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Structural Analogs of Leukotrienes C and D avd 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 C_4 (LTC_4) and leukotriene D_4 (LTD_4) retained substantial contractile activities. Amide analogs of LTD_4 and 5-*O*-methyl-LTC_4 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 C_4; leukotriene D_4; leukotriene analog; contractile activity; guinea pig pulmonary parenchymal strips

Each of the leukotriene constituents of slow-reacting substance of anaphylaxis (SRS–A), leukotrienes C_4 (LTC_4) and D_4 (LTD_4), 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 LTC_4 and LTD_4 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.

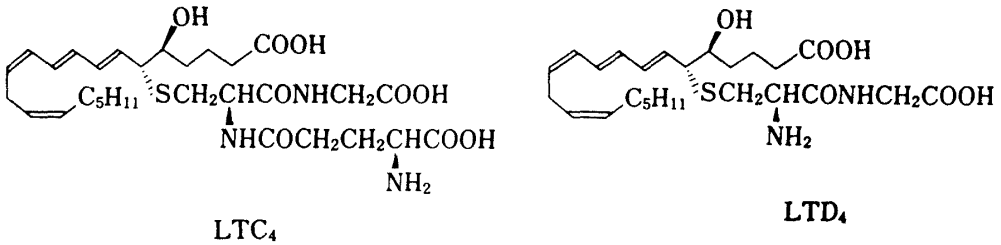


Chart 1

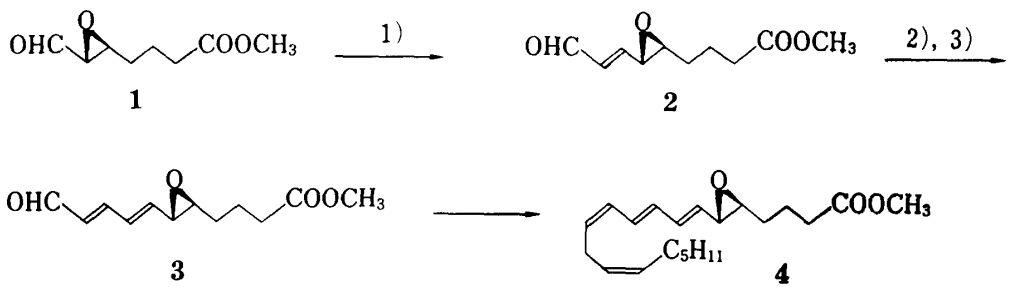
We prepared thirteen analogs of LTC_4 and LTD_4, in which the hydrophobic and hydrophilic regions are modified, and tested them for contractile activities on guinea pig pulmonary parenchymal strips.

Materials and Method

LTC_4 and LTD_4 were prepared by Corey's method[2] 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*)-oxidohexanoate (**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–NEt_3 in CH_2Cl_2 afforded methyl 10-formyl 5(*S*),6(*S*)-

oxido-7(*E*),9(*E*)-decadienoate (**3**), mp 57—59°C, which was converted to LTA_4 methyl ester (**4**) in 93% yield by Corey's method.



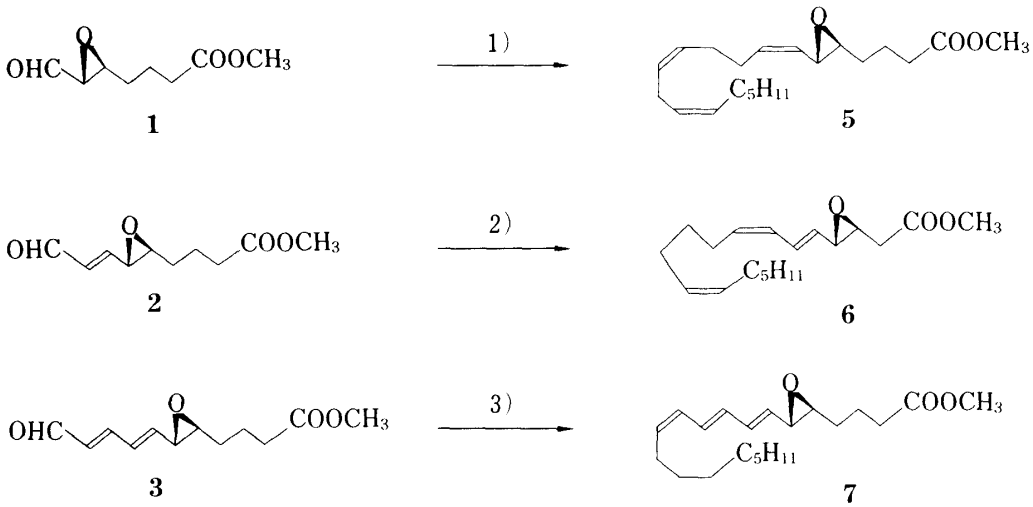
1 2 3 4

1) $Ph_3P=CHCHO$, benzene 2) EtO ⟨ Li, THF 3) MsCl, Et_3N

Chart 2

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.

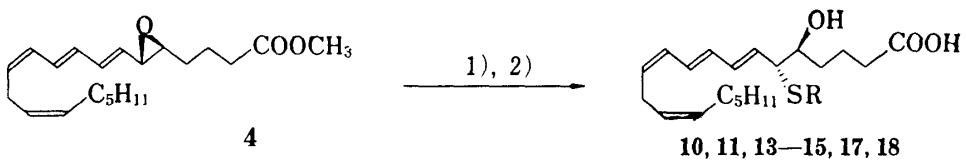


1 5 2 6 3 7

1) *n*-$C_5H_{11}CH=CHCH_2CH=CH-(CH_2)_2-CH=PPh_3$, HMPA, THF
2) *n*-$C_5H_{11}CH=CH-(CH_2)_3-CH=PPh_3$, 3) *n*-$C_8H_{17}CH=PPh_3$

Chart 3

Reaction of LTA_4 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.



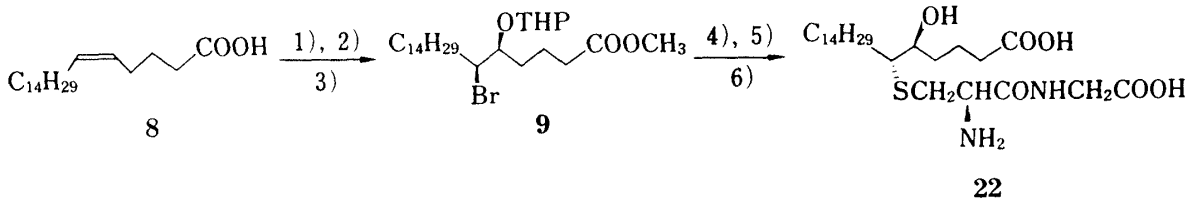
4 **10, 11, 13—15, 17, 18**

1) HSR, Et_3N, CH_3OH 2) 0.2M K_2CO_3, CH_3OH

Chart 4

Reactions of LTA analogs (**5**—**7**) with *N*-trifluoroacetyl-L-cysteinylglycine methyl ester[2] or *N*-trifluoroacetylgluthathione dimethyl ester[2] 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 CH_2Cl_2 at r.t. for 1 h gave the bromo-lactone. 2) Methanolysis of the bromo-lactone followed by treatment with dihydropyran in CH_2Cl_2 in the presence of a catalytic amount of *p*-TsOH afforded methyl *threo*-5-tetrahydro-pyranyloxy-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 K_2CO_3 in MeOH) afforded a diastereomixture of perhydro LTD (**22**).



1) NBS
2) CH_3OH, *p*-TsOH
3) DHP
4) Tfa-Cys-Gly-OMe
NaH, DME, HMPA
5) Py-*p*-TsOH, CH_3OH
6) 0.3M K_2CO_3, CH_3OH

Chart 5

LTD_4 bisamide (**12**) was prepared from *N*-trifluoroacetyl-LTD_4 dimethyl ester with ammonia/ammonium chloride at r.t. overnight. Deamino LTD_4 bisdimethylamide (**13**) was prepared from the methyl ester of deamino LTD_4 glycinedimethylamide prepared from LTA_4 methyl ester (**4**) and 3-mercaptopropionylglycinedimethylamide (**25**), and diemethylamine 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 (ED_{50}) 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 LTC_4 or 1×10^{-9} g/ml LTD_4.

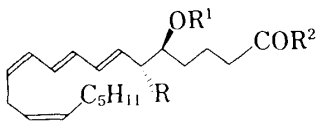
Modification in the Hydrophilic Region

The effect of replacement of an amino acid in the peptide part of LTC_4 or LTD_4 by another amino acid was studied by using six analogs (**10**, **11**, **14**, **15**, **17**, **18**).

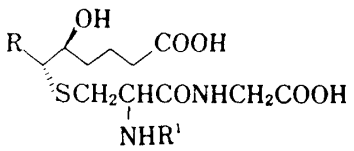
Deamino LTC_4 (**10**) and deamino LTD_4 (**11**) retained almost all the contractile activities of the parent compounds. The analog (**14**), in which the glycine unit of LTC_4 had been removed, still showed substantial activity. The analog (**15**) in which the glycine unit of LTD_4 was replaced by L-glutamic acid lost most of the activity (3 orders of magnitude, decrease).

LTD_4 bisamide (**12**) and deamino LTD_4 bisdimethylamide (**13**) were virtually inactive on

TABLE I. Contractile Activities of Analogs of Leukotrienes C_4 and D_4



	R		R^1	R^2	Contractile activity[a] ED_{50} (g/ml)
	$SCH_2\underset{\underset{NHCOCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH}{\vert}}{\overset{\overset{NH_2}{\vert}}{C}}HCONHCH_2COOH$	(LTC_4)	H	OH	5×10^{-9}
	$SCH_2\underset{\underset{NH_2}{\vert}}{C}HCONHCH_2COOH$	(LTD_4)	H	OH	1×10^{-9}
10	$SCH_2\underset{\underset{NHCOCH_2CH_2COOH}{\vert}}{C}HCONHCH_2COOH$		H	OH	1×10^{-8}
11	$SCH_2CH_2CONHCH_2COOH$		H	OH	1×10^{-9}
12	$SCH_2\underset{\underset{NH_2}{\vert}}{C}HCONHCH_2CONH_2$		H	NH_2	Inactive at 10^{-6}
13	$SCH_2CH_2CONHCH_2CON(CH_3)_2$		H	$N(CH_3)_2$	Inactive at 10^{-6}
14	$SCH_2\underset{\underset{NHCOCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH}{\vert}}{C}HCOOH$		H	OH	1×10^{-8}
	$\underset{\underset{NH_2}{\vert}}{C}H_2CH_2COOH$		H	OH	1×10^{-6}
15	$SCH_2\underset{\underset{NH_2}{\vert}}{C}HCONHCHCOOH$				
16	$SCH_2\underset{\underset{NHCOCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH}{\vert}}{\overset{\overset{NH_2}{\vert}}{C}}HCONHCH_2COOH$		CH_3	OH	5×10^{-7}
17	$SCH_2CH_2CH_2NHCH_2COOH$		H	OH	1×10^{-7}
18	$SCH_2CH_2CH_2N(CH_3)_2$		H	OH	1×10^{-6}



	R	R^1	Contractile activity[a] ED_{50} (g/ml)
19	$n\text{-}C_5H_{11}\overset{Z}{C}H{=}CH(CH_2)_3\overset{Z}{C}H{=}CH\overset{E}{C}H{=}CH-$	$COCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH$	1×10^{-8}
20	$n\text{-}C_8H_{17}\overset{Z}{C}H{=}CH\overset{E}{C}H{=}CH\overset{E}{C}H{=}CH-$	$COCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH$	1×10^{-8}
21	$n\text{-}C_5H_{11}\overset{Z}{C}H{=}CHCH_2\overset{Z}{C}H{=}CH(CH_2)_2\overset{Z}{C}H{=}CH-$	$COCH_2CH_2\underset{\underset{NH_2}{\vert}}{C}HCOOH$	5×10^{-7}
22	$n\text{-}C_{14}H_{29}$-[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 LTC_4 (**16**) had only 1/100 of the activity of LTC_4. The analogs (**17**, **18**) showed greatly decreased activity relative to LTC_4 (2 orders of magnitude or more).

Modification in the Hydrophobic Region

9(*Z*)-Δ^{11}-dihydro LTC_4 (**19**) and Δ^{14}-dihydro LTC_4 (**20**) showed only slightly less contractile

activity than LTC_4. However, 7(*Z*)-Δ^9-dihydro LTC_4 (**21**) in which three double bonds were non-conjugated had only 1/100 of the activity of LTC_4. Perhydro LTC (**22**) was virtually inactive.

Discussion

It is clear that the amino group of LTC_4 and LTD_4 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 LTC_4 and LTD_4 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 LTD_4 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*)-oxidohexanoate[2] (**1**, 1.0 g) in 20 ml of ahydrous 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% NEt_3) to afford 965 mg (83%) of **2** as a colorless oil. $[\alpha]_D$ −23.4° (c=0.1, $CHCl_3$). 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 ($CDCl_3$) ppm: 2.97 (1H, dt, J=2, 6 Hz, $\underline{H}_5$), 3.35 (1H, dd, J=2, 6 Hz, $\underline{H}_6$), 3.68 (3H, s), 6.23—6.68 (2H, m) and 9.57 (1H, dd, J=1, 6.5 Hz, -C$\underline{H}$O).

Methyl 10-Formyl-5(*S*),6(*S*)-oxido-7(*E*),9(*E*)-decadienoate (**3**)——A 1.45 M *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, NEt_3 (1.5 ml) and MsCl (193 μl) were added. The reaction solution was stirred for 1 h at −78°C and poured into aqueous $NaHCO_3$. After being vigorously stirred at r.t. for 10 min, the reaction mixture was extracted twice with CH_2Cl_2. The organic phase was washed with saturated NaCl, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2: Et_2O/30: 1, containing 0.1% NEt_3) to afford 429 mg (76%) of the title compound. mp 57—59°C (ether–*n*-hexane). $[\alpha]_D$ −33.1° (c=1.97, $CHCl_3$). 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 ($CDCl_3$) ppm: 2.92 (1H, dt, J=2, 7 Hz, $\underline{H}_5$), 3.23 (1H, dt, J=2, 7 Hz, $\underline{H}_6$), 3.68 (3H, s), 5.98 (1H, dd, J=8, 16 Hz, $\underline{H}_7$), 6.17 (1H, dd, J=8, 16 Hz, $\underline{H}_{10}$), 6.64 (1H, dd, J=11, 16 Hz, $\underline{H}_8$), 7.10 (1H, dd, J=11, 16 Hz, $\underline{H}_9$) and 9.58 (1H, d, J=8 Hz, -C$\underline{H}$O).

Methyl 5(*S*),6(*S*)-oxido-7(*Z*),11(*Z*),14(*Z*)-eicosatrienoate (**5**)——A 1.5 M 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 Na_2SO_4 and concentrated *in vacuo*.

The residue was purified by column chromatography on $AgNO_3$–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 ($CDCl_3$) ppm: 0.89 (3H, t, J=7 Hz), 2.65—2.95 (3H, m), 3.34 (1H, dd, J=2, 9 Hz, $\underline{H}_6$), 3.67 (3H, s), 5.05 (1H, dd, J=9, 11 Hz, $\underline{H}_7$) and 5.1—5.90 (5H, m). High resolution MS *m/e*: Calcd for $C_{21}H_{34}O_3$, 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, -N<u>CH_2</u>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 $C_{33}H_{52}N_2O_9S$: 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, -N<u>CH_2</u>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 $C_{27}H_{43}NO_6S$: 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}, -S<u>CH_2</u><u>CH_2</u>CO-), 3.15 (1H, dd, J=4, 10 Hz, H_6), 4.05 (2H, d, J=4 Hz, -N<u>CH_2</u>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 $C_{29}H_{59}N_3O_4S$: 535.34436 Obsd: 535.34628
14	0.9 (3H, t, J=7 Hz), 2.7—3.1 (4H, m, H_{13}, -S<u>CH_2</u>-), 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_5), 4.5—5.0 (2H, m, -N<u>CH</u>CO-×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 $C_{33}H_{49}N_2O_9SF_3$: 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}, -S<u>CH_2</u>-), 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, -N<u>CH</u>CO-×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 $C_{33}H_{49}N_2O_9SF_3$: 706.31106 Obsd: 706.31183
16	0.89 (3H, t, J=7 Hz), 2.7—3.1 (4H, m, H_{13}, -S<u>CH_2</u>-), 3.4 (3H, s), 3.66 (3H, s), 3.75 (3H, s), 3.95—4.15 (2H, m, -N<u>CH_2</u>-CO-), 5.1—7.0 (10H, m, olefinic proton, NH×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 $C_{36}H_{54}N_3O_{10}SF_3$: 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<u>CH_2</u>N-), 3.66 (3H, s), 3.75 (3H, s), 4.04 (2H, br s, -N<u>CH_2</u>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 $C_{29}H_{44}NO_6SF_3$: 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}, -S<u>CH_2</u>-), 3.53 (1H, dd, J=4, 10 Hz, H_6), 3.67 (3H, s), 3.6—3.8 (1H, m, H_5), 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 $C_{26}H_{45}NO_3S$: 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, -N<u>CH_2</u>CO-), 4.5—4.75(2H, m, -N<u>CH</u>CO-×2), 5.31—6.87 (6H, m, olefinic proton)	3310, 2900, 1730, 1660, 1550, 1210	236(28000)	765(M^+), 747, 734, 635, 546, 431, 430, 131, 129 Calcd for $C_{35}H_{54}N_3O_{10}SF_3$: 765.34817 Obsd: 765.34790
20	0.88 (3H, t, J=6 Hz), 2.75—2.98 (2H, m, -S<u>CH_2</u>-), 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, -N<u>CH_2</u>CO-), 5.25—6.8 (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 $C_{35}H_{54}N_3O_{10}SF_3$: 765.34817 Obsd: 765.34884
21	0.89 (3H, t, J=6 Hz), 2.65—3.0 (4H, m, H_{13}, -S<u>CH_2</u>-), 3.04—3.2 (1H, m, H_6), 3.67(3H, s), 3.78 (3H, s), 4.04 (2H, d, J=5 Hz, -N<u>CH_2</u>CO-), 5.08—5.8 (6H, m, olefinic proton)	3450, 3275, 2900, 1735, 1700, 1630, 1210		765(M^+), 747, 734, 733, 702, 635 Calcd for $C_{35}H_{54}N_3O_{10}SF_3$: 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, -S<u>CH_2</u>-), 3.6—3.8 (1H, m, H_5), 3.62 (3H, s), 3.73 (3H, s), 4.05 (2H, d, J=5 Hz, -N<u>CH_2</u>CO-)	3340, 2890, 1740, 1650, 1180		628(M^+), 610, 597, 578, 565, 497, 483, 450, 385, 131 Calcd for $C_{29}H_{51}N_2O_7SF_3$: 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 Propected Peptides

		mp (°C)	$[\alpha]_D$ (MeOH)	NMR ($CDCl_3$) δppm	Analysis (%) Calcd (Found) C	H	N	S
23	$HSCH_2\underset{\underset{NHCOCH_2CH_2CH_2COOCH_3}{\vert}}{CH}CONHCH_2COOCH_3$	106—108	−35.8° (c=1.00)	2.7—3.3 (2H, m, $-CO\underline{CH_2}-$), 3.6 (3H, s), 3.67 (3H, s), 3.97 (2H, d, $-N\underline{CH_2}CO-$), 4.5—4.87 (1H, m, $-N\underline{CH}CO-$)	45.00 (45.11	6.25 6.23	8.75 8.79	10.00 10.23)
24	$HSCH_2CH_2CONHCH_2COOCH_3$	Oil		1.7 (1H, t, J=8 Hz, HS–), 2.77—3.1 (4H, m, $-S\underline{CH_2}\underline{CH_2}CO-$), 3.72 (3H, s), 4.0 (2H, d, J= 4 Hz, $-NCH_2CO-$)	40.68 (40.65	6.21 6.32	7.91 7.88	18.08 18.02)
25	$HSCH_2CH_2CONHCH_2CON(CH_3)_2$	Oil		1.63 (1H, t, J=8 Hz, HS–), 2.5—3.0 (4H, m, $-S\underline{CH_2}\underline{CH_2}CO-$), 2.97 (6H, s), 4.0 (2H, d, J= 4 Hz, $-NCH_2CO-$)	44.21 (44.24	7.37 7.40	14.74 14.75	16.84 16.81)
26	$HSCH_2\underset{\underset{NHCOCH_2CH_2\underset{\underset{NHCOCF_3}{\vert}}{CH}COOCH_3}{\vert}}{CH}COOCH_3$	100—101	−23.7° (c=0.67)	1.65 (1H, t, J=8 Hz), 2.69—2.9 (2H, m, $-S\underline{CH_2}-$), 3.65 (3H, s), 3.68 (3H, s), 4.25—4.6 (2H, m, $-N\underline{CH}CO-\times 2$)	38.50 (38.51	4.55 4.33	7.49 7.53	8.56 8.24)
27	$HSCH_2\underset{\underset{NHCOCF_3}{\vert}}{CH}CONH\underset{\underset{CH_2CH_2COOCH_3}{\vert}}{CH}COOCH_3$	Oil	−126.0 (c=1.00)	1.7 (1H, t, J=8 Hz, HS–), 2.4 (2H, t, J=7 Hz, $-\underline{CH_2}CO-$), 3.02—3.25 (2H, m, $-S\underline{CH_2}-$), 3.59 (3H, s), 3.63 (3H, s), 4.16—4.9 (2H, m, $-N\underline{CH}CO-\times 2$)	38.5 (38.50	4.55 4.52	7.49 7.58	8.56 8.81)
28	$HSCH_2CH_2CH_2N\underset{\underset{COCF_3}{\vert}}{CH_2}COOCH_3$	Oil		1.4 (1H, t, J=8 Hz, HS–), 1.7—2.8 (4H, m, $-S\underline{CH_2}\underline{CH_2}-$), 3.55 (2H, t, J=7 Hz, $-\underline{CH_2}N-$), 3.73 (3H, s), 4.03 (2H, bs, $-N\underline{CH_2}CO-$)	37.07 (37.00	4.63 4.61	5.41 5.76	12.36 12.44)
29	$HSCH_2CH_2CH_2N(CH_3)_2$	Oil		1.62 (1H, t, J=8 Hz, HS–), 1.8 (2H, q, J=7 Hz, $-\underline{CH_2}-$), 2.25 (6H, s), 2.23—2.72 (4H, m, $-S\underline{CH_2}-$, $-\underline{CH_2}N-$)	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° (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, $\underline{H}_5$), 3.14 (1H, dd, J=2, 6 Hz, $\underline{H}_6$), 3.67 (3H, s), 5.20—5.60 (4H, m), 5.98 (1H, br t, J=11 Hz, $\underline{H}_9$) and 7.70 (1H, dd, J=11, 16 Hz, $\underline{H}_8$). High resolution MS *m/e*: Calcd for $C_{21}H_{34}O_3$, 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, $\underline{H}_5$), 3.13 (1H, dd, J=2, 8 Hz, $\underline{H}_6$), 3.67 (3H, s) and 5.2—6.75 (6H, m, olefinic proton). High resolution MS *m/e*: Calcd for $C_{21}H_{34}O_3$, 334.25078. Obsd, 334.25072.

5(*S*)-Hydroxy-6(*R*)-glycinocarbonylethylthio-7(*E*),9(*E*),11(*Z*),14(*Z*)-eicosatetraenoic Acid (Deamino LTD$_4$, 11)——A solution of leukotriene A_4 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 LTD_4 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 LTD_4 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)[2] [Nucleosil C_{18} column (Macherey Nagel Co., Düran, Germany, 4.6ϕ×250 mm, 5 μm particles), solvent, 65 MeOH/35 H_2O/0.1 AcOH buffered to pH 5.6 with 2 N NH_4OH; flow rate, 1.0 ml/min] to afford deamino LTD_4 (0.31 mg). Retention volume: R_v=15.0 (11-*trans* isomer: R_v=17.5). UV (MeOH–H_2O) 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 C_{18} Column (Macherey, Nagel Co., Duran, Germany, 5 μm particles, 4.6×250 mm) eluted with 65 CH_3OH/35 H_2O/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 acid[4] (**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, –COOC$\underline{H}_3$) and 3.75—4.20 (2H, m, $\underline{H}_5$ and $\underline{H}_6$).

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, –COOC$\underline{H}_3$) and 3.50—4.20 (2H, m, $\underline{H}_5$ and $\underline{H}_6$).

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 siilca 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, :COOC$\underline{H}_3$), 4.55—4.75 (1H, m, –O–C$\underline{H}$–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 NH_4Cl, and extracted with ether. The organic phase was washed with water, dried over Na_2SO_4 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 NEt_3. The residue was purified by column chromatography on silica gel (CH_2Cl_2: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**.

LTD_4 Bisamide (12)——*N*-Trifluoroacetyl LTD_4 dimethyl ester[2] (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 LTD_4 bisamide (**12**, 300 μg), MS *m/e*: 494 (M^+). UV (MeOH–H_2O) nm: 270, 280 (ε, 40000) and 290.

Deamino LTD_4 Bisdimethylamide (13)——A solution of LTA_4 methyl ester (**4**, 2.5 mg) in 0.75 ml of methanol containing NEt_3 (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% NEt_3) 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 ($CDCl_3$) 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, $-SCH_2CH_2CO-$, H_{13}), 3.51 (1H, dd, J=4, 10 Hz, H_6), 3.66 (3H, s), 4.05 (2H, d, J=4 Hz, $-NCH_2CO-$), 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% NEt_3) to afford 1.1 mg of the title compound. Spectral data are summarized in Table II.

5-*O*-Methyl LTC_4 (16)——*N*-Trifluoroacetyl LTC_4 trimethyl ester[2] (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 LTC_4 trimethyl ester (13 mg), MS *m/e*: 777 (M^+), 745, 713 and 430.

Hydrolysis of *N*-trifluoroacetyl-5-*O*-methyl LTC_4 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 NEt_3 (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:H_2O/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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