

Synthesis and 5-Lipoxygenase Inhibitory Activity of 5-Hydroperoxy-6,8,11,14-eicosatetraenoic Acid Analogues

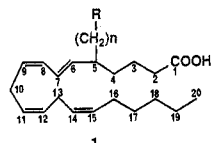
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A series of eicosatetraenes (2-24) were designed, synthesized, and evaluated in vitro for inhibitory activity against 5-lipoxygenase (20000g supernatant from homogenized rat basophilic leukemia cells). All compounds were found to be active with the potencies (IC_{50} 's) ranging from 0.19 to 97 μ M. Compounds containing the hydroxamic acid functionality (10-12) exhibited the best activity (IC_{50} = 0.19-2.8 μ M). The most potent inhibitor was 5-[[[(hydroxyamino)carbonyl]methyl]-6,8,11,14-eicosatetraenoic acid (11), which was 10 times more active than the C-1 hydroxamates of arachidonic acid or 5-HETE. Cyclization of the linear eicosanoids 2 and 14 in the C-1 to C-5 region produced compounds (21 and 24, respectively) with several-fold greater potency.

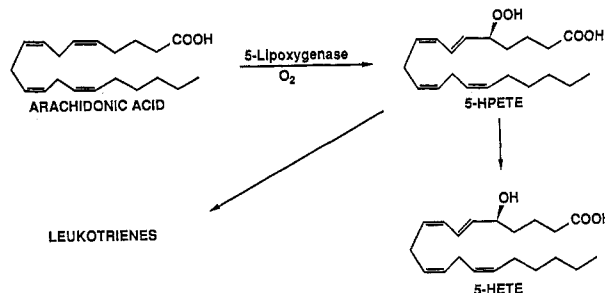
The metabolism of arachidonic acid (AA), catalyzed by the enzyme 5-lipoxygenase, produces 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE), which undergoes further bioconversions to 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and to the leukotrienes (LTA_4 , LTB_4 , LTC_4 , LTD_4 , and LTE_4). These potent biological substances have been implicated as important mediators of inflammation and allergic reactions¹ (Scheme I), and consequently, inhibitors of 5-lipoxygenase may be of therapeutic value in the treatment of inflammatory and allergic diseases. On the basis of current knowledge of the enzymatic mechanisms of related lipoxygenases,² it is reasonable to assume that the reaction of oxygen with AA to form 5-HPETE requires a metal species, putatively iron, in the active site of the enzyme.

Considering this premise, a series of eicosatetraenoic acids 1 were synthesized in which various functional groups capable of interacting with iron or other functional groups in the active site of the enzyme were incorporated at the C-5 position, and these analogues were evaluated for 5-lipoxygenase inhibitory activity. For example, the hy-

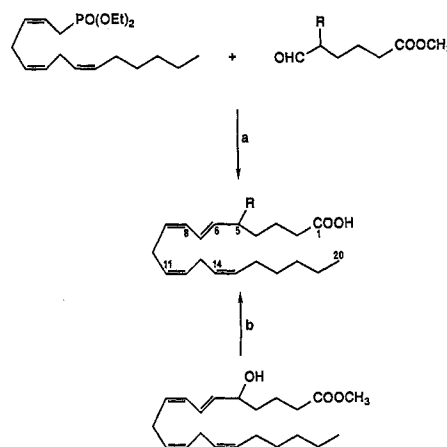


droxamic acid functionality was chosen to explore the binding of bidentate ligands known to chelate strongly with iron.⁴ An acid functionality or amine was selected to examine the possibility for ionic binding. Other groups such as hydroxyl and thiol had the potential for H-bonding interactions. Insertion of a double bond at C-5 provided possible charge-transfer interactions, making the C-7 position particularly susceptible to attack, in a Michael type reaction, by nucleophilic groups potentially in that vicinity of the enzyme. The position of the functionality was also varied from direct attachment at C-5 to extension by one to three methylene groups in order to evaluate potentially important spatial relationships for effective inhibition. Wherever possible, the eicosanoid skeleton

Scheme I



Scheme II



from C-8 to C-20 was preserved to maximize possible important binding interactions associated with this region. Also considered in this study was the effect of restricting the conformational mobility of the C-1 to C-5 region by cyclization as in the synthesis and evaluation of the ketone, lactone,⁵ and lactam analogues.

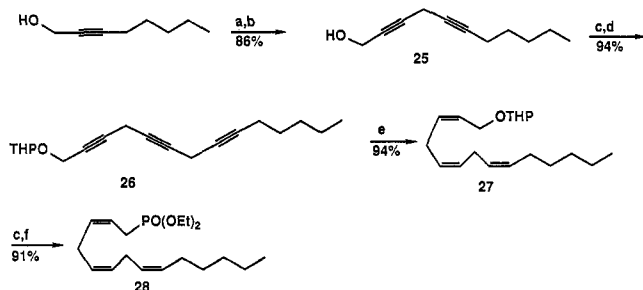
Chemistry

Two synthetic routes were developed to prepare the eicosanoids. The first approach involved a multistep total synthesis of the compounds while the second approach b utilized direct transformation of 5-HETE methyl ester (Scheme II).

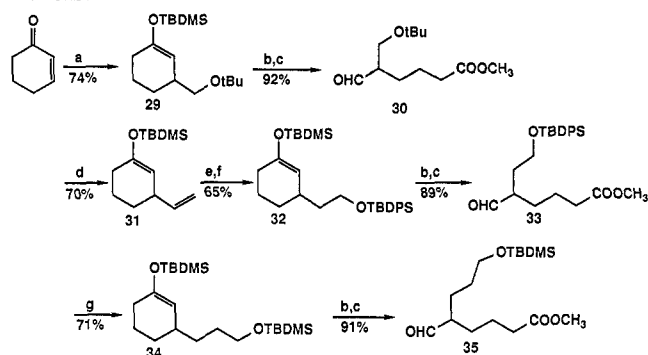
The retrosynthetic analysis for the total synthesis disconnects each compound at the C-6 double bond into aldehyde and phosphonate precursors. The advantages of this approach include (a) a convergent construction employing a common C-7 to C-20 fragment containing the three cis double bonds, (b) high geometrical control of the

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Scheme III^a

^a (a) PBr_3 ; (b) $\text{HOCH}_2\text{C}\equiv\text{CH}$, EtMgBr , CuI ; (c) $\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2$, 2Br_2 ; (d) EtMgBr , CuI , $\text{THPOCH}_2\equiv\text{CH}$; (e) H_2 /Lindlar catalyst; (f) P(OEt)_3 .

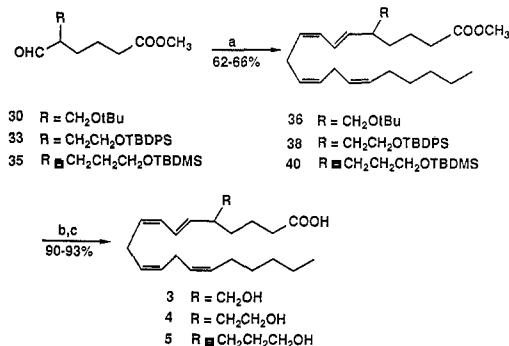
Scheme IV^a

^a (a) $\text{LiCH}_2\text{O}-t\text{-Bu}$, TBDMSOTf ; (b) O_3 , CH_3SCH_3 ; (c) CH_2N_2 ; (d) $\text{CH}_2=\text{CHMgBr}$, TBDMSOTf , CuI ; (e) 9-BBN; (f) TBPDSOTf ; (g) $\text{LiCu}(\text{CH}_2\text{CH}_2\text{CH}_2\text{OTBDMS})$, TBDMSOTf .

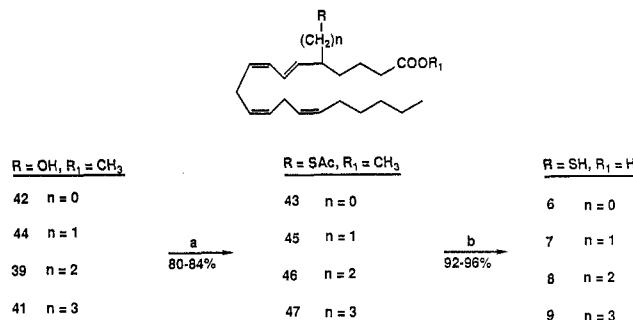
C-6,7 trans olefin by a Wittig–Horner–Emmons coupling, and (c) flexibility to construct other novel and potentially useful analogues. The second route takes advantage of the readily accessible 5-HETE methyl ester⁶ and involves conventional hydroxyl functional group transformations.

The common intermediate in the total synthesis of these eicosatetraenoic acids, namely diethyl 2(*Z*),5(*Z*),8(*Z*)-tetradecatrienylphosphonate (28), was prepared as follows (Scheme III). Commercially available 2-octyn-1-ol was brominated with phosphorus tribromide and the resulting halide coupled with propargyl alcohol to give 2,4-undecadiyn-1-ol. Diyne 25 was subsequently halogenated with 1,2-bis(diphenylphosphino)ethane tetrabromide and the resulting bromide was coupled with the tetrahydropyranyl ether of propargyl alcohol to yield the triyne 26. Triyne 26 was then subjected to controlled hydrogenation employing Lindlar catalyst. Traces of overreduced or underreduced products were removed by argentation chromatography utilizing 20% silver nitrate on silica gel. The resulting pure cis triene 27 was deprotected and brominated in one step with 1,2-bis(diphenylphosphino)ethane tetrabromide and finally treated with triethyl phosphite to afford 28 as a colorless oil in an overall yield of 69% from 2-octyn-1-ol.

The aldehyde precursors 30, 33, and 35 for the target eicosanoids were prepared from 2-cyclohexenone as illustrated in Scheme IV. Conjugate addition of (*tert*-butoxymethyl)lithium⁷ to the α,β -unsaturated ketone followed by trapping of the enolate with *tert*-butyldimethylsilyl triflate gave the (silyloxy)alkene 29. The (silyloxy)alkene 29 was treated with ozone⁸ followed by reduction with

Scheme V^a

^a (a) LDA , 28; (b) Ac_2O , FeCl_3 or $n\text{-Bu}_4\text{NF}$; (c) LiOH .

Scheme VI^a

^a (a) Ph_3P , $i\text{-PrOOCN}=\text{NCOO}-i\text{-Pr}$, CH_3COSH ; (b) LiOH .

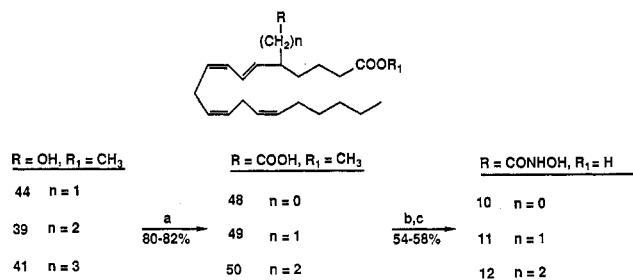
dimethyl sulfide and esterification with diazomethane to afford aldehyde 30. The preparation of aldehyde 33 commenced with conjugate addition of vinylmagnesium bromide⁹ to 2-cyclohexenone and subsequent trapping of the enolate with *tert*-butyldimethylsilyl triflate to yield the (silyloxy)alkene 31. Hydroboration of 31 with 9-BBN¹⁰ gave, after alkaline hydrogen peroxide workup and silylation, compound 32. Subjection of 32 to ozone followed by dimethyl sulfide reduction and esterification with diazomethane provided 33. The synthesis of aldehyde 35 involved treatment of 2-cyclohexenone with $\text{LiCu}(\text{CH}_2\text{CH}_2\text{CH}_2\text{OSi}(\text{CH}_3)_2-t\text{-C}_4\text{H}_9)_2$ ¹¹ followed by trapping of the enolate with the silyl triflate to provide the (silyloxy)alkene 34. The (silyloxy)alkene 34 was reacted with ozone, reduced, and esterified to give aldehyde 35.

The preparation of the hydroxy eicosanoid 3 proceeded as follows (Scheme V). Wittig coupling of the anion derived from phosphonate 28 with aldehyde 30 gave, after flash chromatography,¹² a 66% yield of adduct 36. No trace of cis isomer was found as evidenced by 300-MHz NMR. The *tert*-butyl protecting group of 36 was removed by treatment with acetic anhydride and anhydrous ferric chloride to produce the acetate 37. Hydrolysis of 37 with lithium hydroxide gave the alcohol 3.¹³ The other hydroxy eicosanoids 4 and 5 were prepared in a similar fashion.

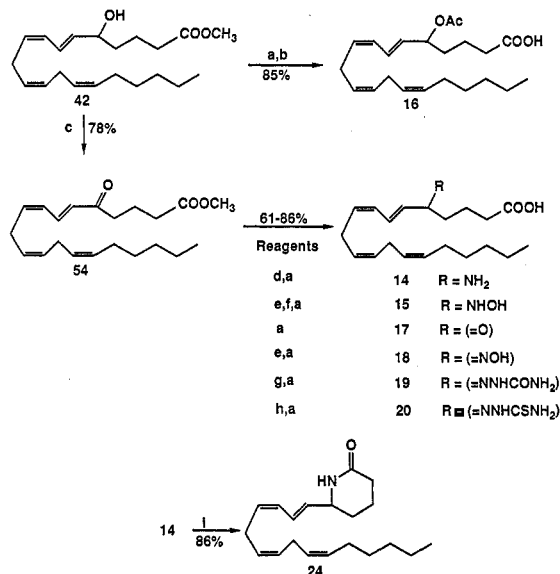
Treatment of the alcohols 42, 44, 39, and 41 with thioacetic acid and the preformed adduct of triphenylphosphine and diisopropyl azodicarboxylate¹⁴ gave the corresponding thiol esters 43, 45, 46, and 47, which were

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Scheme VII^a

^a (a) PDC; (b) ClOCCOCl, NH₂OH; (c) LiOH.

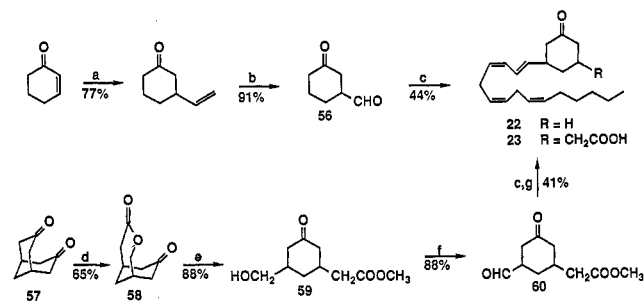
Scheme VIII^a

^a (a) LiOH; (b) Ac₂O; (c) MnO₂; (d) NH₄OAc, NaCNBH₃; (e) NH₂OH; (f) NaCNBH₃; (g) NH₂NHCONH₂; (h) NH₂NHCSNH₂; (i) *p*-TsOH.

hydrolyzed with lithium hydroxide to provide the thiol acids 6, 7, 8, and 9 (Scheme VI).

The eicosanoids containing the hydroxamic acid functionality, 10–12, were obtained from the corresponding alcohols 44, 39, and 41, in a reaction sequence involving (a) oxidation with pyridinium dichromate, (b) successive treatment of the resulting acids 48, 49, and 50, with oxalyl chloride and hydroxylamine, and (c) hydrolysis of the methyl esters 51, 52, and 53 with lithium hydroxide (Scheme VII).

The eicosanoid targets 14–20 were prepared from the readily accessible 5-HETE methyl ester (42) (Scheme VIII). Treatment of 5-HETE (2) with acetic anhydride and pyridine afforded the acetate 16. Oxidation of 5-HETE methyl ester (42) with manganese dioxide followed by hydrolysis of the resulting ketone 54¹³ with lithium hydroxide gave 5-KETE (17). Reaction of 54 with hydroxylamine provided the oxime methyl ester 55, which was reduced with sodium cyanoborohydride¹⁵ and hydrolyzed with lithium hydroxide to give the hydroxylamine 15. Treatment of 54 with ammonium acetate and sodium cyanoborohydride gave, after hydrolysis with lithium hydroxide, the amine 14. The oxime 18, semicarbazide 19, and thiosemicarbazide 20 were synthesized from 54 via conventional methods of preparation of these ketone derivatives. 5-HETE δ -lactone (21) was synthesized ac-

Scheme IX^a

^a (a) CH₂=CHMgBr, CuI; (b) O₃, CH₃SCCH₃; (c) LDA, 28; (d) MCPBA; (e) Et₃N, CH₃OH; (f) PDC; (g) LiOH.

Table I. RBL-1 5-Lipoxygenase Inhibitory Activities of 5-HPETE Analogues

compd	R	IC ₅₀ (95% CL), μ M
2	OH	77 (70–85)
3	CH ₂ OH	29 (23–37)
4	CH ₂ CH ₂ OH	22 (18–33)
5	CH ₂ CH ₂ CH ₂ OH	97 (81–108)
6	SH	7.6 (6.8–8.5)
7	CH ₂ SH	11 (10–13)
8	CH ₂ CH ₂ SH	25 (23–27)
9	CH ₂ CH ₂ CH ₂ SH	36 (32–40)
10	CONHOH	1.4 (1.2–1.7)
11	CH ₂ CONHOH	0.19 (0.18–0.20)
12	CH ₂ CH ₂ CONHOH	2.8 (2.5–3.1)
13	CH ₂ COOH	96 (85–108)
14	NH ₂	25 (22–29)
15	NHOH	21 (19–23)
16	OCOCH ₃	68 (57–81)
17	=O	18 (14–24)
18	=NOH	11 (9.1–14.1)
19	=NNHCONH ₂	7.0 (6.0–8.1)
20	=NNHCSNH ₂	7.9 (7.1–8.8)

cording to a reported procedure from arachidonic acid.⁶ The lactam 24 was prepared from the corresponding amino acid 14 by acid-catalyzed cyclization utilizing *p*-toluenesulfonic acid.

The ketone 22 was obtained through total synthesis as follows (Scheme IX). Reaction of 2-cyclohexenone with vinylmagnesium chloride⁹ catalyzed by cuprous iodide gave, after ozonolysis and reduction with dimethyl sulfide, the aldehyde 56.¹⁶ Coupling of the ketone 56 with phosphonate 28 afforded the ketone 22. Since the ketone had limited solubility in our biological assay, the keto acid 23 was prepared as follows. The diketone 57¹⁷ was reacted with *m*-chloroperbenzoic acid¹⁸ to yield the lactone 58, which was opened with triethylamine to provide the ester alcohol 59. The ester alcohol 59 was oxidized to aldehyde 60 with pyridinium dichromate. Aldehyde 60 was coupled with phosphonate 28 to give the keto ester 61, which was treated with lithium hydroxide to yield the keto acid 23.

Enzyme Inhibition

Evaluation of the synthesized analogues as inhibitors of 5-lipoxygenase in vitro was conducted according to the method of Dyer et al.¹⁹ using the 20000g supernatant from

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Table II. RBL-1 5-Lipoxygenase Inhibitory Activities of Cyclic 5-HPETE Analogues

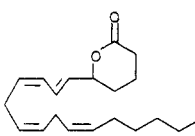
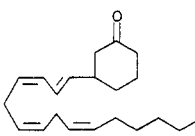
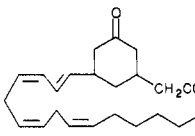
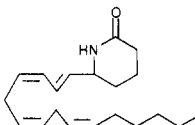
compd	structure	IC ₅₀ (95% CL), μ M
21		27 (23–31)
22		27% at 30
23		59 (54–65)
24		10 (9.6–10.8)

Table III. Comparison of the 5-Lipoxygenase Inhibitory Potency of Compound 11 with Several Common Reference Inhibitors

inhibitor	IC ₅₀ (95% CL), μ M
11	0.19 (0.18–0.20)
BW-755C	1.3 (1.2–1.5)
phenidone	2.1 (2.0–2.2)
5-HETE hydroxamic acid	2.1 (1.7–2.5)
arachidonohydroxamic acid	2.2 (2.0–2.4)
15-HETE	7.3 (6.8–7.8)
Rev-5901	>45
5,6-DHA	55 (46–64)

rat basophilic leukemia cells, and the results are shown in Tables I and II. For comparative purposes, the inhibitor activity of several commonly cited reference 5-lipoxygenase inhibitors is presented in Table III.

The compounds exhibited a wide range of 5-lipoxygenase inhibitory activities (IC₅₀ = 0.19–97 μ M). In the case of the hydroxyl substitution at C-5, the methyl and ethyl derivatives 3 and 4 exhibited more than twice the inhibitory activity of 5-HETE (2) whereas the propyl analogue 5 was less potent. Replacement of the 5-HETE hydroxyl group by a thiol group (6) resulted in a 10-fold increase in activity. However, in contrast to what was observed with the hydroxyl derivatives, extension of the thiol moiety by one, two or three methylene units (7–9) progressively decreased potency compared to direct attachment at C-5 (6). By far the most potent inhibitors were the hydroxamic acids 10–12, which exhibited IC₅₀'s of 0.19–2.8 μ M. In this series of inhibitors, the position of the hydroxamate group was extremely important for activity. The most potent inhibitor, compound 11, contains a hydroxamate group extended by one methylene from position C-5. Direct attachment of the hydroxamate group at C-5 as in 10 or homologation by two methylene groups as in 12 resulted in a sevenfold and 14-fold decrease in potency, respectively. In addition, hydroxamic acid 11 was not only more potent than the reference 5-lipoxygenase inhibitors but was also 10 times more active than the C-1 hydroxamates of arachidonic acid²⁰ and 5-HETE, which had IC₅₀'s of 2.2 and

2.1 μ M, respectively. These results show that the position of the hydroxamic acid moiety on the eicosanoid template is highly important for 5-lipoxygenase inhibition.

Comparison of the activity of hydroxamic acid 11 with the corresponding carboxylic acid 13 indicated the extreme differences in potency (approximately 500 times) between these closely related compounds. The amine 14 and hydroxylamine 15 were more than twice as potent as the corresponding hydroxyl analogue 2. Likewise the carbonyl derivatives 17–20 were at least fourfold more potent than 2 with the semicarbazide derivative 19 exhibiting an IC₅₀ of 7.0 μ M.

The cyclic eicosanoids 21–24 were also found to inhibit the enzyme. Ketone 22 in which C-1 and C-5 are joined together by a carbon bond produced 27% inhibition at 30 μ M; however, due to solubility problems with the compound, higher concentrations could not be tested. Insertion of an acetic acid side chain on the cyclohexanone portion of the molecule improved solubility, and this keto acid 23 was active in the potency range of 5-HETE (IC₅₀'s of 59 and 77 μ M, respectively). Cyclization of 5-HETE to form the lactone 21 increased potency threefold (IC₅₀'s of 77 and 27 μ M, respectively). Lactam 24 was also found to be about 3 times more active than the corresponding linear analogue, the amine 10 (IC₅₀'s of 10 and 25 μ M, respectively). The increased inhibitor activity following cyclization of C-1 to C-5 may be attributed to the formation of a more favorable conformation for binding. Not only are the cyclized eicosanoids more rigid but they are also more stable than the linear analogues.

Conclusion

A synthetic scheme for preparing a variety of 5-substituted eicosanoid analogues has been presented. This study shows that 5-lipoxygenase inhibitors can be rationally designed by substitution of various functional groups at C-5 with an eicosanoid as a template. Compounds containing a hydroxamic acid group provide the most potent inhibitors, and the location of this group on the eicosanoid template has a substantial effect on potency. The known affinity of hydroxamic acids for Fe³⁺ suggests a possible mechanism of inhibition involving binding to the putative iron moiety in the 5-lipoxygenase active site. The lactone and lactam inhibitors demonstrate that cyclization of C-1 and C-5 results in enhanced activity and a free carboxylate is not required for inhibitory activity. The potent inhibition provided by hydroxamate-containing analogues and the activities of the more conformationally constrained inhibitors suggests a possible strategy to designing pharmaceutically useful inhibitors of 5-lipoxygenase.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) was distilled from sodium benzophenone immediately prior to use. All reactions were carried out under an atmosphere of argon or nitrogen. ¹H NMR spectra were obtained on a General Electric QE-300 NMR instrument at 300 MHz and on a Varian T-60 NMR instrument at 60 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. ¹H NMR data are tabulated in the following order: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. Mass spectra were recorded with an HP5985 A spectrometer and high-resolution mass spectra were obtained on a Kratos MS50 instrument. Merck TLC plates were used for

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analytical TLC and Merck Kieselgel 60 was used for column chromatography. Microanalyses were performed by the Abbott Analytical Department. All products were obtained as colorless oils unless otherwise noted.

2,4-Undecadiyn-1-ol (25). Phosphorus tribromide (93 g, 0.344 mol) was added dropwise to a mixture of 2-octyn-1-ol (130 g, 1.03 mol) in anhydrous ether (300 mL) and pyridine (5 mL) under argon at -30°C . The reaction mixture was allowed to warm gradually to 23°C and maintained at this temperature for 3 h. It was then poured into saturated aqueous NaCl (500 mL). The organic layer was removed and the aqueous layer extracted with ether (2×100 mL). The combined ethereal solutions were dried (MgSO_4) and vacuum distilled (bp 40°C at 0.1 mmHg) to afford 1-bromo-2-octyne (181 g, 94%).

A solution of bromoethane (20.3 g, 0.16 mol) in dry THF (150 mL) was added dropwise to magnesium (4.06 g, 0.167 mol) in THF (60 mL) at 0°C under argon. After the bromoethane addition was completed, the reaction temperature was allowed to rise to 23°C and the mixture was stirred until all the magnesium metal dissolved. 3-Butyn-1-ol (5.96 g, 0.085 mol) in THF (75 mL) was added dropwise at 0°C . The reaction mixture was then stirred at 23°C for 12 h. Copper(I) iodide (0.20 g, 0.002 mol) was added to the gray reaction slurry. After the mixture was stirred for 15 min, 1-bromo-2-octyne (10.0 g, 0.053 mol) in THF (10 mL) was added and the reaction mixture refluxed for 12 h. The reaction mixture was recharged with copper(I) iodide (0.2 g) and refluxed an additional 6 h. The reaction mixture was poured into a mixture of 2 N H_2SO_4 (250 mL) and ice (100 g). The resulting solution was extracted with ether (3×50 mL), and the ether extracts were washed with saturated aqueous NH_4Cl and saturated aqueous Na_2CO_3 and dried (MgSO_4). The solvent was removed and the crude oil was chromatographed (silica gel; pentane/ether, 2/1) to give 2,4-undecadiyn-1-ol (25) (8.6 g, 91%): ^1H NMR (60 MHz, CDCl_3) δ 4.20 (m, 2 H), 3.20 (m, 2 H), 2.10 (m, 2 H), 1.80 (br s, 1 H), 1.10–1.60 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 164 (M^+). Anal. ($\text{C}_{11}\text{H}_{16}\text{O}$) C, H.

1-[(3,4,5,6-Tetrahydro-2-pyranil)oxy]tetradeca-2,5,8-triyn-1-ol (26). A solution of bromine (46.5 g, 0.29 mol) in CH_2Cl_2 (150 mL) was slowly added dropwise to a solution of 1,2-bis(diphenylphosphino)ethane (60 g, 0.15 mol) in CH_2Cl_2 (725 mL) under argon at 0°C . Diyne 25 (25 g, 0.14 mol) in CH_2Cl_2 (150 mL) was added to this mixture and the resulting solution stirred at 23°C for 2 h. The volume of the reaction mixture was reduced in vacuo to 60 mL and ether (1.5 L) added. Pentane (3 L) was added to the ether solution and the organic solution dried (MgSO_4). The mixture was filtered through a pad of silica gel (Merck, 40–60 μm). The solvent was removed in vacuo to afford 1-bromo-2,4-undecadiyne (33 g, 99%) as a colorless oil. Next a solution of bromoethane (15.8 g, 0.145 mol) in dry THF (100 mL) was added dropwise to magnesium (3.16 g, 0.13 mol) in THF (200 mL) at 0°C under argon. After the addition was completed, the reaction mixture was stirred at ambient temperature until all the magnesium dissolved. A solution of 1-[(tetrahydro-2-pyranil)oxy]-2-propyne (17.0 g, 0.12 mol) in THF (75 mL) was slowly added to the cooled ethylmagnesium bromide solution. After the mixture was stirred for 10 h at 23°C , copper(I) iodide (1.0 g) was added and the resulting mixture stirred an additional 15 min. 1-Bromo-2,4-undecadiyne (27.2 g, 0.12 mol) in THF (75 mL) was added and the resulting mixture stirred at 23°C for 2 h. The reaction mixture was poured into a solution of 2 N H_2SO_4 (250 mL) and ice (100 mL). The resulting solution was extracted with ether (3×100 mL), and the ether extracts were washed with saturated aqueous NH_4Cl (100 mL) and saturated aqueous Na_2CO_3 (100 mL) and dried (MgSO_4). The solvent was removed and the residue was chromatographed (silica gel; ether/pentane; 1/9) to provide triyne 26 (32.8 g, 95%): ^1H NMR (60 MHz, CDCl_3) δ 4.70 (m, 1 H), 4.21 (s, 2 H), 3.32–4.00 (m, 2 H), 3.12 (m, 4 H), 2.10 (m, 2 H), 1.10–1.91 (m, 12 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 286 (M^+). Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_2$) C, H.

1-[(3,4,5,6-Tetrahydro-2-pyranil)oxy]tetradeca-2(Z),5-(Z),8(Z)-triene (27). A mixture of 10% Pd/ BaSO_4 (0.128 g) in ethyl acetate (90 mL) and quinoline (2.6 mL) was treated with hydrogen at 1 atm and 23°C for 2 h followed by the addition of 1-[(tetrahydro-2-pyranil)oxy]undeca-2,5,8-triyn-1-ol (26) (2.4 g, 8.3 mmol) in ethyl acetate (5 mL). After the uptake of the theoretical amount of hydrogen was observed (45 min), the ethyl acetate was

removed in vacuo and the residue dissolved in ether (100 mL). The ether layer was washed with aqueous 3 N HCl (50 mL) and saturated aqueous NaHCO_3 (50 mL) and dried (MgSO_4). The solution was filtered through a pad of silica gel (Baker, 25–40 mesh) and the solvent removed in vacuo. The crude residue was chromatographed (silica gel; pentane/ether; 17/1) to give triene 27 (2.0 g, 91%): ^1H NMR (60 MHz, CDCl_3) δ 5.12–5.81 (m, 6 H), 4.62 (m, 1 H), 4.10 (m, 2 H), 3.31–4.04 (m, 2 H), 2.83 (m, 4 H), 2.10 (m, 2 H), 1.71 (m, 6 H), 1.32 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 292 (M^+). Anal. ($\text{C}_{19}\text{H}_{32}\text{O}_2$) C, H.

Diethyl 2(Z),5(Z),8(Z)-Tetradecatrienylphosphonate (28). A solution of bromine (0.22 g, 1.36 mmol) was slowly added dropwise to a solution of 1,2-bis(diphenylphosphino)ethane (0.27 g, 0.68 mmol) in CH_2Cl_2 (2 mL) under argon at 0°C . A slight excess of 1,2-bis(diphenylphosphino)ethane was added back to the solution until the light yellow color disappeared. Triene 27 was added in one portion. The mixture was stirred at 23°C for 9 h. The solvent was removed in vacuo and the residue dissolved in hexane/ether (2/1) (60 mL). The solution was filtered through a pad of silica gel to afford 1-bromo-2,5,8-tetradecatriene (170 mg, 93%). The bromo compound (1.30 g, 4.8 mmol) in CH_3CN (75 mL) under argon was treated with ethyl phosphite (1.20 g, 7.2 mmol) and the resulting solution warmed to 70 – 75°C for 7 h. The solution was cooled to 23°C and the solvent removed. The crude residue was chromatographed (silica gel; ether/ethyl acetate, 1/1) to provide the product 28 (1.54 g, 98%): ^1H NMR (60 MHz, CDCl_3) δ 5.2–5.6 (m, 6 H), 3.8–4.4 (m, 4 H), 2.4–3.0 (m, 6 H), 1.9–2.3 (m, 2 H), 1.3–1.6 (t, 6 H, $J = 7$ Hz), 1.1–1.6 (m, 6 H), 0.9 (t, 3 H, $J = 7$ Hz); MS, m/e 328 (M^+). Anal. ($\text{C}_{18}\text{H}_{33}\text{O}_3\text{P}$) C, H.

3-(tert-Butoxymethyl)-1-[(tert-butoxydimethylsilyl)-oxy]-1-cyclohexene (29). A solution of $\text{CuBr} \cdot (\text{CH}_3)_2\text{S}$ (159.5 mg) in *tert*-butyl methyl ether (1 mL) and isopropyl sulfide (2 mL) was added to a solution of *t*-BuOCH₂Li (200 mg, 2.15 mmol) in THF (10 mL) at -78°C under argon. After the mixture was stirred at -78°C for 40 min, 2-cyclohexen-1-one (68 mg, 0.7 mmol) in TBME (2 mL) was added dropwise and the mixture was warmed to -30°C . After 30 min at this temperature, *tert*-butoxydimethylsilyl triflate (528 mg, 2 mmol) in THF (5 mL) was added and the reaction mixture allowed to warm to 23°C and stirred at this temperature for 1 h. The solution was then cooled and poured into aqueous NH_4Cl . Ammonium hydroxide was added to give a pH of 8 and the solution was extracted with ether (3×10 mL). The ether layers were dried (MgSO_4), and the solvent was evaporated. Chromatography (silica gel; pentane/ether, 3/1) provided 29 (154 mg, 74%): ^1H NMR (60 MHz, CDCl_3) δ 4.8 (d, $J = 1$ Hz, 1 H), 3.6 (m, 2 H), 2.4–1.5 (m, 7 H), 1.0 (s, 9 H), 0.9 (s, 9 H); MS, m/e 270 (M^+). Anal. ($\text{C}_{17}\text{H}_{34}\text{O}_2\text{Si}$) C, H.

Methyl 6-tert-Butoxy-5-formylhexanoate (30). A solution of 29 (596 mg, 2 mmol) in methanol (20 mL) and methylene chloride (5 mL) under nitrogen was ozonized at -78°C for 1 h. After the solution turned blue, the ozone was removed and the reaction mixture flushed with oxygen until clear. The reaction mixture was quenched with dimethyl sulfide (5 mL) and allowed to warm to 23°C . The solvent was removed and the crude product dissolved in ether (10 mL) and washed with dilute acetic acid and water. The ether layer was dried (Na_2SO_4) and ethereal diazomethane added at 0°C . The solvent was removed and the crude residue was chromatographed (silica gel; ether/pentane, 1/3) to give 30 (419 mg, 91%): ^1H NMR (60 MHz, CDCl_3) δ 9.6 (d, 1 H), 3.6 (s, 3 H), 3.2 (d, 2 H), 2.3 (m, 3 H), 1.3–1.9 (m, 6 H), 1.0 (s, 9 H); MS, m/e 230 (M^+). Anal. ($\text{C}_{12}\text{H}_{22}\text{O}_4$) C, H.

1-[(tert-Butyldimethylsilyl)oxy]-3-ethenyl-1-cyclohexene (31). CuI (0.40 g, 21.1 mmol) was added in one portion to a stirred solution of vinylmagnesium bromide (30.0 mL, 30 mmol) in THF (10 mL) at 0°C under argon. The resulting brown solution was immediately cooled to 0°C and 2-cyclohexen-1-one (2.00 g, 20.2 mmol) in THF (5 mL) was slowly added dropwise. After stirring at 50°C for 30 min, the mixture was cooled to -60°C and TMEDA (7.0 mL, 20 mmol) was slowly added dropwise followed by the addition of lutidine (4.7 mL, 40 mmol). *tert*-Butyldimethylsilyl methanesulfonate (7.00 mL, 30.0 mmol) was then added. The reaction mixture was stirred at -60°C for 1 h, -20°C for 2 h, and finally 23°C for 2 h. The reaction mixture was quenched by being poured into a mixture of saturated aqueous NaHCO_3 (100 mL) and ice (100 mL). The aqueous layer was extracted with pentane

(4 × 100 mL). The combined pentane extracts were dried (MgSO₄), and the solvent was removed in vacuo to provide crude product. Chromatography (silica gel; pentane/ether, 9/1) of the crude residue gave **31** (3.36 g, 70%): ¹H NMR (60 MHz, CDCl₃) δ 5.0–5.2 (m, 1 H), 4.7–4.9 (m, 2 H), 1.1–2.8 (m, 7 H), 0.9 (s, 9 H), 0.08 (s, 6 H); MS, *m/e* 238 (M⁺). Anal. (C₁₄H₂₆O₂Si) C, H.

1-[(*tert*-Butyldimethylsilyl)oxy]-3-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-1-cyclohexene (32). 9-BBN (10.0 mL of 0.5 M 9-BBN in THF) was added dropwise to a solution of compound **31** (1.19 g, 5 mmol) in THF (25 mL) at 23 °C. The reaction was stirred at 23 °C under argon for 2.5 h. The resulting organoborane was oxidized by adding EtOH (3 mL), 6 N NaOH (1 mL), and 30% H₂O₂ (2 mL). The mixture was heated for 1 h at 50 °C and then cooled to 23 °C. The aqueous layer was saturated with sodium carbonate. The organic layer was separated and the aqueous layer extracted with ether (2 × 20 mL). The organic layers were combined, dried (MgSO₄), and evaporated. Chromatography (SiO₂; pentane/ether, 1/1) of the crude material yielded the alcohol (750 mg, 66%). The alcohol (700 mg, 2.7 mmol) was dissolved in methylene chloride (25 mL) containing (dimethylamino)pyridine (100 mg) and imidazole (243 mg, 2.7 mmol). *tert*-Butyldiphenylsilyl chloride (742 mg, 2.7 mmol) in methylene chloride (10 mL) was added and the reaction mixture was stirred at 23 °C for 4 h. Removal of solvent followed by chromatography (silica gel; pentane/ether, 3/1) gave **32** (1.04 g, 98%): ¹H NMR (60 MHz, CDCl₃) δ 7.5–7.7 (m, 4 H), 7.2–7.5 (m, 6 H), 3.6 (t, 2 H, *J* = 7 Hz), 4.8 (d, 1 H, *J* = 1 Hz), 1.2–2.8 (m, 9 H), 1.0 (s, 9 H), 0.1 (s, 6 H); MS, *m/e* 494 (M⁺). Anal. (C₃₀H₄₆O₂Si₂) C, H.

Methyl 7-[(*tert*-Butyldiphenylsilyl)oxy]-5-formylheptanoate (33). Compound **33** was prepared from **32** in a manner similar to **30**. Chromatography (silica gel; ether/pentane, 1/1) gave **33** (758 mg, 89%): ¹H NMR (60 MHz, CDCl₃) δ 9.6 (d, 1 H), 7.5–7.7 (m, 4 H), 7.2–7.5 (m, 6 H), 3.6 (s, 3 H), 3.6 (t, 3 H, *J* = 7 Hz), 2.30 (m, 3 H), 1.3–1.9 (m, 6 H), 1.1 (s, 9 H); MS, *m/e* 426 (M⁺). Anal. (C₂₅H₃₄O₄Si) C, H.

1-[(*tert*-Butyldimethylsilyl)oxy]-3-[3-[(*tert*-butyldimethylsilyl)oxy]propyl]-1-cyclohexene (34). LiCH₂CH₂OTBDMS (360 mg, 2 mmol), prepared from *n*-butyllithium and 3-iodo-1-[(*tert*-butyldimethylsilyl)oxy]propane in ether, was added to CuI (380 mg, 2 mmol) in dry ether (50 mL) at –40 °C under argon. The solution was stirred at –40 °C for 10 min and then cooled to –70 °C. 2-Cyclohexenone (86 mg, 1 mmol) in ether (10 mL) was added dropwise to the solution at –70 °C. After the mixture was stirred for 1 h, the resulting enolate was quenched by the consecutive addition of HMPA (179 mg, 1 mmol), NEt₃ (202 mg, 2 mmol), and TBDMSiEt (528 mg, 2 mmol). The reaction mixture was stirred at –20 °C for 1 h and then warmed to 23 °C and stirred at that temperature for 1 h. The reaction mixture was extracted with chloroform, and the organic extracts were washed with water and brine. The organic layer was dried (Na₂SO₄) and evaporated. Chromatography (silica gel; pentane/ether, 3/1) of the crude residue gave **34** (252 mg, 71%): ¹H NMR (60 MHz, CDCl₃) δ 2.8 (d, 1 H, *J* = 1 Hz), 3.6 (t, 2 H, *J* = 7 Hz), 1.2–2.8 (m, 11 H), 1.0 (s, 18 H), 0.1 (s, 12 H); MS, *m/e* 356 (M⁺). Anal. (C₂₁H₄₄O₂Si₂) C, H.

Methyl 8-[(*tert*-Butyldimethylsilyl)oxy]-5-formyloctanoate (35). Compound **35** was prepared from **34** in the same fashion as described for **30**. Chromatography (silica gel; ether/pentane, 1/3) gave **35** (569 mg, 90%): ¹H NMR (60 MHz, CDCl₃) δ 9.6 (d, 1 H), 3.6 (t, 1 H, *J* = 7 Hz), 2.1–2.4 (m, 3 H), 1.3–1.9 (m, 8 H), 1.0 (s, 9 H), 0.02 (s, 6 H); MS, *m/e* 316 (M⁺). Anal. (C₁₆H₃₂O₄Si) C, H.

Methyl 5-(*tert*-Butoxymethyl)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-tetraeicosanoate (36). A solution of lithium diisopropylamide (2.16 mmol in THF) was added dropwise to a stirred solution of phosphonate **28** (656 mg, 2 mmol) in THF (15 mL) under argon at –78 °C. The reaction mixture was stirred for 1 min and aldehyde **30** (414 mg, 1.8 mmol) in THF (5 mL) was added. The reaction mixture was stirred at –78 °C for 1 h and then allowed to warm to 23 °C and stirred 20 h. Ether (100 mL) was added followed by aqueous saturated NH₄Cl solution (10 mL). The organic layer was washed with water (30 mL) and brine (30 mL) and dried (MgSO₄). Removal of solvent gave a residue, which was chromatographed (silica gel; pentane/ether, 3/1) to provide **36** (533 mg, 66%): ¹H NMR (300 MHz, CDCl₃) δ 6.45 (dd, 1 H, *J* = 11 and 15 Hz), 5.99 (dd, 1 H, *J* = 11 and 11 Hz), 5.28–5.49

(m, 6 H), 3.68 (s, 3 H), 3.53–3.64 (m, 1 H), 3.41–3.50 (m, 1 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.32 (t, 2 H, *J* = 7 Hz), 2.25–2.35 (m, 1 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.42–1.48 (m, 5 H), 1.22–1.40 (m, 6 H), 1.00 (s, 9 H), 0.88 (t, 3 H, *J* = 7 Hz); MS, *m/e* 404 (M⁺). Anal. (C₂₆H₄₄O₃) C, H.

Methyl 5-(Acetoxymethyl)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoate (37). Acetic anhydride (1 mL) and anhydrous ferric chloride (4 mg, 0.025 mmol) were added to the *tert*-butyl ester **36** (202 mg, 0.5 mmol) in THF (15 mL) at 0 °C under argon. The reaction mixture was stirred for 15 min and then poured into water (150 mL) and extracted with ether (3 × 50 mL). Drying (MgSO₄) and removal of solvent gave a crude oil, which was chromatographed (silica gel; pentane/ether, 4/1) to provide the acetate **37** (187 mg, 96%): ¹H NMR (300 MHz, CDCl₃) δ 6.45 (dd, 1 H, *J* = 11 and 15 Hz), 5.99 (dd, 1 H, *J* = 11 and 11 Hz), 5.26–5.49 (m, 6 H), 3.68 (s, 3 H), 3.53–3.64 (m, 1 H), 3.41–3.51 (m, 1 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.00 (s, 3 H), 1.42–1.78 (m, 5 H), 1.22–1.40 (m, 6 H), 0.88 (t, 3 H, *J* = 7 Hz); MS, *m/e* 390 (M⁺). Anal. (C₂₄H₃₈O₄) C, H.

5-(Hydroxymethyl)-6(*E*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (3). A solution of lithium hydroxide (184 mg, 4.6 mmol) dissolved in water (10 mL) was added dropwise to ester **37** (180 mg, 0.46 mmol) in *i*-PrOH (20 mL) at 23 °C under argon. After 4 h at 23 °C, the reaction mixture was poured into water (100 mL) and the pH adjusted to 5 with 2 N HCl. The solution was extracted with ethyl acetate (3 × 25 mL). The organic extracts were dried (Na₂SO₄) and removed in vacuo. The crude residue was chromatographed (silica gel; pentane/ether/formic acid, 9/1/0.02) to give **3** (144 mg, 94%): ¹H NMR (300 MHz, CDCl₃) δ 6.45 (dd, 1 H, *J* = 11 and 15 Hz), 6.00 (dd, 1 H, *J* = 11 and 11 Hz), 5.30–5.50 (m, 6 H), 3.45–3.50 (m, 1 H), 3.55–3.65 (m, 1 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.25–2.35 (m, 1 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 5 H), 1.20–1.40 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 334 (M⁺); HRMS (C₂₁H₃₄O₃) calcd 334.2508, found 334.2507.

Methyl 5-[2-[(*tert*-Butyldiphenylsilyl)oxy]ethyl]-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoate (38). Compound **38** was prepared from **33** in a manner similar to that for **36**. Chromatography (silica gel; pentane/ether, 3/1) gave **38** (702 mg, 65%): ¹H NMR (300 MHz, CDCl₃) δ 7.61–7.70 (m, 4 H), 7.35–7.45 (m, 6 H), 6.20 (1 H, dd, *J* = 15 and 11 Hz), 5.96 (dd, 1 H, *J* = 11 and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (br t, 2 H, *J* = 7 Hz), 3.65 (s, 3 H), 2.95 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.38 (t, 2 H, *J* = 7 Hz), 2.10 (t, 2 H, *J* = 7 Hz), 2.05 (m, 1 H), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 6 H), 1.10 (s, 9 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 600 (M⁺). Anal. (C₃₉H₅₆O₃Si) C, H.

Methyl 5-(2-Hydroxyethyl)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoate (39). *n*-Bu₄NF (52.4 mg, 0.2 mmol) in THF (2 mL) was added dropwise to methyl ester **38** (120 mg, 0.2 mmol) in THF (10 mL) at 0 °C under argon. The reaction mixture was warmed to 23 °C and stirred for 1 h. Removal of solvent and chromatography (silica gel; pentane/ether, 3/2) of the residue gave **39** (70 mg, 96%): ¹H NMR (300 MHz, CDCl₃) δ 6.31 (dd, 1 H, *J* = 11 and 15 Hz), 5.96 (dd, 1 H, *J* = 11 and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (br t, 2 H, *J* = 7 Hz), 3.65 (s, 3 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.25–2.40 (m, 1 H), 2.06 (m, 3 H), 1.20–1.70 (m, 11 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 362 (M⁺). Anal. (C₂₃H₃₈O₃) C, H.

5-(2-Hydroxyethyl)-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (4). A solution of lithium hydroxide (21 mg, 0.51 mmol) dissolved in water (5 mL) was added dropwise to methyl ester **39** (60 mg, 0.17 mmol) in *i*-PrOH (10 mL) at 23 °C under argon. After 1 h, the reaction mixture was poured into water (80 mL) and the pH adjusted to 5 with 2 N HCl. The solution was extracted with ethyl acetate (3 × 25 mL). The organic extracts were dried (Na₂SO₄) and removed in vacuo. The crude residue was chromatographed (silica gel; pentane/ether/formic acid, 9/1/0.02) to give **4** (56 mg, 95%): ¹H NMR (300 MHz, CDCl₃) δ 6.31 (dd, 1 H, *J* = 11 and 15 Hz), 5.95 (dd, 1 H, *J* = 11 and 11 Hz), 5.25–5.50 (m, 6 H), 3.63 (br t, 2 H, *J* = 7 Hz), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.32 (t, 2 H, *J* = 7 Hz), 2.06 (m, 3 H), 1.20–1.70 (m, 11 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 348 (M⁺); HRMS (C₂₂H₃₆O₃) calcd 348.2457, found 348.2459.

Methyl 5-[3-[(*tert*-Butyldimethylsilyl)oxy]propyl]-6(*E*),8(*Z*),11(*Z*),14(*Z*)-eicosatetraenoate (40). Compound **40** was prepared from **35** as described for **36**. Chromatography (silica

gel; pentane/ether, 3/1) gave **40** (608 mg, 62%): ^1H NMR (300 MHz, CDCl_3) δ 6.31 (dd, 1 H, $J = 11$ and 15 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (s, 3 H), 3.63 (t, 2 H, $J = 7$ Hz), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.32 (t, 2 H, $J = 7$ Hz), 2.06 (m, 3 H), 1.20–1.70 (m, 13 H), 0.91 (s, 9 H), 0.90 (t, 3 H, $J = 7$ Hz), 0.15 (s, 6 H); MS, m/e 440 (M^+). Anal. ($\text{C}_{30}\text{H}_{54}\text{O}_3\text{Si}$) C, H.

Methyl 5-(3-Hydroxypropyl)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (41). Compound **41** was prepared from **40** in the manner described for **39**. Chromatography (silica gel; pentane/ether, 3/2) provided **41** (71 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 6.31 (dd, 1 H, $J = 11$ and 15 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (s, 3 H), 3.63 (t, 2 H, $J = 7$ Hz), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.32 (t, 2 H, $J = 7$ Hz), 2.06 (m, 3 H), 1.20–1.70 (m, 13 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 376 (M^+). Anal. ($\text{C}_{24}\text{H}_{40}\text{O}_3$) C, H.

5-(3-Hydroxypropyl)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (5). Compound **5** was prepared from **41** in the manner described for **4**. Chromatography (silica gel; pentane/ether/formic acid, 9/1/0.02) gave **5** (59 mg, 96%): ^1H NMR (300 MHz, CDCl_3) δ 6.31 (dd, 1 H, $J = 11$ and 15 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 3.63 (t, 2 H, $J = 7$ Hz), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.32 (t, 2 H, $J = 7$ Hz), 2.06 (m, 3 H), 1.20–1.70 (m, 13 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 362 (M^+); HRMS ($\text{C}_{23}\text{H}_{38}\text{O}_3$) calcd 362.2782, found 362.2784.

Methyl 5-(Acetylthio)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (43). Diisopropyl azodicarboxylate (40.4 mg, 0.2 mmol) was added to a stirred solution of triphenylphosphine (52 mg, 0.2 mmol) in THF (4 mL) at 0°C for 30 min. A white precipitate resulted. Alcohol **42**⁶ (33.4 mg, 0.1 mmol) and thiolacetic acid (15.2 mg, 0.2 mmol) in THF (1 mL) was added dropwise and the mixture was stirred for 1 h at 0°C and at 23°C for 1 h. A clear yellow solution resulted. The mixture was dissolved in ether (25 mL) and washed with water. The ether layer was dried (Na_2SO_4) and evaporated. The crude residue was chromatographed (silica gel; pentane/ether, 9/1) to give **43** (33 mg, 84%): ^1H NMR (300 MHz, CDCl_3) δ 6.28 (dd, 1 H, $J = 15$ and 11 Hz), 6.01 (dd, 1 H, $J = 11$ and 11 Hz), 5.30–5.65 (m, 6 H), 3.67 (s, 3 H), 2.95 (t, 2 H, $J = 7$ Hz), 2.81 (t, 2 H, $J = 7$ Hz), 2.30 (s, 3 H), 2.35 (t, 2 H, $J = 7$ Hz), 2.06 (q, 2 H, $J = 7$ Hz), 1.60–1.80 (m, 3 H), 1.21–1.42 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 392 (M^+); HRMS ($\text{C}_{23}\text{H}_{36}\text{O}_3\text{S}$) calcd 392.2385, found 392.2381.

5-Mercapto-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (6). Lithium hydroxide (13 mg, 0.32 mmol) dissolved in water (1.5 mL) was added dropwise to ester **43** (30 mg, 0.08 mmol) in *i*-PrOH (3 mL) at 23°C under argon. The reaction mixture was stirred at 23°C for 1 h. The reaction mixture was added to water (50 mL) and the pH adjusted to pH 5 with 2 N HCl. The solution was extracted with ethyl acetate (3×20 mL). The organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The crude oil was chromatographed (silica gel; pentane/ether/formic acid, 9/1/0.02) to provide **6** (25 mg, 99%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 15$ and 11 Hz), 6.01 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.70 (m, 6 H), 3.50 (m, 1 H), 2.87 (t, 2 H, $J = 7$ Hz), 2.80 (t, 2 H, $J = 7$ Hz), 2.38 (t, 2 H, $J = 7$ Hz), 2.06 (t, 2 H, $J = 7$ Hz), 2.00–2.20 (m, 1 H), 1.60–1.82 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 336 (M^+); HRMS ($\text{C}_{20}\text{H}_{32}\text{O}_3\text{S}$) calcd 336.2123, found 336.2126.

Methyl 5-[(Acetylthio)methyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (45). Compound **45** was prepared from **44** as described for **43**. Chromatography (silica gel; pentane/ether, 9/1) gave **45** (33 mg, 82%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.30–5.55 (m, 6 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.53 (t, 2 H, $J = 7$ Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.20–2.30 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 2.30 (s, 3 H), 1.55–1.75 (m, 5 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 406 (M^+); HRMS ($\text{C}_{24}\text{H}_{38}\text{O}_3\text{S}$) calcd 406.2542, found 406.2544.

5-[(Acetylthio)methyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (7). Compound **7** was prepared from **45** as described for **6**. Chromatography (silica gel; pentane/ether/formic acid, 9/1/0.02) gave **7** (23 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.30–5.55 (m, 6 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.53 (dd, 2 H, $J = 7$ and 7 Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.20–2.30 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.55–1.75 (m, 5 H),

1.20–1.45 (m, 7 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 350 (M^+); HRMS ($\text{C}_{21}\text{H}_{34}\text{O}_3\text{S}$) calcd 350.2280, found 350.2276.

Methyl 5-[2-(Acetylthio)ethyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (46). Compound **46** was prepared from **39** as described for **43**. Chromatography (silica gel; pentane/ether, 9/1) gave **46** (34 mg, 82%): ^1H NMR (300 MHz, CDCl_3) δ 6.32 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.55–2.75 (m, 2 H), 2.35 (t, 2 H, $J = 7$ Hz), 2.30 (s, 3 H), 2.20–2.30 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.50–1.80 (m, 5 H), 1.20–1.5 (m, 8 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 420 (M^+); HRMS ($\text{C}_{25}\text{H}_{40}\text{O}_3\text{S}$) calcd 420.2468, found 420.2469.

5-[2-(Acetylthio)ethyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (8). Compound **8** was prepared from **46** as described for **6**. Chromatography (silica gel; pentane/ether/formic acid, 9/1/0.02) gave **8** (24 mg, 96%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.53 (t, 2 H, $J = 7$ Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.20–2.30 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.50–1.75 (m, 4 H), 1.2–1.45 (m, 8 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 364 (M^+); HRMS ($\text{C}_{22}\text{H}_{36}\text{O}_3\text{S}$) calcd 364.2436, found 364.2439.

Methyl 5-[3-(Acetylthio)propyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (47). Compound **47** was prepared from **41** as described for **43**. Chromatography (silica gel; pentane/ether, 9/1) provided **47** (37 mg, 85%): ^1H NMR (300 MHz, CDCl_3) δ 6.36 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.45 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.65 (br t, 2 H, $J = 7$ Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.30 (s, 3 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.40–1.75 (m, 4 H), 1.20–1.40 (m, 10 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 434 (M^+); HRMS ($\text{C}_{26}\text{H}_{42}\text{O}_3\text{S}$) calcd 434.2855, found 434.2858.

5-[3-(Acetylthio)propyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (9). Compound **9** was prepared from **47** as described for **6**. Chromatography (silica gel; pentane/ether/formic acid, 9/1/0.02) gave **9** (29 mg, 96%): ^1H NMR (300 MHz, CDCl_3) δ 6.36 (dd, 1 H, $J = 11$ and 15 Hz), 5.98 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.65 (br t, 2 H, $J = 7$ Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.06 (q, 2 H, $J = 7$ Hz), 1.95–2.20 (m, 1 H), 1.40–1.75 (m, 5 H), 1.20–1.40 (m, 10 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 378 (M^+); HRMS ($\text{C}_{23}\text{H}_{38}\text{O}_3\text{S}$) calcd 378.2592, found 378.2594.

Methyl 5-Carboxy-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (48). Pyridinium dichromate (395 mg, 1.05 mmol) in DMF (5 mL) was added to alcohol **44** (104 mg, 0.3 mmol) in DMF (5 mL) at 0°C under argon. The reaction mixture was stirred at 23°C for 20 h and then poured into water (120 mL). The aqueous mixture was extracted with ether (3×50 mL), and the organic layers were dried (Na_2SO_4). The ether layer was removed in vacuo and the crude residue was chromatographed (silica gel; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9/1) to give **48** (89 mg, 82%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 15$ and 11 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.50 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.30 (t, 2 H, $J = 7$ Hz), 2.06 (q, 2 H, $J = 7$ Hz), 1.45–1.76 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 362 (M^+); HRMS ($\text{C}_{22}\text{H}_{34}\text{O}_4$) calcd 362.2457, found 362.2459.

Methyl 5-(Carboxymethyl)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (49). Compound **49** was prepared from **39** as described for **48**. Chromatography (silica gel; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9/1) provided **49** (91 mg, 81%): ^1H NMR (300 MHz, CDCl_3) δ 6.38 (dd, 1 H, $J = 15$ and 11 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.55 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.40 (t, 2 H, $J = 7$ Hz), 2.30 (t, 2 H, $J = 7$ Hz), 2.20–2.35 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.45–1.76 (m, 4 H), 1.20–1.45 (m, 6 H), 0.88 (t, 3 H, $J = 7$ Hz); MS, m/e 376 (M^+); HRMS ($\text{C}_{23}\text{H}_{36}\text{O}_4$) calcd 376.2613, found 376.2617.

Methyl 5-(Carboxyethyl)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (50). Compound **50** was prepared from **41** as described for **48**. Chromatography (silica gel; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9/1) provided **50** (93 mg, 93%): ^1H NMR (300 MHz, CDCl_3) δ 6.40 (dd, 1 H, $J = 15$ and 11 Hz), 5.95 (dd, 1 H, $J = 11$ and 11 Hz), 5.28–5.53 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.35 (t, 2 H, $J = 7$ Hz), 2.31 (t, 2 H, $J = 7$ Hz), 2.20–2.30 (m, 1 H), 2.06 (q, 2 H, $J = 7$ Hz), 1.45–1.76 (m, 4 H), 1.20–1.45 (m, 8 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 390 (M^+); HRMS

(C₂₄H₃₈O₄) calcd 390.2770, found 390.2774.

5-(Carboxymethyl)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (13). Compound 13 was prepared from 49 as described for 6. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 13 (16 mg, 91%): ¹H NMR (300 MHz, CDCl₃) δ 6.40 (dd, 1 H, *J* = 15 and 11 Hz), 5.95 (dd, 1 H, *J* = 11 and 11 Hz), 5.28–5.55 (m, 6 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.40 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.25–2.35 (m, 1 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.45–1.76 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 362 (M⁺); HRMS (C₂₂H₃₄O₄) calcd 362.2457, found 362.2460.

Methyl 5-[(Hydroxyamino)carbonyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (51). Oxalyl chloride (25 mg, 0.2 mmol) was added dropwise to acid 48 (36 mg, 0.1 mmol) in THF (1 mL) and DMF (7 mg, 0.1 mmol) at 0 °C under argon. The reaction mixture was stirred for 1 h and then added to a solution of hydroxylamine hydrochloride (28 mg, 0.4 mmol) in THF/H₂O (2/1) (5 mL) containing triethylamine (60 mg, 0.6 mmol) at 0 °C and the reaction mixture stirred for 2 h. The reaction mixture was poured into aqueous ammonium chloride (40 mL) and extracted with ether (3 × 10 mL). The ether layers were dried (Na₂SO₄) and removed in vacuo to give a crude oil, which was chromatographed (silica gel; CH₂Cl₂/MeOH, 9/1) to provide 51 (25 mg, 68%): ¹H NMR (300 MHz, CDCl₃) δ 8.30 (br s, 1 H), 6.41 (dd, 1 H, *J* = 11 and 15 Hz), 5.95 (br t, 1 H, *J* = 11 Hz), 5.30–5.65 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.25–2.40 (m, 1 H), 2.06 (q, 1 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 377 (M⁺); HRMS (C₂₂H₃₅NO₄) calcd 377.2566, found 377.2569.

5-[(Hydroxyamino)carbonyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (10). Compound 10 was prepared from 51 as described for 6. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 10 (17 mg, 94%): ¹H NMR (300 MHz, CDCl₃) δ 8.30 (br s, 1 H), 6.41 (dd, 1 H, *J* = 11 and 15 Hz), 5.95 (br t, 1 H, *J* = 11 Hz), 5.30–5.65 (m, 6 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 363 (M⁺); HRMS (C₂₁H₃₃NO₄) calcd 363.2410, found 363.2414.

Methyl 5-[(Hydroxyamino)carbonyl]methyl-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (52). Compound 52 was prepared from 49 as described for 51. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 52 (27 mg, 69%): ¹H NMR (300 MHz, CDCl₃) δ 8.30 (br s, 1 H), 6.42 (dd, 1 H, *J* = 11 and 15 Hz), 5.45 (br t, 1 H, *J* = 11 Hz), 5.30–5.55 (m, 6 H), 3.65 (t, 2 H, *J* = 7 Hz), 1.95–2.25 (m, 3 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 391 (M⁺); HRMS (C₂₃H₃₇NO₄) calcd 391.2723, found 391.2725.

5-[(Hydroxyamino)carbonyl]methyl-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (11). Compound 11 was prepared from 52 as described for 6. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 11 (21 mg, 92%): ¹H NMR (300 MHz, CDCl₃) δ 8.30 (br s, 1 H), 6.42 (dd, 1 H, *J* = 11 and 15 Hz), 5.45 (br t, 1 H, *J* = 11 Hz), 5.30–5.55 (m, 6 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 1.95–2.25 (m, 3 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 8 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 377 (M⁺); HRMS (C₂₂H₃₅NO₄) calcd 377.2566, found 377.2562.

Methyl 5-[2-[(Hydroxyamino)carbonyl]ethyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoate (53). Compound 53 was prepared from 50 as described for 51. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) provided 53 (28 mg, 66%): ¹NMR (300 MHz, CDCl₃) δ 8.31 (br s, 1 H), 6.42 (dd, 1 H, *J* = 11 and 15 Hz), 5.95 (br t, 1 H, *J* = 11 Hz), 5.30–5.55 (m, 6 H), 3.65 (s, 3 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 1.95–2.25 (m, 3 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 8 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 405 (M⁺); HRMS (C₂₄H₃₉NO₄) calcd 405.2879, found 405.2878.

5-[2-[(Hydroxyamino)carbonyl]ethyl]-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (12). Compound 12 was prepared from 53 as described for 6. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 12 (18 mg, 92%): ¹H NMR (300 MHz, CDCl₃) δ 8.28 (br s, 1 H), 6.42 (dd, 1 H, *J* = 11 and 15 Hz), 5.95 (br t, 1 H, *J* = 11 Hz), 5.30–5.55 (m, 6 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 1.95–2.25 (m, 3

H), 2.06 (q, 2 H, *J* = 7 Hz), 1.50–1.80 (m, 4 H), 1.20–1.45 (m, 8 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 391 (M⁺); HRMS (C₂₃H₃₇NO₄) calcd 391.2723, found 391.2726.

5-Amino-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (14). A solution of ketone 54¹³ (166 mg, 0.5 mmol), ammonium acetate (385 mg, 5 mmol), and sodium cyanoborohydride (22 mg, 0.35 mmol) in absolute methanol (10 mL) was stirred 24 h at 23 °C. HCl (2 N) was added to give a pH of 4. The reaction mixture was poured into water (100 mL) and extracted with ethyl acetate. The aqueous portion was adjusted to pH 10 with 2 N KOH and extracted with ether. The organic layers were combined and dried (MgSO₄). Removal of solvent gave a crude residue, which was dissolved in *i*-PrOH/H₂O (2/1) (15 mL) containing LiOH (42 mg, 1 mmol), and the mixture was stirred at 23 °C for 30 min. The reaction mixture was poured into water (50 mL) and acidified to pH 5. The mixture was extracted with ethyl acetate and dried (Na₂SO₄). Removal of solvent and chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) gave 14 (75 mg, 67%): ¹H NMR (300 MHz, CDCl₃) δ 6.48 (br s, 2 H), 6.42 (dd, 1 H, *J* = 11 and 15 Hz), 6.01 (dd, 1 H, *J* = 11 and 11 Hz), 5.68 (m, 1 H), 5.30–5.50 (m, 5 H), 4.08 (m, 1 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.36 (t, 2 H, *J* = 7 Hz), 2.06 (q, 2 H, *J* = 7 Hz), 1.55–1.80 (m, 4 H), 1.22–1.42 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 319 (M⁺); HRMS (C₂₀H₃₃NO₂) calcd 319.2511, found 319.2513.

5-(Hydroxyamino)-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (15). Sodium cyanoborohydride (42 mg, 0.34 mmol) in methanol (2 mL) was added to a solution of methyl ester 55 (174 mg, 0.5 mmol) in methanol (10 mL) at 0 °C under argon. HCl (3 N) was added to give a pH of 4 and the reaction mixture stirred at 23 °C for 3 h. The reaction mixture was poured into cold water and the pH adjusted to 9 with 6 N LiOH. After the mixture was stirred for 30 min, the pH was lowered to 6 and the solution extracted with ethyl acetate (3 × 50 mL). The ethyl acetate extracts were dried (MgSO₄), and the solvent was removed. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) of the crude residue provided the product 15 (71%, 118 mg): ¹H NMR (300 MHz, CDCl₃) δ 6.45 (dd, 1 H, *J* = 11 and 15 Hz), 6.00 (dd, 1 H, *J* = 11 and 11 Hz), 5.30–5.60 (m, 6 H), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.35 (t, 2 H, *J* = 7 Hz), 2.06 (q, 2 H, *J* = 7 Hz), 1.60–1.81 (m, 4 H), 1.21–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 335 (M⁺); HRMS (C₂₀H₃₃NO₃) calcd 335.2460, found 335.2455.

5-Acetoxy-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (16). Acetic anhydride (0.43 g, 1.4 mmol) was added dropwise at 0 °C under argon to 5-HETE (377 mg, 1.18 mmol) in pyridine (160 mg, 2 mmol). The reaction mixture was stirred at 23 °C for 8 h. Ether (10 mL) was added and the solution was washed with saturated NH₄Cl (5 mL), saturated NaHCO₃ (5 mL), and water (5 mL). The ether solvent was dried (Na₂SO₄) and removed in vacuo. Chromatography (silica gel; CH₂Cl₂/MeOH, 9/1) of the crude residue gave the product 16 (362 mg, 85%): ¹H NMR (300 MHz, CDCl₃) δ 6.53 (dd, 1 H, *J* = 15 and 11 Hz), 6.00 (dd, 1 H, *J* = 11 and 11 Hz), 5.69 (m, 1 H), 5.28–5.50 (m, 5 H), 4.21 (q, 1 H, *J* = 7 Hz), 2.96 (t, 2 H, *J* = 7 Hz), 2.83 (t, 2 H, *J* = 7 Hz), 2.42 (t, 2 H, *J* = 7 Hz), 2.10 (s, 3 H), 2.06 (q, 2 H, *J* = 7 Hz), 1.55–1.80 (m, 4 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, *J* = 7 Hz); MS, *m/e* 362 (M⁺). Anal. (C₂₂H₃₄O₄) C, H.

5-Oxo-6(E),8(Z),11(Z),14(Z)-eicosatetraenoic Acid (17). A solution of ketone 54¹³ (90 mg, 0.27 mmol) in *i*-PrOH (3 mL) under argon was treated at 23 °C with a solution of LiOH (20 mg, 0.48 mmol) in H₂O (1.5 mL). The resulting solution was stirred for 23 °C for 40 min. Saturated aqueous NH₄Cl (4 mL) was added followed by adjusting the pH of the solution to 6. The aqueous portion was extracted with EtOAc (3 × 10 mL). The organic fractions were dried (MgSO₄), and the solvent was evaporated. Chromatography (silica gel; CH₂Cl₂/CH₃OH, 9/1) of the crude product gave 17 (74 mg, 86%): ¹H NMR (300 MHz, CDCl₃) δ 7.58 (dd, 1 H, *J* = 15 and 11 Hz), 6.15 (m, 1 H), 5.87 (m, 1 H), 5.30–5.50 (m, 5 H), 3.10 (t, 2 H, *J* = 7 Hz), 2.84 (t, 2 H, *J* = 7 Hz), 2.65 (t, 2 H, *J* = 7 Hz), 2.39 (t, 2 H, *J* = 7 Hz), 2.06 (q, 2 H, *J* = 7 Hz), 1.96 (t, 2 H, *J* = 7 Hz), 1.20–1.42 (m, 6 H), 0.60 (t, 3 H, *J* = 7 Hz); MS, *m/e* 318; HRMS (C₂₀H₃₀O₃) calcd 318.2195, found 318.2196.

Oxime of 5-KETE (18). Hydroxylamine hydrochloride (140 mg, 2.0 mmol) and sodium acetate (544 mg, 4.0 mmol) were added to ketone 54¹³ (300 mg, 0.9 mmol) in MeOH (15 mL) at 0 °C under

argon. The reaction mixture was stirred for 1 h. The methanol was removed and the residue dissolved into ethyl acetate (50 mL) and washed with saturated NH_4Cl and water. Drying (MgSO_4) and evaporation of the solvent gave a crude product, which was chromatographed (silica gel; ethyl acetate/pentane, 15/85) to give the methyl ester **55** (265 mg, 85%). The methyl ester **55** (100 mg, 0.28 mmol) was added in *i*-PrOH/water (2/1) (10 mL) containing lithium hydroxide (24 mg, 0.56 mmol) and stirred at 23 °C for 1 h. The reaction mixture was added to water (100 mL), acidified to pH 5, extracted with ethyl acetate (3 \times 50 mL), and dried (Na_2SO_4). Removal of solvent and chromatography (silica gel; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9/1) gave **18** (88 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 6.95 (dd, 1 H, $J = 11$ and 15 Hz), 6.15 (dd, 1 H, $J = 11$ and 11 Hz), 5.25–5.52 (m, 6 H), 3.07 (t, 2 H, $J = 7$ Hz), 2.85 (t, 2 H, $J = 7$ Hz), 2.06 (q, 2 H, $J = 7$ Hz), 2.62 (t, 2 H, $J = 7$ Hz), 1.70–1.85 (m, 2 H), 1.25–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 333 (M^+). Anal. ($\text{C}_{20}\text{H}_{31}\text{NO}_3$) C, H, N.

Semicarbazide of 5-KETE (19). Sodium acetate (50 mg) was added to semicarbazide hydrochloride (33 mg, 0.3 mmol) in methanol (10 mL). The ketone **54**¹³ (100 mg, 0.3 mmol) in methanol (2 mL) was added at 0 °C under argon. The mixture was then heated at 50 °C for 30 min. The solvent was cooled and evaporated. Chromatography (silica gel; EtOAc/pentane, 1/3) gave the methyl ester of **19** (96 mg, 82%). The methyl ester of **19** (90 mg, 0.23 mmol) was dissolved in *i*-PrOH/ H_2O (2/1) (10 mL) containing lithium hydroxide (39 mg, 0.92 mmol) at 23 °C under argon and the mixture stirred for 1 h. The solution was poured into water (100 mL) and acidified to pH 5 with 2 N HCl and extracted with ethyl acetate (3 \times 50 mL). The ethyl acetate extracts were dried (Na_2SO_4) and removed in vacuo. The residue was chromatographed (silica gel; $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, 9/1) to give **19** (82 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 9.54 (br s, 1 H), 7.10–7.20 (m, 1 H), 6.54 (br s, 2 H), 6.08–6.22 (m, 1 H), 5.25–5.60 (m, 6 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.80 (t, 2 H, $J = 7$ Hz), 2.62 (t, 2 H, $J = 7$ Hz), 2.36 (t, 2 H, $J = 7$ Hz), 1.96 (t, 2 H, $J = 7$ Hz), 1.60–1.85 (m, 2 H), 1.28–1.42 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 375 (M^+). Anal. ($\text{C}_{21}\text{H}_{33}\text{N}_3\text{O}_3$) C, H, N.

Thiosemicarbazide of 5-KETE (20). Thiosemicarbazide (50 mg, 0.55 mmol) and sodium acetate (80 mg, 0.60 mmol) in methanol (10 mL) was added to ketone **54**¹³ (165 mg, 0.5 mmol) in methanol (10 mL) at 0 °C under argon. The reaction mixture was allowed to warm to 23 °C and finally stirred at 50 °C for 30 min. The solvent was cooled and removed in vacuo. The residue was chromatographed (silica gel; ethyl acetate/pentane, 1/3) to give the methyl ester of **20** (170 mg, 84%). The methyl ester of **20** (100 mg, 0.25 mmol) was added to *i*-PrOH/ H_2O (2/1) (15 mL) at 23 °C under argon containing lithium hydroxide (42 mg, 1 mmol) and stirred for 1 h. The solution was poured into water (150 mL) and acidified to pH 5 with 2 N HCl. The solution was extracted with ethyl acetate (3 \times 50 mL). The organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The crude residue was chromatographed (silica gel; $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 9/1) to provide **20** (93 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 9.45 (br s, 1 H), 7.31 (br s, 1 H), 6.35 (br s, 1 H), 6.60–6.78 (m, 1 H), 6.10–6.25 (m, 1 H), 5.85–5.95 (m, 1 H), 5.28–5.55 (m, 5 H), 2.94 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.25–2.33 (m, 5 H), 2.06 (q, 2 H, $J = 7$ Hz), 2.78 (t, 2 H, $J = 7$ Hz), 1.70–1.85 (m, 2 H), 1.21–1.31 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 391; HRMS ($\text{C}_{21}\text{H}_{33}\text{N}_3\text{O}_2\text{S}$) calcd 391.2294, found 391.2297.

3-Formyl-1-cyclohexanone (56). Copper(I) iodide (1.44 g, 0.0076 mol) was added to a solution of vinylmagnesium bromide (100 mL of 0.90 M solution in THF, 0.090 mol) under argon at 0 °C, and the resulting black solution was stirred at 0 °C for 15 min. A solution of 2-cyclohexen-1-one (4.30 g, 0.045 mol) in dry THF (50 mL) was slowly added dropwise over a 15-min period. The resulting solution was stirred at 5 °C for 1 h. The reaction mixture was then added to a cold aqueous solution (pH 8) of ammonia and ammonium chloride. The organic layer was separated and the aqueous layer extracted with ether. The combined organic extracts were dried (MgSO_4), and the solvent was evaporated. The crude residue was chromatographed (silica gel; pentane/ether, 3/1) to give 3-vinylcyclohexanone¹⁹ (4.3 g, 77%). Ozonolysis was added to 3-vinylcyclohexanone (4.0 g, 32 mmol) in dry CH_3OH (120 mL) and CH_2Cl_2 (36 mL) at –78 °C until the reaction mixture turned blue. Nitrogen was then bubbled into the reaction mixture until the blue color was removed. Dimethyl sulfide (10

mL, 146 mmol) was added to quench the reaction. The reaction mixture was allowed to warm to 23 °C and the solvent was removed in vacuo. The crude residue was dissolved in *p*-dioxane/water (1/1) (100 mL) followed by the addition of glacial acetic acid (0.6 g). The mixture was stirred at 23 °C for 6 h and quenched with saturated NaHCO_3 (40 mL). The mixture was extracted with ether (3 \times 30 mL), the combined extracts were dried (MgSO_4), and the solvent was evaporated. The crude residue was chromatographed (silica gel; pentane/ether, 1/3) to provide **56**¹⁶ (3.7 g, 91%): ^1H NMR (60 MHz, CDCl_3) δ 9.7 (s, 1 H), 2.8 (m, 1 H), 1.5–2.7 (m, 8 H); MS, m/e 126 (M^+).

5-(Hydroxymethyl)-3-oxocyclohexane-1-acetic Acid Methyl Ester (59). A solution of bicyclo[3.3.1]nonane-3,7-dione (**57**)¹⁷ (0.47 g, 3.1 mmol) and *m*-chloroperbenzoic acid (0.65 g, 3.0 mmol) in CH_2Cl_2 (15 mL) was stirred in the dark for 3 h. The reaction mixture was washed with saturated NaHCO_3 (50 mL), 5% NaHSO_4 (50 mL), and water (50 mL) and dried (MgSO_4). The solvent was removed in vacuo to provide 3-oxabicyclo[4.3.1]nonane-4,8-dione (**58**) (0.54 g, 65%), which was carried on without further purification. A solution of 3-oxabicyclo[4.3.1]nonane-4,8-dione (**58**) (0.41 g, 1.5 mmol) and triethylamine (0.91 g, 9 mmol) in methanol (30 mL) was stirred at 23 °C for 18 h. The solution was removed in vacuo and the residue chromatographed (silica gel; ethyl acetate) to give **59** (0.29 g, 95%): ^1H NMR (60 MHz, CDCl_3) δ 3.65 (s, 3 H), 3.55 (d, 2 H, $J = 4.8$ Hz), 1.71–2.62 (m, 10 H), 1.51 (br s, 1 H); MS, m/e 188 (M^+). Anal. ($\text{C}_9\text{H}_{16}\text{O}_4$) C, H.

5-Formyl-3-oxocyclohexane-1-acetic Acid Methyl Ester (60). A solution of **59** (60 mg, 0.3 mmol) in dry CH_2Cl_2 (1 mL) was added to a suspension of pyridinium dichromate (111 mg, 0.32 mmol) in CH_2Cl_2 (10 mL) and the reaction mixture stirred at 23 °C for 12 h. The reaction mixture was then diluted with ether (25 mL) and filtered through a pad of Celite. Removal of solvent gave a crude oil. Chromatography (silica gel, ether) provided **60** (52 mg, 88%): ^1H NMR (60 MHz, CDCl_3) δ 9.71 (s, 1 H), 3.65 (s, 3 H), 1.4–2.62 (m, 10 H); MS, m/e 198 (M^+). Anal. ($\text{C}_{10}\text{H}_{14}\text{O}_4$) C, H.

3-(1(E),3(Z),6(Z),9(Z)-Pentadecatetraenyl)-1-cyclohexanone (22). Compound **22** was prepared from **56** as described for **36**. Chromatography (silica gel; pentane/ether, 3/1) provided **22** (24 mg, 44%): ^1H NMR (300 MHz, CDCl_3) δ 6.37 (dd, 1 H, $J = 15$ and 11 Hz), 5.99 (dd, 1 H, $J = 11$ and 11 Hz), 5.65 (dd, 1 H, $J = 15$ and 11 Hz), 5.31–5.50 (m, 5 H), 2.97 (t, 2 H, $J = 7$ Hz), 2.85 (t, 2 H, $J = 7$ Hz), 2.59 (m, 1 H), 2.21–2.52 (m, 4 H), 2.08 (m, 2 H), 1.40–1.95 (m, 4 H), 1.25–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 300 (M^+); HRMS ($\text{C}_{21}\text{H}_{32}\text{O}$) calcd 300.2453, found 300.2449.

5-(1(E),3(Z),6(Z),9(Z)-Pentadecatetraenyl)-3-oxocyclohexane-1-acetic Acid Methyl Ester (61). The methyl ester **61** was prepared from **60** as described for **36**. Chromatography (silica gel; pentane/ether, 2/1) gave **61** (60 mg, 43%): ^1H NMR (300 MHz, CDCl_3) δ 6.36 (dd, 1 H, $J = 15$ Hz), 5.31–5.52 (m, 5 H), 3.65 (s, 3 H), 2.95 (t, 2 H, $J = 7$ Hz), 2.82 (t, 2 H, $J = 7$ Hz), 2.00–2.61 (m, 12 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 372 (M^+). Anal. ($\text{C}_{24}\text{H}_{36}\text{O}_3$) C, H.

5-(1(E),3(Z),6(Z),9(Z)-Pentadecatetraenyl)-3-oxocyclohexane-1-acetic Acid (23). Compound **23** was prepared from the methyl ester **61** in the manner described for **4**. Chromatography (silica gel; pentane/ether/formic acid, 9/1/0.02) of the residue gave **23** (55 mg, 95%): ^1H NMR (300 MHz, CDCl_3) δ 6.36 (dd, 1 H, $J = 15$ and 11 Hz), 5.96 (dd, 1 H, $J = 11$ and 11 Hz), 5.60 (dd, 1 H, $J = 11$ and 15 Hz), 5.31–5.52 (m, 5 H), 2.95 (t, 2 H, $J = 7$ Hz), 2.82 (t, 2 H, $J = 7$ Hz), 2.00–2.61 (m, 12 H), 1.20–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 358 (M^+); HRMS ($\text{C}_{23}\text{H}_{34}\text{O}_3$) calcd 358.2508, found 358.2505.

6-(1(E),3(Z),6(Z),9(Z)-Pentadecatetraenyl)-2-piperidone (24). *p*-Toluenesulfonic acid (19 mg, 0.1 mmol) was added to compound **14** (32 mg, 0.1 mmol) in benzene (5 mL) under argon and the reaction mixture stirred at 23 °C for 6 h. The solvent was removed in vacuo and the residue chromatographed (silica gel; pentane/ether, 3/1) to give **24** (26 mg, 86%): ^1H NMR (300 MHz, CDCl_3) δ 6.62 (dd, 1 H, $J = 15$ and 11 Hz), 6.01 (dd, 1 H, $J = 11$ and 11 Hz), 5.70 (dd, 1 H, $J = 15$ and 11 Hz), 5.28–5.55 (m, 5 H), 4.20 (m, 1 H), 2.96 (t, 2 H, $J = 7$ Hz), 2.83 (t, 2 H, $J = 7$ Hz), 2.40–2.65 (m, 2 H), 1.80–2.21 (m, 9 H), 1.25–1.45 (m, 6 H), 0.90 (t, 3 H, $J = 7$ Hz); MS, m/e 301 (M^+); HRMS ($\text{C}_{20}\text{H}_{31}\text{NO}$)

calcd 301.2406, found 301.2402.

RBL-1 5-Lipoxygenase Inhibition Assay. 5-Lipoxygenase activity was measured in the 20000g supernatant from homogenized rat basophilic leukemia (RBL-1) cells. Inhibitors or vehicle (2% Me₂SO) were preincubated for 20 min with the RBL-1 supernatant (7.5×10^6 cell equivalents/mL) at 37 °C in pH 6.8 buffer (10 mM BES, 10 mM PIPES, 1 mM EDTA, 0.1 M NaCl, 0.7 mM CaCl₂) prior to initiating the 5-lipoxygenase reaction by addition of 66 μM [¹⁴C]arachidonic acid. [³H]-5-HETE added to the reaction mixture served as a recovery standard. Reactions were terminated by acidification to pH 3 and the mixtures were extracted with diethyl ether. The ether extracts were evaporated under nitrogen and the reaction products were separated from nonconverted substrate by thin-layer chromatography. Radioactivity comigrating with 5-HETE was measured by liquid scin-

tillation counting and corrected for recovery of [³H]-5-HETE. Inhibition was calculated as the percent reduction from control levels of [¹⁴C]-5-HETE formation. Concentrations causing 50% inhibition (IC₅₀'s) and their 95% confidence limits were calculated as the 50% intercept and their fiducial limits from linear regression analysis²² of percent inhibition vs. log concentration plots.

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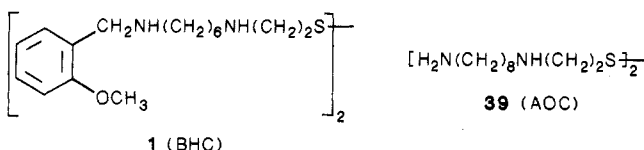
Structure-Activity Relationships among Di- and Tetramine Disulfides Related to Benextramine

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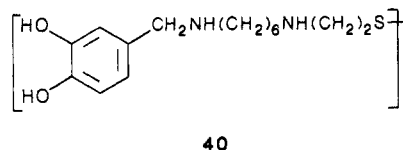
The synthesis and irreversible α-blocking activity in the rat vas deferens of a series of tetra- and diamine disulfides 2-38, structural analogues of benextramine (BHC), are described. All compounds containing a central cystamine moiety displayed an irreversible α-adrenergic blockade at concentrations ranging from 10⁻⁴ to 6 × 10⁻⁶ M. Potency was increased in cystamines N,N'-disubstituted with 6-aminoethyl groups, especially when the outer nitrogen atoms bear arylalkyl substituents or are enclosed in a ring. However, N,N,N',N'-tetrasubstituted cystamines were poor blockers. Structural specificity in the outer portion of the tetramine disulfide is low, since many types of substituents gave rise to potent α-blockers. Even replacement of the outer amines with nonbasic ethers or amides was observed to maintain irreversible α-blockade.

In a series of structure-activity relationship (SAR) studies of tetramine disulfides carried out by Melchiorre and Belleau,¹ optimum α-adrenergic blocking potency was found for compounds N,N'-bis[6-[(*o*-methoxybenzyl)-amino]hexyl]cystamine (BHC, 1) and N,N'-bis(8-amino-octyl)cystamine, (AOC, 39). From the structural features of these compounds, the authors proposed a topographical model for the α-adrenoceptor in the rat vas deferens.



Accordingly, BHC would interact with a set of four anionic centers and two flat areas complementary to the aromatic rings, and AOC would bind to a different set of four anionic centers. These two binding areas would be symmetrically disposed on the surface of the receptor and share a common central thiol group. According to the hypothesis, the initial electrostatic interactions between the four cationic nitrogen atoms and the anionic centers would lead to a conformational change unmasking the thiol

group. This would allow a disulfide-thiol exchange reaction, which in turn results in a covalent blockade of the α-adrenoceptor. The analogues of tetramine disulfides BHC and AOC showed different SAR. Thus, the optimum chain length between the "inner" and "outer" nitrogen atoms was found to be six carbon atoms in BHC and eight methylene groups in AOC. Substitution on the outer nitrogen atom led to a reduced potency in AOC, while in BHC a benzylic substituent (especially an *o*-methoxybenzyl group) on this position gave maximal potency. On the other hand, methylation of the inner nitrogen atoms led to a marked reduction in potency in the BHC series while the AOC methylated analogues showed almost no changes in activity. On the basis of the high α-blocking activity of the catechol-containing disulfide 40, which was



equiactive with BHC, Melchiorre also suggested a common binding site for the outer (3,4-dihydroxybenzyl)amino moiety of 40, as well as the (*o*-methoxybenzyl)amino group of BHC and the catecholamine neurotransmitters, in spite of the benzylic nature of the N-substituent and the lack of a benzylic hydroxyl group found in catecholamines.

Aiming to extend and evaluate the above possibilities and especially the predicted relationship between the catecholamine and BHC binding sites, we undertook the synthesis of a new series of polyamine disulfides containing terminal moieties structurally related in some instances to α-adrenergic drugs.² Thus, compounds 2-9 (Table I)

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