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Structural Analogs of Leukotrienes C and D and Their Contractile Activities

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Thirteen structural analogs of leukotrienes C and D were prepared and tested for contractile activities on guinea pig pulmonary parenchymal strips. The analogs differed from the native structures in the peptide moiety, the 5-hydroxyl group, the carboxyl group and in the number and geometry of ethylenic bonds.

Deamino analogs of leukotriene C₄ (LTC₄) and leukotriene D₄ (LTD₄) retained substantial contractile activities. Amide analogs of LTD₄ and 5-O-methyl-LTC₄ showed some activity. Modification of the peptide moiety caused a 1–3 orders of magnitude decrement. Analogs in which the various ethylenic bonds were saturated retained substantial contractile activity. However, perhydro LTD had no contractile activity.

Keywords—slow-reacting substance (SRS); leukotriene C₄; leukotriene D₄; leukotriene analog; contractile activity; guinea pig pulmonary parenchymal strips

Each of the leukotriene constituents of slow-reacting substance of anaphylaxis (SRS-A), leukotrienes C₄ (LTC₄) and D₄ (LTD₄), has significant biological potency as a nonvascular smooth muscle spasmogen for guinea pig pulmonary parenchymal strips and guinea pig ileum.¹⁾ The contractile activities of LTC₄ and LTD₄ are ~500 and ~10000 times greater on a molar basis than that of histamine on guinea pig pulmonary parenchymal strips and 70–200 times greater on guinea pig ileum.¹⁾

The molecular structures of these leukotrienes are divided into two regions, a hydrophilic region (from the eicosanoid carboxyl to the C-6 peptide) and a hydrophobic region (C-7 to C-20). The chemical modification of these parts of leukotriene should provide useful information on the relation of the structure to the biological activity.

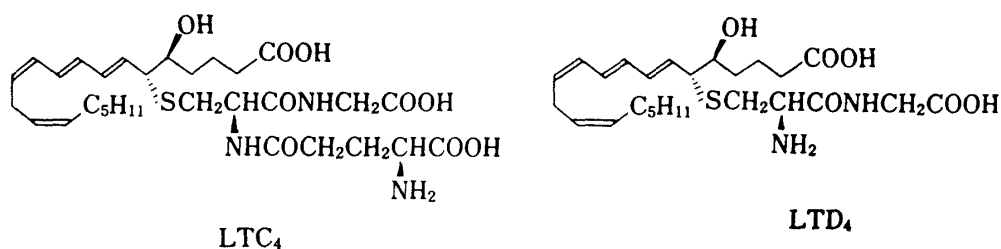


Chart 1

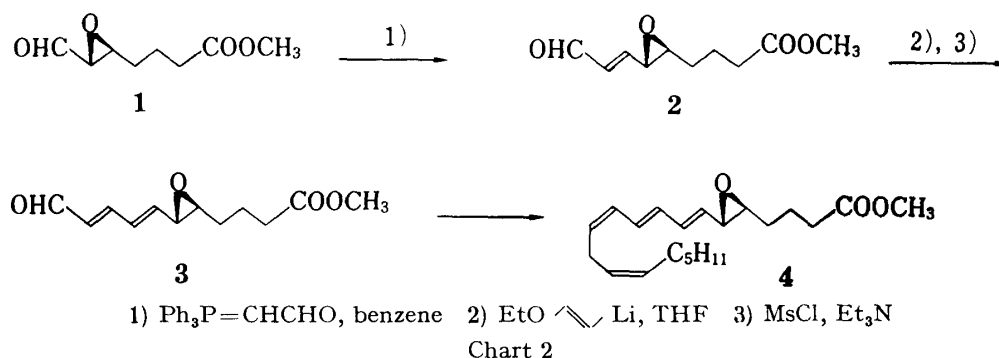
We prepared thirteen analogs of LTC₄ and LTD₄, in which the hydrophobic and hydrophilic regions are modified, and tested them for contractile activities on guinea pig pulmonary parenchymal strips.

Materials and Method

LTC₄ and LTD₄ were prepared by Corey's method²⁾ with some modification.

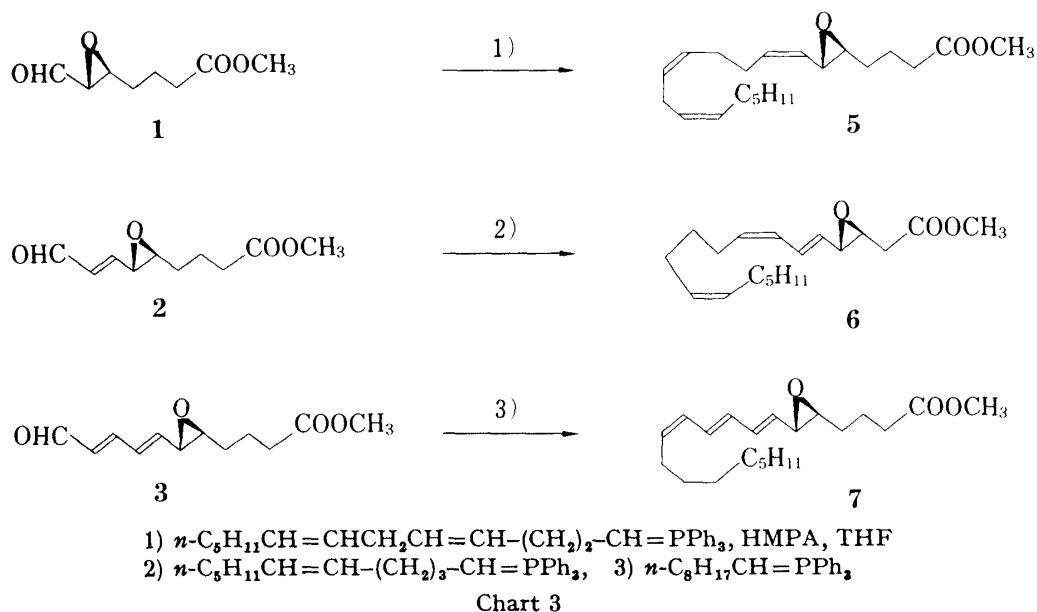
Methyl 8-formyl-5(S),6(S)-oxido-7(E)-octenoate (2) was prepared from methyl 6-formyl-5(S),6(S)-oxidoheptanoate (1) by Wittig reaction with formylmethylidenetriphenylphosphorane in benzene. Reaction of 2 with 1-lithio-2-ethoxyethylene in tetrahydrofuran (THF) at -78°C for 1 h followed by treatment with MsCl-NEt₃ in CH₂Cl₂ afforded methyl 10-formyl 5(S),6(S)-

oxido-7(*E*),9(*E*)-decadienoate (**3**), mp 57—59°C, which was converted to LTA₄ methyl ester (**4**) in 93% yield by Corey's method.

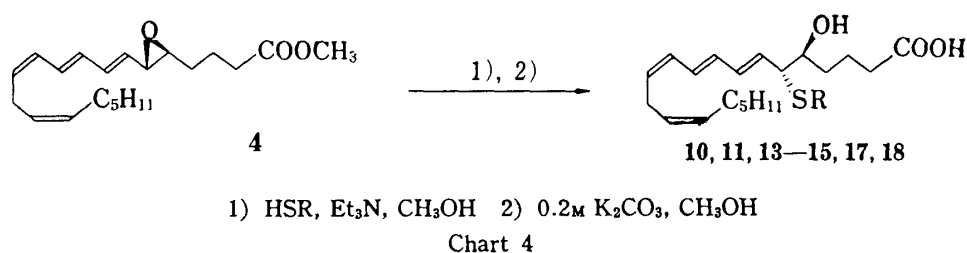


Synthesis of LTA analogs was accomplished as follows. Methyl 5(*S*),6(*S*)-oxido-7(*Z*),11(*Z*),14(*Z*)-eicosatrienoate (**5**) was prepared by the reaction of **1** with the ylide, generated from 4(*Z*),7(*Z*)-tridecadien-1-yltriphenylphosphonium bromide and *n*-butyllithium in THF-hexamethylphosphoramide (HMPA) at 0°C for 1 h.

In the same manner, methyl 5(*S*),6(*S*)-oxido-7(*E*),9(*Z*),14(*Z*)-eicosatrienoate (**6**) and methyl 5(*S*),6(*S*)-oxido-7(*E*),9(*E*),11(*Z*)-eicosatrienoate (**7**) were prepared from **2** and **3** by Wittig reaction with the ylides generated from 5(*Z*)-undecen-1-yltriphenylphosphonium bromide and 1-nonyltriphenylphosphonium bromide, respectively.



Reaction of LTA₄ methyl ester with the peptide analogs and the amine analogs (**23**—**29** in Table III) containing an HS group in methanol- NEt_3 (3 eq) at r.t. under an Ar atmosphere, followed by hydrolysis with 0.2 M K_2CO_3 in methanol, afforded the leukotriene analogs (**10**, **11**, **14**, **15**, **17**, **18**) as shown in Chart 4.



Reactions of LTA analogs (5—7) with *N*-trifluoroacetyl-L-cysteinylglycine methyl ester²⁾ or *N*-trifluoroacetylglutathione dimethyl ester²⁾ were accomplished in the same manner, affording the leukotriene analogs (19—21).

Perhydroleukotriene D (22) was prepared as follows. 1) Bromolactonization of 5(*Z*)-eicosaenoic acid (8) with *N*-bromosuccinimide (NBS) in CH₂Cl₂ at r.t. for 1 h gave the bromo-lactone. 2) Methanolysis of the bromo-lactone followed by treatment with dihydropyran in CH₂Cl₂ in the presence of a catalytic amount of *p*-TsOH afforded methyl *threo*-5-tetrahydropyranyloxy-6-bromoeicosanoate (9). 3) Reaction of 9 with sodium salt of *N*-trifluoroacetyl-L-cysteinylglycine methyl ester in dimethoxyethane (DME)–HMPA at 70°C for 17 h followed by deprotection reactions (pyridine–*p*-TsOH in MeOH, 0.2 M K₂CO₃ in MeOH) afforded a diastereomixture of perhydro LTD (22).

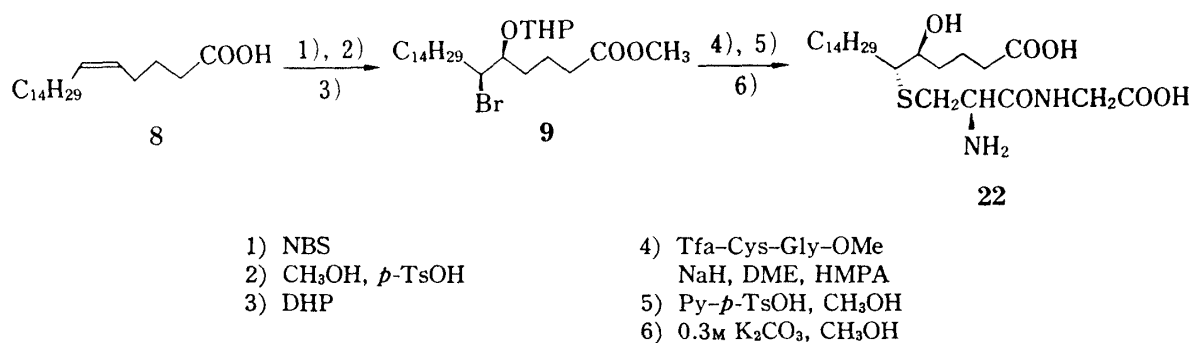


Chart 5

LTD₄ bisamide (12) was prepared from *N*-trifluoroacetyl-LTD₄ dimethyl ester with ammonia/ammonium chloride at r.t. overnight. Deamino LTD₄ bisdimethylamide (13) was prepared from the methyl ester of deamino LTD₄ glycinedimethylamide prepared from LTA₄ methyl ester (4) and 3-mercaptopropionylglycinedimethylamide (25), and diethylamine in the presence of dimethylammonium chloride at r.t.

Biological Measurement—The contractile activities of the leukotriene analogs on guinea pig pulmonary parenchymal strips were measured according to the method described in ref. 3. The concentrations of naturally occurring leukotriene or analogs required to achieve a response equal to 50% of the 10^{−5} g/ml histamine response (ED₅₀) were determined by interpolation from the concentration–effect relationship.

Results

The contractile activities of leukotriene analogs on guinea pig pulmonary parenchymal strips are summarized in Table I.

A contractile response representing 50% of that produced by 10^{−5} g/ml histamine on guinea pig pulmonary parenchymal strips was achieved with a concentration of 5 × 10^{−9} g/ml LTC₄ or 1 × 10^{−9} g/ml LTD₄.

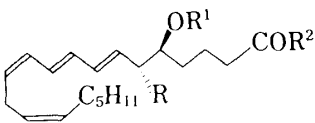
Modification in the Hydrophilic Region

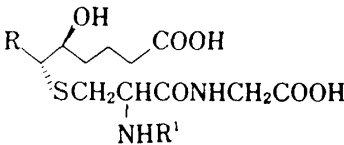
The effect of replacement of an amino acid in the peptide part of LTC₄ or LTD₄ by another amino acid was studied by using six analogs (10, 11, 14, 15, 17, 18).

Deamino LTC₄ (10) and deamino LTD₄ (11) retained almost all the contractile activities of the parent compounds. The analog (14), in which the glycine unit of LTC₄ had been removed, still showed substantial activity. The analog (15) in which the glycine unit of LTD₄ was replaced by L-glutamic acid lost most of the activity (3 orders of magnitude, decrease).

LTD₄ bisamide (12) and deamino LTD₄ bisdimethylamide (13) were virtually inactive on

TABLE I. Contractile Activities of Analogs of Leukotrienes C₄ and D₄

				
R	R ¹	R ²	Contractile activity ^{a)} ED ₅₀ (g/ml)	
SCH ₂ CHCONHCH ₂ COOH NHCOCH ₂ CH ₂ CHCOOH NH ₂	(LTC ₄)	H	OH	5 × 10 ⁻⁹
SCH ₂ CHCONHCH ₂ COOH NH ₂	(LTD ₄)	H	OH	1 × 10 ⁻⁹
10 SCH ₂ CHCONHCH ₂ COOH NHCOCH ₂ CH ₂ COOH		H	OH	1 × 10 ⁻⁸
11 SCH ₂ CH ₂ CONHCH ₂ COOH		H	OH	1 × 10 ⁻⁹
12 SCH ₂ CHCONHCH ₂ CONH ₂ NH ₂		H	NH ₂	Inactive at 10 ⁻⁶
13 SCH ₂ CH ₂ CONHCH ₂ CON(CH ₃) ₂		H	N(CH ₃) ₂	Inactive at 10 ⁻⁶
14 SCH ₂ CHCOOH NHCOCH ₂ CH ₂ CHCOOH NH ₂		H	OH	1 × 10 ⁻⁸
15 SCH ₂ CHCONHCHCOOH NH ₂		H	OH	1 × 10 ⁻⁶
16 SCH ₂ CHCONHCH ₂ COOH NHCOCH ₂ CH ₂ CHCOOH NH ₂		CH ₃	OH	5 × 10 ⁻⁷
17 SCH ₂ CH ₂ CH ₂ NHCH ₂ COOH		H	OH	1 × 10 ⁻⁷
18 SCH ₂ CH ₂ CH ₂ N(CH ₃) ₂		H	OH	1 × 10 ⁻⁶

		
R	R ¹	Contractile activity ^{a)} ED ₅₀ (g/ml)
19 n-C ₅ H ₁₁ CH=CH(CH ₂) ₃ CH=CHCH=CH- ^Z ^E ^E	COCH ₂ CH ₂ CHCOOH NH ₂	1 × 10 ⁻⁸
20 n-C ₈ H ₁₇ CH=CHCH=CHCH=CH- ^Z ^E ^E	COCH ₂ CH ₂ CHCOOH NH ₂	1 × 10 ⁻⁸
21 n-C ₅ H ₁₁ CH=CHCH ₂ CH=CH(CH ₂) ₂ CH=CH- ^Z ^Z ^Z	COCH ₂ CH ₂ CHCOOH NH ₂	5 × 10 ⁻⁷
22 n-C ₁₄ H ₂₉ - ^{b)}	H	Inactive at 10 ⁻⁶

a) Contractile response representing 50% of that produced by 10⁻⁵ g/ml histamine on guinea pig pulmonary parenchymal strips.

b) Diastereomixture (about 1: 1).

pulmonary parenchymal strips. 5-*O*-Methyl LTC₄ (16) had only 1/100 of the activity of LTC₄. The analogs (17, 18) showed greatly decreased activity relative to LTC₄ (2 orders of magnitude or more).

Modification in the Hydrophobic Region

9(*Z*)-Δ¹¹-dihydro LTC₄ (19) and Δ¹⁴-dihydro LTC₄ (20) showed only slightly less contractile

activity than LTC₄. However, 7(*Z*)-Δ⁹-dihydro LTC₄ (**21**) in which three double bonds were non-conjugated had only 1/100 of the activity of LTC₄. Perhydro LTC (**22**) was virtually inactive.

Discussion

It is clear that the amino group of LTC₄ and LTD₄ is not critical for the contractile activity on guinea pig pulmonary parenchymal strips. However, the hydroxyl group at C-5 and the carboxyl groups are critical for the contractile activity.

Replacement of the peptide part of LTC₄ and LTD₄ by other peptides, amino acid or amine resulted in a 1—3 orders of magnitude decrement in the contractile activity.

As saturation of all ethylenic bonds of LTD₄ resulted in complete loss of activity, it is clear that the ethylenic bonds are critical for the activity. However saturation of the 11, 12 or 14, 15 ethylenic bond caused only a slight decrease in activity, while saturation of the 9, 10 ethylenic bond caused a 2 orders of magnitude decrement in activity. These data suggest that the conjugation of ethylenic bonds is important for the contractile activity and minor geometrical changes in the hydrophobic part are unimportant.

Experimental

Melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-100 machine and signals are given in δ units downfield from TMS as an internal standard. Infrared (IR) and ultraviolet (UV) spectra were measured on Hitachi 69—30 and Hitachi 124 spectrometers. Mass spectra (MS) and specific rotation (20°) were taken on JMS-01SG and Jasco DIP-4 machines, respectively.

Methyl 8-Formyl-5(*S*),6(*S*)-oxido-7(*E*)-octenoate (2)—Formylmethylidenetriphenylphosphorane (1.8 g) was added to a solution of methyl 6-formyl-5(*S*),6(*S*)-oxidohexanoate²¹ (**1**, 1.0 g) in 20 ml of anhydrous benzene under an Ar atmosphere. The resulting solution was stirred at 60°C for 1.5 h and concentrated *in vacuo*. The residue was separated by column chromatography on silica gel (cyclohexane: ethyl acetate/3: 1, containing 0.1% NEt₃) to afford 965 mg (83%) of **2** as a colorless oil. $[\alpha]_D -23.4^\circ$ ($c=0.1$, CHCl₃). UV (EtOH) nm: 229 (ϵ , 18000). IR (film) cm⁻¹: 2950, 1740, 1690 and 1640. MS m/e : 199 ($M^+ + 1$), 198 (M^+) and 167. NMR (CDCl₃) ppm: 2.97 (1H, dt, $J=2, 6$ Hz, H₆), 3.35 (1H, dd, $J=2, 6$ Hz, H₆), 3.68 (3H, s), 6.23—6.68 (2H, m) and 9.57 (1H, dd, $J=1, 6.5$ Hz, -CHO).

Methyl 10-Formyl-5(*S*),6(*S*)-oxido-7(*E*),9(*E*)-decadienoate (3)—A 1.45M *n*-butyllithium/*n*-hexane solution (1.7 ml) was added to a solution of 2-ethoxyvinyl-tri-*n*-butyltin (904 mg) in anhydrous THF at -78°C under an Ar atmosphere. The resulting solution was stirred at -78°C for 1 h and a solution of methyl 8-formyl-5(*S*),6(*S*)-oxido-7(*E*)-octenoate (**2**, 496 mg) in anhydrous THF (3 ml) was added in one portion. Thirty minutes later, NEt₃ (1.5 ml) and MsCl (193 μ l) were added. The reaction solution was stirred for 1 h at -78°C and poured into aqueous NaHCO₃. After being vigorously stirred at r.t. for 10 min, the reaction mixture was extracted twice with CH₂Cl₂. The organic phase was washed with saturated NaCl, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH₂Cl₂: Et₂O/30: 1, containing 0.1% NEt₃) to afford 429 mg (76%) of the title compound. mp 57—59°C (ether-*n*-hexane). $[\alpha]_D -33.1^\circ$ ($c=1.97$, CHCl₃). UV (EtOH) nm: 273 (ϵ , 32000). IR (film) cm⁻¹: 1720, 1670 and 1635. MS m/e : 224 (M^+), 208, 195, 193 and 155. NMR (CDCl₃) ppm: 2.92 (1H, dt, $J=2, 7$ Hz, H₆), 3.23 (1H, dt, $J=2, 7$ Hz, H₆), 3.68 (3H, s), 5.98 (1H, dd, $J=8, 16$ Hz, H₇), 6.17 (1H, dd, $J=8, 16$ Hz, H₁₀), 6.64 (1H, dd, $J=11, 16$ Hz, H₈), 7.10 (1H, dd, $J=11, 16$ Hz, H₉) and 9.58 (1H, d, $J=8$ Hz, -CHO).

Methyl 5(*S*),6(*S*)-oxido-7(*Z*),11(*Z*),14(*Z*)-eicosatrienoate (5)—A 1.5M solution of *n*-butyllithium in hexane (2.46 ml) was added to a solution of 4(*Z*),7(*Z*)-tridecadien-1-yltriphenylphosphonium bromide (1.92 g) in anhydrous THF at -78°C under an Ar atmosphere. The resulting solution was stirred at 0°C for 5 min, and then 9 ml of HMPA and a solution of methyl 6-formyl-5(*S*),6(*S*)-oxidohexanoate (**1**, 635 mg) in anhydrous THF (7 ml) were added. After being stirred for 1 h at 0°C, the reaction solution was poured into 100 ml of phosphate buffer (pH 7.0) and extracted three times with ether. The organic phase was washed with saturated NaCl, dried over Na₂SO₄ and concentrated *in vacuo*.

The residue was purified by column chromatography on AgNO₃-impregnated silica gel (ether:*n*-hexane/1: 3) to afford 622 mg (51%) of the title compound as a colorless oil. IR (film) cm⁻¹: 1735 and 1430. MS m/e : 334 (M^+), 316, 303 and 131. NMR (CDCl₃) ppm: 0.89 (3H, t, $J=7$ Hz), 2.65—2.95 (3H, m), 3.34 (1H, dd, $J=2, 9$ Hz, H₆), 3.67 (3H, s), 5.05 (1H, dd, $J=9, 11$ Hz, H₇) and 5.1—5.90 (5H, m). High resolution MS m/e : Calcd for C₂₁H₃₄O₃, 334.25078. Obsd 334.25091.

Methyl 5(*S*),6(*S*)-oxido-7(*E*),9(*Z*),14(*Z*)-eicosatrienoate (6)—The title compound was prepared from methyl 8-formyl-5(*S*),6(*S*)-oxido-7(*E*)-octenoate (**2**) and 5(*Z*)-undecen-1-yltriphenylphosphonium bromide

TABLE II. Spectral Data for Protected Leukotriene Analogs^{a)}

	NMR(CDCl ₃) δ ppm	IR(film) cm ⁻¹	UV(MeOH) nm	MS m/e
10	0.89 (3H, t, $J=6$ Hz), 3.3–3.9 (2H, m, H ₅ , H ₆), 3.65 (3H, s), 3.75 (3H, s), 3.79 (3H, s), 4.05 (2H, d, $J=5$ Hz, -NCH ₂ CO-), 5.2–7.2 (8H, m, olefinic proton)	3640, 3300, 1740, 1650, 980	270 (30000), 281 (40000), 291 (31000)	652 (M ⁺), 634, 620, 523, 491, 332, 315, 301 Calcd for C ₃₃ H ₄₉ N ₃ O ₉ S ₃ : 625.33933 Obsd: 652.33815
11	0.89 (3H, t, $J=6$ Hz), 3.1–3.8 (2H, m, H ₅ , H ₆), 3.66 (3H, s), 3.76 (3H, s), 4.06 (2H, d, $J=5$ Hz, -NCH ₂ CO-), 5.0–6.6 (8H, m, olefinic proton)	3400, 2910, 1725, 1665, 1540, 1200	270 (31000), 281 (40000), 290 (31000)	509 (M ⁺), 491, 477, 379, 347, 333, 315, 301, 368, 235 Calcd for C ₂₉ H ₄₃ NO ₆ S ₃ : 509.28109 Obsd: 509.28413
13^{b)}	0.89 (3H, t, $J=6$ Hz), 2.95 (6H, s), 2.98 (6H, s), 2.35 (2H, t, $J=7$ Hz), 2.55–3.06 (6H, m, H ₁₃ , -SCH ₂ CH ₂ CO-), 3.15 (1H, dd, $J=4, 10$ Hz, H ₆), 4.05 (2H, d, $J=4$ Hz, -NCH ₂ CO-), 5.2–6.65 (8H, m, olefinic proton)	3410, 1675, 1650, 1440, 1240, 1180	271 (31000), 281.5 (43000), 291 (30000)	535 (M ⁺), 520, 517, 497, 391, 346, 328 Calcd for C ₂₉ H ₄₉ N ₃ O ₄ S ₃ : 535.34436 Obsd: 535.34628
14	0.9 (3H, t, $J=7$ Hz), 2.7–3.1 (4H, m, H ₁₃ , -SCH ₂ -), 3.3–3.6 (1H, m, H ₆), 3.67 (3H, s), 3.76 (3H, s), 3.79 (3H, s), 3.6–3.8 (1H, m, H ₆), 4.5–5.0 (2H, m, -NCHCO- $\times 2$), 5.2–6.9 (8H, m, olefinic proton)	3300, 1720, 1650, 1200, 985	270 (30000), 281 (39000), 291 (31000)	701 (M ⁺), 688, 675, 674, 662, 657, 649, 648, 578, 550, 465 Calcd for C ₃₃ H ₄₉ N ₃ O ₉ SF ₃ : 706.31106 Obsd: 706.31212
15	0.89 (3H, t, $J=6$ Hz), 2.35 (2H, t, $J=7$ Hz), 2.4 (2H, t, $J=7$ Hz), 2.8–3.05 (4H, m, H ₁₃ , -SCH ₂ -), 3.52 (1H, dd, $J=4, 9.5$ Hz, H ₆), 3.66 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 4.2–4.8 (2H, m, -NCHCO- $\times 2$), 5.2–6.73 (8H, m, olefinic proton)	3410, 1730, 1670, 1440, 1240, 1180	271 (30000), 281 (41000), 291 (31000)	706 (M ⁺), 688, 675, 576, 550, 465, 374, 332, 315, 301 Calcd for C ₃₃ H ₄₉ N ₃ O ₉ SF ₃ : 706.31106 Obsd: 706.31183
16	0.89 (3H, t, $J=7$ Hz), 2.7–3.1 (4H, m, H ₁₃ , -SCH ₂ -), 3.4 (3H, s), 3.66 (3H, s), 3.75 (3H, s), 3.95–4.15 (2H, m, -NCH ₂ CO-), 5.1–7.0 (10H, m, olefinic proton, NH $\times 2$)	3300, 1730, 1650, 1535, 1430, 1205	270 (31000), 281 (41000), 291 (32000)	777 (M ⁺), 745, 713, 656, 430, 398, 366, 347, 315, 145 Calcd for C ₃₈ H ₅₄ N ₃ O ₁₀ SF ₃ : 777.34817 Obsd: 777.34920
17	0.89 (3H, t, $J=7$ Hz), 3.1–3.8 (2H, m, H ₅ , H ₆), 3.53 (2H, t, $J=7$ Hz, -CH ₂ CH ₂ N-), 3.66 (3H, s), 3.75 (3H, s), 4.04 (2H, br s, -NCH ₂ CO-), 5.1–6.6 (8H, m, olefinic proton)	3300, 1730, 1700, 1200	272 (30000), 281 (40000), 292 (31000)	591 (M ⁺), 573, 559, 480, 461, 448, 350 Calcd for C ₂₉ H ₄₄ NO ₆ SF ₃ : 591.28412 Obsd: 591.28493
18	0.89 (3H, t, $J=6.5$ Hz), 2.18 (6H, s), 2.6–3.05 (4H, m, H ₁₃ , -SCH ₂ -), 3.53 (1H, dd, $J=4, 10$ Hz, H ₆), 3.66 (3H, s), 3.75 (3H, s), 3.67 (3H, s), 3.6–3.8 (1H, m, H ₆), 5.18–6.75 (8H, m, olefinic proton)	3500, 1740, 1260, 1170	271 (32000), 281 (43000), 290.5 (32000)	451 (M ⁺), 436, 433, 420, 368, 332, 301, 131, 129 Calcd for C ₂₉ H ₄₅ NO ₃ S ₃ : 451.31200 Obsd: 451.31182
19	0.88 (3H, t, $J=7.5$ Hz), 2.88 (2H, t, $J=7$ Hz), 3.48 (1H, dd, $J=4, 10$ Hz, H ₆), 3.66 (3H, s), 3.75 (3H, s), 3.78 (3H, s), 4.03 (2H, d, $J=5$ Hz, -NCH ₂ CO-), 4.5–4.75 (2H, m, -NCHCO- $\times 2$), 5.31–6.87 (6H, m, olefinic proton)	3310, 2900, 1730, 1660, 1550, 1210	747, 734, 635, 546, 431, 131, 129	765 (M ⁺), 733, 636, 467, 432, 400, 335, 317, 303 Calcd for C ₃₃ H ₅₄ N ₃ O ₁₀ SF ₃ : 765.34817 Obsd: 765.34790
20	0.88 (3H, t, $J=6$ Hz), 2.75–2.98 (2H, m, -SCH ₂ -), 3.52 (1H, dd, $J=4, 8$ Hz, H ₆), 3.66 (3H, s), 3.75 (3H, s), 3.78 (3H, s), 4.03 (2H, d, $J=5$ Hz, -NCH ₂ CO-), 5.08–5.8 (6H, m, olefinic proton)	3600, 3300, 1735, 1700, 1650, 1230	271 (29000), 280 (39000), 290 (30000)	765 (M ⁺), 733, 636, 467, 432, 400, 335, 317, 303 Calcd for C ₃₃ H ₅₄ N ₃ O ₁₀ SF ₃ : 765.34817 Obsd: 765.34884
21	0.89 (3H, t, $J=6$ Hz), 2.65–3.0 (4H, m, H ₁₃ , -SCH ₂ -), 3.04–3.2 (1H, m, H ₆), 3.67 (3H, s), 3.78 (3H, s), 4.04 (2H, d, $J=5$ Hz, -NCH ₂ CO-), 5.08–5.8 (6H, m, olefinic proton)	3450, 3275, 2900, 1735, 1700, 1630, 1210	765 (M ⁺), 747, 734, 733, 702, 635	765 (M ⁺), 747, 734, 733, 702, 635 Calcd for C ₃₃ H ₅₄ N ₃ O ₁₀ SF ₃ : 765.34817 Obsd: 765.34772
22	0.89 (3H, t, $J=6.2$ Hz), 2.3 (2H, t, $J=7$ Hz), 2.65–2.45 (3H, m, H ₆ , -SCH ₂ -), 3.6–3.8 (1H, m, H ₆), 3.62 (3H, s), 3.73 (3H, s), 4.05 (2H, d, $J=5$ Hz, -NCH ₂ CO-)	3340, 2890, 1740, 1650, 1180	628 (M ⁺), 610, 597, 578, 565, 497, 483, 450, 385, 131	628 (M ⁺), 610, 597, 578, 565, 497, 483, 450, 385, 131 Calcd for C ₂₉ H ₅₁ N ₃ O ₇ SF ₃ : 628.33688 Obsd: 628.33725

a) The amino and carboxyl groups protected with *N*-trifluoroacetyl and methyl ester moieties, respectively. b) Final product.

TABLE III. Spectral Data for Projected Peptides

	mp (°C)	[α] _D (MeOH)	NMR (CDCl ₃) δ ppm	Analysis (%)			
				C	H	N	S
23	106—108	-35.8° (c=1.00)	2.7—3.3 (2H, m, -COCH ₂ -), 3.6 (3H, s), 3.67 (3H, s), 3.97 (2H, d, -NCH ₂ CO-), 4.5—4.87 (1H, m, -NCHCO-)	45.00 (45.11)	6.25 6.23	8.75 8.79	10.00 10.23
24	Oil		1.7 (1H, t, J=8 Hz, HS-), 2.77—3.1 (4H, m, -SCH ₂ CH ₂ CO-), 3.72 (3H, s), 4.0 (2H, d, J=4 Hz, -NCH ₂ CO-)	40.68 (40.65)	6.21 6.32	7.91 7.88	18.08 18.02
25	Oil		1.63 (1H, t, J=8 Hz, HS-), 2.5—3.0 (4H, m, -SCH ₂ CH ₂ CO-), 2.97 (6H, s), 4.0 (2H, d, J=4 Hz, -NCH ₂ CO-)	44.21 (44.24)	7.37 7.40	14.74 14.75	16.84 16.81
26	100—101	-23.7° (c=0.67)	1.65 (1H, t, J=8 Hz), 2.69—2.9 (2H, m, -SCH ₂ -), 3.65 (3H, s), 3.68 (3H, s), 4.25—4.6 (2H, m, -NCHCO- $\times$ 2)	38.50 (38.51)	4.55 4.33	7.49 7.53	8.56 8.24
27	Oil	-126.0 (c=1.00)	1.7 (1H, t, J=8 Hz, HS-), 2.4 (2H, t, J=7 Hz, -CH ₂ CO-), 3.02—3.25 (2H, m, -SCH ₂ -), 3.59 (3H, s), 3.63 (3H, s), 4.16—4.9 (2H, m, -NCHCO- $\times$ 2)	38.5 (38.50)	4.55 4.52	7.49 7.58	8.56 8.81
28	Oil		1.4 (1H, t, J=8 Hz, HS-), 1.7—2.8 (4H, m, -SCH ₂ CH ₂ -), 3.55 (2H, t, J=7 Hz, -CH ₂ N-), 3.73 (3H, s), 4.03 (2H, bs, -NCH ₂ CO-)	37.07 (37.00)	4.63 4.61	5.41 5.76	12.36 12.44
29	Oil		1.62 (1H, t, J=8 Hz, HS-), 1.8 (2H, q, J=7 Hz, -CH ₂ -), 2.25 (6H, s), 2.23—2.72 (4H, m, -SCH ₂ - $\times$ 2, -CH ₂ N-)	50.42 (50.45)	10.92 10.90	11.76 11.54	26.89 27.02

according to the procedure described for the preparation of **5**. $[\alpha]_D -19.2^\circ$ ($c=0.34$, cyclohexane). UV (EtOH) nm: 243 (ϵ , 28000). IR (film) cm^{-1} : 1740 and 1420. MS m/e : 334 (M^+), 318, 316 and 303. NMR (CDCl_3) ppm: 0.88 (3H, t, $J=7.5$ Hz), 2.75–2.96 (1H, m, H_5), 3.14 (1H, dd, $J=2, 6$ Hz, H_6), 3.67 (3H, s), 5.20–5.60 (4H, m), 5.98 (1H, br t, $J=11$ Hz, H_9) and 7.70 (1H, dd, $J=11, 16$ Hz, H_8). High resolution MS m/e : Calcd for $\text{C}_{21}\text{H}_{34}\text{O}_3$, 334.25078. Obsd 334.25067.

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-eicosatrienoate (7)—The title compound was prepared from methyl 10-formyl-5(S),6(S)-oxido-7(E),9(E)-decadienoate (**3**) and nonyltriphenylphosphonium bromide according to the procedure described for the preparation of **5**. IR (film) cm^{-1} : 1740 and 1425. UV (EtOH) nm: 271, 280 (ϵ , 50000) and 290. MS m/e : 344 (M^+), 316 and 303. NMR (CDCl_3) ppm: 0.89 (3H, t, $J=6$ Hz), 2.38 (2H, t, $J=6.5$ Hz), 2.90 (1H, dt, $J=2, 5$ Hz, H_5), 3.13 (1H, dd, $J=2, 8$ Hz, H_6), 3.67 (3H, s) and 5.2–6.75 (6H, m, olefinic proton). High resolution MS m/e : Calcd for $\text{C}_{21}\text{H}_{34}\text{O}_3$, 334.25078. Obsd, 334.25072.

5(S)-Hydroxy-6(R)-glycinocarbonylethylthio-7(E),9(E),11(Z),14(Z)-eicosatetraenoic Acid (Deamino LTD₄, 11)—A solution of leukotriene A₄ methyl ester (5.0 mg) in 0.15 ml of methanol containing NEt_3 (3 eq) was added to a flask containing methyl 3-mercaptopropionylglycinate (**24**, 8.4 mg) under an Ar atmosphere at r.t. The solution was stirred for 3 h at r.t. and concentrated *in vacuo*. The residue was separated by preparative thin layer chromatography on silica gel (ethyl acetate:*n*-hexane/2:1, containing 0.1% NEt_3) to afford 4.2 mg of deamino LTD₄ dimethyl ester as an oil. Spectral data are summarized in Table II.

Aqueous potassium carbonate (0.2 M, 0.75 ml) was added to a solution of deamino LTD₄ dimethyl ester (0.6 mg) in 0.2 ml of methanol under an Ar atmosphere and the resulting solution was stirred for 20 h at r.t.. The solution was diluted with 1.01 ml of pH 6.8 phosphate buffer (0.1 M), taken to pH 6.9 by addition of 1.0 M acetic acid and concentrated in the frozen state under reduced pressure. The residue was purified by reverse phase high performance liquid chromatography (HPLC)²¹ [Nucleosil C₁₈ column (Macherey Nagel Co., Düren, Germany, 4.6 ϕ $\times$ 250 mm, 5 μm particles), solvent, 65 MeOH/35 H₂O/0.1 AcOH buffered to pH 5.6 with 2 N NH_4OH ; flow rate, 1.0 ml/min] to afford deamino LTD₄ (0.31 mg). Retention volume: $R_v=15.0$ (11-*trans* isomer: $R_v=17.5$). UV (MeOH–H₂O) nm: 270 (31000), 281 (40000) and 290 (31000) (11-*trans* isomer: 268, 278 and 288 nm).

Other analogs (**10**, **14**, **15**, **17**–**21**) of LTC and LTD were prepared in the same manner. Spectral data for these analogs are summarized in Table II and Table IV. Purified analogs were stored in 50% aqueous methanol at below -20°C .

TABLE IV. Retention Volumes^{a)} of Leukotriene Analogs

Retention volume		Retention volume	
LTC ₄	5.2	16	12.5
LTD ₄	6.1	17	6.3
10	5.7	18	16.0
11	8.8	19	6.0
12	11.2	20	6.1
13	15.5	21	6.2
14	5.6	22	12.7
15	5.8		

a) Retention volume data were obtained on a Nucleosil C₁₈ Column (Macherey, Nagel Co., Düren, Germany, 5 μm particles, 4.6 $\times$ 250 mm) eluted with 65 CH₃OH/35 H₂O/0.1 AcOH, buffered to pH 5.6 with 2 N NH_4OH at a flow rate of 1 ml/min. Detected by UV and RI.

5,6-erythro-5-Hydroxy-6-[cysteinylglycin-(S)-yl]eicosanoic Acid (Perhydro LTD, 22)—A solution of NBS (427 mg) in CH_2Cl_2 (10 ml) was added to a solution of 5(Z)-eicosaenoic acid⁴¹ (**8**, 620 mg) in CH_2Cl_2 (10 ml) in one portion at r.t. under an Ar atmosphere. After being stirred for 1 h at r.t. the solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2) to afford the bromo-lactone (335 mg). IR (KBr) cm^{-1} : 1720, 1405 and 1258. MS m/e : 390, 388, 370, 308 and 291. NMR (CDCl_3) ppm: 3.60 (3H, s, $-\text{COOCH}_3$) and 3.75–4.20 (2H, m, H_5 and H_6).

A solution of the bromo-lactone (873 mg) and *p*-TsOH (10 mg) in methanol (10 ml) was stirred for 1 h at r.t. and quenched with NEt_3 (50 μl). The solution was concentrated *in vacuo* to afford the crude product, which was purified by column chromatography on silica gel (CH_2Cl_2) to give the bromo-hydrin ester (926 mg: 98%). IR (film) cm^{-1} : 3540 and 1720. MS m/e : 422, 420 (M^+), 380, 341, 323 and 309. NMR (CDCl_3) ppm: 3.60 (3H, s, $-\text{COOCH}_3$) and 3.50–4.20 (2H, m, H_5 and H_6).

A solution of the bromo-hydrin ester (926 mg) and dihydropyran (0.6 ml) in CH_2Cl_2 (10 ml) containing *p*-TsOH (5 mg) was stirred for 30 min at r.t. then quenched with NEt_3 (50 μl). The solution was concentrated *in vacuo* to afford the crude product, which was purified by column chromatography on silica gel (cyclohexane: CH_2Cl_2 /1:1) to give a diastereomixture of the bromo-ester (**9**, 992 mg) in 89% yield. IR (film) cm^{-1} : 1740, 1460 and 1430. MS m/e : 506, 504, 475, 473 and 424. NMR (CDCl_3) ppm: 3.50–4.40 (4H, m), 3.68 (3H, s, $-\text{COOCH}_3$), 4.55–4.75 (1H, m, $-\text{O}-\text{CH}-\text{O}-$).

The bromo-ester (**9**, 51 mg) and methyl *N*-trifluoroacetyl-L-cysteinylglycinate (59 mg) were dissolved in anhydrous DME (1.0 ml), and then 63% NaH (7.6 mg) and HMPA (0.3 ml) were added at r.t. The resulting solution was heated at 70°C for 17 h, cooled to r.t. poured into aqueous NH₄Cl, and extracted with ether. The organic phase was washed with water, dried over Na₂SO₄ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate:cyclohexane/1:1) to afford the *N*-trifluoroacetyl-5-tetrahydropyranyloxy dimethyl ester of **22** (24 mg, MS *m/e* 712).

The *N*-trifluoroacetyl-5-tetrahydropyranyloxy dimethyl ester of **22** (12 mg) was treated with methanol (5 ml) and a catalytic amount of pyridinium-*p*-toluene sulfonate (PPTS) at 40°C for 3.5 h and then concentrated *in vacuo* after quenching with NEt₃. The residue was purified by column chromatography on silica gel (CH₂Cl₂:ethyl acetate/2:1) to afford the *N*-trifluoroacetyl dimethyl ester of **22** (5 mg). Spectral data are summarized in Table II.

Hydrolysis of the *N*-trifluoroacetyl dimethyl ester of **22** and purification by HPLC were done according to the methods described for the preparation of **11**.

LTD₄ Bisamide (12)—*N*-Trifluoroacetyl LTD₄ dimethyl ester²¹ (1.0 mg) was treated with liquid ammonia (0.2 ml) in the presence of ammonium chloride (1.0 mg) in a sealed tube at r.t. for 24 h. The reaction solution was carefully concentrated at atmospheric pressure. The residue was purified by reverse phase HPLC (under the conditions described before) to afford LTD₄ bisamide (**12**, 300 μg), MS *m/e*: 494 (M⁺). UV (MeOH-H₂O) nm: 270, 280 (ε, 40000) and 290.

Deamino LTD₄ Bisdimethylamide (13)—A solution of LTA₄ methyl ester (**4**, 2.5 mg) in 0.75 ml of methanol containing NEt₃ (3 eq) was added to a flask containing 3-mercaptopropionylglycinedimethylamide (**25**, 4.4 mg) under an Ar atmosphere at r.t. The resulting solution was stirred for 3 h at r.t. then concentrated *in vacuo*. The residue was separated by preparative thin layer chromatography (TLC) on silica gel (ethyl acetate:*n*-hexane/2:1, containing 0.1% NEt₃) to afford 2.3 mg of the methyl ester of the title compound, MS *m/e*: 522 (M⁺), UV (EtOH) nm: 271, 281 (ε, 40000) and 291, NMR (CDCl₃) ppm: 0.89 (3H, t, *J*=6 Hz), 2.98 (6H, s), 2.35 (2H, t, *J*=7 Hz), 2.55–3.05 (6H, m, –SCH₂CH₂CO–, H₁₃), 3.51 (1H, dd, *J*=4, 10 Hz, H₆), 3.66 (3H, s), 4.05 (2H, d, *J*=4 Hz, –NCH₂CO–), and 5.20–6.65 (8H, m, olefinic proton).

The methyl ester of **13** (2.3 mg) was treated with dimethylamine (0.2 ml) in the presence of dimethylammonium chloride (0.5 mg) in a sealed tube at r.t. for 24 h. The reaction solution was carefully concentrated and the residue was purified by preparative TLC (ethyl acetate containing 0.1% NEt₃) to afford 1.1 mg of the title compound. Spectral data are summarized in Table II.

5-*O*-Methyl LTC₄ (16)—*N*-Trifluoroacetyl LTC₄ trimethyl ester²¹ (40 mg) was allowed to react with diazomethane (400 eq) in ethyl acetate (5 ml) in the presence of silica gel (400 mg) at r.t. for 18 h. After filtration of silica gel, the filtrate was concentrated *in vacuo* to afford *N*-trifluoroacetyl-5-*O*-methyl LTC₄ trimethyl ester (**13** mg), MS *m/e*: 777 (M⁺), 745, 713 and 430.

Hydrolysis of *N*-trifluoroacetyl-5-*O*-methyl LTC₄ trimethyl ester followed by purification by reverse phase HPLC was done according to the method described for the preparation of **11**.

Methyl 3-Mercaptopropionylglycinate (24)—Phosphorus pentachloride (1.98 g) was added to a suspension of 3,3'-dithiodipropionic acid (1.00 g) in anhydrous ether in one portion at 0°C. The resulting mixture was stirred for 1 h at 0°C then concentrated *in vacuo* to afford crude 3,3'-dithiodipropionyl chloride.

A solution of 3,3'-dithiodipropionyl chloride in ether (10 ml) was added dropwise to a solution of methyl glycinate (932 mg) and NEt₃ (1.06 g) in anhydrous ether (30 ml) at 0°C. The resulting solution was stirred for 30 min at 0°C. The precipitate was collected by filtration, washed with water and dried *in vacuo* to afford crude dimethyl 3,3'-dithiodipropionylglycinate, which was purified by column chromatography on silica gel (ethyl acetate) to give 1.17 g of the title compound.

Dimethyl 3,3'-dithiodipropionylglycinate (1.17 g) was treated with triphenylphosphine (1.04 g) in 12 ml of DME:H₂O/5:1 at r.t. for 24 h under an Ar atmosphere. The precipitate was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate: ether/2:1) to afford 500 mg of methyl 3-mercaptopropionylglycinate (**24**). Spectral data are summarized in Table III.

Other peptides were also prepared in the same manner and their spectral data are summarized in Table II.

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