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Synthesis and biological properties of hydroxythioether fatty acids related to leukotrienes: antagonists and agonists of slow-reacting substance of anaphylaxis (SRS-A)

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Summary — A series of 6-hydroxy-7-thioether and 6-thioether-7-hydroxy derivatives of commercially available petroselinic acid and 5-hydroxy-6-thioether derivatives of fatty acids containing an aromatic moiety were synthesized. Several of the compounds have exhibited SRS-A antagonist (*eg*, **5**, **10**)/agonist (*eg*, **34**, **35**) activity. Compound **5** antagonized SRS-A-induced contractions of the isolated guinea pig ileum with $IC_{50} = 0.09\ \mu M$.

hydroxythioether fatty acids / SRS-A / antagonist / agonist / peptidoleukotriene analogs

Introduction

Leukotrienes (LTs) are a family of substituted fatty acids derived from arachidonic acid *via* a 5-lipoxygenase biosynthetic pathway. The peptide-linked eicosanoids, LTC_4, LTD_4 and LTE_4, which arise by conjugation of glutathione to epoxide intermediate LTA_4, are the principal constituents of slow-reacting substance of anaphylaxis (SRS-A) [1–5]. SRS-A has been viewed for many years as a crucial mediator of immediate hypersensitivity reactions. The peptidoleukotrienes are potent contractile agents on airway smooth muscle and probably account for most of the biological activity of SRS-A [1–6]. The strong association of these eicosanoids with the pathophysiology of allergic asthma and other immediate hypersensitivity reactions [7] has sparked intense activity among medicinal chemists [6, 8–10].

Interference with the production or action of the key leukotrienes (or SRS-A) could result in therapeutically useful anti-asthmatic drugs. Thus, we initiated a program in 1980 directed towards the discovery of leukotriene (SRS-A) antagonists. Our target structures centered on diverse hydroxythioether fatty acids, and the synthetic efforts evolved in several synthetic directions. In this paper, we describe the synthesis and biological examination of 6-hydroxy-7-thioether and 6-thioether-7-hydroxy derivatives of commercially available petroselinic acid and 5-hydroxy-6-thioether derivatives of fatty acids containing an aromatic moiety. Several of the compounds have exhibited SRS-A antagonist/agonist activity in the guinea pig ileum and parenchymal strip [11].

Chemistry

The synthesis of 6,7-substituted saturated LT analogs was readily achieved by using commercially available petroselinic acid. We hoped that the 6,7-substitution would impart antagonistic activities. Known *Z*-epoxide **18** was synthesized from petroselinic acid in two steps by esterification and epoxidation with *m*-chloroperbenzoic acid (MCPBA) (72% yield). The known *E*-epoxide **19** [12] was prepared from **18** using the procedure of Sonnet and Oliver [13], involving sequential treatment with *m* Ph_3PBr_2, zinc/acetic acid, and MCPBA (64% yield). Various hydroxythioethers (scheme 1) were prepared by opening each epoxide

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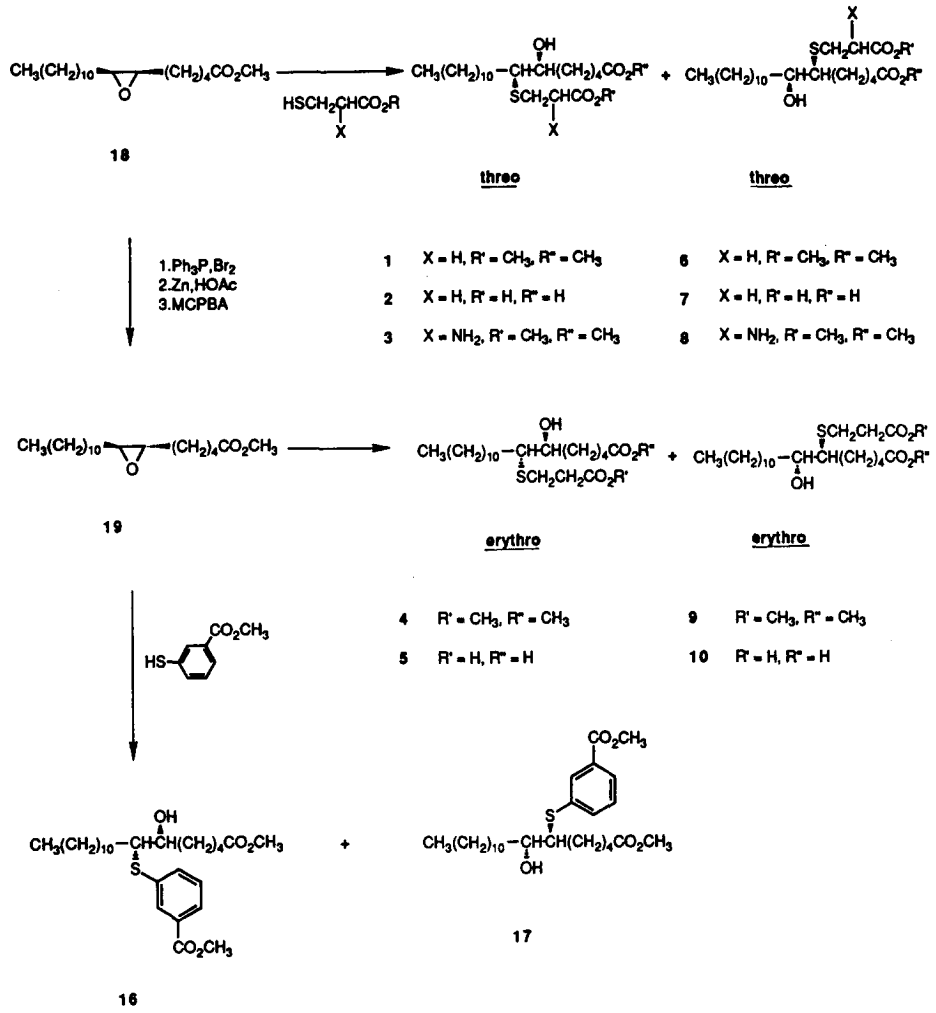
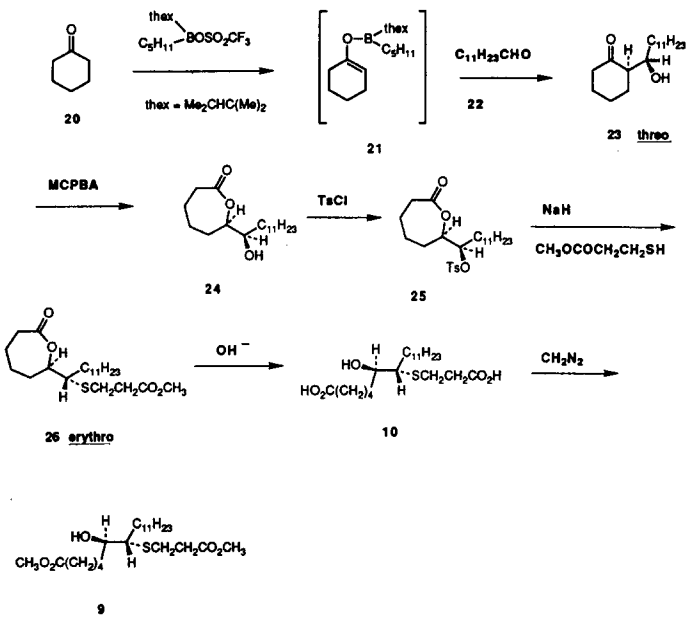


Scheme 1.

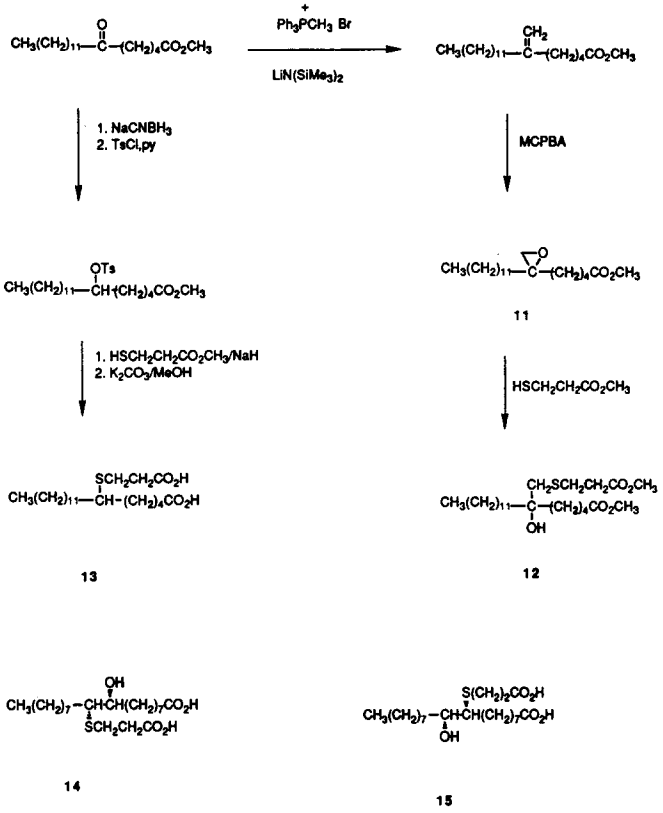
with a suitable thiol compound in the presence of triethylamine. The resulting mixture of regioisomers, in each case, was separated by Waters Prep-500 liquid chromatography (LC). The structure of each regioisomer of a pair was established by the mass spectral fragmentation pattern, and desulfurization with Raney nickel to known alcohols. For each compound, the parent ion corresponds to cleavage of the C–C bond between positions 6 and 7.

Because of the interesting biological activity observed for **9** and **10**, we developed a regioselective and stereoselective synthesis (scheme 2). Thus, the boron enolate of cyclohexanone **21** was subjected to a *threo*-selective aldol condensation with dodecanal according to the procedure of Evans *et al* [14]. Baeyer–Villiger oxidation of the aldol product furnished hydroxylactone **24** with complete retention of configuration at the migrating center (50% yield overall). Displacement of the tosyloxy group from **25** (formed in 80% yield with *p*-toluene sulfonyl chloride and pyridine) with 3-mercaptopropionate proved to be problematic due to side reactions (elimination and lactone opening). Eventually, a process was developed that afforded **26** in *ca* 30% yield. Diacid **10** was obtained *via* basic hydrolysis in quantitative yield. Similar synthetic procedures were applied to oleic acid to give compounds **14** and **15**. In addition, analogs **12** and **13**, with a different arrangement of the thiopropionic acid residues (scheme 3), were synthesized and tested.

Several analogues of leukotrienes, in which an aromatic unit was substituted for carbon–carbon double bonds, were also prepared. We hoped that replacement of the polyene portion of the leukotrienes



Scheme 2.



Scheme 3.

with a related pi-electron system, couched in the aromatic moiety, might afford SRS-A antagonists (or inhibitors of the lipoxygenase pathway) [15–19]. Also, enhanced chemical stability was anticipated for the target molecules by replacement of the sensitive polyene network. The compounds prepared for testing are presented in tables I and II.

4-Octyloxybenzaldehyde, from alkylation of 4-hydroxybenzaldehyde with 1-bromooctane (96%), was subjected to a Wittig reaction with 4-carboxybutylidenetriphenylphosphorane, involving lithium base in tetrahydrofuran (THF) (scheme 4). A white solid THF complex of the desired lithium carboxylate, **27**, was collected. Upon acidification, this material supplied the *E*-olefinic product exclusively. Investigation of the Wittig process revealed that anomalously high *E*-stereoselectivity (*E*/*Z* = 95/5) occurred in the reaction itself, and this led to an extensive mechanistic study [21–23]. Isolation of the lithium salt complex served to fractionate the isomers, leading to pure *E*-product **28**. Oxidation of methyl ester **29** with MCPBA resulted in substantial, if not exclusive, production of benzoate adduct **31**. This side reaction probably stems from the elevated lability of a 4-alkoxystyrene oxide to reaction at the benzylic carbon [24]. We were able to diminish this difficulty by using two-phase buffered conditions. After examining various mild bases, we elected to conduct the MCPBA oxidation with aqueous sodium bicarbonate, whereby the side reaction was held to *ca* 10% [25].

E-Epoxide **30**, readily isolated by crystallization, was reacted with thiols in the conventional manner [26] to afford cysteine (**32** and **34**) and cys–gly (**33** and **35**) adducts. The compounds possessed the desired *erythro* stereochemistry at positions 5 and 6, but were a mixture of diastereomers by virtue of the stereogenic center (*R* configuration) in the L-cysteine fragment.

4-Octylbenzaldehyde was similarly converted to an *E*-epoxide (**37**) for condensation with thiols (scheme 5). The alkene isomer ratio from the Wittig reaction was *Z*/*E* = 13/87. Thus, the thiopropionate diester (**38**) and diacid salt (**39**) were prepared as a mixture of *threo* and *erythro* isomers in a 13/87 ratio. In this case, the MCPBA oxidation did not pose a problem.

Phthalanol (**40**), obtained from the reduction of phthalide with diisobutylaluminum hydride [27], was reacted with 4-carboxybutylidenetriphenylphosphorane to give a mixture of isomeric alkenes (**41**, *E*/*Z* = 35/65; scheme 6). Interestingly, the *E*-alkene did not predominate in this case [21–23]. This may be due to the presence of an *ortho*-substituent but, more particularly, we believe that it is related to the

Table I. Physical properties and biological activities of aliphatic hydroxythio analogues.

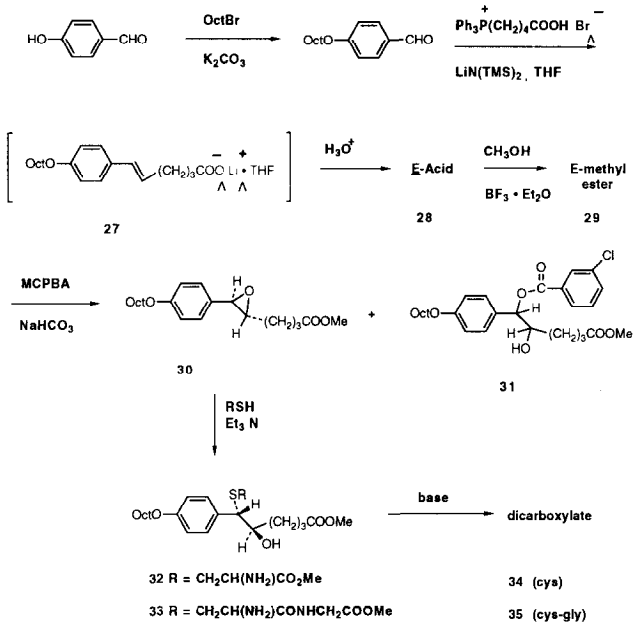
Cmpd	*Isomer*	*Yield (%)*[a]	*mp (°C)*	*Formula*	*Analysis*	*Guinea pig ileum*[b] *(IC_{50}, μM)*	*Guinea pig lung*[c] *(IC_{50}, μM)*	*LTD_4 binding guinea pig lung (K_i, nM)*
1	*threo*	29	Oil	$C_{23}H_{44}O_5S$	C, H, S	1.0	>100	
2	*threo*	100	Oil	$C_{21}H_{40}O_5S$	C, H, S	9.6	>100[d]	
3	*threo*	35	Oil	$C_{23}H_{45}NO_5S$	C[e], H, N, S	12.7	>100	
4	*erythro*	26	Oil	$C_{23}H_{44}O_5S$	C, H, S	>10	1.1	
5	*erythro*	77	91.5–93	$C_{21}H_{40}O_5S$	C, H, S	0.09[d]	4[d]	
6	*threo*	20	Oil	$C_{23}H_{44}O_5S$	C[f], H, S	1	>100	
7	*threo*	80	Oil	$C_{21}H_{40}O_5S{\cdot}0.5H_2O$	C, H, S	0.24[d]	61.5[d]	
8[g]	*threo*	35	Oil	$C_{23}H_{45}NO_5S$	C, H, N, S	1.6	>100	
9	*erythro*	11	Oil	$C_{23}H_{44}O_5S$	C, H, S	>10	9	
10	*erythro*	69	65	$C_{21}H_{40}O_5S$	C, H, S	0.15	4	
12	–	86	Oil	$C_{24}H_{46}O_5S$	C, H, S		>100	
13	–	80	54–57	$C_{21}H_{40}O_4S$	C, H, S		>100	
14	*erythro*	75	79–81	$C_{21}H_{40}O_5S$	C, H, S		>100	
15	*erythro*	79	80.5–84.5	$C_{21}H_{40}O_5S$	C, H, S		>100	
16	*erythro*	36	Oil	$C_{27}H_{44}O_5S$	C, H, S		>100	
17	*erythro*	26	Oil	$C_{27}H_{44}O_5S$	C, H, S		>100	
FPL-55712						0.011	72.4	
SKF-104353[h]								3.2[h]
LY-170680[h]						0.002[h]		

[a]Purified yield of last step. [b]IC_{50} of >10 μM is considered inactive. [c]Parenchymal strip, IC_{50} of >100 μM is considered inactive. [d]Compound appears to have some agonist activity. [e]C: calculated, 61.71; found, 62.81. [f]C: Calculated, 63.85; found, 64.08. [g]Contaminated with 30% of compound **3**. [h][20].

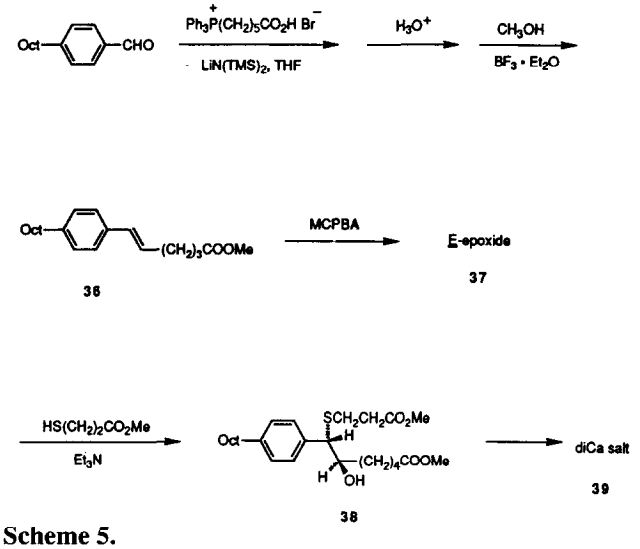
Table II. Physical properties and biological activities of aromatic hydroxy thio analogues.

Cmpd	*Isomer*	*Yield (%)*[a]	*mp (°C)*	*Formula*	*Analysis*	*Guinea pig ileum*[b] *(IC_{50}, μM)*	*Guinea pig lung*[c] *(IC_{50}, μM)*
28	–	28	77–78.5	$C_{20}H_{30}O_3$	C, H	35	>100
30	–	80	60–62	$C_{21}H_{32}O_4$	C, H	125	>100
32	*erythro*	42	Oil	$C_{25}H_{41}NO_6S$	C[d], H, N, S	14	>100[e]
34	*erythro*		193–196	$C_{23}H_{35}NO_6SK_2{\cdot}1.2H_2O{\cdot}0.2KHCO_3$	C, H, N, S, K	–	IC_{50} >100 EC_{50} = 34[f]
35	*erythro*	90	166–180	$C_{25}H_{38}N_2O_7SLi_2{\cdot}2.3H_2O{\cdot}1.4LiHCO_3$	C[g], H, N, S, Li, H_2O	–	IC_{50} >100 EC_{50} = 11[f]
38	*erythro*	59	Oil	$C_{26}H_{42}O_5S$	C, H, S	–	60% inhibition at 100 μM[e]
39	*erythro*	60	230–280	$C_{24}H_{36}O_5SCa{\cdot}0.5H_2O$	C, H, S, H_2O[h]	–	89% inhibition at 100 μM[e]
43	–	36	Oil	$C_{20}H_{36}O_3{\cdot}0.2CHCl_3$	C, H	–	–

[a]Purified yield of last step. [b]IC_{50} of >10 μM is considered inactive. [c]IC_{50} of >100 μM is considered inactive. [d]C: calculated 62.08; found 62.78. [e]Compound appears to have some agonist activity. [f]Compound has agonist activity, EC_{50} of LTC_4 = 0.77 μM. [g]C: calculated 47.97; found 46.15. [h]H_2O: calculated 1.88; found 4.24.

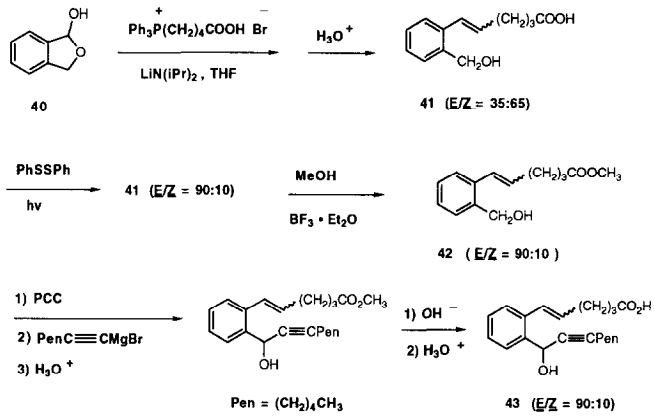


Scheme 4.



Scheme 5.

presence of a lithio-oxido group in proximity to the site of reaction. After condensation to the oxaphosphetane intermediate, the lithio-oxido group may, in some way, counteract the tendency of the lithiocarboxy group to induce anomalous *E*-stereoselectivity. The acid mixture was photoisomerized in the presence of diphenyl disulfide to enhance the *E*-isomer, and then transformed into the methyl ester (**42**, *E*/*Z* = 90/10) [28]. The corresponding aldehyde,



Scheme 6.

formed by pyridinium chlorochromate oxidation, was combined with heptynylmagnesium bromide to furnish hydroxy ester **43** [29]. Attempted epoxidation of the alkene group with MCPBA appeared to result in intramolecular ring formation involving the neighboring hydroxy group.

Although the cyclization problem could be avoided by protection of the hydroxy group in **43** as a *tert*-butyldimethylsilyl ether, we did not pursue this sequence further to peptidoleukotriene analogues.

Biological results

Several of the compounds have exhibited *in vitro* SRS-A antagonist/agonist activity in the guinea pig ileum and lung parenchymal strip tests (tables I and II) [30, 31]. In general, *erythro*-isomers of aliphatic compounds were more active than the *threo*-isomers which is not surprising since the naturally-formed SRS-A also consists of *erythro*-isomers (table I, **5** *vs* **2**; **10** *vs* **7**). Compounds **5** and **10** showed reasonable antagonist activity, but both also exhibited some agonist activity. The octahydro analogues of leukotrienes C, D, and E were reported in the literature to exhibit agonist activity [32]. It is interesting to note that compounds **5** and **10** (being 6-hydroxy, 7-thio substituted instead of 5,6 substitution) exhibited mostly antagonist activity. The oleic acid analogues (compounds **14** and **15**), as expected, were inactive. Compound **13**, which lacks a hydroxyl group, was also inactive. Compounds containing an aromatic moiety (table II) generally showed weak or no antagonist activity in the lung parenchymal test. However, two analogues exhibited agonist activity (**34** and **35**). No *in vivo* activity was detected in these compounds, using a guinea pig lung anaphylaxis model pretreated with indomethacin, pyrilamine and propranolol. Consequently, these compounds were not pursued further.

Experimental protocols

Chemistry

The procedure for the synthesis of compounds **1**, **2**, **4**, **6** and **9** exemplifies the method for the synthesis of compounds **3**, **5**, **7**, **8**, **10**, **14**, **15**, **16**, **17**, which were characterized satisfactorily by ^{1}H-NMR, MS and elemental analyses.

*threo-Methyl-6-hydroxy-7{[2-(methoxycarbonyl)ethyl]thio}-octadecanoate **1** and threo-methyl-7-hydroxy-6{[2-(methoxycarbonyl)ethyl]thio}octadecanoate **6***

To a solution of methyl 6,7-*cis*-epoxyoctadecanoate [33] (5.44 g, 0.017 mol) in absolute MeOH (10 ml), at room temperature under an argon atmosphere, was added Et_3N (9.67 ml, 0.070 mol) and methyl-3-mercaptopropionate (8.35 g, 7.7 ml, 0.070 mol). The reaction mixture was refluxed for 18 h, cooled, diluted with water (100 ml), and extracted into ether. The organic extracts were washed with 1 N HCl and water, and dried over $MgSO_4$. The organic layer was filtered and evaporated *in vacuo* to give the crude product (10 g). This crude product contained equal amounts of **1** and **6**. Purification *via* Waters Prep-500 LC gave 2.17 g (29%) of **1** as a golden oil. 90 MHz ^{1}H-NMR ($CDCl_3$) δ 3.70 (3, s, CO_2CH_3), 3.66 (3, s, CO_2CH_3), 3.46 (1, m), 2.25–2.85 (8, m, 1-exchangeable), 1.27–1.72 (26, broad m) and 0.90 ppm (3, t, CH_3); MS (70 eV) 417 (m, 15), 401, 295, 288, 201, 145. Pure **6** was also obtained (1.47 g, 20%) as a golden oil. 90-MHz ^{1}H-NMR ($CDCl_3$) δ 3.70 (3, s, CO_2CH_3), 3.67 (3, s, CO_2CH_3), 3.50 (1, m), 2.25– 2.91 (8, m, 1-exchangeable), 1.25–1.75 (26, broad m) and 0.88 ppm (3, t, CH_3); MS (70 eV) 417 (m, 15), 401, 295, 263, 248, 161.

*threo-7-(2-Carboxyethyl)thio-6-hydroxyoctadecanoic acid **2***

A solution of *threo*-methyl-6-hydroxy-7{[2-(methoxycarbonyl)ethyl]thio}octadecanoate (2.15 g, 0.005 mol) in 5:1 MeOH/H_2O (50 ml, v/v) was treated with K_2CO_3 (6.87 g, 0.050 mol) and heated to reflux for 2 h. The reaction mixture was cooled, diluted with water (100 ml) and acidified to pH = 2.0 with 6 N HCl. The aqueous mixture was extracted with ether (3 x 100 ml) and the organic extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo* to afford 2.0 g (100%) of pure diacid. 90-MHz ^{1}H-NMR ($CDCl_3$) δ 7.73 (3, broad s, OH, CO_2H exchangeable), 3.52 (1, m), 2.25–2.90 (7, m), 1.25–1.80 (26, broad m) and 0.90 ppm (3, t, CH_3).

*erythro-Methyl-6-hydroxy-7{[2-(methoxycarbonyl)ethyl]thio}-octadecanoate **4** and erythro-methyl-7-hydroxy 6{[2-(methoxycarbonyl) ethyl]thio}-octadecanoate **9***

Similarly, 10.0 g (0.032 mol) of methyl 6,7-*trans*-epoxyoctadecanoate **19** [12] gave 28.0 g of crude product. This crude product contains equal amounts of **4** and **9**. Purification *via* Waters Prep-500 LC gave 3.64 g of pure **4** as an oil. 90 MHz ^{1}H-NMR ($CDCl_3$) δ 3.69 (s, CH_3), 3.67 (m, 1H), 3.65 (s, CH_3), 2.20–2.90 (m, 8H, 1 exchangeable), 1.26–1.80 (broad m, 26H), 0.89 (t, 3H). Pure **9** was also obtained (1.59 g) as an oil. 90 MHz ^{1}H-NMR ($CDCl_3$) δ 3.69 (s, 3H), 3.67 (m, 1H), 3.65 (s, CH_3), 2.30–-2.90 (m, 8H), 1.25–1.80 (broad m, 26H), 0.90 (t, 3H).

*Methyl-2-dodecyl-2-oxiranepentanoate **11***

A solution of methyl-6-methyleneoctadecanoate (4.33 g, 0.014 mol), 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide (0.86 g) and MCPBA (4.28 g, 0.035 mol) in CH_2Cl_2 (250 ml) was refluxed for 3 h. The reaction mixture was cooled and filtered. After the filtrate was evaporated, the residue was dissolved in ether and washed with 10% $NaHCO_3$ (2 x 300 ml), H_2O (1 x 100 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give an oil (4.8 g) which was purified *via* Waters Prep-500 LC (hexane/ether, 10/1) to yield pure **11** as a viscous oil (3.4 g, 75%). 90 MHz ^{1}H-NMR ($CDCl_3$) δ 1.0–1.8 (m, 31H) 2.2–2.4 (t, 2H), 2.42 (s, 2H), 3.62 (s, 3H). Anal calcd for $C_{20}H_{38}O_3$: C, 73.57; H, 11.73. Found: C, 73.51; H, 11.73.

*Methyl-{6-hydroxy-6-[(2-methoxycarbonylethyl)-thio]methyl}-octadecanoate **12***

A solution of methyl-2-dodecyl-2-oxiranepentanoate (2.08 g, 6.38 mol), methyl-mercaptopropionate (2.2 ml, 19.1 mmol), and triethylamine (3.55 ml, 25.5 mmol) in MeOH (10 ml) was refluxed for 10.5 h. The reaction mixture was taken up in ether and washed with 1 N HCl and H_2O. The ether layer was dried ($MgSO_4$) and evaporated *in vacuo* to yield a clear oil. Purification by dry column chromatography (1/1, hexane/ether, silica gel) afforded the desired product with a yield of 2.46 g (86%) of methyl-6-hydroxy-[(2-methyl-carbonylethyl)thio]-methyl octadecanoate. 60 MHz ^{1}H-NMR ($CDCl_3$) δ 3.68 (3, s, CO_2CH_3), 3.65 (3, s, $-CO_2CH_3$), 2.72 (6, m), 2.65 (3, m), 2.25 (2, m), 1.8–0.85 (31, broad m).

Methyl-6-methyleneoctadecanoate

To a suspension of methyl triphenylphosphonium bromide (32.13 g, 0.09 mol) was added lithium diisopropylamide (LDA) (10.7 g, 0.1 mol) dissolved in THF (100 ml). The resulting deep-yellow solution was stirred at room temperature for 2 h. Methyl-6-oxo-octadecanoate [34] (25 g, 0.08 mol) in dry THF (250 ml) was then added. The reaction mixture was stirred for 18 h and was filtered through Celite. The filtrate was concentrated *in vacuo* to give 40 g of an oily solid which was purified *via* Waters Prep-500 LC to give methyl-6-methyleneoctadecanoate (7.8 g).

*6-[(2-Carboxyethyl)thio]octadecanoic acid **13***

A solution of methyl-6-hydroxy octadecanoate [34] 4.97 g (0.016 mol) in pyridine (20 ml) was treated with *p*-toluenesulfonyl chloride (9.02 g, 0.048 mol) and stirred at 23°C for 4.5 h. The reaction solution was diluted with H_2O (100 ml), extracted with ether (200 ml), washed with 10% HCl solution, saturated NaCl solution, and dried ($MgSO_4$). The solvent was removed to give the crude tosylate which was purified by column chromatography on silica gel (1/1, hexane/ether) to yield 6.34 g of pure tosylate as an oil (85% yield). A solution of the tosylate (3.0 g, 0.0064 mol) in THF (6 ml) was added portionwise to a mixture containing NaH (0.77 g, 0.032 mol) and methyl-mercaptopropionate (7.69 g, 0.064 mol) in THF (50 ml). The reaction mixture was stirred at ambient temperature for 10 days. The reaction mixture was diluted with H_2O (200 ml), extracted with ether (300 ml), washed with 1 N NaOH solution and H_2O and dried over $MgSO_4$ to give the crude thioether which was purified by dry column chromatography on silica gel (5/1, hexane/ether) to give 1.66 g of the methyl ester of **13** (62% yield). A solution of the ester (1.58 g, 0.0038 mol) was dissolved in MeOH/H_2O (60 ml, 5/1) and treated with K_2CO_3 (5.24 g, 0.038 mol) and refluxed for 2.5 h. The mixture was cooled, diluted with H_2O (50 ml) and neutralized with 1 N HCl to pH ~4.0. A milky white suspension resulted. It was extracted with ether (300 ml), dried over $MgSO_4$, and the solvent was removed to give **13** (1.17 g, 80% yield) mp: 54–57°C. 60 MHz ^{1}H-NMR ($CDCl_3$) δ 0.8–1.1 (t, CH_3), 1.1–2 (m, 28H), 2.2–2.6 (m, 2H), 2.6–3.0 (m, 5H). Anal calcd for $C_{21}H_{40}O_4S$: C, 64.91; H, 10.37; S, 8.25; Found: C, 64.87; H, 10.39; S, 8.29.

threo-2-(1-Hydroxydodecyl)cyclohexanone ***23***

To 24 ml of BH_3–$(CH_3)_2S$ (10.0 M) at 0°C in a 3-neck, 2-liter flask under N_2 was added 2,3-dimethylbutene (33.2 ml, 0.28 mol). The solution was stirred at 0°C for 2 h. The reaction was cooled to –30°C after the addition of THF (90 ml). Cyclopentene (24.6 ml, 0.28 mol) was added, the reaction mixture was stirred for 2 h, and allowed to warm to room temperature. The reaction mixture was then cooled to –78°C, and CF_3SO_3H (23 ml, 0.26 mol) was added over a 20 min period. The reaction was stirred at –78°C for 2 h, and a solution of THF (100 ml), diisopropylethylamine (45.28 ml, 0.26 mol) and cyclohexanone (22.8 ml, 0.22 mol) was added and the resulting mixture was stirred at –78°C for 0.5 h. Dodecylaldehyde (70.52 ml, 0.32 mol) in THF (400 ml) was added over a 20 min period. The resulting white suspension was stirred 0.5 h at –78°C and 1.5 h at 0°C. The reaction mixture was quenched by pouring onto 1 l of phosphate buffer (pH = 6.8) and extracting into ether (2 x 1000 ml). The ether was washed with saturated NaCl, dried over $MgSO_4$ and filtered. The solvent was removed to give 140.9 g of a yellow oil which was dissolved in 500 ml of MeOH/*i*-PrOH (1/1) at 0°C and treated with 30% H_2O_2 (200 ml). The solution was stirred at room temperature for 2 h, diluted with H_2O (600 ml) and extracted into ether (2 x 750 ml). The ether layer was washed with saturated $NaHCO_3$ (2 x 400 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give crude **23** (153.2 g).

threo-7-(1-Hydroxydodecyl)-2-oxepanone ***24***

Crude **23** (51.0 g, 0.18 mol) was dissolved in $CHCl_3$ (750 ml) and treated with MCPBA (46.8 g, 0.27 mol) and $NaHCO_3$ (22.8 g, 0.27 mol). The mixture was stirred at room temperature under N_2 in the dark for 5 h. The mixture was then cooled and the solid filtered. The filtrate was evaporated under reduced pressure and the residue dissolved in ether and washed with 1 M K_2CO_3 solution and saturated NaCl. The organic layer was dried over $MgSO_4$, filtered and the solvent removed. The residue was purified by Waters Prep-500 LC (2/1, Hex/EtOAc) to yield pure **24** (13.02 g) as a white solid, mp: 53–54.5°C. 60 MHz ^{1}H-NMR ($CDCl_3$) δ 0.88 (t, CH_3) 1.0–2.0 (m, 26H), 2.3 (m, OH), 2.5–2.8 (m, 2H), 3.3–3.7 (m, 1H), 3.8–4.25 (m, 1H). Anal calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48. Found: 72.09, H, 11.34.

threo-7-[1-(4-Methylphenyl)sulfonyldodecyl]-2-oxepanone ***25***

A solution of **24** (4.97 g, 0.016 mol) and *p*-toluenesulfonyl chloride (9.02 g, 0.48 mol) in pyridine (20 ml) was kept at room temperature for 4 h. The reaction mixture was poured into H_2O (100 ml), extracted with ether (200 ml) and washed with dilute HCl solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue was purified *via* column chromatography (silica gel: 1/1, hexane/ether) to give 6.34 g of the desired product as an oil. 60 MHz ^{1}H-NMR ($CDCl_3$) δ 0.7–2.2 (m, 29H), 2.5 (s, CH_3) 2.7 (m, CH_2CO_2), 4.5 (m, 2H), 7.35 (d, 2H), 7.8 (d, 2H).

erythro-Methyl-3-[[1-(7-oxo-2-oxepanyl)dodecyl]thio]propanoate ***26***

A solution of **25** (3.0 g, 0.0064 mol) in THF (6 ml) was added to a solution of methyl mercaptopropionate (7.69 g, 0.064 mol) and NaH (0.77 g, 0.032 mol) in THF (50 ml). The reaction mixture was kept at room temperature for 13 days and was quenched with H_2O (200 ml) and extracted with ether (300 ml). The ether layer was extracted with 450 ml of 1 N NaOH and 450 ml of H_2O and dried over $MgSO_4$. The solvent was removed to give an oily product which was purified by column chromatography on silica gel (5/1, hexane/ether). A pure product (1.66 g) was obtained as a colorless oil.

E-6-(4-Octyloxyphenyl)-5-hexanoic acid ***28***

4-Octyloxybenzaldehyde was prepared from 4-hydroxybenzaldehyde (49 g, 0.4 mol), 1-bromooctane (123 g, 0.64 mol), and K_2CO_3 (180 g, 1.2 mol) in dimethylformamide (DMF) (400 ml) [35]. After heating at reflux for 6 h, the cooled mixture was poured into water (800 ml) and extracted (hexane/ether, 2/1, 2 x 500 ml). The organic solution was rinsed with 5% aqueous NaOH, water, and 2% HCl, then dried ($MgSO_4$) and concentrated. The residue was distilled (Kugelrohr, at 0.5 torr) to furnish a low boiling fraction up to 125°C (pot temp), then the desired product at 135–155°C, as a pale yellow oil (90.6 g, 95%).

4-Carboxybutyltriphenylphosphonium bromide (110.8 g, 0.25 mol, dried *in vacuo*) was suspended in dry THF (400 ml) under nitrogen. With water-bath cooling, a solution of lithium hexamethyldisilazide (87.8 g, 0.525 mol) in THF (350 ml) was added slowly. The reaction turned deep red and the solid dissolved. After 1 h at ambient temperature, a solution of the aldehyde (35.1 g, 0.15 mol) in THF (150 ml) was added slowly. The reaction was treated with water (20 ml), to hydrate the salts, then dry ether (1 l) was added. The off-white solid which separated second was collected, whereas the initially separated gum was left behind. The solid was rinsed with dry ether, mixed with warm ethyl acetate and filtered; then it was partitioned between 10% HCl and methylene chloride (500 ml). The organic phase was rinsed with water, dried (Na_2SO_4) and concentrated to a waxy, crystalline solid, contaminated with some $Ph_3P^+(CH_2)_4COOH$ Cl^- and $Ph_3P{=}O$. The material was combined with warm dry ether (450 ml), filtered, diluted with pentane (400 ml), and cooled to 0°C. An off-white flaky solid (19.7 g, 41%) was obtained. Treatment of the mother liquor with cyclohexylamine afforded solid acid salt (21 g), which was partitioned between 10% HCl and ether. The ethereal solution was rinsed with water, dried (Na_2SO_4), and concentrated to a flaky solid (15.2 g, 31%). Recrystallization of the first sample from ether/pentane gave colorless leaflets (13.4 g, 28%), which were homogeneous by thin-layer chromatography (TLC) (ethyl acetate/hexanes, 1/1), mp: 77–78.5°C. 90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.7–2.0 (m, 17), 2.0–2.5 (m, 4), 3.93 (t, 2, OCH_2), 5.8–6.15 (dt, 1, vinyl H_5, J = 16, 6.5 Hz), 6.2–6.45 (d, 1, vinyl H_6, J = 15.5 Hz), 6.7–7.3 (ABq, 4, arom, 2 groups of peaks centered at δ 6.8 and 7.25), 9.1–10.8 (br, s, 1, COOH). UV (CH_3OH) λ_{max} (ε) 30.6 (1810, shl), 294 (3100, shl), 259 (25200). IR (4% in $CHCl_3$) 2932, 2859, 1712 (C=O), 1609, 1511, 1242, 1174 cm^{-1}.

Methyl-E-6-(4-octyloxyphenyl)-5-hexenoate ***29***

The acid (10.0 g) in methanol (250 ml) was treated with BF_3-etherate (0.2 ml). Esterification was 95% complete in 3 days at 23°C. The solution was concentrated to one-half volume and saturated $NaHCO_3$ (50 ml) was added. The combined ethereal extracts (2 x 100 ml) were rinsed with water, dried (Na_2SO_4), and concentrated to a pale yellow oil (9.8 g, 96%), homogeneous by TLC (ethyl acetate/hexanes, 2/1). 90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.7–2.5 (m, 21), 3.63 (s, 3), 3.8–4.1 (pseudo t, 2), 5.7–6.5 (m, 2, vinyl), 6.7–7.3 (AB q, 4).

Methyl-E-3-(4-octyloxyphenyl)oxiranebutanoate ***30***

Olefin ester **29** (11.0 g, 33.1 mmol) in methylene chloride (150 ml) was combined with 1 M $NaHCO_3$ (132 ml, 132 mmol). The mixture was stirred rapidly, cooled in a water bath, and treated with a solution of MCPBA (6.9 g, 34 mmol, 85% assay) in methylene chloride (50 ml). Addition required 40 min; the reaction was complete after an additional 60 min. The organic phase was separated and rinsed with 5% NaOH

containing Na_2SO_4 (1.0 g), then rinsed with water. The organic solution was dried (Na_2SO_4) and concentrated to an off-white solid (11.7 g). ^{1}H-NMR indicated the presence of 10% benzoate **31**. The material was recrystallized from hexanes to furnish a white solid (9.2 g, 80%), containing <5% benzoate. Recrystallization of 2.0 g of this solid from 95% aqueous methanol (25 ml) gave a pure-white solid (1.56 g), mp: 60–62°C. 90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.8–1.0 (m, 3), 1.2–2.0 (m, 16), 2.3–2.5 (m, 2, CH_2CO), 2.8–3.05 (m, 1, $CHCH_2$), 3.54 (d, 1, ArCH, J = 2 Hz, *E*-isomer), 3.65 (s, 3, OCH_3), 3.94 (t, 2, OCH_2, J = 6.5 Hz), 6.83 (d, 2, *o*-arom, J = 8.5 Hz), 7.15 (d, 2, *m*-arom, J = 8.5 Hz).

Methyl-6-[(3-chlorobenzoyl)oxy]-5-hydroxy-(4'-octyloxybenzene)hexanoate ***31***

Olefin ester **29** (8.4 g, 0.025 mol) in dry methylene chloride (20 ml) was treated with a solution of MCPBA (5.5 g, 0.027 mol, 85% assay) in CH_2Cl_2 (30 ml). After 1 h, the reaction mixture was washed with 5% aqueous Na_2CO_3, then water. The organic layer was dried (Na_2SO_4) and concentrated to a tan oil (12 g; essentially one spot by TLC, hexane/ethyl acetate, 2/1). Part of this oil (5.0 g) was chromatographed on a dry column of silica gel (300 g) with petroleum ether/ethyl acetate (5/2). The major band was extracted with methylene chloride to afford a pale-yellow oil (3.5 g) on evaporation. The viscous material, homogeneous by TLC, was dried *in vacuo* at 60°C. 360 MHz ^{1}H-NMR ($CDCl_3$) δ 0.7–2.6 (m, 22, CH_3 + $(CH_2)_6$ + $(CH_2)_3$ + OH), 3.58 (s, 3, OCH_3), 3.8–4.15 (m, 3, OCH_2 + CHOH, t for OCH_2 at δ 3.92), 5.78 (d, 1, CHOC(O), J = 7 Hz), 6.85 (d, 2, J = 8 Hz), 7.2–7.6 (m, 4, d for OAr at δ 7.29), 7.8–8.1 (m, 2, *o*-arom, s at δ 8.00 and d at δ 7.93); approximately 3 mol% of methylene chloride was present. The moderate vicinal coupling (J = 7 Hz) between H_5 and H_6 is suggestive of the *erythro*-configuration, resulting from inversion of configuration at C_6 of the *E*-epoxide intermediate.

erythro-5-[2-Hydroxy-5-methoxycarbonyl-1-(4-octyloxyphenyl)-1-pentyl]-L-cysteine methyl ester ***32***

A solution of **30** (5.3 g, 15.23 mmol), L-cysteine methyl ester (5.65 g, 41.54 mmol) and triethylamine (8.47 ml, 60.9 mmol) in MeOH (130 ml) was stirred at ambient temperature for 8 h. The solvent was then removed and water was added to the residue. The resultant solution was extracted with ether, and the combined organic extracts were then washed with water. The ethereal solution was dried ($MgSO_4$), filtered and concentrated to an oily residue. Chromatography on dry silica gel (525 g, $CHCl_3$/MeOH; 8/1), followed by chromatography on a Waters Prep-500 high-performance liquid chromatography (HPLC) ($CHCl_3$/MeOH; 60/1), gave pure **32** as an oil (3.1 g, 42%). $[\alpha]_D^{25}$ = +6.54° (c = 0.4, MeOH). 90 MHz ^{1}H-NMR ($CDCl_3$) δ 0.9 (t, 3H), 1.1–1.9 (m, 14H), 2.1–2.4 (m, 4H), 2.7 (m, 2H), 3.6–4.0 (m, 11H), 6.82 (d, 2H), 7.28 (d, 2H).

Hydrolysis of dimethyl esters ***32–34***

Amino diester **32** (1.57 g, 3.2 mmol) in methanol/water (60 ml, 5/1) was treated with anhydrous K_2CO_3 (0.51 g, 6.3 mmol). The yellow solution was stirred at 23°C for 6 h. The methanol was evaporated *in vacuo* and the aqueous solution was lyophilized to a foam. To remove most of the $KHCO_3$, the material was heated with absolute ethanol (50 ml), left to cool and filtered. The ethanolic solution was concentrated *in vacuo* to a pale-yellow, amorphous solid, which was dried at 60°C *in vacuo* (0.90 g), mp: 70–190°C, darkened at 193–196°C, and turned orange-brown. TLC on silica gel with ethyl acetate/ methanol/acetic acid/water (25/5/1/1) showed two spots of equal intensity (I_2 staining), R_f = 0.24 and 0.36, for the two diastereomers. 90 MHz ^{1}H-NMR (CD_3OD) δ 0.75–1.0 (m, 3), 1.0–2.0 (m, 16), 2.0–2.25 (m, 2, CH_2COO), 2.65–2.8 (m, 2, CH_2S), 3.75–4.05 (m, 4, CH_2OAr + CHOH + CHN), 4.25–4.4 (m, ArCHS), 6.7–6.9 (d, 2), 7.2–7.4 (d, 2).

erythro-[S-[2-Hydroxy-5-methoxycarbonyl-1-(4-octyloxyphenyl)-1-pentyl]-L-cysteinyl]glycine methyl ester ***33***

A solution of **30** (2.61 g, 7.5 mmol), *N*-(trifluoroacetyl)-L-cysteinylglycine methyl ester [37] (4.32 g, 15.0 mmol), and triethylamine (4.17 ml, 22.5 mmol) in MeOH (20 ml) was stirred at ambient temperature for 3 h. The MeOH was removed on a rotary evaporator, water was added and the product was extracted with ether (3x). The combined ethereal layers were washed 3 times with water, dried ($MgSO_4$), filtered and concentrated. Compound **33** was purified by Waters Prep-500 HPLC ($CHCl_3$/MeOH; 40/1) to give pure **33** (2.0 g, 42% yield). 60 MHz ^{1}H-NMR ($CDCl_3$) δ 1.0 (m, 3H), 3.8–4.8 (m, 11H), 6.7 (d, 2H), 7.3 (d, 2H), 7.7 (br s, 2H). MS (CI-CH_4) m/e 637 (m + 1), 319 (M-H_2O + 1).

erythro-N-[S-[5-Carboxy-2-hydroxy-1-(4-octyloxyphenyl)-pentyl]-L-cysteinyl]glycine dilithium salt ***35***

Compound **33** (1.87 g, 2.9 mmol) and LiOH·H_2O (0.47 g, 10.2 mmol) were dissolved in a 3/1 solution of MeOH/H_2O (80 ml). After stirring at ambient temperature for 2 h, the MeOH was removed on a rotary evaporator, and additional water (50 ml) was added. The solution was washed with $CHCl_3$ (2 x 30 ml), and the product was extracted into butanol (7 x 70 ml). The organic layers were concentrated, and the resulting powder was dissolved in water, filtered, and lyophilized to give pure **35** (1.4 g, 90% yield), mp: 166–180°C. 360 MHz ^{1}H-NMR (DMSO-d_6) δ 0.89 (t, 3H), 1.3–1.9 (m, 16H), 2.5 (br s, 4H), 3.4 (br s, 15H), 3.71 (dd, 2H), 3.86 (dd, 2H) 5.0 (br s, 1H), 6.86 (d, 2H), 7.10 (dd, 2H), 7.89 (s, 1H), 7.97 (br s, 7.97). ^{13}C-NMR (D_2O) δ 15.1, 23.8 (2C), 27.0, 30 (3C), 32.9, 35.2, 36.8, 37.8, 55.3 (3C), 56.5, 69.4, 74.3, 115.7 (2C), 131.5 (2C), 132.7, 159.1, 176.3, 177.6, 184.4.

erythro-Methyl 7-hydroxy-6-[(3-methoxy-3-oxopropyl)thio]-4-octylbenzeneheptanoate

To 5-carboxypentyltriphenylphosphonium bromide [38] (17.6 g, 38.3 mmol) under N_2 was added 84.5 ml of a 1 M solution of lithium hexamethyldisilazide in THF (84.5 mmol). After 45 min, *p*-octylbenzaldehyde (8.3 g, 38.3 mmol) was added. After an additional 1.5 h, 1 N HCl was added (100 ml), and the product was extracted into hexane. The organic layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and evaporated to give an oil. This material was added to MeOH (100 ml) and BF_3·OEt_2 (*ca* 1 ml) was added. After refluxing for 4 h, the solution was cooled and then added to saturated aqueous $NaHCO_3$. The product (**36**) was extracted into CH_2Cl_2, and the organic layer was then washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. Although the majority of this crude preparation of **36** was carried on to the next step, an analytical sample was purified by preparative TLC (EtOAc/hexane; 1/9). Gas–liquid chromatography (GLC)/MS analysis of this material revealed a 13/87 mixture of *Z*/*E*-isomers. 90 MHz ^{1}H-NMR ($CDCl_3$): δ 0.9 (t, 3H), 1.0–1,8 (m, 16H), 1.8–2.7 (m, 6H), 3.6 (br s, 3H), 5.8–6.5 (m, 2H), 7.1 (m, 4H).

Compound **36**, prepared as described above, was dissolved in 200 ml of CH_2Cl_2 and teated with 4,4-thiobis-(6-*tert*-butyl-3-methylphenol) [39] (*ca* 1 mg) and 85% MCPBA. The solution was stirred for 18 h at ambient temperature and 3 h at reflux. The solution was then added to saturated aqueous sodium sulfite, and the product was extracted into hexane. The organic

layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated to afford **37** as an oil (13.2 g).

To crude **37** (4.4 g) in MeOH (40 ml) was added triethylamine (6.1 ml, 43.8 mmol) and methyl 3-mercaptopropionate (3.5 ml, 31.6 mmol). After refluxing for 18 h, the solvent was removed and the residue was purified by Waters Prep-500 HPLC (EtOAc/hexane; 1/3) to give **38** as an oil pure by TLC (3.5 g, 59% yield from *p*-octylbenzaldehyde). 60 MHz ^{1}H-NMR ($CDCl_3$): δ 1.2 (t, 3H), 1.35 (m, 18H), 2.4 (m, 9H), 3.6 (s, 6H), 3.7 (s, 2H), 7.0 (dd, J = 4.9 Hz).

7-[(2-Carboxyethyl)thio]-6-hydroxy-4-octylbenzeneheptanoic acid calcium salt ***39***

A solution of **38** (2.4 g, 5.3 mmol) in MeOH/H_2O (100 ml, 6/1) was treated with LiOH (0.28 g, 11.7 mmol) and stirred for 18 h at ambient temperature and 4 h at reflux. To the mixture was added EtOAc (200 ml) and 0.3 N HCl (until pH = 1). The organic layer was dried ($MgSO_4$), filtered and concentrated. This material was dissolved in water (20 ml) containing NaOH (0.32 g, 8 mmol). 1 N HCl (*ca* 5 drops) was added until the pH was adjusted to 7. To this solution was added $CaCl_2$ (481 mg, 4.3 mmol) dissolved in water (10 ml). The flocculent white precipitate was filtered and washed with water (5 ml) and ether (2 x 10 ml). This produced a white solid (1.7 g, 60% yield) **39**, mp: 230–280°C (dec). 360 MHz ^{1}H-NMR (DMF–d_7) δ 0.9 (t, 3H), 1.1–2.0 (m, 21H), 2.55 (m, 6H), 3.0 (m, 2H), 3.6 (m, 2H), 7.05 (d, 2H), 7.15 (d, 2H).

Methyl-6-[(2-hydroxymethyl)phenyl]-5-hexenoate ***42***

To a suspension of 4-carboxybutyltriphenylphosphonium bromide (192 g, 0.43 mol) in THF (1.5 l) was added lithium diisopropylamide (100 g, 0.95 mol) under a stream of N_2. After 1.5 h, lactol **40** [27] (26.8 g, 0.20 mol) was added dropwise in THF (500 ml). The solution was allowed to stir overnight. The mixture was then poured into water (1.5 l), and the product was washed with EtOAc (3 x 1 l). The aqueous layer was then acidified (1 N HCl) and the product was extracted into ether. The organic layers were combined, dried, filtered and concentrated. A small fraction of this material was treated with diazomethane and analyzed by GLC. A 35/65 ratio of *E*/*Z*-isomers of methyl esters **42** was observed. A 60 MHz ^{1}H-NMR on acid **41** supported this assignment.

The sample of **41** prepared as described above was dissolved in MeOH (1.25 l), and PhSSPh (30.6 g, 0.14 mol) was added. Nitrogen gas was bubbled in for 1 h. The mixture was irradiated with light from two flood lamps for 12 h, after which time GLC indicated a 90/10 *E*/*Z* ratio. The solution was filtered and the filtrate was concentrated. The residue was treated with EtOAc, and the product was extracted into 1 N NaOH. The aqueous layer was acidified with 1 N HCl, and the product was extracted with ether. The ether layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in MeOH (400 ml) and treated with BF_3•OEt_2 (*ca* 1 ml). After standing overnight, the solution was poured into saturated aqueous $NaHCO_3$. The product was extracted into ether. The organic layer was dried ($MgSO_4$), filtered and concentrated. The product was distilled on a Kugelrohr apparatus (160–170°C, 0.3 mm) to give **42** (11.2 g, 24% from **40**). 60 MHz ^{1}H-NMR ($CDCl_3$) δ 1.6–2.4 (m, 6H), 3.5 (s, *ca* 3H), 4.8 (s, 2H), 5.6–6.8 (m, 2H), 7.1–7.9 (m, 4H).

6-[2-(1-Hydroxy-2-octynyl)phenyl]-5-hexanoic acid ***43***

To a solution of **42** (11.2 g, 47.8 mmol) in CH_2Cl_2 (30 ml) was added pyridinium chlorochromate (16.7 g, 77.3 mmol) in CH_2Cl_2 (110 ml). The mixture was allowed to stir overnight. It was then treated with 2 volumes of ether. The solution was decanted from the precipitate, which was washed 2x with ether. The combined organic layers were passed through a pad of CC-4 silica gel, concentrated and distilled on a Kugelrohr apparatus (150°C, 0.1 mm) to give the aldehyde as a yellow oil (7.9 g, 71%). 60 MHz ^{1}H-NMR ($CDCl_3$): δ 1.7–2.7 (m, 6H), 3.6 (s, *ca* 0.3 H), 3.7 (s, *ca* 2.7H), 5.6–6.5 (m, 2H), 7.0–8.0 (m, 4H).

To a solution of EtMgBr (4.4 ml of 3 M EtMgBr, 13.3 mmol) in ether (40 ml) under N_2 was added 1-heptyne (2.61 ml, 20 mmol). The mixture was stirred for 0.5 h at ambient temperature and 0.5 h at reflux. The aldehyde described above (3.11 g, 13.3 mmol) was dissolved in 60 ml of THF and added to the reaction mixture. After stirring overnight, 0.1 N HCl was added and the solution was allowed to stir for 1 h. The product was extracted into ether, the ether layer was washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in MeOH (25 ml); KOH (2.14 g KOH pellets, 33.3 mmol) and water (*ca* 2 ml) were added. The solution was stirred at ambient temperature for 4 h, followed by refluxing for 3 h. The mixture was then poured into water, which was then acidified with 1 N HCl. The product was extracted into ether. The organic layer was dried ($MgSO_4$), filtered and concentrated. The product was then purified by Waters Prep-500 HPLC to give **43** as an oil (1.5 g, 36%). 90 MHz ^{1}H-NMR ($CDCl_3$) δ 7.1–7.7 (m, 4H), 5.6–7.0 (m, 4H), 1.1–2.6 (m, 14H), 0.9 (t, 3H).

Biological testing

SRS-A was prepared using a modified version of the method described by Orange and Moore [30]. Female Sprague–Dawley rats (Charles River) weighing 300–400 g were administered indomethacin (1 mg/kg), in Tris buffer (pH 7.8–8.1). One hour later, 10 mM L-cysteine in Krebs buffer (1 ml) was administered intraperitoneally followed 2.5 min later by the calcium ionophore A-23187 (5 μg/ml) in Krebs solution (5 ml) containing heparin (50 μg/ml). Approximately 5 min later, the animals were killed and the peritoneal fluid was harvested into cold polycarbonate centrifuge tubes, which were centrifuged at 150 *g* for 10 min at 4°C. The supernatant fraction was extracted with 80% ethanol for 30 min at 4°C and centrifuged at 40000 *g* for 30 min at the same temperature. The supernatant fraction was then evaporated to dryness at 45°C under nitrogen and stored at –70°C. Activity was expressed in biological units and was determined using a modified version of the bioassay described by Brocklehurst [31] in the guinea pig ileum. One unit of activity was defined as the amount of material required to produce an isometric contraction equivalent to 5 ng/ml of histamine (in the presence of 0.1 μM atropine and 1 μM chlorpheniramine).

Guinea pig ileum assay

A modified version of the procedure described by Brocklehurst [31] was used for compound evaluation. Male guinea pigs weighing 225–250 g were killed by cervical dislocation. The peritoneal cavity was exposed and a section of the terminal ileum was removed and gently rinsed with 30–50 ml of warm Krebs buffer. The tissue was then placed in warm (37°C) Krebs solution oxygenated with 95% O_2:5% CO_2 and cut into 1.5-cm segments. The tissues were suspended in 10-ml siliconized isolated tissue baths, and attached to Grass FTO3 isometric force-displacement transducers under a 1-g load and allowed to stabilize for 15–30 min. The tissues were treated with 1 μM chlorpheniramine and 0.1 μM atropine for 2 min and then exposed to SRS-A, 1–2 biological units at 20-min intervals

until a reproducible submaximal contraction was obtained (2–3 exposures). A test compound was added to the bath, incubated for 15 min, and the effect on the SRS-A response was recorded as percent inhibition relative to the average of the two previous control responses. Increasing compound concentrations were evaluated until complete inhibition was observed, or until a concentration of 100 μM of the test compound was reached.

Immunologically mediated contraction of the guinea pig lung parenchymal strip (IMCPS) [40]

Male Hartley guinea pigs (Hazelton Dutchland), weighing 200–250 g were sensitized by the subcutaneous administration of chicken egg albumin (1.0 mg), and *Bordetella pertussis*, 11 x 10^9 cells (Massachusetts Public Health Biologics Laboratories) 3–4 weeks prior to the experiments. The heart and lungs were removed together. Peripheral lung strips were prepared (2 from each animal). The lung strips were suspended in a 10-ml siliconized isolated organ bath containing oxygenated (95% O_2:5% CO_2) Krebs buffer and attached to a Grass FTO3 isometric force displacement transducer under an initial tone of 1 g. The tissues were allowed to equilibrate for 45–60 min. Following the equilibration period, chlorpheniramine (10 μM) and indomethacin (100 μM) were added to eliminate the contribution of histamine and prostaglandins to the contractile response. The resultant contraction was then attributed to the formation and release of SRS-A. Tissues originating from the same animal were matched so that one tissue served as a vehicle control tissue. Test drug was added and the tissues were incubated for another 30 min. Egg albumin (0.1 μg/ml) was added to each bath and the contractile response was recorded in milligrams of developed force.

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