Apomorphine-Induced Hypothermia. The method has recently been described.[32] Male Sprague–Dawley rats (200–250 g) were used and were housed at 21 ± 0.5 °C. Groups of rats (*n* = 6) were pretreated ip with saline or test compounds 60 min prior to the injection of apomorphine hydrochloride (1 mg/kg sc). Core temperature was measured by a rectal probe. The temperature was recorded before administration of the test compounds as well as before apomorphine and 15 min after apomorphine. The means of the temperature variations were calculated and the changes were expressed in percent of respective control mean value. The ED_{50} value, calculated by regression analysis using Fieller's theorem for calculation of the 95% confidence limit,[43] refers to the dose level which blocks the hypothermic effect of apomorphine by 50%.

Measurement of Catalepsy. Catalepsy was measured in the horizontal bar test 20, 40, 60, 90, 120, and 240 min after ip injection of the test compound. In addition, a control group receiving saline was tested in the same manner as drug-treated groups. A test cage (Macrolon type III) fitted with a horizontal bar (diameter of 1.5 cm) placed 7 cm above the cage floor was used. The rat was placed with both front limbs extended over the bar, and the catalepsy interval was defined as the time (descent latency) required for both front limbs to be removed from the bar. The test was limited to a cutoff time if the rat had not moved after 60 s. A rat was considered to be cataleptic if it showed a mean time of more than 15 s from the three test occasions showing the longest descent latencies. The proportion of animals that were cataleptic according to this criterion (≥15 s) was calculated for each dose level of the drugs and was presented in dose–response curves. The ED_{50}, determined by probit analysis, is defined as the dose at which 50% of the animals are cataleptic.

Design, Synthesis, and 5-Lipoxygenase-Inhibiting Properties of 1-Thio-Substituted Butadienes

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The synthesis of novel 1-thio-substituted butadienes, designed as mechanism-based 5-lipoxygenase inhibitors, is described. The structure of these compounds closely resembles a proposed high-energy intermediate during the lipoxygenation of arachidonic acid. They demonstrate 5-lipoxygenase inhibition in vitro and in vivo. The most potent compound is 15a with an IC_{50} of 1.8 μM in vitro. LTC_4 release was inhibited by 80% after intraperitoneal administration of 15c at a dose of 2 mg/kg.

There is strong evidence for the role of leukotrienes as mediators of asthma and inflammation.[1,2] As a key role in their synthesis is played by 5-lipoxygenase,[3] much effort has been directed toward the identification and development of inhibitors of this enzyme. Numerous compounds of diverse chemical structure have been described, many of which have been found to possess inadequate potency or unacceptable toxicity.[4] This latter effect could be explained by a lack of specificity, perhaps resulting from their antioxidant activity[5] or structural features leading to blood cell toxicity, specifically methemoglobinemia.[6] Second generation inhibitors of 5-lipoxygenase will therefore require both better selectivity and enhanced potency. As a rational approach we considered the possibility of designing transition-state analogue[7] inhibitors of 5-lipoxygenase.

Central to the transition-state analogue theory is the hypothesis that, during the course of an enzyme-catalyzed reaction, intermediates and transition states are formed that are bound more tightly by the enzyme than either substrate or product.[7] Chemical structures mimicking the transition state or a high-energy intermediate might reasonably be expected to act as selective and potent inhibitors of the enzyme.

Since the enzymatic conversion of arachidonic acid by 5-lipoxygenase proceeds through a cascade of complex reaction steps, it raises the question whether the transition-state analogue concept, originally formulated by

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(1) Dahlen, S. E.; Kumlin, M.; Granstroem, E.; Hedqvist, P. *Respiration* **1986**, *50* (Suppl. 2), 22.
(2) Feuerstein, G.; Hallenbeck, J. M. *FASEB J.* **1987**, *1*, 186.
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(4) The Scripp leukotriene report: an overview of research in the leukotriene field with a report on the UK leukotriene symposium. PJB Publications: Surrey, England, 1985.
(5) Taylor, G. W.; Clarke, S. *Trends Pharm. Sci.* **1986**, *7*, 100. (a) Salmon, J. A. *Adv. Drug. Res.* **1986**, *15*, 111.
(6) Wintroke, M. M., Lee, G. R., et al., Eds. In *Clinical Hematology*, Lea and Febiger: Philadelphia, 1975; p 1009.
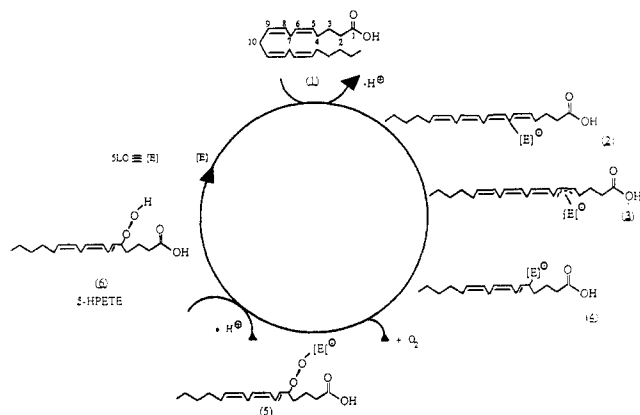(7) Pauling, L. *Chem. Eng. News* **1946**, *24*, 1375.

Figure 1. Hypothetical intermediates of the 5-lipoxygenation of arachidonic acid.
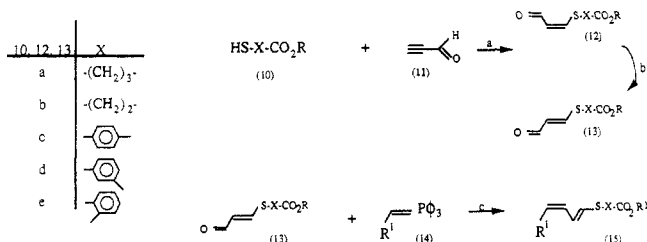
Figure 2. Synthetic approach to thio-substituted butadienes. Conditions: (a) NaOAc, toluene, RT; (b) 0.1% I_2, CH_2Cl_2, RT; (c) THF, DMEU, $-78\,°C \to 0\,°C$.

Pauling[7] and Lienhard[8] for single-step reactions, can be applied. This question has been discussed in detail by Rich[9] in relation to protease inhibition. Despite the difficulties encountered both in selecting the optimal transition state for analogue design and in the experimental verification of the hypothesis that an inhibitor works as a transition-state analogue, Rich concludes that "the idea to use the transition-state analogue concept as a point of departure for designing novel enzyme inhibitors remains a valuable approach".[9]

A detailed consideration of the 5-lipoxygenase reaction gives some evidence that lipoxygenation of arachidonic acid proceeds through several steps involving organoiron compounds.[10] We hypothesize a plausible reaction scheme shown in Figure 1 to illustrate the multiple steps that may occur during catalysis.

During the course of these reaction steps, transition states should be formed which have negative charges at C-5 and C-7. We therefore assume that the replacement of C-5 in structure 4, or C-7 in structure 2, by a more electronegative atom should lead to stable analogues of 4 or 2 possessing good inhibitory activity for 5-lipoxygenase. Structures like 7 (X = sulfur) have already been described by Corey[11] as inhibitors for 5-lipoxygenase. We attempted to establish whether greater potency and selectivity could be achieved through analogues of structure 8 where X is sulfur, i.e., 9. These butadienes, having the C-8 cis and C-6 trans stereochemistry, are new, although related structures have been described by Funk and Alteneder[12]

Figure 3. Synthesis of phosphonium ylides 18. Conditions: (d) NEt_3, DMAP, TsCl, CH_2Cl_2, $-4\,°C$; (e) PPh_3, $120\,°C$; (f) n-BuLi, THF, $-20\,°C$; (g) (1) n-BuLi, THF, $-20\,°C$; (2) n-C_8H_{17} (20), DMF, $-20\,°C \to$ RT; (h) Amberlyst (H^+); (i) THF, DMEU, $-78\,°C \to 0\,°C$; (j) $Bu_4N^+F^-$, CH_2Cl_2.

as inhibitors, albeit weak, of 15-lipoxygenase. We developed a flexible and efficient synthetic approach to their synthesis as outlined in Figure 2.
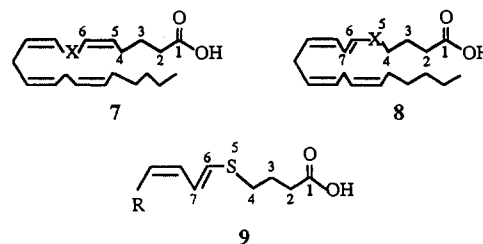
Chemistry

The retrosynthetic analysis for the total synthesis disconnects 15 at the cis double bond into Wittig and aldehyde precursors 14 and 13. This approach offers three advantages: (1) it provides a highly convergent access to the butadiene system using readily available reagents; (2) it allows stereochemical control of the double bonds; and (3) it includes a high degree of flexibility to synthesize a variety of structural analogues.

The aldehyde precursors 13 are easily available: the reaction of mercapto compounds 10 with propynal (11) gave a mixture of the cis- and trans-acroleins 12 and 13, which were converted to the pure trans isomer 13 by treatment with iodine in dichloromethane.

The synthesis of the phosphonium ylides 18 proceeded as shown in Figure 3: Coupling of THP ether 19 with n-octyl bromide (20) followed by deprotection (and for 16c followed by hydrogenation) gave the alcohols 16b,c. Alcohol 16a was synthesized by a different strategy, starting with Wittig olefination of 3-[(tert-butyldimethylsilyl)-oxy]propanal (22)[13] with ylide 23 to give 24. Subsequent desilylation with fluoride gave 16a. The alcohols 16 were converted to the phosphonium salts 18 by tosylation and following treatment with triphenylphosphine. These phosphonium salts 18 can be smoothly deprotonated at $-20\,°C$ by n-butyllithium to generate the ylides 14. No double deprotonation was observed under these conditions, and after addition of the corresponding aldehyde 13 the

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Table I. Inhibition of RBL-1 5-Lipoxygenase by Thiobutadienes

$$R^1 \diagup\!\!=\!\!\diagdown\!\!\diagup\!\!\diagdown\!\!\diagup^{S}_{R^2}$$

compound	R^2	R^1	IC_{50} (95% CL),[a] μM
15a	$(CH_2)_3CO_2CH_3$	$CH_2CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$ (cis, cis)	1.8 (0.8–4.1)
15b	$(CH_2)_3CO_2CH_3$	$CH_2C{\equiv}C(CH_2)_7CH_3$	5.2 (4.3–6.4)
15c	$(CH_2)_3CO_2CH_3$	$CH_2CH{=}CH(CH_2)_7CH_3$ (cis)	6.2 (5.1–7.6)
15d	$(CH_2)_3CO_2CH_3$	$CH_2C{\equiv}CCH_3$	32% at 40 μM
15e	$(CH_2)_3CO_2CH_3$	$(CH_2)_{10}CH_3$	22.0 (15.8–30.7)
15f	$(CH_2)_2CO_2CH_3$	$(CH_2)CH{=}CH(CH_2)_7CH_3$ (cis)	47% at 40 μM
15g	$C_6H_5CO_2CH_3$ (ortho)	$CH_2CH{=}CH(CH_2)_7CH_3$ (cis)	2.7 (0.8–9.5)
15h	$C_6H_5CO_2CH_3$ (meta)	$CH_2CH{=}CH(CH_2)_7CH_3$ (cis)	7.4 (6.4–8.6)
15i	$C_6H_5CO_2CH_3$ (para)	$CH_2CH{=}CH(CH_2)_7CH_3$ (cis)	11.2 (7.1–17.5)
15j	$C_6H_5CO_2CH_3$ (ortho)	$CH_2C{\equiv}C(CH_2)_7CH_3$	45% at 40 μM
15k	$C_6H_5CO_2CH_3$ (meta)	$CH_2C{\equiv}C(CH_2)_7CH_3$	9.0 (5.8–13.9)
15l	$C_6H_5CO_2CH_3$ (para)	$CH_2C{\equiv}C(CH_2)_7CH_3$	21% at 40 μM
15m	$(CH_2)_3CO_2H$	$CH_2CH{=}CH(CH_2)_7CH_3$ (cis)	7.2
nafazatrom			6

[a] Concentration of inhibitor required to give 50% inhibition with 95% confidence limits in parentheses ($n = 4$).
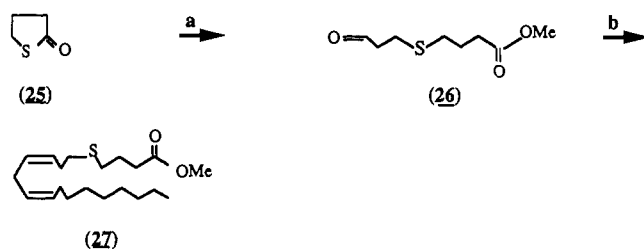

Figure 4. Synthesis of structural analogues of the thiobutadienes 15. Conditions: (a) (1) NaOMe, MeOH; (2) acrolein; (b) 14c, THF, DMEU, −78 °C → 0 °C.
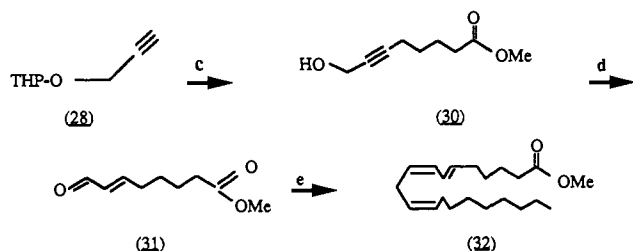

Figure 5. Synthesis of structural analogues of thiobutadienes 15. Conditions: (c) (1) *n*-BuLi, THF, −20 °C; (2) 1-[4-bromo-butyl]-4-ethyl-2,6,7-trioxabicyclo[2.2.2]octane (29), (3) Amberlyst (H⁺), MeOH, (4) K₂CO₃, MeI, DMF; (d) (1) H₂, Ni; (2) MnO₂; (e) 14c, THF, DMEU, −78 °C → 0 °C.

butadienes 15 were stereoselectively obtained in moderate to good yields.

In order to establish structure–activity relationships, we synthesized compounds **27** and **32** as outlined in Figures 4 and 5.

The synthesis of the thio compound **27** was performed via the ylide **14** and the aldehyde **26** by the same methodology as described for **15**. Aldehyde **26** is easily accessible from γ-thiobutyrolactone (**25**) and acrolein as outlined in Figure 4.

The arachidonic acid analogue **32** was obtained through a total synthesis as described in Figure 5. The acetylenic alcohol **30** was obtained by nucleophilic addition of the anion, generated from **28** with *n*-butyllithium, to the ortho ester **29** and subsequent deprotection and esterification following a procedure developed by Corey.[14] Subsequent hydrogenation of **30** over a nickel catalyst and oxidation with manganese dioxide gave the unsaturated alcohol **31**, which was converted to the arachidonic acid analogue **32** by Wittig olefination with **14c**.
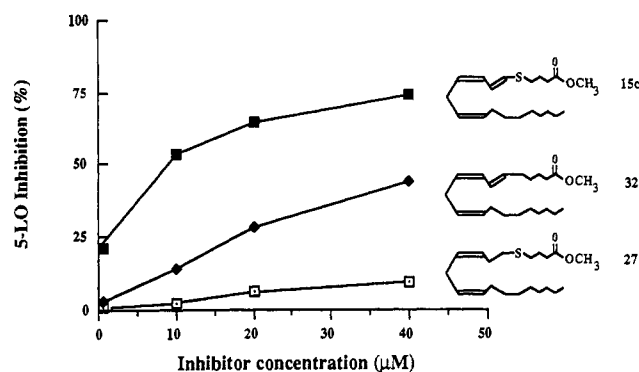
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(14) Corey, E. J.; Raju, N. *Tetrahedron Lett.* **1983**, *24*, 5571.

Figure 6. Testing the concept by "removal" of sulfur (32) or the C_6 double bond (27) from inhibitor structure 15c.

Results

Enzyme Inhibition. The synthesized compounds **15a–m** were evaluated as inhibitors of 5-lipoxygenase by using the 100000*g* supernatant from RBL-1 cells. The results are shown in Table I. The compounds show a wide range of activities with IC_{50} values from 2 μM for **15a** to values beyond 40 μM for **15l**. The structures **15a–f** were used to investigate the influence of length and bond configuration of the lipophilic "tail" (R^1) on activity. Greatest inhibition was produced by **15a**. This compound is the most similar in structure to the proposed intermediate **4**.

The ester function seems not to be of crucial importance for activity, as the acid **15m** shows a similar activity profile to its corresponding ester **15c**. Deleting bonds (**15e**), shortening the "tail" (**15d**), or substituting triple bonds for the C_{11} double bond (**15b**) lead to compounds with reduced activity.

In order to explore the structure–activity relationships in greater detail, we were interested in examining the effect of conformational restriction of the "head" part of **15**. Therefore, in compounds **15g–l** benzoic acid "head" regions were incorporated into the **15c** structure. Activity in this series was retained; the most active compound, **15g**, was substituted in the ortho configuration. As with **15b** in the nonaromatic series, changing the C_{11} bond arrangement (**15j–l**) did not improve inhibitory potency of the benzoic acid "head" compounds.

To examine whether the inhibitory activity of **15c** is due to an unspecific effect exerted by the sulfur atom or the productlike arrangement of the double bonds, we synthesized the carbon analogue **32** and the dihydro analogue **27**. The order of potency for these three compounds was **15c** > **32** ≫ **27** (Figure 6), indicating that the activity of
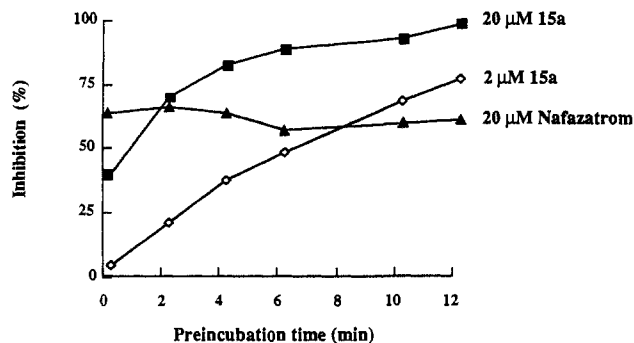
Figure 7. Effect of preincubation time on inhibition of 5-LO by 15a and nafazatrom.

Table II. Inhibition of Zymosan-Stimulated Release of Leukotriene C_4 from Intact Mouse Peritoneal Macrophages following Pretreatment with Thiobutadienes

	in vitro (60-min PT/ 4-h challenge)	intraperitoneal administration (15-min PT/45-min challenge)	
compd	IC_{50} (μM)	dose, mg/kg	% inhibition
15c	22	20	80
15e	>60	20	10

15c is not an unspecific effect due to the mercaptobutyric acid unit.

In order to investigate the time dependence of enzyme inhibition by these thiobutadienes, we preincubated 15a with the RBL-1 enzyme preparation for various times before the addition of the substrate. This experiment demonstrated a slow onset for maximal inhibition (Figure 7). By contrast, nafazatrom produced the same level of inhibition independent of preincubation time.

In Vitro Evaluation in Mouse Peritoneal Macrophages. For compounds 15c and -e inhibitory action in vitro was assessed by measurement of the zymosan-induced release of leukotriene C_4 from mouse peritoneal macrophages. Cells were pretreated with 15c or -e for 60 min, and LTC_4 release was determined by radioimmunoassay (Table II). The compounds showed the same order of potency as that seen in the broken cell enzyme test.

In Vivo Evaluation in Mice. Additional confirmation of 5-lipoxygenase inhibition by 15c was demonstrated in vivo by measurement of LTC_4 release after intraperitoneal pretreatment of mice with a dose of 20 mg/kg. There was clear inhibition of leukotriene synthesis by 15c, but not by 15e, which is in agreement with the corresponding in vitro data for these compounds (Table II).

Specificity. Selected compounds were examined for specificity by using (1) a cyclooxygenase preparation from bull vesicles, (2) a supernatant preparation from human polymorphonuclear cells expressing 5-LO, 12-LO, and 15-LO activity, (3) a methemoglobin induction assay, and (4) a xanthine oxidase assay.

Two of the most potent inhibitors (15a and -c), together with the least active one (15e), were evaluated for activity in the cyclooxygenase assay and for their ability to induce the formation of methemoglobin, which is a common toxic effect of a number of chemical classes of 5-lipoxygenase inhibitors (Table III). All were inactive at concentrations higher than those required to inhibit 5-lipoxygenase. By contrast, BW 755C, equipotent to 15a as an inhibitor of 5-lipoxygenase, induced the formation of unacceptably high levels of methemoglobin.

Parallel determinations of 5-LO, 12-LO, and 15-LO products in a supernatant preparation from human PMN cells clearly demonstrated a specificity toward 5-LO by 15c

Table III. Comparison of Thiobutadienes 15 with Reference Compounds in Tests of 5-Lipoxygenase Inhibition, Cyclooxygenase Inhibition, and Methemoglobin Induction

compound	5-LO IC_{50}, μM	COI, % at 20 μM	MetHb induction % at 10^{-4} M
15a	2	13	nt
15c	6	11	<3
15e	22.0	1	<3
BW 755C	2	30	17
nafazatrom	6	(12) [10 μM]	<3
indomethacin	≫20	90 [5 μM]	nt

Table IV. Specificity Comparison between 15c and Nafazatrom against Different Mammalian Lipoxygenases[a]

compound	concn, μM	LTB_4	5-HETE	12-HETE	15-HETE
15c	20	60	58	0	(20)
nafazatrom	20	65	68	58	30

[a] Parentheses indicate potentiation of activity.

(Table IV). Nafazatrom,[15] in contrast, inhibited all three lipoxygenases to a similar extent.

When 15c was compared with nafazatrom and phenidone for antioxidant activity by using the xanthine oxidase assay, 15c at 30 μM was without effect, while both nafazatrom and phenidone tested at the same concentration produced more than 85% inhibition.

Discussion

The butadienes presented in this paper are clearly potent inhibitors of 5-lipoxygenase. The most potent compound of the series, 15a, has activity beginning at submicromolar concentrations. We conclude from the data that for optimal activity we must incorporate both sulfur substitution at C_5 and the butadiene structure.

Shortening the aliphatic side chain or removing double or triple bonds at the 11-position produces compounds with markedly reduced activity. Substitution of the carboxylic side chain by an aromatic ring gives insight into structure–activity relationships on the conformation of the inhibitor on the enzyme surface. The ortho-substituted compound 15h is a more potent inhibitor than either the meta- or para-substituted analogues. From this we propose that the carboxylic group of 15a and -c lies near the sulfur position in its active conformation. There is clearly demonstrated specificity for 5-lipoxygenase over other lipoxygenases and cyclooxygenase. In intact cell preparations these butadienes show the same order of inhibitory potency both in vitro and when administered intraperitoneally 15 min prior to the zymosan stimulus. These experiments indicated a persistence of effect for at least 4 h postchallenge in vitro and 45 min postchallenge in vivo. Finally, unlike nafazatrom and phenidone, the inhibitor 15c was inactive in tests designed to identify antioxidants and substances that may induce methemoglobinemia formation.

There is evidence for slow binding of 15a to 5-lipoxygenase. Whether this is indicative of the fact that 15a represents a transition-state analogue is still unknown. Detailed kinetic studies will be necessary to clarify the mode of binding for 15a.

Conclusion

Our work shows that the transition-state analogue approach offers a feasible heuristic alternative for the design

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of inhibitors of leukotriene synthesis and has led to the discovery of a new class of 5-lipoxygenase inhibitors. However, we cannot ultimately prove whether these compounds bind to the catalytic site and resemble a transition state like conformation on the protein surface, because X-ray data for 5-lipoxygenase–inhibitor complexes are not yet available. We are currently examining the kinetics of inhibition and synthesizing analogues of other possible transition states in order to develop a better understanding of the mode of inhibition of the presented thiobutadienes.

Experimental Section

Chemistry. Unless stated otherwise, chemical reagents were obtained from commercial sources and were used directly. Reactions were routinely conducted under a dry argon atmosphere. THF was distilled from LiAlH$_4$, commercial anhydrous Et$_2$O was used directly, and CH$_2$Cl$_2$ was distilled from CaH$_2$. Elemental combustion analyses were performed by BAYER-Elementaranalytik. E. Merck silica gel 60 (230–400 mesh ASTM) was utilized for flash chromatography. Final purification of products was carried out on silica gel 60 (Grace, 20 μm) at a solvent pressure of 20 bar. Analytical thin-layer chromatography was conducted with E. Merck silica gel 60F-254 precoated TLC plates. ^{1}H NMR spectra were obtained with Bruker WP 200SY, AM 250, or AM 300 spectrometers, and chemical shift values δ are given in ppm.

Preparation of Aldehydes 13. Methyl (2E)-8-Oxo-5-thia-6-octenoate (13a). Methyl 4-mercaptobutyrate was prepared in situ from γ-thiobutyrolactone by treatment with sodium methoxide as follows: γ-Thiobutyrolactone (5.5 g, 50 mmol) was added to a solution of 3.42 g (60 mmol) of sodium methoxide in 50 mL of CH$_3$OH. The resulting mixture was stirred for 2 h at 45 °C, then cooled to 20 °C, and quenched by the addition of 4.6 mL (55 mmol) of concentrated HCl and 285 μL (5 mmol) of glacial acetic acid. This reaction mixture was directly used for the synthesis of 13a as follows: A 65% solution of propynal in toluene (50 mmol, 4.1 g) was added to the reaction mixture at 0 °C. The mixture was stirred for 2 h at 0 °C and then at 20 °C for 16 h. Toluene (30 mL) was added, and the solvents were removed under reduced pressure. The residue was partitioned between 50 mL of CH$_2$Cl$_2$ and 50 mL of H$_2$O, and the organic layer was separated, washed with saturated NaHCO$_3$, washed with brine, and dried over MgSO$_4$. The solvent was removed in vacuo, and the crude mixture of the trans and cis isomer 12 and 13 was dissolved in 100 mL of CH$_2$Cl$_2$ followed by the addition of 13 mg of I$_2$ and stirred at 20 °C until the cis isomer was no longer detectable (6 h; TLC control, solvent: CH$_2$Cl$_2$/Et$_2$O, 10:1). The solvent was removed under reduced pressure, and the residue was chromatographed (silica gel; CH$_2$Cl$_2$/Et$_2$O/petroleum ether/Et$_3$N, 10:1:10:0.01) to give 13a (7.23 g, 77%).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 2.0 (q; J = 7 Hz, 2 H, 3-H), 2.4 (t; J = 7 Hz, 2 H, 4-H), 2.9 (t; J = 7 Hz, 2 H, 2-H), 3.6 (s; 3 H, OCH_3), 6.2 (dd; $^3J_{7,6}$ = 15.6 Hz, $^3J_{7,8}$ = 8.0 Hz, 1 H, 7-H), 7.5 (d; J = 15.6 Hz, 1 H, 6-H), 9.4 (d; J = 8.0 Hz, 1 H, 8-H). Anal. (C$_8$H$_{12}$O$_3$S) C, H.

Methyl (2E)-7-Oxo-4-thia-5-heptenoate (13b). Methyl 3-mercaptopropionate (6.0 g, 50 mmol) was reacted with 4.1 g of a 65% solution of propynal (50 mmol) in toluene containing 40 mg (0.5 mmol) of NaOAc according to the procedure for 13a to give 13b (5.4 g, 62%).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 2.6 (t, J = 7 Hz, 2 H, 2-H), 3.1 (t; J = 7 Hz, 2 H, 3-H), 3.6 (s; 3 H, OCH_3), 6.0 (dd; $^3J_{6,5}$ = 15.2 Hz, $^3J_{6,7}$ = 7.6 Hz, 1 H, 6-H), 7.5 (d; J = 5.2 Hz, 1 H, 5-H), 9.3 (d; J = 7.6 Hz, 1 H, 7-H). Anal. (C$_7$H$_{10}$O$_3$S) C, H.

Methyl (1E)-4-(3-Oxo-1-propenylthio)benzoate (13c). Methyl 4-mercaptobenzoate (8.4 g, 50 mmol) was reacted with 4.1 g of a 65% solution of propynal (50 mmol) in toluene containing 40 mg (0.5 mmol) of NaOAc according to the procedure for 13a to give 13c (6.7 g, 60%).

^{1}H NMR (CD$_2$Cl$_2$; 200 MHz) δ 3.9 (s; 3 H, OCH_3), 6.1 (dd; $^3J_{2',1'}$ = 15 Hz, $^3J_{2',3'}$ = 7.2 Hz, 1 H, 2'-H), 7.6 (d; J = 10 Hz, 2 H, 2-H, 6-H), 7.7 (d; J = 15 Hz, 1 H, 1'-H), 8.0 (d; J = 10 Hz, 2 H, 3-H, 5-H), 9.5 (d; J = 7.2 Hz, 1 H, 3'-H).

Methyl (1E)-3-(3-Oxo-1-propenylthio)benzoate (13d). Methyl 3-mercaptobenzoate (8.4 g, 50 mmol) was reacted with 4.1 g of a 65% solution of propynal (50 mmol) in toluene con-

taining 40 mg (0.5 mmol) of NaOAc according to the procedure for 13a to give 13d (7.8 g; 70.2%).

^{1}H NMR (CD$_2$Cl$_2$; 300 MHz) δ 3.9 (s; 3 H; OCH_3), 6.0 (dd; $^3J_{2',1'}$ = 15 Hz, $^3J_{2',3'}$ = 7 Hz, 1 H, 2'-H), 7.5 (t; J = 8 Hz, 1 H, 5-H), 7.7 (d; J = 15 Hz, 1 H, 1'-H), 7.7 (d; J = 8 Hz, 1 H, 6-H), 8.1 (d; J = 8 Hz, 1 H, 4-H), 8.2 (s; 1 H, 2-H), 9.8 (d; J = 7 Hz, 1 H, 3'-H). Anal. (C$_{11}$H$_{10}$O$_3$S) C, H.

Methyl (1E)-2-(3-Oxo-1-propenylthio)benzoate (13e). Methyl 2-mercaptobenzoate (8.4 g, 50 mmol) was reacted with 4.1 g of a 65% solution of propynal (50 mmol) in toluene containing 40 mg (0.5 mmol) of NaOAc according to the procedure for 13a to give 13e (8.2 g, 73.8%).

^{1}H NMR (CD$_2$Cl$_2$; 200 MHz) δ 3.9 (s; 3 H, OCH_3), 6.3 (dd; $^3J_{2',1'}$ = 15 Hz, $^3J_{2',3'}$ = 7 Hz, 1 H, 2'-H), 7.4–7.6 (m; 3 H, ar CH), 7.7 (d; J = 15 Hz, 1 H, 1'-H), 8.0 (d; J = 7 Hz, 1 H, 2-H), 9.5 (d; J = 7 Hz, 1 H, 3'-H). Anal. (C$_{11}$H$_{10}$O$_3$S) C, H.

Preparation of Butadienes 15. Methyl (6E,8Z,11Z,14Z)-5-Thia-6,8,11,14-eicosatetraenoate (15a). A 1.61 M solution of *n*-butyllithium (930 μL, 1.5 mmol) was added at –20 °C to 898 mg (1.5 mmol) of 18a in 6 mL of THF. The blood-red solution was stirred at –20 °C for 4 min and was subsequently cooled to –78 °C followed by the addition of 1 mL of DMEU and 282 mg (1.5 mmol) of 13a in 0.5 mL of THF. The reaction mixture was stirred for 3 h at –78 °C and 1 h at –40 °C and was then allowed to warm to 20 °C. Hexane (6 mL) was added, the layers were separated, and the lower layer was extracted with 10 mL of hexane. The combined hexane layers were freed from solvent, and the residue was chromatographed (silica gel, 20 μm, deactivated with petroleum ether containing 0.1% Et$_3$N; petroleum ether/Et$_2$O, 10:1) to give 15a (170 mg, 34%); R_f 0.76 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH_3), 1.2 [b; 6 H, (CH_2)$_3$CH$_3$], 1.9 (q; J = 6 Hz, 2 H, 3-H), 2.0 (dt; $^3J_{16,15}$ = $^3J_{16,17}$ = 7 Hz, 2 H, 16-H), 2.4 (t; J = 6 Hz, 2 H, 2-H), 2.7 (t; J = 6 Hz, 2 H, 4-H), 2.8, 2.9 (ea dd; J = 7 Hz, 4 H, 10-H, 13-H), 3.6 (s; 3 H, OCH_3), 5.2–5.4 (m; 5 H, 9-H, 11-H, 12-H, 14-H, 15-H), 5.9 (dd; $^3J_{8,7}$ = 11.0 Hz, $^3J_{8,9}$ = 10.8 Hz, 1 H, 8-H), 6.10 (d; J = 14.9 Hz, 1 H, 6-H), 6.4 (dd; $^3J_{7,6}$ = 14.9 Hz, $^3J_{7,8}$ = 11.0 Hz, 1 H, 7-H). Anal. (C$_{20}$H$_{32}$O$_2$S) C; H: calcd, 9.6; found, 10.6; S: calcd, 9.5; found, 10.0.

Methyl (6E,8Z)-5-Thia-6,8-eicosadien-11-ynoate (15b). 18b (1.198 g, 2.0 mmol) in 11 mL of THF was reacted with 1.3 mL of a 1.6 M *n*-butyllithium solution (2.0 mmol) and 376 mg (2.0 mmol) of 13a as described for 15a to give 15b (238 mg, 35%); R_f 0.76 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH_3), 1.2–1.4 [b; 12 H, (CH_2)$_6$CH$_3$], 1.9 (q; J = 7 Hz, 2 H, 3-H), 2.1 (m; 2 H, 13-H), 2.4 (t; J = 7 Hz, 2 H, 4-H), 2.7 (t; J = 7 Hz, 2 H, 2-H), 3.0 (m; 2 H, 10-H), 3.6 (s; 3 H, OCH_3), 5.2 (dt; $^3J_{9,8}$ = 10.7 Hz, $^3J_{9,10}$ = 7.0 Hz, 1 H, 9-H), 5.9 (dd; $^3J_{8,9}$ = $^3J_{8,7}$ = 10.7 Hz, 1 H, 8-H), 6.15 (d; J = 15.0 Hz, 1 H, 6-H), 6.3 (dd; $^3J_{7,6}$ = 14.7 Hz, $^3J_{7,8}$ = 11.2 Hz, 1 H, 7-H). Anal. (C$_{20}$H$_{32}$O$_2$S) C; H: calcd, 9.6; found, 10.3; S: calcd, 9.5; found, 8.1.

Methyl (6E,8Z,11Z)-5-Thia-6,8,11-eicosatrienoate (15c). 18c (6.01 g, 10.0 mmol) in 22 mL of THF was reacted with 6.2 mL of a 1.6 M *n*-butyllithium solution (10.0 mmol) and 1.88 g (10.0 mmol) of 13a as described for 15a to give 15c (2.48 g, 73.5%); R_f 0.85 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 300 MHz) δ 0.8 (t; J = 7 Hz, 3 H, CH$_2$CH_3), 1.2 [b; 12 H, (CH_2)$_6$CH$_3$], 1.9 (q; J = 7 Hz, 2 H, 3-H), 2.0 (dd; J = 7 Hz, 2 H, 13-H), 2.4 (t; J = 7 Hz, 2 H, 2-H), 2.7 (t; J = 7 Hz, 2 H, 4-H), 2.8 (dd; J = 7 Hz, 2 H, 10-H), 3.6 (s; 3 H, OCH_3), 5.30 (dt; $^3J_{11,12}$ = 10.2 Hz, J = 7.7 Hz, 1 H, 11-H or 12-H), 5.35 (m; 1 H, 11 H or 12 H), 5.4 (dt; $^3J_{9,8}$ = 10.2 Hz, $^3J_{9,10}$ = 8.3 Hz, 1 H, 9-H), 5.95 (dd; $^3J_{8,7}$ = $^3J_{8,9}$ = 10.2 Hz, 1 H, 8-H), 6.20 (d; J = 14.7 Hz, 1 H, 6-H), 6.5 (dd; $^3J_{7,6}$ = 14.7 Hz, $^3J_{7,8}$ = 10.2 Hz). Anal. (C$_{20}$H$_{34}$O$_2$S) C, S; H: calcd, 10.1; found, 10.8.

Methyl (6E,8Z)-5-Thia-6,8-tridecadien-11-ynoate (15d). 18d (1.597 g, 3.19 mmol) in 12 mL of THF was reacted with 1.98 mL of a 1.61 M *n*-butyllithium solution (3.2 mmol) and 600 mg (3.19 mmol) of 13a as described for 15a to give 15d (283 mg; 37%); R_f 0.82 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 1.7 (t; J = 2 Hz, 3 H, 13-H), 1.9 (q; J = 6 Hz, 2 H, 3-H), 2.4 (t; J = 6 Hz, 2 H, 2-H), 2.7 (t; J = 6 Hz, 2 H, 4-H), 2.9 (m; 2 H, 10-H), 3.6 (s; 3 H, OCH_3), 5.2 (dt; $^3J_{9,8}$ = 10.5 Hz, $^3J_{9,10}$ = 7.4 Hz, 2 H, 19-H), 5.9 (dd; $^3J_{8,7}$ = 10.7

Hz, $^3J_{8,9}$ = 10.5 Hz, 1 H, 8-H), 6.15 (d; J = 14.9 Hz, 1 H, 6-H), 6.30 (dd; $^3J_{7,6}$ = 14.9 Hz, $^3J_{7,8}$ = 10.7 Hz, 1 H, 7-H). Anal. ($C_{13}H_{18}O_2S$) C, H, S.

Methyl (6E,8Z)-5-Thia-6,8-eicosadienoate (15e). 18e (2.17 g, 4.2 mmol) in 25 mL of THF was reacted with 2.6 mL of a 1.62 M n-butyllithium solution (4.2 mmol) and 800 mg (4.3 mmol) 13a as described for 15a to give 15e (870 mg; 60%); R_f 0.67 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 300 MHz) δ 0.9 (t; J = 7 Hz, 3 H, CH$_2$CH$_3$), 1.3 [b; 18 H, (CH$_2$)$_9$CH$_3$], 1.9 (q; J = 7 Hz, 2 H, 3-H), 2.1 (dt; $^3J_{10,9}$ = $^3J_{10,11}$ = 7 Hz, 2 H, 10-H), 2.4 (t; J = 7 Hz, 2 H, 2-H), 2.8 (t; J = 7 Hz, 2 H, 4-H), 3.6 (s; 3 H, OCH$_3$), 5.3 (dt; $^3J_{9,10}$ = 7.5 Hz, $^3J_{9,8}$ = 10.5 Hz, 1 H, 9-H), 5.95 (dd; $^3J_{8,7}$ = 11.2 Hz, $^3J_{8,9}$ = 10.5 Hz, 1 H, 8-H), 6.15 (d; J = 15 Hz, 1 H, 6-H), 6.45 (dd; $^3J_{7,6}$ = 15 Hz, $^3J_{7,8}$ = 11.2 Hz, 1 H, 7-H). Anal. (C$_{20}$H$_{36}$O$_2$S) C, H, S.

Methyl (5E,7Z,10Z)-4-Thia-5,7,10-nonadecatrienoate (15f). 18b (601 mg, 1.0 mmol) in 11 mL of THF was reacted with 382 μL of a 2.6 M n-butyllithium solution (1.0 mmol) and 174 mg (1.0 mmol) 13b according to the procedure given for 15a to give 15f (118 mg; 36%); R_f 0.74 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH$_3$), 1.2 [m; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (dt; $^3J_{12,13}$ = 6 Hz, 2 H, 12-H), 2.6 (t; J = 7 Hz, 2 H, 3-H), 2.8 (t; J = 7 Hz, 2 H, 9-H), 2.9 (t; J = 7 Hz, 2 H, 2-H), 3.6 (s; 3 H, OCH$_3$), 5.2 (m; 2 H, 10-H, 11-H), 5.35 (dt; $^3J_{8,7}$ = 10.6 Hz, $^3J_{8,9}$ = 7.0 Hz, 1 H, 8-H), 5.90 (dd; $^3J_{7,8}$ = 10.6 Hz, $^3J_{7,6}$ = 10.6 Hz, 1 H, 7-H), 6.10 (d; J = 14.9 Hz, 1 H, 5-H), 6.40 (dd; $^3J_{6,5}$ = 15.0 Hz, $^3J_{6,7}$ = 11.0 Hz, 1 H, 6-H). Anal. (C$_{19}$H$_{32}$O$_2$S) C; H: calcd, 9.9; found, 10.5; S: calcd, 9.9; found, 10.6.

Methyl (1E,3Z,6Z)-2-(1,3,6-Pentadecatrienylthio)benzoate (15g). 18c (1.20 g, 2.0 mmol) in 12 mL of THF was reacted with 1.3 mL of a 1.6 M solution of n-butyllithium (2.0 mmol) and 445 mg (2.0 mmol) of 13c according to the procedure given for 15a to give 15g (241 mg; 32%); R_f 0.82 (petroleum ether/ether, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.7 (t; J = 6 Hz, 3 H, CH$_2$CH$_3$), 1.2 [m; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (dt; $^3J_{8,9}$ = $^3J_{8,7}$ = 6 Hz, 2 H, 8'-H), 2.9 (dd; $^3J_{5,6}$ = $^3J_{5,4}$ = 7 Hz, 2 H, 5'-H), 3.8 (s; 3 H, OCH$_3$), 5.3 (m; 2 H, 6'-H, 7'-H), 5.4 (dt; $^3J_{4,3}$ = 10.7 Hz, $^3J_{4,5}$ = 7.7 Hz, 1 H, 4'-H), 6.1 (dd, $^3J_{3,4}$ = $^3J_{3,2}$ = 10.9 Hz, 1 H, 3'-H), 6.3 (d; J = 14.8 Hz, 1 H, 2'-H), 7.1, 7.4 (ea dd; $^3J_{4,3}$ = $^3J_{4,5}$ = $^3J_{5,4}$ = $^3J_{5,6}$ = 7 Hz, 2 H, 4-H, 5-H), 7.3, 7.9 (ea d; J = 7 Hz, 2 H, 3-H, 6-H). Anal. (C$_{23}$H$_{32}$O$_2$S) C, H, S.

Methyl (1E,3Z,6Z)-3-(1,3,6-Pentadecatrienylthio)benzoate (15h). 18c (1.80 g, 3.0 mmol) in 8 mL of THF was reacted with 1.12 mL of a 2.68 M solution of n-butyllithium (3.0 mmol) and 734 mg (3.3 mmol) of 13d according to the procedure for 15a to give 15h (975 mg; 87%); R_f 0.81 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH$_3$), 1.3 [m; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (dt; $^3J_{8,9}$ = 6 Hz, $^3J_{8,7}$ = 6 Hz, 2 H, 8'-H), 3.9 (s; 3 H, OCH$_3$), 5.35–5.50 (m; 3 H, 4'-H, 6'-H, 7'-H), 6.10 (dd; $^3J_{3,4}$ = $^3J_{3,2}$ = 10.8 Hz, 1 H, 3'-H), 6.40 (d; J = 14.8 Hz, 1 H, 1'-H), 6.80 (dd; $^3J_{2,1}$ = 14.7 Hz, $^3J_{2,3}$ = 11.2 Hz, 1 H, 2'-H), 7.40 (dd; $^3J_{5,4}$ = $^3J_{5,6}$ = 7.7 Hz, 1 H, 5-H), 7.55, 7.85 (ea ddd; $^3J_{4,5}$ = $^3J_{6,5}$ = 7.7 Hz, $^4J_{4,2}$ = $^4J_{6,2}$ = 1.8 Hz, $^4J_{4,6}$ = $^4J_{6,4}$ = 1.1 Hz, 2 H, 4-H, 6-H), 8.0 (dd; $^4J_{2,4}$ = $^4J_{2,6}$ = 1.8 Hz, 1 H, 2-H). Anal. (C$_{23}$H$_{32}$O$_2$S) C, H; S: calcd, 8.6; found, 7.6.

Methyl (1E,3Z,6Z)-4-(1,3,6-Pentadecatrienylthio)benzoate (15i). 18c (1.20 g, 2.0 mmol) in 12 mL of THF was reacted with 1.3 mL of a 1.61 M solution of n-butyllithium (2.1 mmol) and 445 mg (2.0 mmol) of 13e according to the procedure for 15a to give 15i (411 mg; 55%); R_f 0.81 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH$_3$), 1.2 [m; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (dt; $^3J_{8,7}$ = $^3J_{8,9}$ = 7 Hz, 2 H, 8'-H), 2.9 (dd; $^3J_{5,4}$ = $^3J_{5,6}$ = 7 Hz, 2 H, 5'-H), 3.8 (s; 3 H, OCH$_3$), 5.3 (m; 2 H, 6'-H, 7'-H), 5.4 (dt; $^3J_{4,3}$ = 11.5 Hz, $^3J_{4,5}$ = 7.7 Hz, 1 H, 4'-H), 6.05 (dd; $^3J_{3,4}$ = $^3J_{3,2}$ = 11.1 Hz, 1 H, 3'-H), 6.3 (d; J = 14.7 Hz, 1 H, 1'-H), 6.75, 6.85 (ea dd; $^3J_{2,3}$ = $^3J_{3,2}$ = 11.3 Hz, $^4J_{2,6}$ = $^4J_{3,5}$ = 1.1 Hz, 4 H, 2-H, 3-H, 5-H, 6-H). Anal. (C$_{23}$H$_{32}$O$_2$S) C, H, S.

Methyl (1E,3Z)-2-(1,3-Pentadecadien-6-ynylthio)benzoate (15j). 18b (1.198 g, 2.0 mmol) in 11 mL of THF was reacted with 1.3 mL of a 1.61 M solution of n-butyllithium (2.1 mmol) and 445 mg (2.0 mmol) of 13c according to the procedure for 15a to give 15j (350 mg; 47%); R_f 0.77 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 7 Hz, 3 H, CH$_2$CH$_3$), 1.2 [b; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (m; 2 H, 8'-H), 3.0 (m; 2 H, 5'-H),

3.8 (s; 3 H, OCH$_3$), 5.45 (dt; $^3J_{4,3}$ = 10.7 Hz, $^3J_{4,5}$ = 7.3 Hz, 1 H, 4'-H), 6.1 (dd; $^3J_{3,4}$ = 10.7 Hz, $^3J_{3,2}$ = 11.2 Hz, 1 H, 3'-H), 6.4 (d; J = 14.8 Hz, 1 H, 1'-H), 6.85 (dd; $^3J_{2,1}$ = 14.8 Hz, $^3J_{2,3}$ = 11.2 Hz, 1 H, 2'-H). Anal. (C$_{23}$H$_{30}$O$_2$S) C, H, S.

Methyl (1E,3Z)-3-(1,3-Pentadecadien-6-ynylthio)benzoate (15k). 18b (1.198 g, 2.0 mmol) in 11 mL of THF was reacted with 1.3 mL of a 1.61 M solution of n-butyllithium (2.1 mmol) and 445 mg (2.0 mmol) of 13d according to the procedure for 15a to give 15k (481 mg; 65%); R_f 0.77 (petroleum ether/ether, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 0.8 (t; J = 6 Hz, 3 H, CH$_2$CH$_3$), 1.2–1.4 [b; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (m; 2 H, 8'-H), 2.9 (m; 2 H, 5'-H), 3.8 (s; 3 H, OCH$_3$), 5.4 (dt; $^3J_{4,3}$ = 10.6 Hz, $^3J_{4,5}$ = 7.2 Hz, 1 H, 4'-H), 6.0 (dd; $^3J_{3,4}$ = $^3J_{3,2}$ = 10.9 Hz, 1 H, 3'-H), 6.35 (d; J = 14.8 Hz, 1 H, 1'-H), 6.6 (dd; $^3J_{2,1}$ = 14.8 Hz, $^3J_{2,3}$ = 11.2 Hz). Anal. (C$_{23}$H$_{30}$O$_2$S) C; H: calcd, 8.2; found, 8.7; S: calcd, 8.7; found, 8.1.

Methyl (1E,3Z)-4-(1,3-Pentadecadien-6-ynylthio)benzoate (15l). 18b (1.198 g, 2.0 mmol) in 11 mL of THF was reacted with 1.3 mL of a 1.61 M solution of n-butyllithium (2.1 mmol) and 445 mg (2.0 mmol) of 13e according to the procedure for 15a to give 15l (453 mg; 61%); R_f 0.78 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 200 MHz) δ 0.8 (t; J = 7 Hz, 3 H, CH$_2$CH$_3$), 1.2–1.4 [b; 12 H, (CH$_2$)$_6$CH$_3$], 2.1 (m; 2 H, 8'-H), 3.1 (m; 2 H, 5'-H), 3.9 (s; 3 H, OCH$_3$), 5.5 (dt; $^3J_{4,3}$ = 10.5 Hz, $^3J_{4,5}$ = 7.4 Hz, 1 H, 4'-H), 6.15 (dd; $^3J_{4,3}$ = 10.5 Hz, $^3J_{4,5}$ = 7.4 Hz, 1 H, 4'-H), 6.15 (dd; $^3J_{3,2}$ = 11.2 Hz, $^3J_{3,4}$ = 10.5 Hz, 1 H, 3'-H), 6.5 (d; J = 14.8 Hz, 1 H, 1'-H), 6.85 (dd; $^3J_{2,1}$ = 14.8 Hz, $^3J_{2,3}$ = 11.2 Hz, 1 H, 2'-H), 7.35, 7.95 (ea d; J = 8.5 Hz, 4 H, ar CH). Anal. (C$_{23}$H$_{30}$O$_2$S) C, H, S.

(6E,8Z,11Z)-5-Thia-6,8,11-eicosatrienoic Acid (15m). 15c (800 mg, 2.36 mmol) was dissolved in 20 mL of MeOH followed by the addition of 471 mg (11.2 mmol) of LiOH·H$_2$O and 2.5 mL of H$_2$O. The resulting mixture was stirred at 20 °C for 2.5 h and was subsequently treated with 110 mL of 0.1 N HCl. The solvents were stripped off, and the residue was chromatographed on silica gel to give 15m (743 mg; 97%); R_f 0.32 (petroleum ether/Et$_2$O, 1:1).

^{1}H NMR (CD$_2$Cl$_2$; 200 MHz) δ 0.9 (t; J = 8 Hz, 3 H, CH$_2$CH$_3$), 1.3 [m; 12 H, (CH$_2$)$_6$CH$_3$], 2.0 (m; 4 H, 13-H, 3-H), 2.5 (t; J = 8 Hz, 2 H, 2-H), 2.8 (t; J = 7 Hz, 2 H, 4-H), 2.9 (t; J = 8 Hz, 2 H, 10-H), 5.2–5.5 (m; 3 H, 9-H, 11-H, 12-H), 6.0 (dd; $^3J_{8,7}$ = 10.3 Hz, $^3J_{8,9}$ = 10.0 Hz, 1 H, 8-H), 6.2 (d; J = 14.1 Hz, 1 H, 6-H), 6.5 (dd; $^3J_{7,6}$ = 14.1 Hz, $^3J_{7,8}$ = 10.3 Hz, 1 H, 7-H). Anal. (C$_{19}$H$_{32}$O$_2$S) C, S; H: calcd, 9.9; found, 10.8.

Preparation of Alcohols 16. (*Z*,*Z*)-3,6-Dodecadien-1-ol (16a). 3-Nonenyltriphenylphosphonium tosylate[16] (21 g, 38 mmol) was dissolved in 150 mL of THF followed by the addition of 23.5 mL of a 1.61 M solution of n-butyllithium (38 mmol) in hexane at −20 °C. After being stirred for 15 min, the reaction mixture was cooled to −78 °C and 20 mL of DMEU was added followed by the addition of 7.1 g (38 mmol) of 3-[(*tert*-butyldimethyl-silyl)oxy]propanal[13] (22) in 20 mL of THF. The reaction mixture was warmed up to −40 °C and stirred at this temperature for 5 h. Hexane (300 mL) was added, and the mixture was warmed up to 20 °C.

The lower layer was extracted twice with 50 mL of hexane, the hexane fractions were combined with the upper layer, the solvents were removed in vacuo, and the residue was chromatographed (silica gel; petroleum ether/Et$_2$O, 100:1, followed by 1:1) to give 24 (6.7 g, 60%), which was subsequently deprotected as follows: 6.7 g of 24 was dissolved in THF and 29 mL (29 mmol) of a 1 M solution of tetrabutylammonium fluoride in THF was added under vigorous stirring. After 2 h 1.4 mL (29 mmol) of glacial acetic acid was added and the solvents were removed in vacuo. The crude residue was flash chromatographed (silica gel; petroleum ether followed by CH$_2$Cl$_2$) to give 16a (3.46 g, 78%), which was used without further purification for the preparation of 17a.

3-Dodecyn-1-ol (16b). But-3-yn-1-yl tetrahydropyranyl ether (33 g, 0.21 mol) was dissolved in 200 mL of THF and cooled to −78 °C. A 2.75 M solution of n-butyllithium (85 mL, 0.24 mol) was added, and the resulting mixture was warmed to 0 °C and then cooled to −78 °C again. LiI (1.5 g, 12 mmol) and 41 mL of octyl bromide were dissolved in 110 mL of DMEU, and the

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mixture was added to the first solution. The reaction mixture was warmed to 20 °C and stirred for 20 h. Hexane (0.5 L) and 0.5 L of H_2O were added, the layers were separated, and the lower one was extracted twice with 100 mL of hexane. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated to give crude 21 (64.8 g), which was used without further purification.

Crude 21 (64 g) was dissolved in 0.5 L of methanol, 3 g of Amberlyst 15 (H$^+$ form) was added, and the resulting mixture was stirred at 40 °C for 20 h and then filtered. K_2CO_3 (0.5 g) was added to the filtrate, the solvent was removed in vacuo, and the residue was flash chromatographed (silica gel; petroleum ether/ Et_2O, 10:1) to give 16b (27.7 g; 72% overall yield), which was used for the synthesis of 16c and 17b without further purification.

(*Z*)-3-Dodecen-1-ol (16c) was synthesized according to ref 17. 16b (4.56 g, 25 mmol) was hydrogenated to give 16c (4.41 g; 96%).

3-Pentyn-1-ol (16d) was purchased from Aldrich.

Preparation of Tosylates 17. (*Z,Z*)-3,6-Dodecadienyl *p*-Toluenesulfonate[18] (17a). TsCl (3.6 g, 19 mmol), 580 mg (4.8 mmol) of DMAP, and 3.2 mL of NEt$_3$ (23 mmol) were dissolved in 45 mL of CH_2Cl_2 and cooled to -10 °C. 16a (3.5 g, 19 mmol) in 45 mL of CH_2Cl_2 was added, and the resulting mixture was stirred at -4 °C (very thorough temperature control is necessary) for 7 h. H_2O (30 mL) was added, and the mixture was warmed to +5 °C. After 10 min, 50 g of crushed ice, 50 mL of H_2O, 50 mL of CH_2Cl_2, and 1.1 mL of HCl were added, and the resulting two layers were separated as quickly as possible. The organic layer was washed immediately with saturated NaHCO$_3$ and then washed with brine and dried over Na$_2$SO$_4$. The solvent was evaporated in vacuo and the residue chromatographed (silica gel; petroleum ether/ether, 10:1) to give 17a (4.52 g; 54%).

3-Dodecynyl *p*-Toluenesulfonate (17b). 16b (18.5 g, 0.1 mol) and 19.1 g (0.1 mol) of TsCl were reacted according to the procedure given for 17a to give 17b (10.0 g; 30%).

^{1}H NMR (CD$_2$Cl$_2$; 300 MHz) δ 0.9 (t; *J* = 9 Hz, 3 H, 12-H), 1.3 [m, b; 12 H, (C*H*$_2$)$_6$CH$_3$], 2.1 (m; 2 H, 5-H), 2.5 (m; 5 H, 2-H, ar C*H*$_3$), 4.0 (t; *J* = 9 Hz, 2 H, 1-H), 7.4 and 7.8 (ea d; *J* = 11 Hz, 4 H, arC*H*).

(*Z*)-3-Dodecenyl *p*-Toluenesulfonate (17c).[18,19] 16c (36.9 g, 0.2 mol) and 38.2 g (0.2 mol) of TsCl were reacted according to the procedure given for 17a to give 17c (66.5 g; 98%).

3-Pentynyl *p*-Toluenesulfonate (17d).[20] Pent-3-ynol (8.4 g, 0.1 mol) and 19.1 g (0.1 mol) of TsCl were reacted according to the procedure given for 17a to give 17d (20.4 g; 86%).

Synthesis of Wittig Salts 18. 3,6-Dodecadienyltriphenylphosphonium *p*-Toluenesulfonate (18a). 17a (4.52 g, 10.2 mmol) and 2.67 g (10.2 mmol) of triphenylphosphine were heated under argon without solvent for 18 h at 120 °C. The resulting mixture was cooled to 20 °C, mixed with 8 mL of toluene and 80 mL of petroleum ether, heated to reflux, and cooled down again. The upper layer was removed, and the lower layer was treated again as described with toluene and petroleum ether. After removal of the upper layer the residue was dried at 10^{-3} mbar for 24 h to give 18a (3.3 g; 54%). The product was sufficiently pure as indicated by the ^{13}C NMR spectrum and was used without further purification.

^{13}C NMR (CD$_2$Cl$_2$; 50 MHz) δ 14 (12-C), 20.5 (11-C), 21 (ar CH$_3$), 21 (d; $^1J_{CP}$ = 65.8 Hz, 1-C), 23 (10-C), 25 (9-C), 27 (8-C), 29 (2-C), 32 (5-C), 118 (d; $^1J_{CP}$ = 86.1 Hz, ar P-*C*), 126, 126.5, 127 (4-C, 6-C, 7-C), 126, 128, 139, 145 (ar *C*-tosyl), 130, 134, 135 (ar *C*-phosphonium), 130.5 (3-C).

3-Dodecynyltriphenylphosphonium *p*-Toluenesulfonate (18b). 17b (10.0 g, 30 mmol) and 7.95 g (30 mmol) of triphenylphosphine were reacted as described for 18a to give 18b (13.6 g; 76%).

^{13}C NMR (CD$_2$Cl$_2$; 75 MHz) δ 13 (d; $^1J_{CP}$ = 4.6 Hz, 2-C), 14 (12-C), 18.5 (5-C), 21 (ar CH$_3$), 21 (d; $^1J_{CP}$ = 52.7 Hz, 1-C), 23, 28.5, 29, 29.2, 29.3, 32 (6-C, 7-C, 8-C, 9-C, 10-C, 11-C), 76.5 (d; $^3J_{CP}$ = 7.4 Hz, 3-C), 85 (4-C), 118 (d; $^1J_{CP}$ = 86.6 Hz, ar P-*C*), 126, 128, 139, 145 (ar *C*-tosyl), 130, 134, 135 (ar *C*-phosphonium).

3-Dodecenyltriphenylphosphonium *p*-Toluenesulfonate (18c). 17c (62 g, 183 mmol) and 48.6 g (183 mmol) of triphenylphosphine were reacted as described for 18a to give 18c (91.0 g; 83%).

^{13}C NMR (CD$_2$Cl$_2$); 62.5 MHz) δ 14 (20-C), 20.5 (19-C), 21 (ar CH$_3$), 22.5 (d; $^1J_{PC}$ = 49.3 Hz, 1-C), 23 (10-C), 27.5 (5-C), 29.5 (2-C), 29.5, 29.6, 29.7, 32 (6-C, 7-C, 8-C, 9-C), 118 (d; $^1J_{PC}$ = 85.8 Hz, ar P-*C*), 126 (4-C), 126.5, 129, 139, 145 (ar *C*-tosyl), 131, 134, 135.5 (ar *C*-phosphonium), 133 (3-C).

3-Pentynyltriphenylphosphonium *p*-Toluenesulfonate (18d). 17d (11.9 g, 50 mmol) and 13.1 g (50 mmol) triphenylphosphine were reacted as described for 18a to give 18d (18.0 g; 71.9%).

^{13}C NMR (CD$_2$Cl$_2$; 75 MHz) δ 3 (5-C), 13 (d; $^2J_{PC}$ = 5.1 Hz, 2-C), 21 (ar CH$_3$), 22 (d; $^1J_{PC}$ = 52.8 Hz, 1-C), 75.5 (d; $^3J_{PC}$ = 8.2 Hz, 3-C), 81 (4-C), 118 (d; $^1J_{PC}$ = 86.0 Hz, ar *C*-P), 126, 128, 138, 145 (ar *C*-tosyl), 130, 134, 135 (ar *C*-phosphonium).

Dodecyltriphenylphosphonium bromide (18e) was purchased from Alfa.

Methyl 8-Oxo-5-thiaoctanoate (26). γ-Thiobutyrolactone (11.0 g, 100 mmol) was added to a solution of 6.7 g (120 mmol) of sodium methoxide in 100 mL of CH$_3$OH. The resulting mixture was stirred for 1.5 h at 45 °C, then cooled down to 20 °C, and neutralized by the addition of 9.1 mL (110 mmol) of HCl and 570 µL (10 mmol) of glacial acetic acid. The mixture was cooled to 0 °C followed by the addition of 5.6 g (100 mmol) of acrolein and then stirred at 20 °C for 7 h. Toluene (50 mL) was added, and the solvents were removed at 50 °C under reduced pressure. The residue was dissolved in 100 mL of toluene and 100 mL of H_2O, and the organic layer was separated, washed with saturated NaHCO$_3$ and brine, and then dried over MgSO$_4$. The organic solvent was removed in vacuo and the crude residue purified by flash chromatography to give 26 (10.5 g, 55%), which was used for the synthesis of 27 without further purification.

^{1}H NMR (CD$_2$Cl$_2$; 250 MHz) δ 1.9 (q; *J* = 7 Hz, 2 H, 3-H), 2.4 (t; *J* = 7 Hz, 2 H, 2-H), 2.5 (t; *J* = 7 Hz, 2 H, 4-H), 2.7 (m; 4 H, 5-H, 6-H), 3.6 (s; 3 H, OCH$_3$), 9.7 (t; *J* = 1 Hz, 1 H, 8-H). Anal. (C$_8$H$_{14}$O$_3$S) C, H.

Methyl (8*Z*,11*Z*)-5-Thia-8,11-eicosadienoate (27). A 1.61 M solution of *n*-butyllithium (1.3 mL, 2.0 mmol) was added at -20 °C to 1.20 g (2.0 mmol) of 3-dodecenyltriphenylphosphonium tosylate (19) in 7 mL of THF. The blood-red solution was stirred for a further 3 h and allowed to warm to 0 °C, and 10 mL of hexane was added. The lower layer was extracted twice with 5 mL of hexane, combined with the upper layer, and dried over MgSO$_4$. The solvents were removed under reduced pressure, and the crude residue was chromatographed (20-µm silica gel; petroleum ether/Et$_2$O, 15:1) to give 27 (368 mg, 54%).

^{1}H NMR (CD$_2$Cl$_2$; 200 MHz) δ 0.9 (b; 3 H, 20-H), 1.3 (b; 12 H, 14-19 H), 1.9 (q; *J* = 6 Hz, 2 H, 3-H), 2.1 (b; 2 H, 13-H), 2.4 (b; 2 H, 7-H), 2.45 (t; *J* = 6 Hz, 2 H, 4-H), 2.55 (m; 4 H, 2-H, 6-H), 2.8 (t; *J* = 5 Hz, 2 H, 10-H), 3.6 (s; 3 H, OCH$_3$), 5.4 (m; 4 H, 8-H, 9-H, 11-H, 12-H). Anal. (C$_{20}$H$_{36}$O$_2$S) C, H.

Methyl 8-Hydroxy-6-octynoate (30). Solution A. A 2.66 M solution of *n*-butyllithium in hexane (133 mL, 0.3 mol) was added to a solution of 42 g (0.3 mol) of propynol tetrahydropyranyl ether (28) in 300 mL of THF at -78 °C. The resulting mixture was warmed up to -20 °C, stirred for 1 h, and cooled down again to -78 °C.

Solution B. LiI (4.4 g, 33 mmol) was dissolved in 300 mL of DMEU at 40 °C. The solution was cooled to 20 °C, and 79.5 g (0.3 mol) of ortho ester 29 was added.

Solution B was added to solution A over a period of 30 min at -78 °C, and the resulting mixture was warmed up to 20 °C and stirred for 16 h. H_2O (200 mL) and 500 mL of petroleum ether were added, the lower layer was extracted three times with 50 mL of petroleum ether, and the combined organic layers were washed with brine and dried over MgSO$_4$. The solvents were removed in vacuo, and the crude residue was purified by flash chromatography (silica gel slurry prepared with petroleum ether and 0.5% NEt$_3$; chromatographed with petroleum ether/ethyl acetate, 20:1) to give 1-[7-(tetrahydropyran-2-yloxy)hept-5-ynyl]-4-ethyl-2,6,7-trioxabicyclo[2.2.2]octane (60.4 g, 62%) which was directly deprotected as follows: 500 mL of CH$_3$OH was added, followed by the addition of 15 g amberlyst 15 (H$^+$ form). The resulting mixture was refluxed for 3 h, cooled to 20 °C, and filtered

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to remove the ion-exchange resin. The solvents were removed in vacuo, and the crude mixture (54 g) was used without delay for the next reaction step: 48 g of the crude mixture was dissolved in 500 mL of CH_3OH, 10 g (0.25 mol) of $LiOH \cdot H_2O$ in 250 mL of H_2O was added, and the resulting mixture was stirred at 40 °C for 6 h and then 16 h at 20 °C. CH_3OH was removed under reduced pressure, and the residue was acidified to pH 2 with 2 N HCl and extracted 10 times with 50-mL portions of Et_2O. The combined organic fractions were dried over $MgSO_4$, and the solvent was removed to give 18.2 g of product, which was esterified without further purification.

The product was dissolved in 100 mL of DMF, 19.2 g (0.14 mol) K_2CO_3 and 19.7 g (0.14 mol) of MeI were added, and the resulting mixture was stirred at 40 °C for 16 h. The solvent was removed (40 °C, 10^{-2} mbar), and the crude residue was treated with 100 mL of CH_2Cl_2 and filtered. The filtrate was concentrated in vacuo, and the residue was flash chromatographed (silica gel; petroleum ether/ethyl acetate, 10:1) to give **30** (16.5 g; 84%, corresponds to 32% yield from **29**). The product was used without further purification.

^1H NMR (CD_2Cl_2; 300 MHz) δ 1.5 (m; 2 H, 3-H or 4-H), 1.7 (m; 2 H, 3-H or 4-H), 1.8 (b; 1 H, O*H*), 2.2 (tt; $^3J_{5,4}$ = 7 Hz, $^5J_{5,8}$ = 2 Hz, 2 H, 5-H), 2.3 (t; J = 7 Hz, 2 H, 2-H), 3.6 (s; 3 H, OC*H₃*), 4.2 (t; $^5J_{8,5}$ = 2 Hz, 2 H, 8-H).

Methyl (6*E*)-8-Oxo-6-octenoate (31). **30** (8.5 g, 50 mmol) was hydrogenated according to the procedure of H. C. Brown[17] to give 7.9 g of product (91%), which was directly oxidized to **31** by the following procedure: 4.3 g of the product described above was dissolved in 200 mL of CH_2Cl_2, 17.4 g (0.2 mol) of MnO_2 was added, and the resulting mixture was refluxed for 16 h. Silica gel (20 g) was added, and the resulting slurry was filtered under argon (pressure filter funnel) and thoroughly washed with ethyl acetate. The solvents were removed under reduced pressure to give **31** (1.9 g, 44%) as a mixture of the cis and the trans isomers. A 674-mg sample of this mixture was dissolved in 30 mL of CH_2Cl_2, and 3 mg of I_2 and 50 μL of DBN were added. The resulting mixture was stirred at 20 °C for 16 h, 100 mg of Al_2O_3 was added, and the solvent was removed in vacuo. The residue was flash chromatographed (silica gel slurry prepared with petroleum ether containing 0.5% Et_3N; chromatographed with petroleum ether/ethyl acetate, 10:1) to give **31** (598 mg; corresponds to 36% total yield from **30**).

31 was used without further purification for the preparation of **32**.

^1H NMR (CD_2Cl_2; 250 MHz) δ 1.5 (m; 4 H, 3-H, 4-H), 2.3 (m; 4 H, 2-H, 5-H), 3.6 (s; 3 H, OC*H₃*), 6.0 (dd; $^3J_{7,6}$ = 15.7 Hz, $^3J_{7,8}$ = 8.0 Hz, 1 H, 7-H), 6.8 (dt; $^3J_{6,7}$ = 15.7 Hz, $^3J_{6,5}$ = 6.7 Hz, 1 H, 6-H), 9.4 (d; $^3J_{8,7}$ = 8.0 Hz, 1 H, 8-H).

Methyl (6*E*,8*Z*,11*Z*)-6,8,11-Eicosatrienoate (32). **31** (438 mg, 2.6 mmol) was reacted with 2.6 mmol 3-dodecenyltriphenylphosphonium tosylate as described for **27**. Chromatography (20-μm silica gel; petroleum ether/Et_2O, 19:1) provided **32** (474 mg, 57%).

^1H NMR (CD_2Cl_2; 200 MHz) δ 0.8 (t; J = 7 Hz, 3 H, 20-H), 1.3 [m; 14 H, 4-H, (C*H₂*)₆CH₃], 1.6 (m; 2 H, 3-H), 2.1 (m; 4 H, 5-H, 13-H), 2.3 (t; J = 8 Hz, 2 H, 2-H), 2.9 (t, J = 7 Hz, 2 H, 10-H), 3.6 (s; 3 H, OC*H₃*), 5.3 (m; 3 H, 9-H, 11-H, 12-H), 5.6 (dt; $^3J_{6,5}$ = 6.0 Hz, $^3J_{6,7}$ = 15.0 Hz, 1 H, 6-H), 5.9 (t; J = 10.8 Hz, 1 H, 8-H), 6.3 (dd; $^3J_{7,6}$ = 15.0 Hz, $^3J_{7,8}$ = 10.8 Hz, 1 H, 7-H); mass spectrum, m/z 320 (parent), 205, 161. Anal. ($C_{21}H_{36}O_2$) C: calcd, 78.7; found, 79.2; H: calcd, 11.3; found, 11.9.

Biological Assays. 5-Lipoxygenase Inhibition Assay. 5-Lipoxygenase was prepared as a 100000g supernatant from RBL-1 cells. Test compound or vehicle (2% DMSO) was preincubated at 25 °C for 5 min with the enzyme (2.5×10^6 cell equivalents) in 0.5 mL of assay buffer (50 mM KH_2PO_4, 50 μM ATP, pH 7.0). Reactions were started by the addition of 30 μM arachidonic acid (containing 0.1 μCi of [^{14}C]arachidonic acid) and stopped after 5 min by precipitation of protein with methanol/$CHCl_3$ (2:1). The samples were then acidified with formic acid and extracted with $CHCl_3$. Reaction products were separated from nonconverted substrate by TLC on silica gel by using petroleum ether/ether/acetic acid (50:50:1) and quantitated by using a Berthold LB 2842 linear analyzer. Inhibition of 5-lipoxygenase activity was calculated as the ratio of the amounts of substrate remaining in the absence and presence of inhibitor. IC_{50} values were determined by linear regression analysis of percentage in-

hibition versus log inhibitor concentration.

Selectivity Assays. The 100000g supernatant was prepared from polymorphonuclear cells isolated to 95% purity according to the method of Boyum.[21] Aliquots of this preparation were preincubated at 20 °C for 10 min in the presence or absence of test compound in phosphate-buffered saline (pH 8.3) containing 0.5 mM $MgCl_2$. Reactions were started by the addition of 120 μM arachidonic acid and were stopped after 5 min by the addition of citric acid. Lipoxygenase products (5-HETE, 12-HETE, 15-HETE, LTB_4) were extracted with Et_2O, the ether phases evaporated, and residues dissolved in acetonitrile/water (1:1). HPLC analysis was performed by using an RP C-18 column using a solvent gradient 40:60 (acetonitrile/H_2O containing 0.1% H_3PO_4) to 70:30 at a flow rate of 2 mL/min. Detection was by UV (272 and 232 nm), and PGB_2 was employed as an internal standard.

Cyclooxygenase was prepared as a microsomal fraction from bovine seminal vesicles according to the method of Yamamoto.[22] Test compound or vehicle (2% DMSO) was preincubated at 25 °C for 5 min with the enzyme in 0.5 mL of assay buffer (50 mM KH_2PO_4, 2 μM hemoglobin, 100 μM phenol) before addition of substrate. The remainder of the assay was as for lipoxygenase except that the solvent system used for TLC was ethyl acetate/trimethylpentane/acetic acid/water (55:25:10:50).

Xanthine oxidase inhibition was monitored as described previously.[23]

Methemoglobin induction was investigated in vitro by monitoring spectrophotometrically the conversion of hemoglobin from lysed human red blood cells to methaemoglobin.[24]

Assay with Intact Mouse Peritoneal Macrophages. In Vitro. Mouse peritoneal macrophages, obtained by lavage, were isolated by adherence and maintained in Dulbecco's modified Eagle medium. Following 60-min pretreatment with test compounds they were challenged with opsonized zymosan. LTC_4 release was determined 3 h later by radioimmunoassay[25] of cell-free supernatant.

In Vivo. Male CD_1 mice (Charles River) weighing 25–30 g were randomly assigned to groups of 10. Each group was given test compound in 1 mL of vehicle [100 μL of poly(ethylene glycol) to 10 mL of Jakshik's medium[26]] or vehicle alone, by intraperitoneal injection. After 15 min each animal was challenged by an intraperitoneal injection of 2 mL of a 1:1 mixture of zymosan (0.5 mg/mL) and L-cysteine (1 mg/mL). Forty-five minutes later each animal was humanely killed, and peritoneal contents were collected by lavage. After centrifugation (200g for 5 min) to remove cells the supernatants were assayed for LTC_4 by radioimmunoassay.[22]

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