 was stirred at room temperature for 5 h. Water was added to the reaction mixture, and the aqueous solution was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with water, dried ($MgSO_4$), and filtered, and the solvent was concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 10:90) gave the desired acetate **96** as a yellow oil (0.13 g, 71%): ^{1}H NMR ($CDCl_3$) δ 7.14−7.18 (m, 1 H, ArH), 6.96−7.02 (m, 2 H, ArH), 2.44 (s, 3 H, CH_3), 2.37 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 167.69, 156.09,

134.35, 126.94, 126.83, 121.63, 121.59, 113.23, 112.98, 20.20, 15.17; HRMS (CI) calcd for $C_9H_{10}FO_2S$ (MH^+) 201.03856, found 201.03867.

2-Acetoxy-3,5-difluorophenyl Methyl Sulfide (103). Step 1. 2,4-Difluoro-1-(methoxymethyl)phenol (98). To a solution of 2,4-fluorophenol (**97**; 2 g, 15.37 mmol) in 30 mL of dry pyridine was added powdered KOH (0.85 g, 15.25 mmol). The resulting solution was treated with methoxymethyl chloride (1.6 g, 19.37 mmol), heated to reflux for 3.5 h, cooled, and partitioned between 1 M NaOH and Et_2O. The organic solution was washed with 1 M HCl (2 × 30 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **98** as a yellow oil (1.5 g, 56%): ^{1}H NMR ($CDCl_3$) δ 7.10−7.16 (m, 1 H, ArH), 6.78−6.88 (m, 2 H, ArH), 5.15 (s, 2 H, CH_2), 3.52 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 159.058, 119.110, 119.01, 110.76, 110.71, 110.46, 110.42, 105.08, 104.78, 104.73, 104.43, 96.31, 56.28.

Step 2. 2,4-Difluoro-3-(trimethylsilyl)-1-(methoxymethyl)phenol (99). To a solution of **98** (0.84 g, 4.8 mmol) in 20 mL of freshly distilled THF at −78 °C was added nBuLi (2.5 M solution in hexanes, 2.2 mL, 5.43 mmol) under argon. The resultant pink solution was stirred at −78 °C for 2 h, and then trimethylsilyl chloride (1.0 M solution in THF, 5.43 mL, 5.43 mmol) was added to the solution which was then stirred at room temperature for 24 h. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 20 mL). The combined organic solution was washed with brine and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **99** as a pale-yellow oil (0.8 g, 67%): ^{1}H NMR ($CDCl_3$) δ 7.09−7.17 (m, 1 H, ArH), 6.68−6.74 (t, 1 H, J = 8.0 Hz, ArH), 5.13 (s, 2 H, CH_2), 3.52 (s, 3 H, CH_3), 0.36−0.37 (t, 9 H, J = 1.4 Hz, $Si(CH_3)_3$).

Step 3. 3,5-Difluoro-2-(methoxymethyleneoxy)-4-(trimethylsilyl)phenyl Methyl Sulfide (100). To a solution of **99** (0.63 g, 2.56 mmol) in 20 mL of freshly distilled THF at −78 °C was added nBuLi (2.5 M solution in hexanes, 1.2 mL, 2.9 mmol) under argon. The resultant yellow solution was stirred at −78 °C for 2 h, and then dimethyl disulfide (0.27 g, 2.9 mmol) was added to the solution which was stirred at room temperature for 24 h. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 20 mL). The combined organic solution was washed with brine and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **100** as a pale-yellow oil (0.51 g, 68%): ^{1}H NMR ($CDCl_3$) δ 6.55−6.58 (t, 1 H, J = 9.0 and 1.2 Hz, ArH), 5.09 (s, 2 H, CH_2), 3.63 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 0.34−0.35 (t, 9 H, J = 1.4 Hz, $Si(CH_3)_3$); FAB-MS 293 (MH^+, 24), 292 (M^+, 70), 262 (66), 261 (100).

Step 4. 3,5-Difluoro-2-(methoxymethyleneoxy)phenyl Methyl Sulfide (101). To a reaction mixture containing **100** (0.51 g, 1.74 mmol) in 10 mL of freshly distilled THF at −78 °C were added trifluoroethanol (0.18 g, 1.82 mmol) and tetrabutylammonium fluoride (1 M solution in THF, 1.74 mL, 1.74 mmol) under argon. The solution was stirred at −78 °C for 20 min and at room temperature for 30 min. The reaction was quenched with water and extracted with Et_2O (2 × 15 mL). The combined ether extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **101** as a colorless oil (0.27 g, 71%): ^{1}H NMR ($CDCl_3$) δ 6.60−6.64 (m, 2 H, ArH), 5.11 (s, 2 H, CH_2), 3.63 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3).

Step 5. 3,5-Difluoro-2-hydroxyphenyl Methyl Sulfide (102). A reaction mixture of **101** (0.27 g, 1.22 mmol) in THF (200 μL), water (1 mL), and 6 M HCl (1 mL) was heated at 60 °C for 4 h. The solution was poured into an equal volume of brine and extracted with Et_2O (3 × 10 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **102** as a pale-yellow oil (0.15 g, 53%): ^{1}H NMR ($CDCl_3$) δ 6.87−6.91 (m, 1 H, ArH), 6.75−6.82 (m, 1 H, ArH), 5.85 (d, 1 H, J = 2.1 Hz, OH), 2.41 (s, 3 H, CH_3).

Step 6. 2-Acetoxy-3,5-difluorophenyl Methyl Sulfide (103). A reaction mixture containing **102** (0.11 g, 0.62 mmol), dry pyridine (60 μL, 0.74 mmol), and Ac_2O (69 μL, 0.74 mmol) in 2 mL of dry CH_2Cl_2 was stirred at room temperature for 10 h. Water was added to the reaction mixture, and the aqueous solution was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with water, dried ($MgSO_4$), and filtered, and the solvent was concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave the desired acetate **103** as a yellow oil (75 mg, 56%): ^{1}H NMR ($CDCl_3$) δ 6.67−6.73 (m, 2 H, ArH), 2.43 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3); FAB-MS 219 (MH^+, 28), 218 (M^+, 30), 176 (84), 157 (70), 149 (42), 121 (46), 79 (100); HRMS (CI) calcd for $C_9H_9F_2O_2S$ (MH^+) 219.02913, found 219.02906.

2-(Bromomethyl)phenyl Heptyl Sulfide (107). Step 1. 2-(Hydroxymethyl)thiophenol (105). To a solution of thiosalicylic acid (**104**; 1.8 g, 11.7 mmol) in 60 mL of dry THF at 0 °C was added borane−THF (1 M solution in THF, 35 mL, 35 mmol), and the reaction mixture was heated under reflux overnight. The solution was cooled to 0 °C, and MeOH was added dropwise until gas evolution ceased. The solution was diluted with EtOAc (100 mL) and washed with 1 N HCl, saturated $NaHCO_3$, and water. Combined aqueous layers were neutralized and reextracted with Et_2O (3 × 100 mL). The combined organic solution was dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 20:80) gave **105** as a pale-yellow oil (1.17 g, 71%): ^{1}H NMR ($CDCl_3$) δ 7.31−7.37 (m, 2 H, ArH), 7.16−7.22 (m, 2 H, ArH), 4.74 (s, 2 H, CH_2), 3.69 (s, 1 H, SH), 1.81 (bs, 1 H, OH).

Step 2. 2-(Hydroxymethyl)phenyl Heptyl Sulfide (106). To a solution of **105** (40 mg, 0.35 mmol) in 1 mL of dry DMF were added $KHCO_3$ (39 mg, 0.39 mmol) and 1-iodoheptane (85 mg, 0.39 mmol). The reaction mixture was stirred overnight, diluted with water, and extracted with Et_2O (3 × 5 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **106** as a pale-yellow oil (82 mg, 97%): ^{1}H NMR ($CDCl_3$) δ 7.35−7.39 (m, 2 H, ArH), 7.18−7.29 (m, 2 H, ArH), 4.77−4.79 (d, 2 H, J = 7.7 Hz, CH_2), 2.90−2.95 (t, 2 H, J = 9.9 Hz, CH_2), 2.21−2.25 (bt, 1 H, OH), 1.60−1.70 (m, 2 H, CH_2), 1.27−1.44 (m, 8 H, CH_2), 0.86−0.90 (t, 3 H, J = 6.7 Hz, CH_3).

Step 3. 2-(Bromomethyl)phenyl Heptyl Sulfide (107). To a solution of **106** (0.25 g, 1.05 mmol) in 4 mL of dry THF at 0 °C was added PBr_3 (1 M solution in CH_2Cl_2, 1 mL, 1 mmol). After the solution stirred for 90 min, the reaction was quenched with MeOH (130 μL) and water (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic solution was washed with saturated $NaHCO_3$ and water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (hexanes) gave **107** as a colorless oil (0.23 g, 72%): ^{1}H NMR ($CDCl_3$) δ 7.35−7.39 (m, 2 H, ArH), 7.23−7.29 (m, 1 H, ArH), 7.14−7.19 (m, 1 H, ArH), 4.69 (s, 2 H, CH_2), 2.93−2.98 (t, 2 H, J = 7.3 Hz, CH_2), 1.62−1.69 (m, 2 H, CH_2), 1.40−1.45 (m, 2 H, CH_2), 1.23−1.35 (m, 6 H, CH_2), 0.85−0.90 (t, 3 H, J = 6.5 Hz CH_3); ^{13}C NMR ($CDCl_3$) δ 137.18, 130.57, 129.53, 129.10, 126.17, 33.93, 32.14, 31.67, 29.03, 28.81, 22.57, 14.06; HRMS (CI) calcd for $C_{14}H_{22}BrS$ (MH^+) 301.06253, found 301.06264.

2-Acetoxyphenyl Heptyl Selenide (111). Step 1. 2-Methoxyphenyl Heptyl Selenide (109). To a solution of anhydrous anisole (**108**; 2.5 g, 23.11 mmol) in 25 mL of freshly distilled THF at 0 °C was added nBuLi (2.5 M solution in hexanes, 10 mL, 25 mmol) under argon. The resultant orange solution was stirred at 0 °C for 1 h and at room temperature for 30 min under argon. Selenium powder (1.97 g, 25 mmol) was added to this solution at −78 °C which was allowed to stir at room temperature for 2 h. The reaction mixture was then cooled to −78 °C and treated with 1-iodoheptane (5.65 g, 25 mmol), and this solution was stirred at room temperature

for 12 h. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 30 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 4:96) gave **109** as a colorless oil (5.34 g, 85%): ^{1}H NMR ($CDCl_3$) δ 7.30−7.33 (d, 1 H, J = 7.5 and 1.4 Hz, ArH), 7.16−7.22 (d of t, 1 H, J = 7.0 and 1.5 Hz, ArH), 6.90−6.93 (t, 1 H, J = 7.5 Hz, ArH), 6.81−6.88 (d, 1 H, J = 8.1 Hz, ArH), 3.88 (s, 3 H, CH_3), 2.86−2.91 (t, 2 H, J = 7.4 Hz, CH_2), 1.67−1.77 (m, 2 H, J = 7.0 Hz, CH_2), 1.37−1.46 (m, 2 H, CH_2), 1.27−1.34 (m, 6 H, CH_2), 0.85−0.89 (t, 3 H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 157.51, 130.45, 127.15, 121.34, 120.16, 112.94, 110.22, 55.76, 31.69, 29.97, 29.70, 28.78, 25.03, 22.59, 14.07.

Step 2. 2-Hydroxyphenyl Heptyl Selenide (110). To a solution of **109** (2 g, 7.02 mmol) in 20 mL of dry CH_2Cl_2 at −78 °C was added boron tribromide (1 M solution in CH_2Cl_2, 8.02 mL, 8.02 mmol) under argon. The solution was stirred overnight at room temperature. The reaction was carefully quenched with water and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 5:95) gave **110** as a pale-yellow oil (1.71 g, 89%): ^{1}H NMR ($CDCl_3$) δ 7.54−7.57 (dd, 1 H, J = 7.6 and 1.5 Hz, ArH), 7.24−7.29 (t, 1 H, ArH), 6.99−7.02 (dd, 1 H, J = 8.1 and 1.1 Hz, ArH), 6.81−6.85 (dt, 1 H, J = 7.6 and 1.3 Hz, ArH), 6.61 (s, 1 H, OH), 2.65−2.72 (t, 2 H, J = 7.5 Hz, CH_2), 1.56−1.66 (m, 2 H, CH_2), 1.24−1.39 (m, 8 H, CH_2), 0.84−0.88 (t, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.64, 137.50, 131.24, 120.73, 115.47, 114.24, 32.35, 31.65, 30.28, 29.53, 28.67, 22.55. 14.04.

Step 3. 2-Acetoxyphenyl heptyl selenide (111) was prepared by the acetylation of **110** with Ac_2O. Title compound was obtained as a colorless oil (0.48 g, 94%) upon purification by chromatography on silica gel (EtOAc/hexanes, 5:95): ^{1}H NMR ($CDCl_3$) δ 7.47−7.50 (dd, 1 H, J = 7.6 and 1.4 Hz, ArH), 7.23−7.29 (t, 1 H, ArH), 7.13−7.19 (t, 1 H, J = 7.4 and 1.3 Hz, ArH), 7.04−7.07 (dd, 1 H, J = 7.9 and 1.1 Hz, ArH), 2.84−2.89 (t, 2 H, J = 7.5 Hz, CH_2), 2.34 (s, 3 H, CH_3), 1.63−1.72 (m, 2 H, CH_2), 1.26−1.43 (m, 8 H, CH_2), 0.85−0.89 (t, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.04, 150.31, 132.86, 130.21, 127.75, 126.55, 122.38, 31.61, 29.77, 29.70, 28.64, 26.95, 22.50, 20.88, 13.98; HRMS (CI) calcd for $C_{15}H_{23}O_2Se$ (MH^+) 315.08631, found 315.08624.

2-Octylphenyl Acetate (116). Step 1. (Methoxymethyleneoxy)phenol (113). To a solution of phenol (**112**; 1 g, 10.63 mmol) in 30 mL of freshly distilled CH_3CN were added potassium fluoride-activated alumina powder (8 g) and methoxymethyl chloride (1.7 g, 21 mmol), and this reaction mixture was allowed to stir at room temperature for 12. The solution was filtered over Celite, and the filtrate was concentrated in vacuo. Chromatography on silica gel (hexanes) gave **113** as a colorless oil (0.8 g, 53%): ^{1}H NMR ($CDCl_3$) δ 7.25−7.32 (m, 2 H, ArH), 7.00−7.05 (m, 3 H, ArH), 5.18 (s, 2 H, CH_2), 3.48 (s, 3 H, CH_3).

Step 2. 1-[2-(Methoxymethyleneoxy)phenyl]octane (114). To a solution containing **113** (713 mg, 5.16 mmol) in 10 mL of freshly distilled THF at 0 °C was added nBuLi (2.5 M solution in hexane, 2.16 mL, 5.4 mmol) under argon. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 15 min. 1-Iodooctane (1.3 g, 5.4 mmol) was added to this solution at −78 °C which was allowed to stir for 12 h at room temperature. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 10 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (hexanes) gave **114** as a colorless oil (0.9 g, 69%): ^{1}H NMR ($CDCl_3$) δ 7.25−7.29 (m, 1 H, ArH), 7.11−7.15 (m, 1 H, ArH), 7.03−7.06 (m, 1 H, ArH), 6.90−6.95 (m, 1 H, ArH), 5.19 (s, 2 H, CH_2), 3.48 (s, 3 H, CH_3), 2.59−2.64 (t, 2 H, J = 7.4 Hz, CH_2), 1.58−1.60 (m, 2 H, CH_2), 1.26−1.31 (m, 10 H, CH_2), 0.85−0.89 (t, 3 H, J = 6.9 Hz, CH_3).

Step 3. 2-Octylphenol (115). A solution of **114** (350 mg, 1.4 mmol) in THF (230 μL), water (2 mL), and 6 M HCl (5 mL) was heated at 60 °C for 6 h. The reaction mixture was poured into an equal volume of brine and extracted with Et_2O (3 × 10 mL). The combined ether solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 2:98) gave **115** as a colorless oil (90 mg, 31%): ^{1}H NMR ($CDCl_3$) δ 7.05−7.12 (m, 2 H, ArH), 6.81−6.89 (m, 1 H, ArH), 6.74−6.77 (m, 1 H, ArH), 4.65 (s, 1 H, OH), 2.56−2.62 (t, 2 H, J = 7.6 Hz, CH_2), 1.58−1.65 (m, 2 H, CH_2), 1.26−1.32 (m, 10 H, CH_2), 0.85−0.89 (t, 3 H, J = 7.0 Hz, CH_3).

Step 4. 2-Octylphenyl acetate (116) was prepared by the acetylation of **115** with Ac_2O. The crude product was purified by chromatography on silica gel (EtOAc/hexanes, 1:99) to afford the desired acetate as a pale-yellow oil (34 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 7.16−7.25 (m, 3 H, ArH), 6.99−7.02 (m, 1 H, ArH), 2.47−2.52 (t, 2 H, J = 7.6 Hz, CH_2), 2.32 (s, 3 H, CH_3), 1.53−1.55 (m, 2 H, CH_2), 1.26−1.29 (m, 10 H, CH_2), 0.85−0.90 (t, 3 H, J = 6.9 Hz, CH_3); HRMS (CI) calcd for $C_{16}H_{25}O_2$ (MH^+) 249.18546, found 249.18551.

2-Acetoxyphenyl-*N*-heptyl-*N*-methylamine (121). Step 1. 2-Hydroxyphenylmethylamine (118). A solution of 2-aminophenol (**117**; 1 g, 9.16 mmol) in dry DMF (5 mL) was treated with $KHCO_3$ (0.95 g, 9.5 mmol) and CH_3I (1.56 g, 10.99 mmol) and stirred at room temperature for 5 h. The solution was diluted with water and extracted with EtOAc (3 × 20 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (hexanes) to afford 2-hydroxyphenyl-*N*,*N*-dimethylamine (**119**; 0.51 g, 40%) as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 7.77 (s, 1 H, OH), 6.68−6.85 (m, 4 H, ArH), 2.63 (s, 6 H, $N(CH_3)_2$). Subsequent elution with EtOAc/hexanes (1:9) afforded **118** as a white solid (0.47 g, 42%): mp = 102−104 °C; ^{1}H NMR (DMSO-d_6) δ 7.59 (s, 1 H, OH), 6.60−6.66 (m, 2 H, ArH), 6.35−6.41 (m, 2 H, ArH), 4.70 (bs, 1 H, NH), 2.67 (s, 3 H, CH_3).

Step 2. 2-Hydroxyphenyl-*N*-heptyl-*N*-methylamine (120). A solution of **118** (200 mg, 1.62 mmol) in 3 mL of dry DMF was treated with $KHCO_3$ (165 mg, 1.65 mmol) and 1-iodoheptane (370 mg, 1.65 mmol). After stirring at room temperature for 10 h, the reaction mixture was diluted with water and extracted with EtOAc (2 × 15 mL). The combined EtOAc layers were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by chromatography on silica gel (EtOAc/hexanes, 10:90) to afford **120** as a pale-yellow oil (245 mg, 68%): ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1 H, OH), 6.76−6.87 (m, 1H, J = 7.7 Hz, ArH), 6.67−6.75 (m, 3 H, ArH), 2.91−2.94 (t, 2 H, J = 7.3 Hz, CH_2), 2.60 (s, 3 H, CH_3), 1.36−1.40 (m, 2 H, CH_2), 1.20−1.25 (m, 8 H, CH_2), 0.81−0.84 (t, 3 H, J = 7.1 Hz, CH_3).

Step 3. 2-Acetoxyphenyl-*N*-heptyl-*N*-methylamine (121) was prepared by the acetylation of **120** with Ac_2O. Title compound was obtained as a pale-yellow oil (0.24 g, 84%) upon chromatography on silica gel (EtOAc/hexanes, 10:90): ^{1}H NMR ($CDCl_3$) δ 7.11−7.15 (t, 1 H, J = 8.2 and 1.7 Hz, ArH), 7.01−7.03 (d, 1 H, J = 8.0 and 1.2 Hz, ArH), 6.95−6.97 (d, 1 H, J = 7.9 and 1.7 Hz, ArH), 6.91−6.93 (t, 1 H, J = 7.2 and 1.3 Hz, ArH), 2.88−2.92 (t, 2 H, J = 7.5 Hz, CH_2), 2.63 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 1.39−1.42 (m, 2 H, CH_2), 1.21−1.25 (m, 8 H, CH_2), 0.81−0.84 (t, 3 H, J = 6.8 Hz, CH_3); HRMS (CI) calcd for $C_{16}H_{26}NO_2$ (MH^+) 264.19635, found 264.19621.

2-Acetoxyphenyl Hept-2-ynyl Ether (126). Step 1. 2-Hydroxyphenyl Hept-2-ynyl Ether (124). To a solution of catechol (**122**; 0.35 g, 3.16 mmol) in 3 mL of dry DMF were added $KHCO_3$ (0.33 g, 3.3 mmol) and 1-bromohept-2-yne (0.5 g, 3.16 mmol), and this mixtue was stirred at room temperature overnight. After dilution with water, the mixture was extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 1:99 then 10:90) afforded **124** as a colorless oil (0.22 g, 34%): ^{1}H NMR ($CDCl_3$) δ 6.83−6.99 (m, 4 H, ArH), 5.64 (s, 1 H, OH), 4.72−4.73 (t, 2 H, J = 2.1 Hz, CH_2), 2.19−2.25 (m, 2 H, CH_2), 1.33−1.51 (m, 4 H, CH_2), 0.87−0.91 (t, 3 H, J = 7.2 Hz, CH_3); FAB-MS 205 (MH^+, 35), 204 (M^+, 100), 157 (70), 93 (40), 79 (90).

Step 2. 2-Acetoxyphenyl hept-2-ynyl ether (126) was prepared by the acetylation of **124** with Ac_2O. Chromatography on silica gel (EtOAc/hexanes, 5:95) afforded the desired acetate **126** as a pale-yellow oil (0.13 g, 83%): ^{1}H NMR ($CDCl_3$) δ 7.17−7.19 (t, 1 H, ArH), 7.11−7.16 (dd, 1 H, ArH), 7.00−7.06 (dd, 1 H, ArH), 6.96−6.99 (t, 1 H, ArH), 4.67−4.69 (t, 2 H, J = 2.1 Hz, CH_2), 2.31 (s, 3 H, CH_3), 2.18−2.22 (m, 2 H, CH_2), 1.35−1.50 (m, 4 H, CH_2), 0.86−0.91 (t, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.08, 149.45, 140.17, 126.62, 122.92, 121.41, 114.43, 88.67, 74.55, 57.33, 30.40, 21.82, 20.70, 18.43, 13.52; FAB-MS 247 (MH^+, 80), 205 (100), 121 (60), 79 (40); HRMS (CI) calcd for $C_{15}H_{19}O_3$ (MH^+) 247.13342, found 247.13332.

2-Acetoxy-3-(heptylthio)naphthalene (132). Step 1. 2-Methoxynaphthalene (128). A solution of β-naphthol (**127**; 5 g, 34.68 mmol) in 25 mL of anhydrous DMF was treated with K_2CO_3 (5.42 g, 39.26 mmol) and CH_3I (5.57 g, 39.26 mmol). The reaction mixture was stirred at room temperature under argon for 24 h. The solution was diluted with water and extracted with EtOAc (3 × 25 mL). The combined EtOAc extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (hexanes) gave **128** as a white solid (1.8 g, 79%): ^{1}H NMR ($CDCl_3$) δ 7.72−7.78 (m, 3 H, ArH), 7.41−7.46 (t, 1 H, J = 7.4 Hz, ArH), 7.31−7.36 (t, 1 H, J = 7.5 Hz, ArH), 7.13−7.16 (m, 2 H, ArH), 3.92 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 157.53, 134.51, 129.34, 128.90, 127.61, 126.69, 126.32, 123.54, 118.67, 105.67, 55.22.

Step 2. 2-Methoxy-3-(heptylthio)naphthalene (129). To a solution of **128** (2.2 g, 13.92 mmol) in 30 mL of freshly distilled THF at 0 °C was added nBuLi (2.5 M solution in hexane, 5.7 mL, 14.2 mmol) under argon. The solution was stirred at 0 °C for 1 h and at room temperature for 30 min. Sulfur powder (460 mg, 14.3 mmol) was added to this solution at −78 °C and allowed to stir at room temperature for 2 h. The reaction mixture was treated with 1-iodoheptane (3.23 g, 14.3 mmol) at −78 °C and allowed to stir overnight at room temperature. The reaction mixture was quenched with saturated NH_4Cl and extracted with Et_2O (3 × 30 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 1:99) gave **129** as a white solid (3.4 g, 85%) which was recrystallized from hexanes: mp = 74−75 °C; ^{1}H NMR ($CDCl_3$) δ 7.68−7.71 (unresolved dd, 2 H, J = 8.1 Hz, ArH), 7.56 (s, 1 H, ArH), 7.30−7.41 (m, 2 H, ArH), 7.09 (s, 1 H, ArH), 4.00 (s, 3 H, OCH_3), 2.98−3.03 (t, 2 H, J = 7.4 Hz, CH_2), 1.70−1.77 (m, 2 H, CH_2), 1.44−1.52 (m, 2 H, CH_2), 1.29−1.36 (m, 6 H, CH_2), 0.86−0.90 (t, 3 H, J = 6.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 154.93, 132.35, 129.12, 128.21, 126.47, 126.41, 125.54, 125.30, 125.25, 124.05, 104.91, 55.86, 31.69, 31.48, 29.04, 28.89, 28.48, 26.63, 22.60, 14.07.

Step 3. 3-(Heptylthio)naphth-2-ol (131). A reaction mixture containing **129** (2.7 g, 9.4 mmol) in 25 mL of dry CH_2Cl_2 was treated with boron tribromide (1 M solution in CH_2Cl_2, 10 mL, 10 mmol) at −78 °C under argon and stirred overnight at room temperature. The solution was quenched with water and then extracted with CH_2Cl_2 (3 × 20 mL). The combined CH_2Cl_2 extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 2:98) yielded **131** as a pale-yellow oil which solidified upon standing (1.8 g, 70%). Recrystallization from hexanes afforded white needles: mp = 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1 H, ArH), 7.68−7.74 (t, 2 H, J = 8.4 Hz, ArH), 7.40−7.46 (t, 1 H, J = 8.0 Hz, ArH), 7.32−7.34 (t, 1 H, J = 4.0 Hz, ArH), 6.83 (s, 1 H, OH), 2.74−2.79 (t, 2 H, J = 7.4 Hz, CH_2), 1.53−1.63 (m, 2 H, CH_2), 1.24−1.37 (m, 8 H, CH_2), 0.83−0.88 (t, 3 H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 153.29, 135.87, 135.39, 128.72, 127.41, 127.10, 126.41, 123.73, 122.35, 108.94, 37.08, 31.65, 29.52, 28.75, 28.52, 22.53, 14.03.

Step 4. 2-Acetoxy-3-(heptylthio)naphthalene (132) was prepared by the acetylation of **131** with Ac_2O. Chromatography on silica gel (EtOAc/hexanes, 2:98) afforded the desired acetate **132** as a colorless oil which solidified upon freezing (0.49 g, 93%): ^{1}H NMR ($CDCl_3$) δ 7.74−7.76 (m, 3 H, ArH), 7.51 (s, 1 H, ArH), 7.42−7.46 (m, 2 H, ArH), 2.95−3.00 (t, 2 H, J = 7.4 Hz, CH_2), 2.40 (s, 3 H, CH_3), 1.65−1.72 (m, 2 H, CH_2), 1.43−1.48 (m, 2 H, CH_2), 1.27−1.32 (m, 6 H, CH_2), 0.86−0.90 (t, 3 H, J = 6.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.22, 146.76, 131.95, 131.79, 129.85, 127.72, 127.31, 126.69, 126.12, 125.91, 119.67, 32.82, 31.63, 28.83, 28.78, 28.59, 22.53, 20.81, 14.02; HRMS (CI) calcd for $C_{19}H_{25}O_2S$ (MH^+) 317.15753, found 317.15767.

1-Acetoxy-2-(heptylthio)naphthalene (136). Step 1. 1-Bromo-2-methoxynaphthalene (134). A solution of 1-bromo-2-naphthol (**133**; 5 g, 22.41 mmol) in 30 mL of anhydrous DMF was treated with K_2CO_3 (3.6 g, 26 mmol) and CH_3I (3.7 g, 26 mmol) and stirred at room temperature for 24 h. The solution was diluted with water and extracted with EtOAc (3 × 30 mL). The combined EtOAc extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (hexanes) gave **134** as a white solid (1.8 g, 79%) which was recrystallized from hexanes: mp = 85−87 °C; ^{1}H NMR ($CDCl_3$) δ 8.21−8.23 (d, 1 H, J = 8.7 Hz, ArH), 7.77−7.84 (m, 2 H, ArH), 7.54−7.59 (t, 1 H, J = 7.6 Hz, ArH), 7.37−7.42 (t, 1 H, J = 7.0 Hz, ArH), 7.27−7.30 (d, 1 H, ArH), 4.04 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 133.08, 129.76, 128.94, 128.00, 127.71, 126.08, 124.28, 113.54, 57.02.

Step 2. 1-(Heptylthio)-2-methoxynaphthalene (130). To a solution of **134** (2.5 g, 11.26 mmol) in 30 mL of freshly distilled THF at −78 °C was added nBuLi (2.5 M solution in hexane, 4.8 mL, 12 mmol) under argon. The solution was allowed to stir at −78 °C for 1 h and at 0 °C for 30 min. Following the addition of sulfur powder (384 mg, 12 mmol) at −78 °C, the reaction mixture was stirred for 2 h at room temperature. The mixture was treated with 1-iodoheptane (2.71 g, 12 mmol) at −78 °C and stirred overnight at room temperature. The reaction was quenched with saturated NH_4Cl and extracted with Et_2O (2 × 40 mL). The combined organic layers were washed with water, dried ($MgSO_4$), and filtered, and the solvent was concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes; 1:99) gave a yellow oil (3.1 g, 95%) which was a 3:1 mixture of desired product **130** and starting material. This mixture was used in the next step without further purification.

Step 3. 1-(Heptylthio)naphth-2-ol (135). A reaction mixture containing **130** (1.5 g, 5.21 mmol) in 20 mL of dry CH_2Cl_2 was treated with boron tribromide (1 M solution in CH_2Cl_2, 5.5 mL, 5.5 mmol) at −78 °C under argon and stirred overnight at room temperature. The solution was carefully quenched with water and then extracted with CH_2Cl_2 (3 × 15 mL). The combined CH_2Cl_2 extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography on silica gel (EtOAc/hexanes, 1:99) yielded **135** as a pale-yellow oil (0.8 g, 57%): ^{1}H NMR ($CDCl_3$) δ 8.32−8.35 (d, 1 H, J = 8.5 Hz, ArH), 7.77−7.80 (d, 2 H, J = 8.7 Hz, ArH), 7.53−7.58 (t, 1 H, J = 7.8 Hz, ArH), 7.40 (s, 1 H, OH), 7.34−7.39 (t, 1 H, J = 7.2 Hz, ArH), 7.24−7.27 (d, 1 H, J = 8.9 Hz, ArH), 2.67−2.72 (t, 2 H, J = 7.3 Hz, CH_2), 1.50−1.57 (m, 2 H, CH_2), 1.22−1.39 (m, 8 H, CH_2), 0.83−0.87 (t, 3 H, J = 7.0 Hz, CH_3).

Step 4. 2-Acetoxy-1-(heptylthio)naphthalene (136) was prepared by the acetylation of **135** with Ac_2O. Chromatography on silica gel (EtOAc/hexanes, 2:98) afforded the desired acetate **136** as a colorless oil (0.15 g, 88%): ^{1}H NMR ($CDCl_3$) δ 8.60−8.63 (d, 1 H, J = 8.6 Hz, ArH), 7.84−7.87 (d, 2 H, J = 8.6 Hz, ArH), 7.57−7.63 (t, 1 H, J = 7.0 Hz, ArH), 7.48−7.53 (t, 1 H, J = 7.0 Hz, ArH), 7.23 (s, 1 H, ArH), 2.76−2.80 (t, 2 H, J = 7.0 Hz, CH_2), 2.42 (s, 3 H, CH_3), 1.41−1.54 (m, 2 H, CH_2), 1.22−1.39 (m, 6 H, CH_2), 0.83−0.87 (t, 3 H, J = 6.9 Hz, CH_3); HRMS (CI) calcd for $C_{19}H_{25}O_2S$ (MH^+) 317.15753, found 317.15745.

Enzymology. Arachidonic acid was purchased from Nu Chek Prep (Elysian, MN). [*1-^{14}C*]Arachidonic acid (∼55−57 mCi/mmol) or [*1-^{14}C-acetyl*]salicylic acid (∼ 55 mCi/mmol) was purchased from NEN DuPont or American Radiolabeled Chemicals (ARC, St. Louis, MO). Hematin, hydrogen peroxide, and guaiacol were purchased from Sigma Chemical Co. (St. Louis, MO). COX-1 was purified from ram seminal vesicles

(Oxford Biomedical Research, Inc., Oxford, MI) as described in earlier reports.[42] The specific activity of the protein was 20 μMO_2/min/mg, and the percentage of holoprotein was 13.5%. ApoCOX-1 was prepared as described earlier.[42] Apoenzyme was reconstituted by the addition of hematin to the assay mixtures. Cyclooxygenase activity (oxygen uptake assay) and peroxidase activity (guaiacol assay) were measured as described in earlier reports.[21,42] Samples of purified human COX-2 (1.62 $\mu g/\mu L$) were generous gifts from Jim Gierse, Monsanto (St. Louis, MO).

Time- and Concentration-Dependent Inhibition of Ovine COX-1 and Human COX-2 Using the Thin Layer Chromatography (TLC) Assay. Cyclooxygenase activity of ovine COX-1 (22 nM) or human COX-2 (88 nM) was assayed by TLC.[21] All assays were conducted in duplicate, and IC_{50} values are the average of duplicate determinations for each compound. Reaction mixtures of 200 μL consisted of hematin-reconstituted protein in 100 mM Tris-HCl, pH 8.0, 500 μM phenol, and [*1-*^{14}*C*]arachidonic acid (50 μM, ~55−57 mCi/mmol). For the time-dependent inhibition assay, hematin-reconstituted COX-1 (22 nM) or COX-2 (88 nM) was preincubated at room temperature for 2 h with inhibitor concentrations ranging from 0 to 1000 μM in DMSO followed by the addition of [*1-*^{14}*C*]arachidonic acid (50 μM) for 30 s at 37 °C. Reactions were terminated by solvent extraction in Et_2O/CH_3OH/1 M citrate, pH 4.0 (30:4:1). The phases were separated by centrifugation at 2000*g* for 2 min, and the organic phase was spotted on a TLC plate (J. T. Baker, Phillipsburg, NJ). The plate was developed in EtOAc/CH_2Cl_2/glacial AcOH (75:25:1) at 4 °C. Radiolabeled prostanoid products were quantitated with a radioactivity scanner (Bioscan, Inc., Washington, DC). The percentage of total products observed at different inhibitor concentrations was divided by the percentage of products observed for protein samples preincubated for the same time with DMSO.

Inhibition of COX-2 Activity in Activated RAW264.7 Cells. Low-passage number murine RAW264.7 cells were grown in DMEM containing 10% heat-inactivated FBS. Cells (6.2×10^6 cells/T25 flask) were activated with 500 ng/mL LPS and 10 U/mL IFN-γ in serum-free DMEM for 7 h. Vehicle (DMSO) or inhibitor in DMSO (0−20 μM) was added for 30 min at 37 °C. Inhibition of exogenous arachidonic acid metabolism or inhibition of PGD_2 synthesis was determined by incubating the cells with 20 μM [*1-*^{14}*C*]arachidonic acid, respectively, for 15 min at 25 °C. Aliquots (200 μL) were removed into termination solution, and total products were quantitated by the TLC assay as described earlier.

Synthesis of [*1-*^{14}*C-acetyl*]-36. 2-Hydroxythioanisole (**2**; 3 mg, 21 μmol) in 300 μL of CH_2Cl_2 containing anhydrous pyridine (2.85 μL, 36 μmol) was allowed to stir at room temperature for 5 min followed by the addition of [*1-*^{14}*C*]acetic anhydride (ARC, St. Louis, MO) (3 μL, 31 μmol, 55 mCi/mmol). The reaction was stirred overnight at room temperature, and the solution was chromatographed on silica gel (hexanes/EtOAc, 2:98) to afford [*1-*^{14}*C-acetyl*]-**36** (1 mg, 35%). TLC analysis (hexanes/EtOAc, 10:90) revealed a single spot (R_f = 0.625); specific activity ~ 55 mCi/mmol.

Trypsin Digestion and Peptide Mapping of COX-2 Labeled with [*1-*^{14}*C-acetyl*]-36, [*1-*^{14}*C-acetyl*]-70, or [*1-*^{14}*C-acetyl*]Salicylic Acid (Aspirin). Hematin-reconstituted human COX-2 (14 μM) in 100 mM Tris-HCl, pH 8.0, containing 500 μM phenol was treated with 1000 equiv of [*1-*^{14}*C-acetyl*]-**36** (3 h at 25 °C resulted in 75% inhibition of COX-2 activity), 25 equiv of [*1-*^{14}*C-acetyl*]-**70** (2 h at 25 °C resulted in 94% inhibition of COX-2 activity), or 30 equiv of [*1-*^{14}*C-acetyl*]-salicylic acid (1.5 h at 25 °C resulted in 79% inhibition). The radiolabeled COX-2 was dialyzed overnight at 4 °C against 2 L of 50 mM Tris-HCl, pH 8.0, 0.4% CHAPS, containing 500 μM phenol. The dialyzed COX-2 samples were injected on a reversed-phase Vydac C4 column (0.46 × 25 cm) and eluted with a solvent system of A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile and a linear gradient from 40% to 60% acetonitrile in 30 min (flow rate, 1 mL/min). The intact protein coeluted with a radioactive peak (retention times of 14−16 min for each of the test compounds). The HPLC system was connected to a Varian 2050 UV detector (λ = 230 nm) and to a radiomatic Flo-one β radioactive flow detector. The protein peaks were collected, lyophilized, dissolved in 100 mM ammonium bicarbonate buffer (pH 7.4), and digested with 44:1 L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)−trypsin (Sigma) for 24 h at 37 °C. The digest was terminated with acid and chromatographed on a Beckman ODS instrument (C18 column). Elution with a solvent system of A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile and a linear gradient from 0% to 50% acetonitrile in 75 min (flow rate, 1 mL/min) revealed a single radioactive peak eluting at ~21−22 min for each of the test compounds.

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Supporting Information Available: Additional NMR and MS data on intermediates and final compounds (13 pages). Ordering information is given on any current masthead page.

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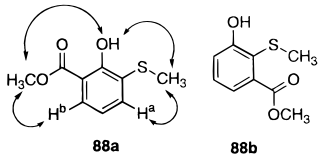
(33) That the lithiation of **86** followed by reaction with methyl chloroformate afforded **88a** and not **88b** was confirmed by NOE experiments: Irradiation of the resonance at δ 2.46 (SCH_3) gave a strong, positive NOE signal at δ 7.38 which is consistent with the presence of an aromatic proton (H^a) in spatial proximity to the SCH_3 moiety (not possible in **88b**); a relatively weak NOE signal also was observed at δ 11.38 which is consistent with the presence of a phenolic OH group in proximity to the SCH_3 moiety. Irradiation of the resonance at δ 3.96 (CO_2CH_3) afforded

strong, positive NOEs at resonances δ 11.38 and 7.67, respectively, indicating that the methyl ester was in spatial proximity to the phenolic OH group and an aromatic proton (H^b). The results of this study thus rule out structure **88b**.



88a **88b**

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