and then individually restrained in Bollman cages for the duration of the experiment. Gastric motility was assessed from the mean amplitude of pressure waves (mean motility index of Bech et al.[27]) recorded via the gastric fistula for four 10-min periods before and after subcutaneous administration of compound. Pressure was monitored by a Bell and Howell 4-422 transducer that, after suitable amplification, was displayed on a hot-wire pen recorder. Only rats with a low pretreatment basal motility (mean amplitude <4 mmHg) were used. Usually four groups of 10 such rats were treated with either a graded dose of test compound or vehicle.

Antagonism of apomorphine-induced climbing behavior was assessed by a modification of the method of Protais et al.[16] Usually four groups of 10 male CD-1 mice (20–25 g) were treated subcutaneously with either a graded dose of test compound or vehicle, 30 min before administration of a submaximal dose of apomorphine hydrochloride (1 mg/kg sc). The degree of antagonism was determined 10, 20, and 30 min later.

ED_{50} values and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.[28]

Acknowledgment. The authors thank R. S. Oliver for the spectroscopic analyses and F. E. Blaney for the computational energy calculations.

Registry No. 2a, 67092-55-1; **2b**, 67092-54-0; **2c**, 98482-26-9; **2d**, 98482-27-0; **2e**, 98482-28-1; **2f**, 98482-29-2; **2g**, 98482-30-5; **2h**, 98482-31-6; **3a**, 10447-21-9; **3a** (oxime), 80220-51-5; **3c**, 27257-46-1; **3c** (oxime), 80220-49-1; **3e**, 23581-42-2; **3e** (oxime), 34893-58-8; **3f**, 67092-64-2; **3f** (oxime), 80220-55-9; **4a**, 98482-32-7; **4b**, 98482-33-8; **4c**, 98482-34-9; **4d**, 98482-35-0; **4e**, 98482-36-1; **4f**, 98482-37-2; **4g**, 98482-38-3; **4h**, 98482-39-4; **5a**, 67092-53-9; **5b**, 67092-52-8; **5c**, 98482-40-7; **5d**, 98482-41-8; **5e**, 98482-42-9; **5f**, 98482-43-0; **5g**, 98482-44-1; **5h**, 98482-45-2; **6**, 98482-46-3; **7**, 98482-47-4; **8**, 98482-48-5; 4-(acetylamino)-5-chloro-2-methoxybenzoyl chloride, 4516-32-9; (2β,9aα)-octahydro-2-methyl-2*H*-quinolizine, 5581-90-8; (2β,6α,9aβ)-octahydro-2,6-dimethyl-2*H*-quinolizine, 98482-49-6; (2β,6α,9aβ)-octahydro-2-ethenyl-6-methyl-2*H*-quinolizine, 98482-50-9; (2β,6α,9aβ)-octahydro-2-(methylcarbonylamino)-6-methyl-2*H*-quinolizine, 98482-51-0.

(27) Bech, K.; Hovendal, C. P.; Anderson, D. *Scand. J. Gastroenterol.* **1982**, *17*, 103.

(28) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

Synthesis and LTD_4 Antagonist Activity of 2-Norleukotriene Analogues

Thomas W. Ku,*,† Mary E. McCarthy,† Barry M. Weichman,‡ and John G. Gleason†

Departments of Medicinal Chemistry and Pharmacology, Division of Research and Development, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101. *Received February 22, 1985*

A series of structural analogues of 4(*R*)-hydroxy-5(*S*)-cysteinylglycyl-6(*Z*)-nonadecenoic acid ((4*R*,5*S*,6*Z*)-2-nor-LTD_1 (**10b**), SK&F 101132) has been synthesized and pharmacologically characterized. (4*R*,5*S*,6*Z*)-2-nor-LTD_1 significantly antagonized LTD_4-induced contractile responses on isolated guinea pig trachea. The cis double-bond geometry appears to be critical for antagonist activity, whereas the trans isomer **17** exhibited weak contractile activity. Replacement of the cysteinylglycyl moiety with cysteine afforded **20**, which retained significant antagonist activity, while lengthening or shortening the lipid tail by five methylene groups resulted in complete loss of activity. The eicosanoid amide **15**, glycinamide **14**, and C-1 carbinol **18** analogues all possessed antagonist activity, whereas the diol derivative **19** exhibited increased intrinsic agonist activity.

Leukotriene C_4, D_4, and E_4 comprise a family of closely related eicosanoic acids derived from arachidonic acid via the 5-lipoxygenase pathway. These leukotrienes possess most of the biological activity attributed to slow-reacting substance of anaphylaxis (SRS-A).[1-4] Released upon antigen provocation of sensitized human and animal lung tissue,[5,6] they induce potent bronchoconstriction, increased microvascular permeability,[7-9] and altered mucus production and transport[10] and have been implicated as important mediators of anaphylaxis.[11] It follows that the discovery of selective leukotriene receptor antagonists may provide new therapeutic approaches to the treatment of allergic asthma and other immediate hypersensitivity diseases.

Structure–activity studies[12-14] on the natural agonist, LTD_4, suggested that the eicosanoid carboxyl region of the molecule was critical for agonist activity on the airway smooth muscle. To define the structural requirements in this region on agonist and antagonist activity, we examined the effect of altering the chain length between the C-1 carboxyl and C-5 hydroxyl groups on intrinsic activity. This study, utilizing the hexahydro analogues of LTD_4 to improve chemical stability and ease of synthesis, resulted in the identification of 2-nor-LTD_1 (**10b**, SK&F 101132) as a chemically stable, selective LTD_4 antagonist. The structure–activity relationship of a series of (4*R*,5*S*,6*Z*)-2-nor-LTD_1 analogues was explored with particular em-

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

(1) Hammarstrom, S.; Murphy, R. C.; Samuelsson, B.; Clark, D. A.; Mioskowski, C.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 1266.
(2) Lewis R. A.; Austen, K. F.; Drazen, J. M.; Clark, D. A.; Marfat, A.; Corey, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3710.
(3) Morris, H. R.; Taylor, G. W.; Piper, P. J.; Samhoun, M. N.; Tippins, J. R. *Prostaglandins* **1980**, *19*, 185.
(4) Murphy, R. C.; Hammarstrom, S.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4275.
(5) Dahlen, S. E.; Hedquist, P.; Hammarstrom, S.; Samuelsson, B. *Nature* **1980**, *288*, 484.
(6) Peck, M. J.; Piper, P. G.; Williams, T. J. *Prostaglandins* **1981**, *21*, 315.
(7) Woodward, D. F.; Weichman, B. M.; Gill, C. A.; Wasserman, M. A. *Prostaglandins* **1983**, *25*, 131.
(8) Marom, Z.; Shelhamer, J. H.; Bach, M. K.; Morton, D. R.; Kaliner, M. *Am. Rev. Respir. Dis.* **1982**, *126*, 449.
(9) Gleason, J. G.; Ku, T. W.; McCarthy, M. E.; Weichman, B. M.; Holden, D.; Osborn, R. R.; Zabko-Potapovich, B.; Berkowitz, B.; Wasserman, M. A. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 732.
(10) Weichman, B. M.; Wasserman, M. A.; Holden, D. A.; Osborn, R. R.; Woodward, D. F.; Ku, T. W.; Gleason, J. G. *J. Pharmacol. Exp. Ther.* **1983**, *227*, 700.
(11) (a) Brocklehurst, W. E. *J. Physiol.* **1960**, *151*, 416. (b) Austin, K. F.; Orange, R. P. *Am. Rev. Respir. Dis.* **1975**, *112*, 423.
(12) Lewis, R. A.; Drazen, J. M.; Austen, K. F.; Toda, M.; Brion, F.; Marfat, A.; Corey, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4579.

0022-2623/85/1828-1847$01.50/0 © 1985 American Chemical Society

Table I. Analytical Data

no.	formula	calcd C	calcd H	calcd N	found C	found H	found N	mp, °C
7a	$C_{20}H_{36}O_3$	74.03	11.18		73.78	11.07		
10a	$C_{24}H_{44}N_2O_6S\cdot1^1/_4H_2O$	56.39	9.17	5.48	56.20	8.88	5.18	141–143
10b	$C_{24}H_{44}N_2O_6S\cdot{}^1/_4H_2O$	58.45	9.10	5.68	58.36	8.87	5.26	144–146
12	$C_{19}H_{34}N_2O_6\cdot2Na(-2\ H)\cdot{}^3/_4H_2O$	47.94	7.09	5.89	47.54	7.38	5.77	88–94
13	$C_{29}H_{54}N_2O_6S\cdot2Na(-2\ H)\cdot1^1/_2H_2O$	56.47	9.31	4.54	56.39	9.11	4.24	142–144
14	$C_{24}H_{45}N_3O_5S\cdot{}^3/_4H_2O$	57.51	9.35	8.38	57.50	9.42	6.72	143–145
15	$C_{24}H_{45}N_3O_5S\cdot Na(-H)\cdot2H_2O$	52.82	8.87	7.70	52.65	8.15	7.72	195–198
16	$C_{24}H_{42}N_2O_5S\cdot1^3/_8CF_3CO_2H$	51.21	6.97	4.46	51.39	7.29	4.39	155–157
17a	$C_{24}H_{44}N_2O_6S\cdot1^1/_8H_2O$	56.64	8.94	5.50	56.99	8.81	5.10	136–139
17b	$C_{24}H_{44}N_2O_6S\cdot{}^7/_8H_2O$	57.14	9.14	5.55	57.59	9.10	5.15	147–150
18	$C_{24}H_{46}N_2O_5S\cdot{}^3/_4H_2O$	59.05	9.81	5.74	59.13	9.78	5.55	110–113
19	$C_{24}H_{48}N_2O_4S\cdot{}^1/_2H_2O$	61.37	10.51	5.96	61.19	10.67	6.00	59–61
20a	$C_{22}H_{40}NO_5S\cdot Na$	58.12	8.87	3.08	57.99	8.85	3.70	141–143
20b	$C_{22}H_{4L}NO_5S_2\cdot3Na(-3H)$	56.69	8.54	3.01	56.40	8.19	3.33	119–122

phasis on the role of the lipid tail, double-bond geometry, C-4 and C-5 absolute stereochemistries, and peptide and carboxyl group on the antagonist potency.

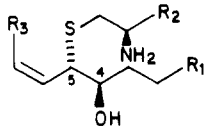
Chemistry. The preparation (Scheme I) of the 2-nor-LTD_1 analogues[15] was keyed to the racemic 6*Z*,4*E* epoxide **7a** which was envisioned to undergo an S_N2 ring-opening reaction by the appropriately substituted mercaptans **8**. Synthesis of the epoxide **7a** took advantage of the Wittig coupling reaction between the ylide derived from the phosphonium salt **6a** and the epoxy aldehyde **5**, which itself was prepared from the readily available succinic acid monomethyl ester (**1**).

Treatment of **1** with oxalyl chloride afforded the acyl chloride **2**, which was directly subjected to Rosenmund reduction conditions to give methyl 3-formylpropionate (**3**). Coupling of **3** with formyl methylenetriphenylphosphorane in toluene gave the trans-α,β-unsaturated aldehyde **4**. Epoxidation[16] yielded the trans epoxy aldehyde **5**, which was condensed with the Wittig reagent from **6a** to give 4,5-epoxy-6(*Z*)-nonadecenoic acid methyl ester (**7a**; J = 11 Hz for vinyl H, J = 2 Hz for oxirane H, assignment confirmed by NOE).[17] Treatment of **7a** with the mercaptan **8a** in $MeOH/Et_3N$ gave, after lactonization, a mixture of diastereomeric γ-lactones **9a** and **9b**, which were separated by preparative HPLC to give the pure lactone **9a** (shorter retention time) and **9b** (longer retention time).[18] The lactones **9a** and **9b** were then saponified to give **10a** and **10b**.

The 4*R* absolute stereochemistry of the hydroxyl functionality of **9b** was determined by reductive desulfurization of **9b** to the known chiral lactone **11b** ($[\alpha]^{22}_D$ −17°, *c* 1, MeOH), establishing the 4*S* chirality of **11b**[19] and hence the 4*R* absolute stereochemistry of **9b** and **10b**. The 4*S* absolute stereochemistry in **10a** was similarly determined by degradation of **9a** to the 4*R* lactone **11a**, which had an equal but opposite specific rotation (+17.5°, *c* 1, MeOH). Assuming that the addition of **8a** to the epoxide **7a** proceeds with inversion of configuration at C-5, then the absolute stereochemistry of **10b** would be of the "unnatural" 4*R*,5*S* chirality relative to LTD_4, and its diastereomer **10a**, the "natural" 4*S*,5*R* stereochemistry.[20]

The diastereomeric nature of **9a** and **9b** was also evidenced by their CD spectra. The isomer **9a** has a positive Cotton effect at 235 nm and a negative extremum at 215 nm, whereas the diastereomer **9b** had the opposite Cotton effects at the respective wavelengths.[21]

Table II. Antagonist Activity of 2-Norleukotriene Analogues on Guinea Pig Tracheal Spiral Strips

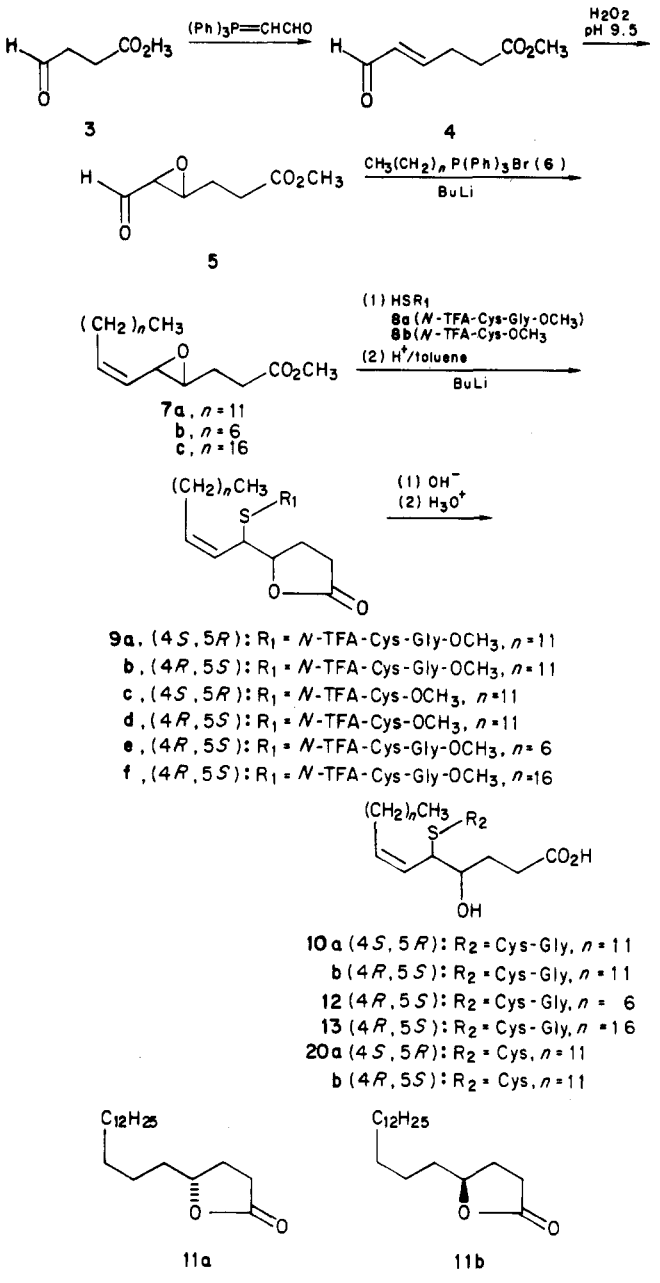


compd	R_1	R_2	R_3	act.: K_B,[a] μM
10a (4*S*,5*R*)	CO_2H	$CONHCH_2CO_2H$	$C_{12}H_{25}$	10.0
10b (4*R*,5*S*)	CO_2H	$CONHCH_2CO_2H$	$C_{12}H_{25}$	6.3
12 (4*R*,5*S*)	CO_2H	$CONHCH_2CO_2H$	C_7H_{15}	inact[e]
13 (4*R*,5*S*)	CO_2H	$CONHCH_2CO_2H$	$C_{17}H_{35}$	inact[e]
14 (4*R*,5*S*)	CO_2H	$CONHCH_2CONH_2$	$C_{12}H_{25}$	3.5
15 (4*R*,5*S*)	$CONH_2$	$CONHCH_2CO_2H$	$C_{12}H_{25}$	7.2
16 (4*R*,5*S*)	γ-lactone	$CONHCH_2CO_2H$	$C_{12}H_{25}$	29.0
17a (4*S*,5*R*,6*E*)	CO_2H	$CONHCH_2CO_2H$	$C_{12}H_{25}$	20[b]
17b (4*R*,5*S*,6*E*)	CO_2H	$CONHCH_2CO_2H$	$C_{12}H_{25}$	40[b]
18 (4*R*,5*S*)	CH_2OH	$CONHCH_2CO_2H$	$C_{12}H_{25}$	14.0
19 (4*R*,5*S*)	CH_2OH	$CONHCH_2CH_2OH$	$C_{12}H_{25}$	*b*
20a (4*S*,5*R*)	CO_2H	CO_2H	$C_{12}H_{25}$	3.4[c]
20b (4*R*,5*S*)	CO_2H	CO_2H	$C_{12}H_{25}$	14.0[d]

[a] In comparison, K_B = 0.1 μM for FPL 55712. [b] Partial agonist. [c] K_B = 8.1 μM vs. LTE_4. [d] K_B = 7.5 μM vs. LTE_4. [e] Neither agonist nor antagonist activity observed.

(13) Drazen, J. M.; Lewis, R. A.; Austen, K. F.; Toda, M.; Brion, F.; Marfat, A.; Corey, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3195.
(14) Okuyama, S.; Miyamoto, S.; Shimoji, K.; Konishi, Y.; Fukushima, D.; Niwa, H.; Arai, Y.; Toda, M.; Hayaski, M. *Chem. Pharm. Bull.* **1982**, *30*, 2453.
(15) All compounds gave satisfactory analyses for C, H, and N and were further characterized by IR, NMR, and mass spectra (Table I).
(16) Gleason, J. G.; Bryan, D. B.; Kinzig, C. M. *Tetrahedron Lett.* **1980**, 1129.
(17) The FT nuclear Overhauser (NOE) experiments were performed in collaboration with Prof. Lloyd Jackman of Pennsylvania State University, on a Bruker IUP 200 spectrometer using the alternating-phase mode. The solution was rigorously degassed by repeated cycles of freezing and thawing. Irradiation of H_7 and H_4 enhances the resonance of H_6, and a large enhancement of signal corresponding to H_5 is observed when H_8 is irradiated, thus establishing trans epoxide and cis olefin relationships.
(18) The initial preparative HPLC work on the separation of the diastereomeric γ-lactones was carried out by K. Erhard of Smith Kline & French Laboratories.
(19) Nishizawa, M.; Yamada, M.; Moyori, R. *Tetrahedron Lett.* **1981**, 247.
(20) (a) Rosenberger, M.; Newkom, C.; Aig, E. R. *J. Am. Chem. Soc.* **1983**, *105*, 3656. (b) Spur, B.; Crea, A.; Peters, W. *Arch. Pharmacol.* **1983**, *315(B)*, 572.
(21) The circular dichorism (CD) spectra of the diastereomeric lactones **9a** and **9b** in methanol (1 mg/mL, 1 mm) were recorded on a Jasco 500-C spectropolarimeter by Dr. W. Holl of Smith Kline & French Laboratories.

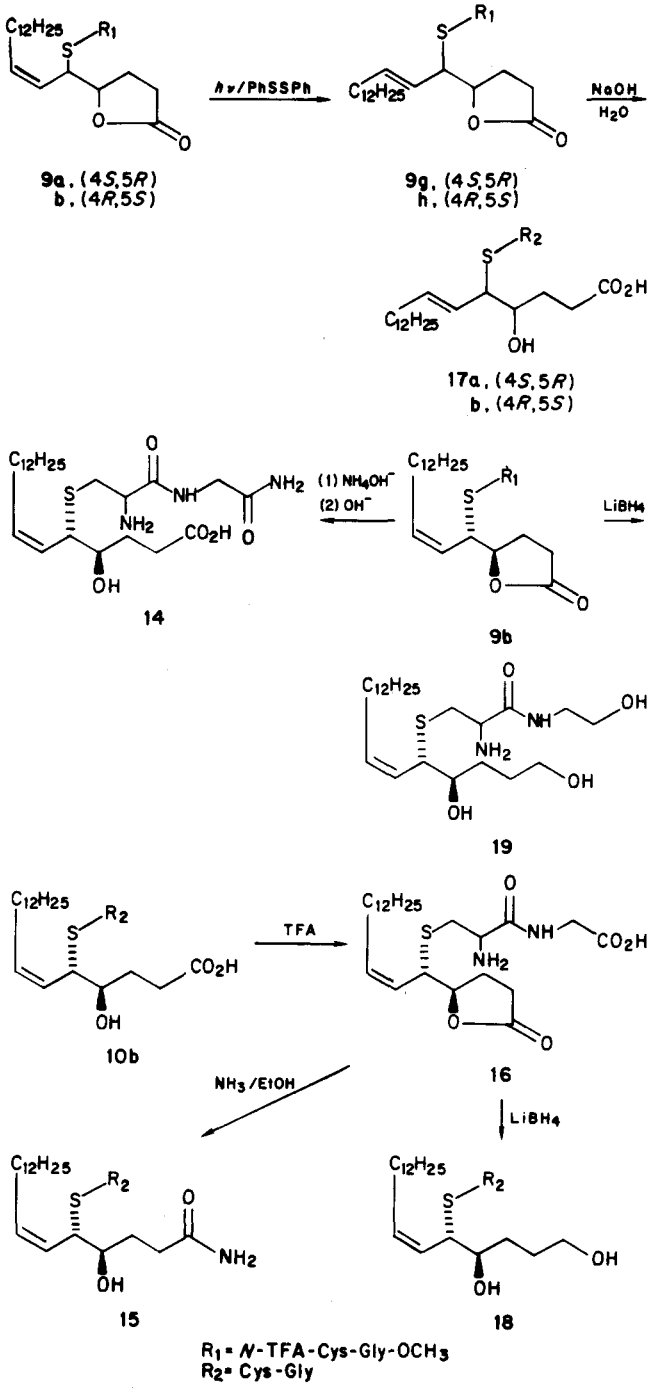
Scheme I



In an analogous fashion, analogues **12** and **13** with shorter and longer lipid tails were prepared from **7b** and **7c**. 2-nor-LTE_1 diastereomeric analogues, **20a** and **20b**, were synthesized from **9c** and **9d** (derived from **7a** and **8b**), respectively, and their stereochemistry was inferred from comparison of CD spectra and HPLC retention times of the precursor lactones with those of **9a** and **9b**.

The chiral lactones **9a**, **9b**, and **16** proved to be useful intermediates for further chemical modification of the eicosanoid and peptide carboxyl groups (Scheme II). Thus, aminolysis of **9b** proceeded exclusively at the peptide carboxyl terminus to give, after hydrolysis, the glycinamide **14**, while borohydride reduction of **9b** afforded the diol **19**. Selective modification of the eicosanoid carboxyl group was achieved by lactonization of **10b** (TFA/CH_2Cl_2) to give the lactone **16**. Aminolysis of **16** afforded the C-1 amide **15**,

Scheme II



while borohydride reduction of **16** provided the C-1 carbinol **18**. The 6*E* isomers **17a** and **17b** were synthesized by photoisomerization of **9a** and **9b**, (J = 15 Hz for vinyl H),[23] respectively, followed by deprotection. Spectral and analytical data on all intermediates and final products were consistent with the proposed structures (Table I).

Results and Discussion

The hexahydro analogues of LTD_4 utilized in this study are listed in Table II. The (4*R*,5*S*,6*Z*)-2-nor-LTD_1 (**10b**, SK&F 101132), when evaluated for LTD_4-like agonist activity on guinea pig tracheal strips,[9,10] exhibited no intrinsic agonist activity at concentrations as high as 10^{-4} M but significantly inhibited the LTD_4-induced guinea pig tracheal contractions[9] with a K_B = 6.3 μM (Table II). A

(22) Perchonock, C. D.; Uzinskas, I.; Ku, T. W.; McCarthy, M. E.; Bondinell, W. E.; Volpe, B. W.; Gleason, J. G.; Weichman, B. M.; Muccitelli, R. M.; DeVan, J. F.; Tucker, S. S.; Vickery, L. M.; Wasserman, M. A. *Prostaglandins*, in press.

(23) Sonnet, P. E. *Tetrahedron* **1980**, *36*, 557 and references cited therein.

detailed description of its pharmacological profile has been documented elsewhere.[10]

This antagonist (**10b**) exhibits a number of significant structural differences from the natural agonist. One difference, which might impact on receptor affinity, is the stereochemistry of the double bond. In **10b**, this bond possesses cis geometry, which contrasts with the 7-trans stereochemistry of the comparable double bond in the naturally occurring leukotrienes. However, the trans isomers **17a** and **17b**, which should more resemble the natural agonists, exhibited weak intrinsic agonist activity, and at 10^{-4} M were less potent as antagonists than the cis isomer **10b**. Deletion of the glycine moiety from the peptide chain in **10** yielded (4*S*,5*R*,6*Z*)-2-nor-LTE_1 (**20a**) and its "unnatural" diastereomer **20b**. The "natural" diastereomer **20a** appeared to be more potent than "unnatural" diastereomer **20b** in antagonizing the LTD_4-induced contractions of the guinea pig trachea; however, both hexahydro LTE_1 analogues possessed equipotent antagonist activity against LTE_4-induced contractions. Thus, present results could not establish a clear relationship of antagonist activity to absolute stereochemistry at C-4 and C-5.

To explore the importance of the ionizable carboxyl group on the LTD_4 antagonist activity, compounds **14–16**, **18**, and **19** were synthesized and evaluated for antagonist activity (Table II). The observed antagonist activity in the eicosanoid monoamide **15** (K_B = 7.2 μM) and C-1 carbinol **18** (K_B = 14 μM) indicates that an ionizable free carboxyl at C-1 is not an absolute requirement for activity on the guinea pig trachea. Lactone **16** (K_B = 29 μM) appears to retain some antagonist activity, suggesting that the fully extended C-1 eicosanoid chain and the free hydroxyl group at C-4 may not be critical structural requirements for potency. However, hydrolysis of **16** during the biological assay cannot be discounted. The slightly improved LTD_4 inhibitory activity of the cysteinylglycyl monoamide **14** (K_B = 3.5 μM) may suggest that the carbonyl group in the carboxyl and amide functions is required for enhanced interaction with the putative LT receptor. The absence of carboxyl groups in the triol **19** results in significant intrinsic contractile activity. The precise role of the free carboxyl at C-1 and hydroxyl group at C-4 remains to be further elucidated.

To assess the importance of the lipid tail on antagonist potency, compounds **12** and **13** were prepared. Neither compound possessed significant antagonist activity. In a related study, we have observed that alteration of the lipid chain length by more than two methylene residues significantly diminishes activity.[22] Thus, the length of the lipid tail is critical for antagonist activity.

In summary, this study demonstrates that (4*R*,5*S*,6*Z*)-2-nor-LTD_1 (**10b**) and several related analogues possess significant leukotriene antagonist activity. The spatial separation of the C-1 carboxyl relative to the C-5 hydroxyl and/or C-6 thioether groups of the peptidoleukotrienes is a critical determinant of both affinity and intrinsic activity at the presumed leukotriene receptor. Saturation of the 9, 11, and 14 double bonds and deletion of one methylene group in the region between C-1 carboxyl and C-5 hydroxyl groups of the natural LTD_4 result in antagonist activity on guinea pig airway smooth muscle. In the 2-nor-LTD_1 series presented here, the "unnatural" 4*R*,5*S* diastereomer appears to have slightly more potent LT antagonist activity, while the isomer with the "natural" absolute stereochemistry exhibited weak intrinsic agonist activity. The cis double bond at C-6 appears to be critical for antagonist activity, whereas the trans isomer exhibited weak contractile activity. Replacement of the peptide carboxyl with a primary amide seems to have marginally enhanced antagonist activity over the parent diacid analogue, while the C-1 primary monoamide retained comparable antagonist activity. Carbinol replacement of the eicosanoid carboxyl diminished activity, while conversion of both carboxyl groups into hydroxyl groups gave rise to an increase in contractile activity on the trachea. Replacement of the Cys-Gly peptide moiety with cysteine residue led to retention of significant antagonist activity, whereas lengthening or shortening the lipid tail by five methylene groups resulted in complete loss of LT antagonist activity.

Studies are under way to further explore the structural requirements for leukotriene antagonist activity. While the precise role of the peptidoleukotrienes in the pathophysiology of asthma remains to be determined, such antagonists may offer new therapeutic opportunities in immediate hypersensitivity diseases, as well as provide tools for the exploration of the role of leukotrienes in disease processes.

Experimental Section

Pharmacological Evaluation.[9,10] Adult male albino Hartley strain guinea pigs weighing 400–600 g were sacrificed by a sharp blow to the head, and the trachea was immediately removed. Tracheal spiral strips of approximate dimensions 2–3 mm cross-sectional width and 3.5-cm length were placed in jacketed 10-mL tissue baths and connected via silk suture to Grass Model FTO3C force displacement transducers for recording isometric tension. The tissue strips were bathed in modified Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.6; $MgSO_4 \cdot 7H_2O$, 1.1: $CaCl_2$, 24.9; KH_2PO_4, 1.0; glucose, 11.1. The tissue baths were maintained at 37.5 °C and continuously aerated with 95% O_2/5% CO_2. The trachea were placed under a resting load of 2.0*g* and equilibrated for 45 min. The trachea were then pretreated for an additional 15 min with 10^{-6} M meclofenamic acid, in order to inhibit the compensatory release of dilator prostaglandins during the LTD_4- and LTE_4-induced contraction.[24] Test compounds (10^{-4} M) or 20 mM sodium carbonate vehicle were incubated with the trachea for 30 min; then, a cumulative concentration–response curve was generated for each trachea by successive increases in the bath concentration of LTD_4 or LTE_4 according to the method of Van Rossum.[25] Solution of different concentrations of LT was added during the plateau of the contraction elicited by the preceding LT concentration. Only one LT concentration–response curve was generated for each trachea. The contractions elicited by the LT were standardized vs. the contraction elicited by the reference agonist, carbachol (10^{-5} M), which did not affect the profile of any of the test compounds studied. When antagonism by a test compound was observed as a parallel shift of the LTD_4 concentration–response curve, the K_B was determined from the ratio (X) of the LTD_4 concentration required to elicit 30% of the carbachol-induced contraction in the presence of the test compound to that in its absence according to the equation K_B = concn. of antag/(X − 1).

Chemistry. Melting points were determined by using a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. IR spectra were taken as Nujol mulls on a Perkin-Elmer Infracord spectrophotometer, and absorptions are reported in wavenumbers. NMR spectra were recorded on a Perkin-Elmer R24 spectrometer and are reported in parts per million downfield from internal Me_4Si. Mass spectra were determined on a Hitachi Perkin-Elmer RMN-6E spectrometer. Preparative high-pressure liquid chromatography was performed by using a Waters Prep LC 500 with 2 Preppak silica cartridges. Unless otherwise indicated, analytical HPLC was done on Lichrosorb, SI-60, column using an RI detector, 1 mL/min flow rate. The circular dichroism (CD) spectra of the diastereomeric lactones in methanol (1 mg/mL, 1 mm) were recorded on a Jasco 500-C spectropolorimeter.

(24) Weichman, B. M.; Muccitelli, R. M.; Osborn, R. R.; Holden, D. A.; Gleason, J. G.; Wasserman, M. A. *J. Pharmacol. Exp. Ther.* **1982**, *222*, 202.
(25) Van Rossum, J. M. *Int. Pharmacodyn. Ther.* **1963**, *143*, 299.

3-Carbomethoxypropionyl Chloride (2). To an ice-cold solution of monomethyl succinate (1) (150 g, 1.135 mol) in 900 mL of methylene chloride and 3 mL of *N,N*-dimethylformamide was added 119 mL (1.26 mol) of oxalyl chloride, while the temperature was kept below 5 °C. After the addition was complete, the reaction mixture was stirred at 0 °C for 1 h and then concentrated in vacuo to give a crude yellow liquid, which was used without purification in the Rosenmund reduction.

3-Carbomethoxypropionaldehyde (3). To the acid chloride 2 (8.9 g, 0.059 mol) in 200 mL of sieve-dried tetrahydrofuran was added 6.9 mL (0.059 mol) of 2,6-lutidine. After standing at room temperature for 30 min, the mixture was filtered and 0.7 g of 10% palladium on carbon was added to the filtrate. The mixture was hydrogenated at 50 psi for 2 h, the solids were filtered off, and the filtrate was concentrated. The residue was dissolved in methylene chloride and washed twice with 10% hydrochloric acid solution and then twice with 5% sodium bicarbonate solution. The organic extract was dried with anhydrous sodium sulfate and concentrated in vacuo. Kugelrohr distillation (40–55 °C (0.05 mmHg)) yielded a colorless liquid: 3.8 g (55% overall yield); mass spectrum (EI) *m/e* 115 ($M^+ - 1$); NMR ($CDCl_3$) δ 2.75 (m, 4 H), 3.75 (s, 3 H, OCH_3), 9.9 (t, CHO).

5-Carbomethoxy-2(*E*)-pentenal (4). To a mechanically stirred solution of 3 (72.1 g, 0.620 mol) in 750 mL of toluene under argon was added 235.9 g (0.776 mol) of (formylmethylene)triphenylphosphorane. The reaction mixture was refluxed for 1.5 h, cooled to room temperature, and then concentrated in vacuo. The residue was left standing under diethyl ether at 0 °C overnight. The mixture was filtered, and the solid was washed with cold ether. The filtrate was concentrated, and the resulting maroon oil was subjected to Kugelrohr distillation. The product (41.2 g (47% yield)) was collected at 65–80 °C (0.05 mmHg): NMR ($CDCl_3$) δ 2.6 (m, 4 H), 3.72 (s, 3 H, OCH_3), 6.2 (m, 1 H, vinyl), 6.9 (m, 1 H, vinyl), 9.5 (d, CHO).

Methyl 4,5-Epoxy-6-oxohexanoate (5). To a solution of 36.4 mL of a 30% hydrogen peroxide solution and 58.2 mL of 1 N sodium bicarbonate in 800 mL of methanol and 400 mL of water under argon was added dropwise over 45 min 41.2 g (0.29 mol) of 4 in 400 mL of methanol. After the addition was complete, the reaction was stirred for 2.5 h at room temperature, while the pH was maintained between 9 and 9.5 by the addition of sodium bicarbonate solution. The reaction mixture was then poured into 1 L of saturated ammonium sulfate solution, the methanol was removed in vacuo, the solids were filtered off, and the product was extracted into methylene chloride. The aqueous layer was back-extracted twice, and the combined extracts were dried with anhydrous sodium sulfate and then concentrated to give a crude golden oil. The product (27 g (60%)) was Kugelrohr distilled at 82–9 °C (0.01 mmHg): NMR ($CDCl_3$) δ 1.97 (m, 2 H), 2.52 (m, 2 H), 3.19 (m, 1 H, oxirane), 3.39 (m, 1 H, oxirane), 3.7 (s, 3 H, OCH_3), 9.0 (d, CHO).

Tridecyltriphenylphosphonium Bromide (6a). A solution of 1-bromotridecane (100 g, 0.4 mol) and triphenylphosphine (100 g, 0.4 mol) in 500 mL of xylenes was refluxed overnight. The reaction mixture was then cooled to room temperature and poured into diethyl ether. The resulting oil was washed three times with ether, dissolved in methylene chloride, and then concentrated in vacuo. The oil was left standing in diethyl ether at 0 °C overnight and then concentrated in vacuo to give 152 g (76%) of a white solid. The phosphonium bromides **6b** and **6c** were similarly prepared.

Methyl 4,5-Epoxy-6(*Z*)-nonadecenoate (7a). To an ice-cold solution of **6a** (97.2 g, 0.185 mol) in 600 mL of sieve-dried tetrahydrofuran under argon was added dropwise 77.2 mL of a 2.4 M solution of *n*-butyllithium in hexane (0.185 mol); the temperature was maintained at 0 °C over 30 min. The reaction mixture was stirred at this temperature for an additional 15 min, cooled to −78 °C (dry ice/2-propanol), and then 26.5 g (0.168 mol) of 5 in 150 mL of dry tetrahydrofuran was added dropwise over 30 min. After the addition was complete, the reaction was stirred for 1 h at −78 °C and then concentrated in vacuo at 24 °C. The residue was triturated with hexane and then left standing at 0 °C overnight. The hexane was decanted, and then the residue was sonicated four times with hexane. The combined extracts were concentrated in vacuo, and the crude product was purified by preparative HPLC (8% ethyl acetate in hexane), giving **7a** as an oil: 23.3 g (43% yield); NMR[17] ($CDCl_3$) δ 2.8 (m, 2 H), 2.81 (dt, 1 H, oxirane, J = 5.6, 2.3 Hz), 3.34 (dd, 1 H, oxirane, J = 2.3, 9.1 Hz), 3.67 (s, OCH_3), 5.01 (m, 1 H vinyl, J = 9.1, 11 Hz), 5.69 (m, 1 H vinyl, J = 11, 7.9 Hz); mass spectrum *m/e* 324 (M^+). The epoxides **7b** [TLC (SiO_2) R_f (10% EtOAc/hexane) 0.43; mass spectrum (CI) *m/e* 255 ($M^+ + 1$)] and **7c** [TLC (SiO_2) R_f (10% EtOAc/hexane) 0.55; mass spectrum (CI) *m/e* 395 ($M^+ + 1$)] were likewise synthesized as above, and their proton NMR spectra were similar.

4(*R*)-Hydroxy-5(*S*)-[[2-(trifluoroacetamido)-3-[(carbomethoxymethyl)amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid, γ-Lactone (9b), and 4(*S*)-Hydroxy-5(*R*)-[[2-(trifluoroacetamido)-3-[(carbomethoxymethyl)amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid γ-Lactone (9a). To the epoxide **7a** (12.9 g, 0.04 mol) under argon at room temperature was added dropwise over 1 h at solution of 14.5 g (0.05 mol) of **8a** in triethylamine (6.7 mL)/methanol (150 mL). The reaction mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was dissolved in a minimum volume of methylene chloride, 100 mL of hexane was added, and then this solution was stored at −15 °C for 1 h. The resulting solid was filtered off, and then the filtrate was concentrated in vacuo, to give a crude oil, consisting of a mixture of products. The mixture (20.4 g) in 200 mL of toluene was heated to 80 °C in the presence of 75 mg of *p*-toluenesulfonic acid for 15 min. The reaction mixture was then concentrated in vacuo, and the resulting crude oil was purified by preparative HPLC (45% ethyl acetate in hexane) to give the desired products as the pure 4*R*,5*S* lactone **9b** (8.1 g, 33% yield) and the pure 4*S*,5*R* lactone **9a** (6.8 g, 28% yield).

9a: TLC (SiO_2) R_f (50% EtOAc/hexane) 0.58; HPLC R_T (40% EtOAc/hexane) 15.44 min; $[\alpha]^{20}_D$ +53.5° (*c* 3, $CHCl_3$); mass spectrum (CI) *m/e* 579 ($M^+ - 1$); NMR identical with that of **9b**.

9b: TLC (SiO_2) R_f 0.53; HPLC R_T 18.06 min; $[\alpha]^{20}_D$ − 9.4° (*c* 3, $CHCl_3$); mass spectrum (CI) *m/e* 579 ($M^+ - 1$); NMR ($CDCl_3$) δ 2.90 (d, 2 H, SCH_2), 3.75 (s, 3 H, OCH_3), 4.10 (m, 3 H), 5.30 (m, 1 H, vinyl, J = 11 Hz), 5.96 (m, 1 H, vinyl, J = 11 Hz), 7.10 (br t, 1 H, NH), 7.50 (br d, 2 H, NH_2).

4-Hydroxy-5(*S*)-[[2-(trifluoroacetamido)-2-carbomethoxyethyl]thio]-6(*Z*)-nonadecenoic Acid, γ-Lactone (9c,d). To methyl 4,5-epoxy-6(*Z*)-nonadecenoate (**7a**; 0.9 g, 0.00277 mol) under argon at room temperature was added dropwise a solution of 1.2 g (0.00519 mol) of 2-(trifluoroacetamido)-2-carbomethoxyethyl mercaptan (**8b**) in triethylamine (0.88 mL)/methanol (15 mL). The reaction mixture was stirred at room temperature for 30 h, concentrated in vacuo, and then filtered through a silica gel bed with chloroform. The chloroform wash was concentrated to give a mixture of hydroxymethyl ester and γ-lactone (2 g) that was heated to 80 °C in 75 mL of toluene in the presence of 27 mg of *p*-toluenesulfonic acid for 20 min. The reaction mixture was then concentrated in vacuo, and the residue was taken up in methylene chloride, washed with 5% sodium bicarbonate solution and then with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated in vacuo. The resulting oil (2 g) was purified by preparative HPLC with 25% ethyl acetate in hexane to give the desired products as the 4*R*,5*S* lactone **9d** and the 4*S*,5*R* isomer **9c**. Each individual diastereomer was purified further on a silica "flash" column, eluting with 30% ethyl acetate in hexane to give 0.3 g each (20% yield).

9c: TLC (SiO_2) R_f (25% EtOAc/hexane) 0.47; HPLC R_T (30% EtOAc/hexane) 7.86 min; $[\alpha]^{20}_D$ +26.3° (*c* 1, $CHCl_3$); NMR ($CDCl_3$) δ 3.10 (d, 2 H, SCH_2), 3.80 (s, 3 H, OCH_3), 3.95 (m, 1 H), 4.63 (m, 1 H), 4.85 (m, 1 H), 5.23 (m, 1 H, vinyl), 5.75 (m, 1 H, vinyl), 7.15 (br s, 1 H).

9d: TLC (SiO_2) R_f 0.33; HPLC R_T 10.0 min; $[\alpha]^{20}_D$ −7.6 (*c* 1, $CHCl_3$); NMR identical with that of **9c**.

4(*R*)-Hydroxy-5(*S*)-[[2-(trifluoroacetamido)-3-[(carbomethoxymethyl)amino]-3-oxopropyl]thio]-6(*Z*)-tetradecenoic Acid, γ-Lactone (9e). The title lactone was synthesized from the epoxide **7b** and the mercaptan **8a** according to the procedure used to make **9b**: TLC (SiO_2) R_f (50% ethyl acetate/hexane) 0.47; HPLC (5 μm, 2 mL/min) R_T 5.59 min; mass spectrum (CI) *m/e* 511 ($M^+ + 1$); NMR ($CDCl_3$) similar to that of **9b**.

4(*R*)-Hydroxy-5(*S*)-[[2-(trifluoroacetamido)-3-[(carbomethoxymethyl)amino]-3-oxopropyl]thio]-6(*Z*)-tetracosenoic Acid, γ-Lactone (9f). The title compound was synthesized from

the epoxide **7c** and the mercaptan **8a** according to the same procedure used to make **9b**: TLC (SiO_2) R_f (50% ethyl acetate/hexane) 0.53; HPLC (5 μ, 2 mL/min) R_T 4.32 min; mass spectrum (CI) m/e 651 ($M^+ + 1$); NMR ($CDCl_3$) similar to that of **9b**.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid (10b). The lactone **9b** (1.9 g, 0.00327 mol) in aqueous sodium hydroxide (0.72 g, 0.018 mol in 50 mL water) was stirred at room temperature overnight. The pH of the reaction mixture was then adjusted to 3.5 with concentrated hydrochloric acid, and the resulting product was collected: 1.45 g (91%); $[\alpha]^{25}_D$ −34.4° (c 0.3, CH_3OH); FD mass spectrum m/e 489 ($M^+ + 1$); NMR (Me_2SO-d_6) δ 0.85 (t, 3 H), 1.25 (br s, 18 H), 1.35 (br s, 2 H), 1.60 (m, 2 H), 2.0 (br s, 2 H), 2.3 (m, 2 H), 2.8 (m, 2 H), 3.3 (dd, 1 H), 3.6 (br s, 1 H), 3.8 (m, 3 H), 5.5 (m, 2 H). Anal. $C_{24}H_{44}N_2O_6S\cdot1^1/_4H_2O$.

4(*S*)-Hydroxy-5(*R*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid (10a). A solution of **9a** (0.6 g, 1 mmol) in aqueous sodium hydroxide (0.22 g, 5.4 mmol in 15 mL water) was stirred at room temperature overnight. The pH was adjusted to 3.5, and the product (0.35 g (73%) was filtered: $[\alpha]^{20}_D$ +39.5° (c 1, CH_3OH); FD mass spectrum m/e 489 ($M^+ + 1$); NMR identical with that of **10b**. Anal. $C_{24}H_{44}N_2O_6S\cdot1^1/_4H_2O$.

4(*R*)-Hydroxynonadecanoic Acid, γ-Lactone (11a). A solution of **9a** (0.7 g, 1.2 mol) in absolute ethanol (0.5 mL) was added to a suspension of freshly activated Raney nickel in absolute ethanol (15 mL). The reaction mixture was heated to reflux for 0.5 h. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The residue was triturated with methylene chloride and hexane and filtered. The filtrate was concentrated and the product purified by two consecutive flash chromatographies (chloroform and 15% ethyl acetate in hexane, respectively): TLC (SiO_2) R_f ($CHCl_3$) 0.42, R_f (15% EtOAc/hexane) 0.32; mass spectrum (CI) m/e 297 ($M^+ + 1$); $[\alpha]^{22}_D$ +17.5° (c 1, CH_3OH);[19] NMR ($CDCl_3$) δ 0.9 (br t, 3 H), 1.25 (br s, 26 H), 1.65 (br s, 2 H), 2.2 (m, 4 H), 4.53 (t, 1 H).

4(*S*)-Hydroxynonadecanoic Acid, γ-Lactone (11b). The title compound was prepared as in **11a**: TLC and NMR identical; mass spectrum (CI) m/e 297 ($M^+ + 1$); $[\alpha]^{22}_D$ −17.2° (c 1, CH_3OH) [lit.[19] $[\alpha]^{26}_D$ −28.7° (c 0.3, CH_3OH)].

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-tetradecenoic Acid (12). The title compound was prepared from **9e** by saponification as in **10b**: TLC (SiO_2) R_f ($CH_2Cl_2/CH_3OH/NH_4OH$ (2:2:1)) 0.60; mass spectrum (FD) m/e 419 ($M^+ + 1$); NMR (Me_2SO-d_6) similar to that of **10b**. Anal. $C_{19}H_{34}N_2O_6\cdot2Na(-2\ H)\cdot^3/_4H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-tetracosenoic Acid (13). The title compound was synthesized from **9f** by saponification as described for **10b**: TLC (SiO_2) R_f ($CH_2Cl_2/CH_3OH/NH_4OH$ (2:2:1)) 0.65; mass spectrum (FD) m/e 559 ($M^+ + 1$); NMR (Me_2SO-d_6) similar to that of **10b**. Anal. $C_{29}H_{54}N_2O_6S\cdot Na(-H)\cdot1^1/_2H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carbamoylmethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid (14). A mixture of lactone methyl ester **9** (0.41 g, 0.7 mmol) in 10 mL of dimethoxyethane, 10 mL of methanol, and 20 mL of concentrated ammonium hydroxide solution was stirred at 0 °C for 2 h. The mixture was neutralized in the cold with concentrated hydrochloric acid to pH 7.5. The crude product was partitioned into methylene chloride. The aqueous phase was further extracted, 2 × 40 mL methylene chloride. The combined extracts were washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to an oil. Flash column chromatography (silica gel, 1.5 in. × 6 in., 3%, $CH_3OH/CHCl_3$) gave the glycinamide lactone: 180 mg (64%); mass spectrum (FD) m/e 582 (M^+). A mixture of 120 mg (0.2 mol) of the γ-lactone in 5 mL of 0.2 M sodium hydroxide solution was stirred at room temperature for 18 h. The mixture was acidified to pH 3 by adding a concentrated hydrochloric acid solution in an ice bath. The resulting precipitates were filtered and washed quickly with cold water and dried at 56 °C for 24 h to give the product: 38 mg (39%); mass spectrum (FD) m/e 487 (M^+); $[\alpha]^{25}_D$ −25.9°. Anal. $C_{24}H_{45}N_3O_5S\cdot^3/_4H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenoic Acid, γ-Lactone (16). A solution of **10b** (0.194 g, 4 mol) and trifluoroacetic acid in methylene chloride (17 mL of 1% solution) was stirred for 2 h at room temperature. The reaction mixture was concentrated, and the residue was azeotroped with diethyl ether to give 0.094 g (50%) of **16**: mass spectrum (FD) m/e 471 ($M^+ + 1$); ^{13}C NMR ($CDCl_3$) δ 72 (OC). Anal. $C_{24}H_{42}N_2O_5\cdot1^3/_8CF_3CO_2H$.

4(*R*)-Hydroxy-5(*S*)-[[3-[(carboxymethyl)amino]-3-oxopropyl]thio]-6(*Z*)-nonadecenamide (15). A mixture of 0.9 g (1.4 mmol) of **16** in 3.8% anhydrous ammonia/ethanol solution (100 mL) was stirred at room temperature for 2 days. The reaction mixture was concentrated to dryness. The residue was azeotroped with methylene chloride to give 0.9 g (100%) of off-white powder. The hygroscopic material was dissolved in 10% NaOH solution (5 mL) and chromatographed on a column of XAD-7 resin. After it was washed with H_2O (100 mL), the column was eluted with aqueous methanol solution (1:1) to give the desired product as a white amorphous powder after lyophylization: mass spectrum (FD) m/e 488 ($M^+ + 1$), 510 ($M^+ + Na$). Anal. $C_{24}H_{44}N_3O_5S\cdot Na(-H)\cdot2H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*E*)-nonadecenoic Acid (17b). A solution of **9b** (1.1g, 1.9 mmol) and diphenyl disulfide (1.7 g, 7.8 mmol) in toluene (1.5 L) was irradiated with cooling with a 210-W lamp (Conrad-Hanovia mercury vapor) through a Pyrex filter for 5.5 h. The crude product after evaporation of toluene was flash chromatographed on silica, eluting with 40% ethyl acetate in hexane to give 450 mg (41%) of the 6*E* lactone **9h**: mass spectrum (CI) m/e 581 ($M^+ + 1$); NMR ($CDCl_3$) δ 3.7 (m, 1 H), 5.3 (m, 1 H, vinyl, $J_{6,7}$ = 15 Hz), 5.78 (m, 1 H, vinyl, $J_{6,7}$ = 15 Hz). A solution of **9h** (0.45 g, 0.78 mmol) in aqueous sodium hydroxide solution (0.17 g, 4.3 mmol, 13 mL) was stirred at room temperature for 24 h. The pH was adjusted to 3.5 with concentrated hydrochloric acid solution to give **17b**: 0.24 g (63%); $[\alpha]^{24}_D$ +67.9° (c 0.5, CH_3OH); mass spectrum (FD) m/e 489 ($M^+ + 1$); NMR (Me_2SO-d_6) δ 5.38 (m, 1 H, vinyl, $J_{6,7}$ = 15 Hz), 5.55 (m, 1 H, vinyl, $J_{6,7}$ = 15 Hz). Anal. $C_{24}H_{44}N_2O_6S\cdot1^1/_8H_2O$.

4(*S*)-Hydroxy-5(*R*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*E*)-nonadecenoic Acid (17a). The title compound was synthesized from **9b** similarly to **17b**. Mass spectrum (FD) m/e 489 ($M^+ + 1$); mp 136–139 °C. Anal. $C_{24}H_{44}O_2O_6S\cdot1^1/_8H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[2-amino-3-[(carboxymethyl)-amino]-3-oxopropyl]thio]-6(*Z*)-nonadecen-1-ol (18). To a suspension of lithium borohydride (0.45 g, 0.02 mol) in diglyme (40 mL) under argon at 0 °C was added dropwise a solution of the lactone **16** (1.9 g, 0.004 mol) in diglyme (30 mL). The reaction mixture was stirred at room temperature overnight, quenched with cold 10% hydrochloric acid solution, extracted with ethyl acetate, dried, and concentrated in vacuo. The crude product was purified on a C_{18} reversed-phase flash column, eluting with 40% acetonitrile in 0.01 M NaH_2PO_4 at pH 6.5. Evaporation of the eluent afforded **18**: 0.43g (24%); HPLC (Lichrosorb RP-18, 5 μ, 210 nm) R_T 5.46 min (50% CH_3CN/0.01 M NaH_2PO_4, pH 6.5, 1 mL/min); mass spectrum (FD) m/e 475 ($M^+ + 1$); NMR (Me_2SO-d_6) δ 0.9 (br t, 3 H), 1.3 (br s, 18 H), 2.1 (m, 4 H), 2.85 (m, 2 H), 3.40 (m, 6 H), 3.7 (m, 5 H), 5.40 (M, 2 H), 8.25 (br s, 1 H). Anal. $C_{24}H_{46}N_2O_5S\cdot^3/_4H_2O$.

4(*R*)-Hydroxy-5(*S*)-[[3-[(hydroxyethyl)amino]-2-amino-3-oxopropyl]thio]-6(*Z*)-nonadecen-1-ol (19). To a suspension of lithium borohydride (0.113 g, 0.051 mol) in diglyme (10 mL) under argon at 0 °C was added dropwise a solution of **9b** (0.5 g, 8.6 mol) in diglyme (15 mL). The reaction mixture was allowed to warm gradually to room temperature over 4 h and then quenched with cold 10% hydrochloric acid solution. The aqueous mixture was then extracted with ethyl acetate, dried, and evaporated. The crude product was purified by flash chromatography through a silica gel column, eluting with 7–10% methanol in methylene chloride solution to give 0.1 g (25%) of product: mass spectrum (CI) m/e 461 ($M^+ + 1$); NMR ($CDCl_3$) δ 0.9 (br t, 3 H), 1.3 (br s, 18 H), 1.69 (m, 4 H), 2.1 (m, 2 H), 3.38 (br s, 6 H), 3.7 (m, 5 H), 5.5 (m, 2 H), 7.8 (br t, 1 H). Anal. $C_{24}H_{48}N_2O_4S\cdot^1/_2H_2O$.

4(*S*)-Hydroxy-5(*R*)-[(2-amino-2-carboxyethyl)thio]-6-(*Z*)-nonadecenoic Acid, Sodium Salt (20a). A partial suspension of γ-lactone **9d** (0.3 g, 0.57 mmol) in aqueous sodium hydroxide (0.205 g, 0.004 mol in 13 mL water) was stirred for 24 h at room temperature. The pH of the reaction mixture was then

adjusted to 3.5 with concentrated hydrochloric acid to give **20a**: 1.32 g (54%); mass spectrum (FD) m/e 432 (M^+ + 1). Anal. $C_{22}H_{41}NO_5S{\cdot}Na(-H)$.

4(*R*)-Hydroxy-5(*S*)-[(2-amino-2-carboxyethyl)thio]-6-(*Z*)-nonadecenoic Acid, Sodium Salt (20b). The reaction was carried out as described for **20a** from **9c** to give **20b**: 0.087 g (35%); mass spectrum (FD) m/e 432 (M^+ + 1). Anal. $C_{22}H_{41}NO_5S{\cdot}1.5Na(-1.5\ H)$.

Acknowledgment. The authors gratefully acknowledge the assistance of D. Holden, L. Vickery, and J. DeVan for conducting the biological testing. Special thanks are due to Dr. C. Perchonock for helpful discussions, K. Erhard and S. Skiendzielewski for their chemical support, E. Reich for microanalysis, G. Roberts, W. Johnson, M. Mentzer, and L. Kilmer for mass spetra, and D. Staiger for some ^1H and ^{13}C NMR studies.

Registry No. **1**, 3878-55-5; **2**, 1490-25-1; **3**, 13865-19-5; **4**, 98303-59-4; **5**, 98303-60-7; **6** (n = 11), 15510-55-1; **6** (n = 6), 13423-48-8; **6** (n = 16), 54907-67-4; **7a**, 98392-67-7; **7b**, 98303-61-8; **7c**, 98303-62-9; **8a**, 75290-62-9; **8b**, 1577-62-4; **9a**, 95405-68-8; **9b**, 95351-90-9; **9b** amide (detrifluoracetylated), 98303-63-0; **9c**, 98461-30-4; **9d**, 98461-31-5; **9e**, 98303-64-1; **9f**, 98330-11-1; **9h**, 98392-68-8; **10a**, 88903-81-5; **10b**, 88477-96-7; **11a**, 98303-65-2; **11b**, 98303-66-3; **12**·2Na, 98330-12-2; **13**·2Na, 98303-67-4; **14**, 95351-98-7; **15**·Na, 98303-68-5; **16**, 98303-69-6; **16**·$x$$CF_3CO_2H$, 98392-69-9; **17a**, 88903-82-6; **17b**, 88903-83-7; **18**, 98303-70-9; **19**, 98303-71-0; **20a**, 88847-39-6; **20a**·Na, 95462-55-8; **20b**, 88903-84-8; **20b**·3Na, 98392-70-2; LTD_4, 73836-78-9; $(Ph)_3P{=}CHCHO$, 2136-75-6; 1-bromotridecane, 765-09-3; triphenylphosphine, 603-35-0.

Factors Affecting Binding of *trans*-*N*-[2-(Methylamino)cyclohexyl]benzamides at the Primary Morphine Receptor

B. Vernon Cheney,* Jacob Szmuszkovicz, Robert A. Lahti, and Dominic A. Zichi

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001. *Received January 11, 1985*

In this paper, we describe the synthesis of a series of *trans*-*N*-[2-(methylamino)cyclohexyl]benzamides possessing morphine-like pharmacological properties. The affinity of the compounds for the agonist and antagonist states of the μ opioid receptor has been established by means of an in vitro binding assay. We have investigated the geometry and electronic structure of the molecules using molecular mechanics and an ab initio SCF-MO procedure with FSGO basis sets. Comparison to naloxone reveals properties of possible importance in receptor association. We have considered both the *S*,*S* and *R*,*R* isomers in the binding model. Statistical analyses imply that three factors play a significant role in binding: (1) membrane–water partitioning, (2) the capacity of the aromatic ring and amine N-substituent to act as electron acceptors, (3) the conformational energy required to attain the binding configuration.

Recent research in our laboratories has led to the discovery of a series of compounds, *trans*-*N*-[2-(methylamino)cyclohexyl]benzamides,[1] that exhibit morphine-like pharmacological properties, although there is little obvious structural resemblance to classical opiate analgesics. Owing to the novel arrangement of key structural features, the benzamide amines serve as useful probes to investigate factors affecting affinity for the primary morphine (μ) receptor. In this report, we discuss the synthesis of these compounds, the results obtained from tests of biological activity (including an in vitro receptor binding assay), and our efforts to determine the properties of these molecules that play an important role in drug–receptor association.

Among the diverse chemical agents with affinity for the morphine receptor, two common structural entities are found: a basic amino group and an aromatic ring.[2-4] Several investigators[3-7] have suggested that effective receptor interactions depend upon the drug assuming a conformation in which the key aromatic ring and basic nitrogen exhibit a spatial relationship similar to that of morphine. In certain classes of these agents, the presence of an allyl or cyclopropylmethyl substituent on the basic nitrogen causes the drug to act as a morphine antagonist or a mixed agonist–antagonist. The orientation of such an N-substituent with respect to other key features has been suggested as a factor in determining the degree of antagonist character.[6-8] These structure–activity relationships provide a basis for comparing the benzamide-amine geometry and electronic structure with that of a reference drug having high specificity for the μ receptor and strong affinity for both the agonist and antagonist states. We have selected naloxone as a suitable reference for structural comparisons and as the radiolabeled marker in the receptor binding assay.

In order to make detailed comparisons of the test and reference molecules, we have examined the geometry and electronic structure of the benzamide amines using the same computational methods employed in a previous study of naloxone.[9] An investigation of the "free-molecule" and "receptor-binding" conformational states was carried out by means of molecular mechanics,[10] a technique utilizing empirical potential functions to represent intramolecular interactions. Characterization of the ground-state elec-

(1) Szmuszkovicz, J.; VonVoigtlander, P. F. *J. Med. Chem.* **1982**, *25*, 1125.
(2) Eddy, N. B.; May, E. L. *Science* **1973**, *181*, 407.
(3) Lewis, J. W.; Bentley, K. W.; Cowan, A. *Ann. Rev. Pharmacol.* **1971**, *11*, 241.
(4) Casy, A. F. "A Guide to Molecular Pharmacology-Toxicology", Part 1; Featherstone, R. M., Ed.; Marcel Dekker: New York, 1973; pp 217–278.
(5) Beckett, A. H.; Casy, A. F. *J. Pharm. Pharmacol.* **1954**, *6*, 986.
(6) Feinberg, A. P.; Creese, I.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4215.
(7) Cheney, B, V.; Duchamp, D. J.; Christoffersen, R. E. "Quantitative Structure–Activity Relationships of Analgesics, Narcotic Antagonists, and Hallucinogens"; NIDA Research Monograph 22; Barnett, G., Trsic, M., Willette, R. E., Eds., National Institute on Drug Abuse: Washington, DC, 1978; pp 218–249.
(8) Loew, G.; Berkowitz, D. *J. Med. Chem.* **1975**, *18*, 656.
(9) Cheney, B. V.; Zichi, D. A. *Int. J. Quantum Chem. Quantum Biol. Symp.* **1981**, *8*, 201.
(10) Duchamp, D. J. "Computer Assisted Drug Design"; Olson, E. C., Christoffersen, R. E., Eds.; American Chemical Society: Washington, DC, 1979; ACS Symp. Ser. No. 112, pp 79–102.

0022-2623/85/1828-1853$01.50/0 © 1985 American Chemical Society