dition of serine borate respond appreciably differently to all spasmogens after addition of serine borate. The tissues were preincubated with test compounds for 5 min before challenge with leukotriene. Values of IC_{50} were reproducible in this assay within an error of less than 30%. FPL 55712, a standard antagonist of LTC_4-induced contractions, had an IC_{50} of 0.51 ± 0.13 μM (mean ± SD; N = 9) against 0.2 nM LTC_4 in this assay.

Leukotriene-Mediated Anaphylaxis in Guinea Pigs. Male Hartley guinea pigs were immunized by ip injections of ovalbumin and *Salmonella typhosa* lipopolysaccharide adjuvant (2.7 mg/kg and 20 μg, ip, respectively). Two weeks later, six guinea pigs were placed into separate compartments of an aerosol chamber and were challenged for 30 s with ovalbumin solution (1% w/v) aerosolized by a DeVilbiss nebulizer. Ten minutes after the start of the challenge, the animals were removed from the aerosol chamber and the incidence of deaths at 30 min postchallenge was recorded. Test compounds were administered orally as solutions in PEG 400 1 h before antigen challenge. Twenty minutes before challenge, the animals were pretreated with pyrilamine (2 mg/kg, ip), indomethacin (10 mg/kg, ip), and propranolol (1 mg/kg, ip). Activity is expressed as the percent inhibition of mortality compared to vehicle-treated animals. Significance was determined by a χ^2 test. The standard inhibitor phenidone (50 mg/kg, ip) inhibited mortality 83% ($p < 0.05$) with this protocol.

Arachidonic Acid Induced Murine Ear Inflammation. This assay was carried out according to Young et al.[12] Topically applied arachidonic acid produces an inflammatory reaction characterized by increased vascular permeability and infiltration of PMNs. One ear of male DBA/2J mice was treated topically with test compound or ethanol vehicle 60 min before the application of arachidonic acid (2 mg/ear). Increases in ear weight due to arachidonic acid treatment were determined 1 h later. Ear swelling, taken as a measure of inflammation, was compared in drug- and vehicle-treated groups. Statistical differences were determined by using Student's t test. AA-861, a standard inhibitor of 5-lipoxygenase, gave an ED_{50} of 0.15 mg/ear by this protocol.

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3,4-Dihydro-2*H*-1-benzopyran-2-carboxylic Acids and Related Compounds as Leukotriene Antagonists

Noal Cohen,*,† Giuseppe Weber,† Bruce L. Banner,† Rocco J. Lopresti,† Beatrice Schaer,† Antonino Focella,† Gladys B. Zenchoff,† Anne-Marie Chiu,† Louis Todaro,† Margaret O'Donnell,‡ Ann F. Welton,‡ Deborah Brown,‡ Ralph Garippa,‡ Herman Crowley,‡ and Douglas W. Morgan‡

Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received January 30, 1989*

Evaluation of a series of 3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acids linked to the 2-hydroxyacetophenone pharmacophore present in the standard peptidoleukotriene antagonist FPL 55712 (1) has led to the discovery of Ro 23-3544 (7), an antagonist possessing greater potency and duration of action vs LTD_4 than the standard (aerosol route of administration, guinea pig bronchoconstriction model). Interestingly, this compound also potently inhibited bronchoconstriction induced by LTB_4 whereas 1 did not. Attempts to establish structure–activity relationships in this series involved modifications in the 2-hydroxyacetophenone moiety, the linking chain, and the chroman system. All variations produced analogues which were either inactive or possessed reduced potency relative to acid 7. Optical resolution of 7 was achieved by two methods. Absolute configurations of the enantiomers were determined via X-ray crystallographic analyses of an intermediate as well as a salt of the *S* enantiomer. Although the enantiomers exhibited similar potencies in in vitro assays and in vivo when administered intravenously, significant differences were observed in the guinea pig bronchoconstriction model vs LTC_4 and LTD_4 when administered by the aerosol route (*S* antipode 15-fold more potent). The properties of 7 have been compared with several recently reported leukotriene antagonists.

Nearly a decade has passed since Samuelsson's elucidation of the peptidoleukotriene (LT) structures and the confirmation that these novel lipid derivatives comprise the slow-reacting substance of anaphylaxis (SRS, SRS-A).[1] During this period, pure LTC_4, LTD_4, LTE_4, and radiolabeled versions thereof have become readily available through total synthesis[2] thus facilitating the establishment of pharmacological assays to detect novel LT antagonists and biosynthesis inhibitors. Not surprisingly, a major worldwide effort to discover such compounds has ensued.[3] The motivation for this explosion in synthetic, medicinal, and biochemical research has been provided by the steadily accumulating evidence that the LTs are intimately involved in the mediation of many serious allergic and inflammatory disorders.[4] On the other hand, since mediators such as histamine, platelet activating factor, thromboxane, and chemotactic peptides may also be involved, the relative importance of the LTs in these disease states will only be defined upon clinical evaluation of selective LT antagonists and biosynthesis inhibitors. It appears that we are finally approaching the time when such information will be available as clinical results involving the first

† Chemistry Research Department.
‡ Pharmacology Department.

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(4) (a) Feuerstein, G.; Hallenbeck, J. M. *FASEB J.* **1987**, *1*, 186. (b) Perchonock, C. D.; Torphy, T. J.; Mong, S. *Drugs Future* **1987**, *12*, 871. (c) Piper, P. J. In ref. 1a, Chapter 8.

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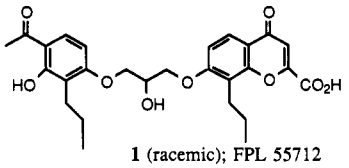
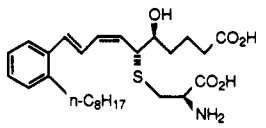
generation of these novel drugs are now being reported.[5]

At the time of our initial studies in this area, two leads existed upon which the design of novel peptido LT antagonists could be based. These were the structures of the agonists themselves, and the prototype SRS-A antagonist FPL 55712 (1).[6] In the early 1980s, as an outgrowth of



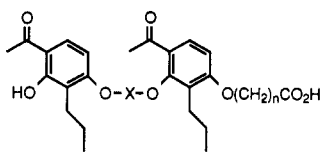
1 (racemic); FPL 55712



2 (5S,6R)

our synthetic work on the natural LTs,[2c,d] we prepared various analogues of LTE_4, such as compound **2**, in which olefinic linkages in the agonist structure were replaced with aromatic rings.[7] Many other variations were incorporated as well in an effort to develop structure–activity relationships for both agonist and antagonist activities. Several of these analogues exhibited in vitro antagonism of SRS-A-induced contractions of guinea pig ileum. Unfortunately, after extensive study, very little in vivo antagonism of LTE_4-induced bronchoconstriction in guinea pigs could be detected and work on this series was abandoned. It should be noted that other aromatic peptido LT analogues have recently been reported, some apparently possessing potent and selective in vivo activity.[8]

Given the disappointing results with our LT analogue program, we sought other structural types and turned to the lead represented by chromone acid **1**. Although **1** itself was never developed clinically, this class has been vigorously investigated and a substantial number of related acidic 2-hydroxyacetophenones have been prepared in recent years, certain of which are currently undergoing clinical trials in asthma.[3a,c,5] We were particularly cognizant of the work of LeMahieu and co-workers in our laboratories, who had found that (aryloxy)alkanoic acids of the type **3** were potent in vivo antagonists of LTE_4



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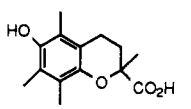
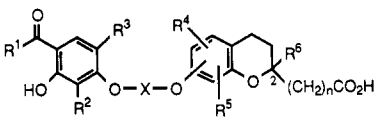


4 (racemic)

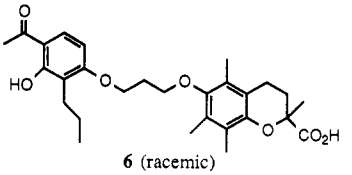
(aerosol route of administration).[9] Thus the concept evolved to prepare acids of the generic structure **5**, related to both **1** and **3**, in which the acidic moiety was part of a 3,4-dihydro-2*H*-1-benzopyran (chroman) ring tethered to the hydroxyacetophenone pharmacophore. Of particular



5

interest, these chromans, possessing a chiral center at C-2, afforded the possibility of probing the stereochemical requirements of the LT receptor sites via antagonists not of the LT structural type. While it had been demonstrated that the activity (agonist vs antagonist) and potency of peptido LTs and their analogues were strongly related to their stereochemistry,[10] nothing was known, in this regard, about the hydroxyacetophenone class of antagonists, all of which were achiral in the vicinity of the important acidic function.[11] Furthermore, the proposed compounds were novel, appeared to be easily preparable, and, hopefully, would exhibit pharmacological properties as peptido LT antagonists superior to the prototypes. Finally, we were encouraged in this direction by our previous extensive experience in chroman chemistry developed through research on the total synthesis of natural α-tocopherol[12] and the availability of potentially useful intermediates from these earlier studies such as the antioxidant acid **4** (Trolox).[13]

With this background, the first compound prepared in this series was acid **6** derived from **4**. It was encouraging



6 (racemic)

to find that **6** was, in fact, an antagonist of SRS-A-induced contraction of guinea pig ileum as well as an inhibitor of exogenous LTD_4-induced bronchoconstriction in guinea pigs when administered by the intravenous and aerosol routes although its potency was not superior to known compounds. We then synthesized and screened as antagonists of LTE_4 various chroman acids of the type **5**, incorporating certain significant SAR results obtained by LeMahieu and co-workers.[9] These efforts led to the discovery of Ro 23-3544 (**7**),[14] an analogue exhibiting sufficient

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(11) Recently, a chiral hydroxyacetophenone-type LT antagonist was prepared, the enantiomers of which were reported to possess very similar pharmacological properties in vitro and in vivo: Young, R. N.; Belanger, P.; Champion, E.; DeHaven, R. N.; Denis, D.; Ford-Hutchinson, A. W.; Fortin, R.; Frenette, R.; Gauthier, J. Y.; Gillard, J.; Guindon, Y.; Jones, T. R.; Kakushima, M.; Masson, P.; Maycock, A.; MacFarlane, C. S.; Piechuta, H.; Pong, S. S.; Rokach, J.; Williams, W. R.; Yoakim, C.; Zamboni, R. *J. Med. Chem.* **1986**, *29*, 1573.

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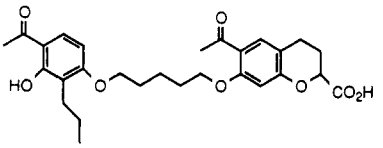
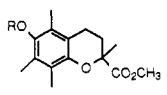


7 (racemic); Ro 23-3544

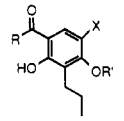
potency to warrant testing vs LTD_4 and LTC_4, which agonists had now become available for screening purposes through scale up of our total syntheses.[2d] Acid **7** was found to be the most potent antagonist of these LTs (aerosol route of administration) seen to that point in time. Herein we describe our attempts to develop structure–activity relationships in this series based on the lead **7**. In addition, we report in some detail on its pharmacology, its optical resolution, and a comparison of the LT antagonist properties of the enantiomers.

Chemistry

Compound **6** was prepared by alkylation of **8**[13] with allyl bromide followed by hydroboration of the terminal olefin



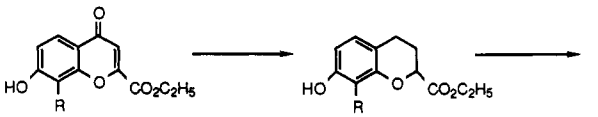
8; R= H
9; R= $CH_2CH=CH_2$
10; R= $(CH_2)_3OH$
11; R= $(CH_2)_3I$



12; R= CH_3; R'= H; X= H
13; R= CH_3; R'= $(CH_2)_3Br$; X= H
14; R= H; R'= H; X= H
15; R= CH_3; R'= H; X= Cl
16; R= CH_3; R'= 1,2-$CH_2C_6H_4(CH_2)_2OTHP$; X= H
17; R= CH_3; R'= 1,2-$CH_2C_6H_4(CH_2)_2OH$; X= H
18; R= CH_3; R'= 1,2-$CH_2C_6H_4(CH_2)_2OSO_2CH_3$; X= H
19; R= CH_3; R'= 1,3-$CH_2C_6H_4CH_2Br$; X= H
20; R= CH_3; R'= $(CH_2)_2C(CH_3)_2(CH_2)_2OTHP$; X= H
21; R= CH_3; R'= $(CH_2)_2C(CH_3)_2(CH_2)_2OH$; X= H
22; R= CH_3; R'= $(CH_2)_2C(CH_3)_2(CH_2)_2OSO_2CH_3$; X= H

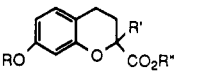
in ether **9**, giving hydroxy ester **10**. This sequence was necessitated by problems encountered with the direct alkylation of **8** with bromide **13** or 1,3-dibromopropane, probably due to steric factors. Coupling of the iodide **11** with dihydroxyacetophenone **12**,[6] in standard fashion, afforded the methyl ester of **6**.

The key 7-substituted chroman intermediates required were prepared starting from chromones **23**[6] and **26**[6] by catalytic hydrogenation,[15] giving **24** and **27**, respectively. Introduction of substituents at C-2 was accomplished by alkylation of the enolate generated from benzyl ether **28** with methyl iodide and allyl bromide. Hydrogenolysis of the resulting products **29** and **30** gave phenolic esters **32** and **31**, respectively. Dibal reduction of ester **25** produced



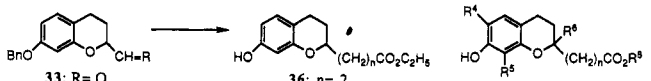
23; R= H
26; R= n-C_3H_7

24; R= H
27; R= n-C_3H_7



25; R= Bn; R'= H; R"= C_2H_5
28; R= Bn; R'= H; R"= CH_3
29; R= Bn; R'= CH_3; R"= CH_3
30; R= Bn; R'= $CH_2CH=CH_2$; R"= CH_3
31; R= H; R'= n-C_3H_7; R"= CH_3
32; R= H; R'= CH_3; R"= CH_3

aldehyde **33**, which upon Wittig–Horner homologation (via **34** and **35**) yielded the chain-elongated esters **36** and **37**.



33; R= O
34; R= $CHCO_2C_2H_5$
35; R= $CHCH=CHCO_2C_2H_5$

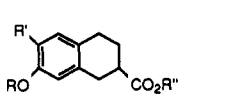
36; n= 2
37; n= 4

38 - 49

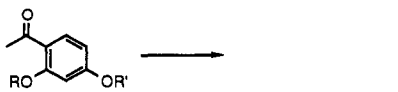
Intermediates bearing substituents in the aromatic portion of the chroman system (**38–49**) are summarized in Table I. Fries rearrangement of 7-acetoxy compounds derived from **24**, **27**, **36**, and **37** with boron trifluoride etherate–acetic acid (method A) afforded mainly the desired 6-acetyl derivatives **38–40**, **45**, and **46** with very minor quantities of the 8-acetyl isomers (e.g. **41**) being isolated. The isomers could be readily separated by column chromatography after reesterification necessitated by the concomitant occurrence of dealkylation. Similar results were obtained by direct treatment of phenols with boron trifluoride etherate–acetic acid (method B), giving **43** and **44** or, by using propionic acid, **42**. Upon chlorination, bromination, and nitration (methods C, D, and E) of **24**, selective electrophilic substitution at C-6 was again observed, allowing compounds **47–49** to be secured although somewhat less efficiently than the acetyl analogues.

The procedures employed for assembling the target acids described in Tables II–V are based on those described previously[6,9] and involve either alkylation of the 7-hydroxychroman with an excess of a 1,*n*-dibromoalkane followed by a subsequent coupling of the resulting (bromoalkoxy)chroman with a 2,4-dihydroxyacetophenone (methods F, G) or, alternatively, attachment of the linking unit first to the dihydroxyacetophenone (**16–22**, see Experimental Section for the synthesis of these intermediates) followed by coupling with **40** (method I, **82–84**). Intermediates were generally not completely characterized and the yields given in the tables refer to overall sequences including saponification of the penultimate esters.

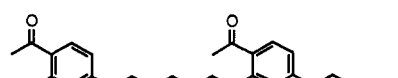
The naphthalene analogue **99** (a CH_2 for O isostere) was synthesized starting with the known hydroxy acid **50**[16] via



50; R= H; R'= H; R"= H
51; R= H; R'= CH_3CO; R"= H
52; R= H; R'= CH_3CO; R"= CH_3



53; R,R'= H
54; R= H; R'= $CH_2CO_2CH_3$
55; R= $Br(CH_2)_5$; R'= $CH_2CO_2CH_3$



56

intermediates **51** and **52**. Compound **56** is related to the LeMahieu series[9] and was viewed as an analogue of **7** in

(14) (a) A part of this work has been reported previously: Cohen, N.; Weber, G.; Banner, B. L.; Lopresti, R. J.; O'Donnell, M.; Welton, A. F.; Brown, D.; Crowley, H.; Zitelli, A. Presented at the 191st National Meeting of the American Chemical Society, April 13–18, New York, NY, 1986; Abstr. MEDI 44. Cohen, N.; Weber, G.; Banner, B. L.; Lopresti, R. J.; O'Donnell, M.; Welton, A. F.; Brown, D.; Garippa, R.; Crowley, H. Presented at the 191st National Meeting of the American Chemical Society, April 13–18, New York, NY, 1986; Abstr. MEDI 45. (b) O'Donnell, M.; Welton, A. F.; Crowley, H.; Brown, D.; Garippa, R.; Cohen, N.; Weber, G.; Banner, B.; Lopresti, R. J. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1987**, *17*, 512. (c) Eur. Pat. Appl. EP 129906 (U.S. Patents 4785017, Nov. 15, 1988 and 4788214, Nov. 29, 1988); F. Hoffmann-La Roche and Co. A. G.; *Chem. Abstr.* **1985**, *103*, 6223s. (d) Other chroman and chromanone leukotriene antagonists have been disclosed: Jpn. Kokai Tokkyo Koho JP 60 42378 (U.S. Patent 4665203, May 12, 1987); G. D. Searle & Co.; *Chem. Abstr.* **1985**, *103*, 160389. Eur. Pat. Appl. 150447 (U.S. Patent 4565882, Jan. 21, 1986); G. D. Searle & Co.; *Chem. Abstr.* **1986**, *104*, 5775. Carnathan, G. W.; Sanner, J. H.; Thompson, J. M.; Prusa, C. M.; Miyano, M. *Agents Actions* **1987**, *20*, 125.

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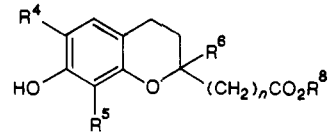
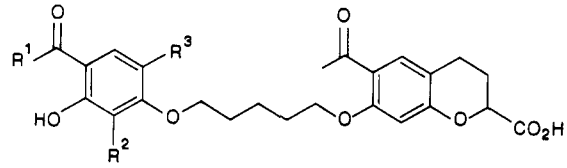
Table I. Data for 3,4-Dihydro-2*H*-1-benzopyran Acid Intermediates



compd	R^4	R^5	R^6	R^8	*n*	method of synthesis	starting material	% yield[a]	mp, °C	formula[b]
38	CH_3CO	H	H	C_2H_5	0	A	**24**	54	93.5–95.5[c]	$C_{14}H_{16}O_5$[d]
39	CH_3CO	n-C_3H_7	H	C_2H_5	0	A	**27**	80	oil	$C_{17}H_{22}O_5$[d]
40	CH_3CO	H	H	CH_3	0	A	**24**	63	135–137[e]	$C_{13}H_{14}O_5$
41	H	CH_3CO	H	CH_3	0	A	**24**	9	81–82.5[f]	$C_{13}H_{14}O_5$
42	C_2H_5CO	H	H	CH_3	0	B	**24**	63	103–104[g]	$C_{14}H_{16}O_5$
43	CH_3CO	H	CH_3	CH_3	0	B	**32**	51	oil[h]	$C_{14}H_{16}O_5$[d]
44	CH_3CO	H	n-C_3H_7	CH_3	0	B	**31**	64	112–115[c]	$C_{16}H_{20}O_5$[j]
45	CH_3CO	H	H	C_2H_5	2	A	**36**	54	oil[h]	$C_{16}H_{20}O_5$[d]
46	CH_3CO	H	H	C_2H_5	4	A	**37**	41	oil[h]	$C_{18}H_{24}O_5$[d]
47	Cl	H	H	CH_3	0	C	**24**	20	solid	$C_{11}H_{11}ClO_4$[d]
48	Br	H	H	C_2H_5	0	D	**24**	33	92–93.5[e]	$C_{12}H_{13}BrO_4$
49	NO_2	H	H	C_2H_5	0	E	**24**	56	105–106[i]	$C_{12}H_{13}NO_6$

[a] Yields of pure (^{1}H NMR, IR, UV, MS, TLC) product isolated by column chromatography and/or recrystallization. [b] C, H analyses when obtained were within ±0.4% of the calculated values. [c] Recrystallized from ethyl acetate–hexanes. [d] A microanalysis was not obtained on this compound. [e] Recrystallized from CCl_4. [f] Recrystallized from methanol–water. [g] Recrystallized from methanol. [h] Samples crystallized on standing. [i] Recrystallized from ethanol. [j] This compound did not provide a satisfactory C, H analysis.

Table II. Data for 3,4-Dihydro-2*H*-1-benzopyran-2-carboxylic Acids: Modifications in *o*-Hydroxyacetophenone Ring

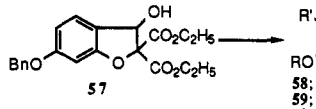


compd	R^1	R^2	R^3	method of synthesis	starting material	% yield[a]	mp, °C	formula[b]	% inhibn of LTD_4 binding at 10 μM (IC_{50}, μM)	% inhibn of LTD_4-induced bronchoconstriction[l] intravenous, 10 mg/kg (ID_{50}, mg/kg)	% inhibn of LTD_4-induced bronchoconstriction[l] aerosol, 1% sol. (IC_{50}, %)
7	CH_3	n-C_3H_7	H	F (G)	**12**, **38** (**40**)	41 (57)	113–115[c]	$C_{28}H_{34}O_8$	70 (4)	93 ± 3 (5)*	89 ± 1 (0.007)*
73	H	n-C_3H_7	H	F	**14**,[j] **40**	38	148–151[d]	$C_{27}H_{32}O_8$	0	0 ± 15[e]	NT
74	CH_3	H	H	F	**53**, **38**	40	183–185.5[d,f]	$C_{25}H_{28}O_8$	0	85 ± 5 (5)*	0 ± 9
75	CH_3	n-C_3H_7	OH	H	**7**	30	120–121[g]	$C_{28}H_{34}O_9$	0	0 ± 3	NT[h]
76	CH_3	n-C_3H_7	Cl	G	**15**,[k] **40**	45	85–102[i]	$C_{28}H_{33}ClO_8$	47	11 ± 19	10 ± 7

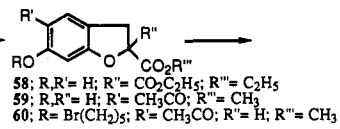
[a] Overall yield of pure (^{1}H NMR, IR, UV, MS, TLC), recrystallized product. [b] C, H analyses were within ±0.4% of the calculated values. [c] Recrystallized from hexanes–ethyl acetate. [d] Material directly precipitated by acidification of the sodium salt. [e] Tested at 1 mg/kg. [f] Recrystallized from ethyl acetate. [g] Recrystallized from acetonitrile. [h] Compound not tested because it decomposed on attempted nebulization. [i] Recrystallized from chloroform–hexanes—compound exhibited a broad melting range despite being pure by all spectral and chromatographic criteria. [j] Eur. Pat. Appl. 61800 (Fisons PLC); *Chem. Abstr.* **1983**, *98*, 89158. [k] U.S. Patent 4550190 (Hoffmann-La Roche Inc.); *Chem. Abstr.* **1986**, *104*, 148569. [l] An asterisk distinguishes values statistically significant using unpaired *t* test ($p < 0.05$); NT, not tested.

which the stereochemical constraints of the chroman ring have been removed, yielding a phenoxyacetic acid. This compound was prepared via **53**–**55**.

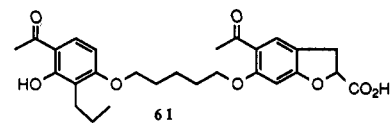
In order to secure the dihydrobenzofuran analogue **61**, we started with readily available hydroxy diester **57**,[17]



57



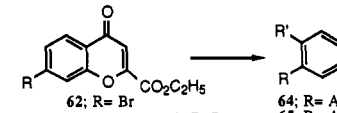
58; R,R'= H; R"= $CO_2C_2H_5$; R'"= C_2H_5
59; R,R"= H; R'= CH_3CO; R'"= CH_3
60; R= $Br(CH_2)_5$; R'= CH_3CO; R"= H; R'"= CH_3



61

which underwent double hydrogenolysis over palladium on carbon, under acidic conditions,[18] giving **58**. Exposure of the latter to Fries rearrangement conditions conveniently induced ester dealkylation and decarboxylation as well as affording, after reesterification, acetophenone **59**. Alkylation with 1,5-dibromopentane (→**60**) and standard completion of the sequence gave the target acid.

For the preparation of **85**, another CH_2 for O isostere, we utilized a palladium-catalyzed coupling[19] in order to obtain a key intermediate. Thus, treatment of bromochromone ester **62**[20] with 5-hexyn-1-ol in the presence of



62; R= Br
63; R= $HO(CH_2)_4C{\equiv}C$

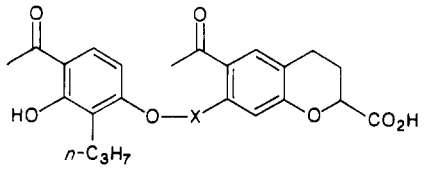
64; R= $AcO(CH_2)_6$; R'= H; R"= C_2H_5
65; R= $AcO(CH_2)_6$; R'= CH_3CO; R"= C_2H_5
66; R= $HO(CH_2)_6$; R'= CH_3CO; R"= CH_3
67; R= $CH_3SO_2O(CH_2)_6$; R'= CH_3CO; R"= CH_3

(17) Reichstein, T.; Oppenauer, R.; Grussner, A.; Hirt, R.; Rhyner, L.; Glatthaar, C. *Helv. Chim. Acta* **1935**, *18*, 816.

(18) Cf.: Plattner, J. J.; Fung, A. K. L.; Parks, J. A.; Pariza, R. J.; Crowley, S. R.; Pernet, A. G.; Bunnell, P. R.; Dodge, P. W. *J. Med. Chem.* **1984**, *27*, 1016.

(19) (a) Takahashi, S.; Kuroyama, Y.; Sonogashira, K.; Hagihara, N. *Synthesis* **1980**, 627. (b) Davies, S. G.; Mobbs, B. E.; Goodwin, C. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2597.

Table III. Data for 3,4-Dihydro-2*H*-1-benzopyran-2-carboxylic Acids: Modifications of Linking Chain

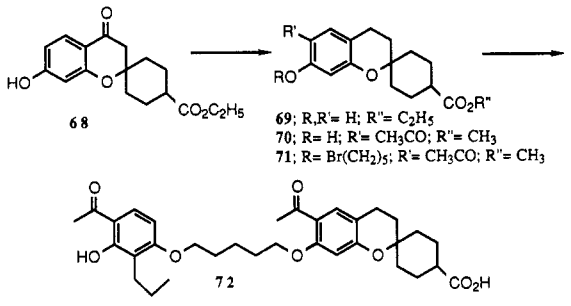


compd	X	method of synthesis	starting material	% yield[a]	mp, °C	formula[b]	% inhibn of LTD_4 binding at 10 μM (IC_{50}, μM)	% inhibn of LTD_4-induced bronchoconstriction[n]: intravenous, 10 mg/kg (ID_{50}, mg/kg)	% inhibn of LTD_4-induced bronchoconstriction[n]: aerosol, 1% sol. (IC_{50}, %)
77	$(CH_2)_3O$	G	**38**[c]	50	176–178.5[d]	$C_{26}H_{30}O_8$	63 (6)	35 ± 13	31 ± 9
78	$(CH_2)_4O$	F	**38**[e]	68	137–140[f]	$C_{27}H_{32}O_8$	26	36 ± 15	37 ± 12
79	$(CH_2)_6O$	F	**38**[g]	52	138.5–142[f]	$C_{29}H_{36}O_8$	96 (5)	66 ± 9 (5)*	36 ± 7
80	$(CH_2)_7O$	F	**38**[h]	39	133–135[f]	$C_{30}H_{38}O_8$	39	60 ± 12 (6)*	44 ± 9
81	$(CH_2)_2O(CH_2)_2O$	F	**38**[i]	60	83.5–86[f]	$C_{27}H_{32}O_9$	8	50 ± 5 (10)*	31 ± 12
82	1,2-$CH_2C_6H_4(CH_2)_2O$	I	**18**, **40**	24	171.5–173.5[j]	$C_{32}H_{34}O_8$	26	24 ± 13	12 ± 8
83	1,3-$CH_2C_6H_4CH_2O$	G	**19**, **40**	30	157–161[j]	$C_{31}H_{32}O_8$	60 (7)	20 ± 2	28 ± 15
84	$(CH_2)_2C(CH_3)_2(CH_2)_2O$	G[m]	**22**, **40**	31	oil	$C_{30}H_{38}O_8$[l]	68 (7)	15 ± 9	23 ± 11
85	$(CH_2)_6$	J	**67**	55	122–124.5[k]	$C_{29}H_{36}O_7$	20	46 ± 7	65 ± 12 (0.3)*

[a] Overall yield of pure (^1H NMR, IR, UV, MS, TLC), recrystallized or chromatographed product. [b] C, H analyses were within ±0.4% of the calculated values. [c] Linking chain introduced by alkylation with 1,3-dibromopropane. [d] Recrystallized from ethyl acetate. [e] Linking chain introduced by alkylation with 1,4-dibromobutane. [f] Recrystallized from ethyl acetate–hexanes. [g] Linking chain introduced by alkylation with 1,6-dibromohexane. [h] Linking chain introduced by alkylation with 1,7-dibromoheptane. [i] Linking chain introduced by alkylation with bis(2-bromoethyl) ether. [j] Recrystallized from ethyl acetate–ether–hexanes. [k] Recrystallized from acetonitrile. [l] This compound did not provide a satisfactory C, H analysis. [m] Mesylate rather than bromide used for alkylation. [n] An asterisk distinguishes values statistically significant using unpaired *t* test ($p < 0.05$).

$PdCl_2(PPh_3)_2$ and cuprous iodide furnished hydroxy ester **63**. Hydrogenation–hydrogenolysis of this acetylenic chromone over palladium on carbon, under acidic conditions (acetic acid containing H_2SO_4), produced chroman diester **64**. Friedel–Crafts acetylation then regioselectively afforded acetophenone **65**, which was converted into mesylate **67**. Coupling of the latter intermediate with **12** gave the methyl ester of **85** (method J).

Condensation of **53** with ethyl 4-oxocyclohexanecarboxylate,[21] in the presence of pyrrolidine,[22] gave chromanone **68** as a mixture of epimers. Reduction with bo-



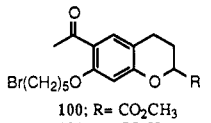
68

69; R,R'= H; R"= C_2H_5
70; R= H; R'= CH_3CO; R"= CH_3
71; R= $Br(CH_2)_5$; R'= CH_3CO; R"= CH_3

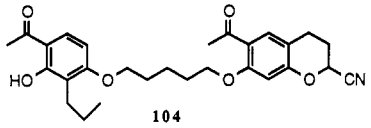
72

rane–boron trifluoride etherate[22] produced the corresponding chroman **69**. Fries rearrangement smoothly introduced the 6-acetyl group and after reesterification, a crystalline product was obtained. Recrystallization gave **70** as a single epimer of unknown relative stereochemistry, which was transformed, via **71**, into **72**, an analogue of **7** in which a spiro-fused cyclohexane ring has been inserted between the chroman ring and the carboxyl moiety.

Tetrazole[23] **91** was synthesized by treatment of nitrile **104** with azide (method K). The nitrile could be best obtained starting from bromo ester **100** via intermediates



100; R= CO_2CH_3
101; R= CO_2H
102; R= $CONH_2$
103; R= CN

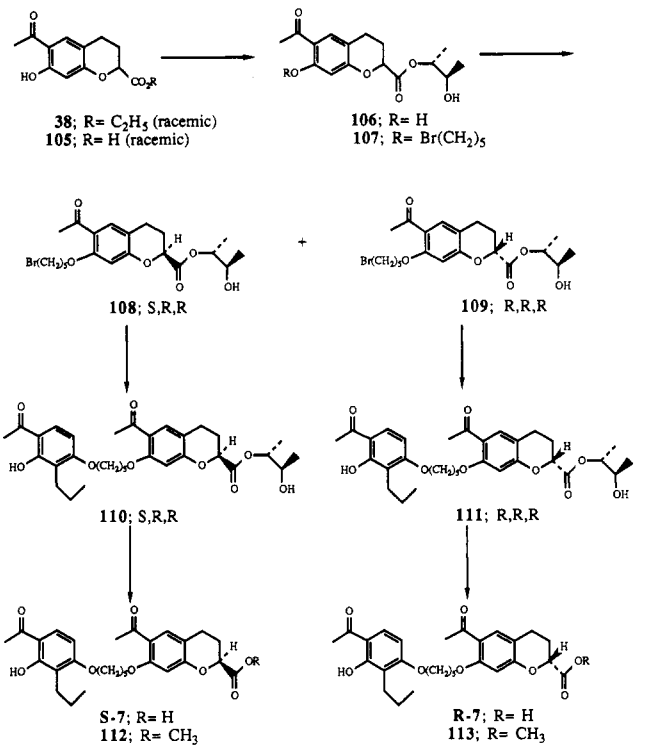


104

101–103. Another acidic derivative of **7**, acyl sulfonamide[24] **92** was produced upon exposure of **7** to benzenesulfonamide in the presence of a carbodiimide (method L). Finally, compound **75**, in which an additional hydroxyl function has been introduced into the hydroxyacetophenone ring, was prepared by oxidation of the sodium salt of **7** with potassium persulfate[25] (method H).

The enantiomers of **7** were available by two alternative approaches. In the first, racemic ester **38** was saponified and acid **105** was condensed (DCC, DMAP) with (2*R*,3*R*)-2,3-butanediol, giving the diastereomeric mixture of monoesters **106**. O-Alkylation of this material with 1,5-dibromopentane and potassium carbonate yielded the corresponding mixture of 5-bromopentyl ethers **107**. Separation of these diastereomers was achieved by preparative HPLC. The purity of the resultant isomers **108** and **109** was determined by LC and ^1H NMR analyses. In particular, the ^1H NMR signals for the methyl groups of the chiral auxiliary are doublets at 1.13 and 1.27 ppm in the 2*S* epimer **108** and at 1.20 and 1.26 ppm in **109**. In order to determine the absolute configurations of these compounds, an X-ray crystallographic analysis was carried out on **109**. A perspective drawing of this molecule is shown in Figure 1.

(20) Ellis, G. P.; Hudson, H. V. *J. Chem. Res. M* **1985**, 3830.
(21) Sanchez, I. H.; Ortega, A.; Garcia, G.; Larraza, M. I.; Flores, H. J. *Syn. Commun.* **1985**, *15*, 141.
(22) Kabbe, H.-J.; Widdig, A. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 247.
(23) Cf.: Marshall, W. S.; Goodson, T.; Cullinan, G. J.; Swanson-Bean, D.; Haisch, K. D.; Rinkema, L. E.; Fleisch, J. H. *J. Med. Chem.* **1987**, *30*, 682.
(24) Cf.: (a) Snyder, D. W.; Giles, R. E.; Keith, R. A.; Yee, Y. K.; Krell, R. D. *J. Pharmacol. Exp. Therap.* **1987**, *243*, 548. (b) Krell, R. D.; Giles, R. E.; Yee, Y. K.; Snyder, D. W. *Ibid.* **1987**, *243*, 557. (c) Eur. Pat. Appl. EP 179619 (ICI Americas Inc.); *Chem. Abstr.* **1986**, *105*, 226346.
(25) (a) Arora, U. *Ind. J. Chem.* **1984**, *23B*, 373. (b) Schock, R. U., Jr.; Tabern, D. L. *J. Org. Chem.* **1951**, *16*, 1772.



Each of the bromopentyl ethers was employed to alkylate 2,4-dihydroxy-3-propylacetophenone,[6] giving esters **110** and **111**, which were shown to be pure by LC and ^1H NMR analyses. Saponification afforded the enantiomeric acids *S*-**7** and *R*-**7**, respectively. Analysis of the methyl esters (**112**, **113**) of these acids by LC using a covalent (*R*)-phenylglycine Pirkle column[26] revealed them to possess enantiomeric purities which corresponded closely to the diastereomeric purities of the precursors, indicating that the synthetic sequence from **108** and **109** had avoided racemization despite the utilization of basic reagents. In this regard, it was found that aqueous solutions of the sodium salts of the antipodal acids exhibited no racemization after several days at room temperature.

The determination of enantiomeric purity of these acids requires some comment. Because of the relatively small $[\alpha]_D$ values exhibited by these compounds, polarimetry was not considered a reliable method.[27] While ^1H NMR studies using chiral lanthanide shift reagents revealed resolution of the C-2 proton in the methyl ester derivative, it was found that enantiomeric impurities of less than 10% would probably go undetected with this method. Thus the preferred procedure for e.e. determination was LC analysis of the methyl ester on the Pirkle column, which provided accurate and reproducible results. Small samples of either the acid or its salts (see below) could be conveniently converted to the methyl ester, without racemization, by treatment with boron trifluoride etherate in methanol.[28]

We also found that acid **1** itself could be resolved using the α-methylbenzylamines. The salts obtained were highly crystalline with the *R*,*S* or *S*,*R* salts being less soluble than the *R*,*R* or *S*,*S* forms. While this resolution was not extremely efficient, it did allow the production of gram quantities of the antipodes without recourse to chromatographic separations. In addition, an X-ray crystallographic analysis was carried out on the *R*-amine *S*-acid salt at 110° K, which provided not only an alternative determination of absolute configuration but also the first such structural study of an LT antagonist of the hydroxyacetophenone class. It was found that several atoms in the linking chain and the propyl moiety are disordered in the crystal. The major rotamer (ca. 65% of the molecules in the crystal) is shown in Figure 2 and the minor rotamer is shown in Figure 3. It is interesting to note, relative to the pharmacological activity of **7**, that in both rotamers, as well as the crystal structure of **109**, the carboxyl group occupies the axial-like conformation in the chroman ring. Also apparent is the skewed relationship of the aromatic rings and a lipophilic region defined by the propyl group and linking chain.

Results and Discussion

Our goal was the identification of an LTD_4 antagonist with greater potency and duration of action than **1** when administered by the most bioavailable route, namely via aerosol delivery directly into the lung. It was hypothesized that this would allow the most efficient testing of the utility of a leukotriene antagonist in asthma. Thus oral activity, while a desirable property, was not considered essential. As it turned out, compound **7** and all of the members of this series of acids, like **1**, were orally inactive. In this context, the compounds described herein were evaluated for their ability to block binding of radiolabeled LTD_4 to receptor sites in homogenized guinea pig lung and to inhibit exogenous, synthetic LTD_4-induced bronchoconstriction in anesthetized guinea pigs when administered by the aerosol and intravenous routes. All of the acids tested were racemic (except for the enantiomers of **7**; see below) or achiral and were screened in vivo as the sodium salts.

In our efforts to develop structure–activity patterns, we systematically varied compound **7** in three regions: (1) the hydroxyacetophenone ring (Table II), (2) the linking fragment (Table III), and (3) the chroman ring (Table IV). It was noted in the binding assay that while many members of this series show activity similar to **1** itself (Table V), their potencies are approximately 1000-fold lower than that exhibited by unlabeled LTD_4 (IC_{50} of 5 nM), suggesting a relatively weak interaction with the LTD_4 receptor. Thus, of the compounds demonstrating significant activity in the binding assay, all exhibit potencies represented by IC_{50} values in the 2–7-μM range. All modifications of the *o*-hydroxyacetophenone ring (Table II) resulted in loss of activity or potency in the binding assay when compared with that of **7**. It is interesting to note that **75** has been isolated upon exposure of **7** to rat liver supernates,[29] suggesting the 5-position as a potential site of metabolic transformation. Regarding the linking unit (Table III), it can be seen that binding activity comparable to that shown by **7** is maintained in **77** (shorter tether), **79** (longer tether), **83**, and **84** (tethers of equivalent length). In contrast, analogues **78**, **82** (shorter tether), **80** (longer tether), **81**, and **85** (isosteric replacement of CH_2 for O or vice versa) lack significant activity.[30] Table IV reveals that binding activity is preserved in acetyl isomer **87**, propanoic acid **89**, acyl sulfonamide derivative **92**, 2-alkylated homologues **97** and **98**, and naphthalene isostere **99**. Of the

(26) Pirkle, W. H.; Schreiner, J. L. *J. Org. Chem.* **1981**, *46*, 4988. A 25 cm × 4.6 mm i.d. covalent (*R*)-phenylglycine column was employed (mobile phase 10% ethanol in heptane; 1 mL/min flow; detection at 254 nm). The *R*-methyl ester is more strongly retained than the *S* antipode.

(27) Samples of the enantiomeric acids exhibited highly variable $[\alpha]^{25}_D$ values in several solvents. We thank Dr. F. Scheidl for these results.

(28) Kadaba, P. K. *Synthesis* **1971**, 316.

(29) Unpublished observations of F. Leinweber and R. Lucek, Department of Drug Metabolism, Hoffmann-La Roche Inc.

(30) Cf.: Gapinski, D. M.; Roman, C. R.; Rinkema, L. E.; Fleisch, J. H. *J. Med. Chem.* **1988**, *31*, 172.

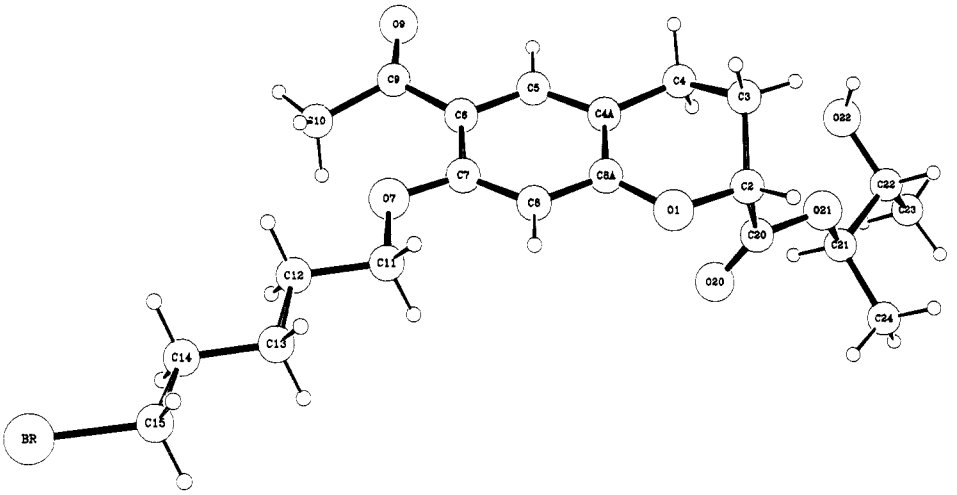


Figure 1. A perspective drawing of a molecule of **109**.

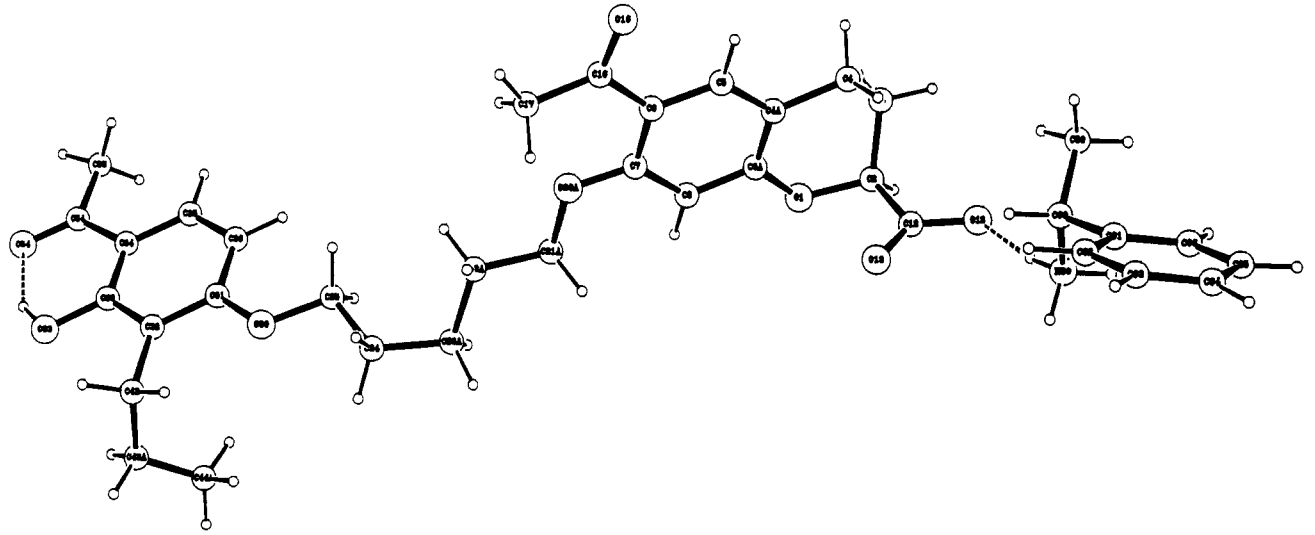


Figure 2. A perspective drawing of the major rotamer of *S*-7 (*R*)-α-methylbenzylamine salt. The dashed bonds indicate hydrogen bonding.

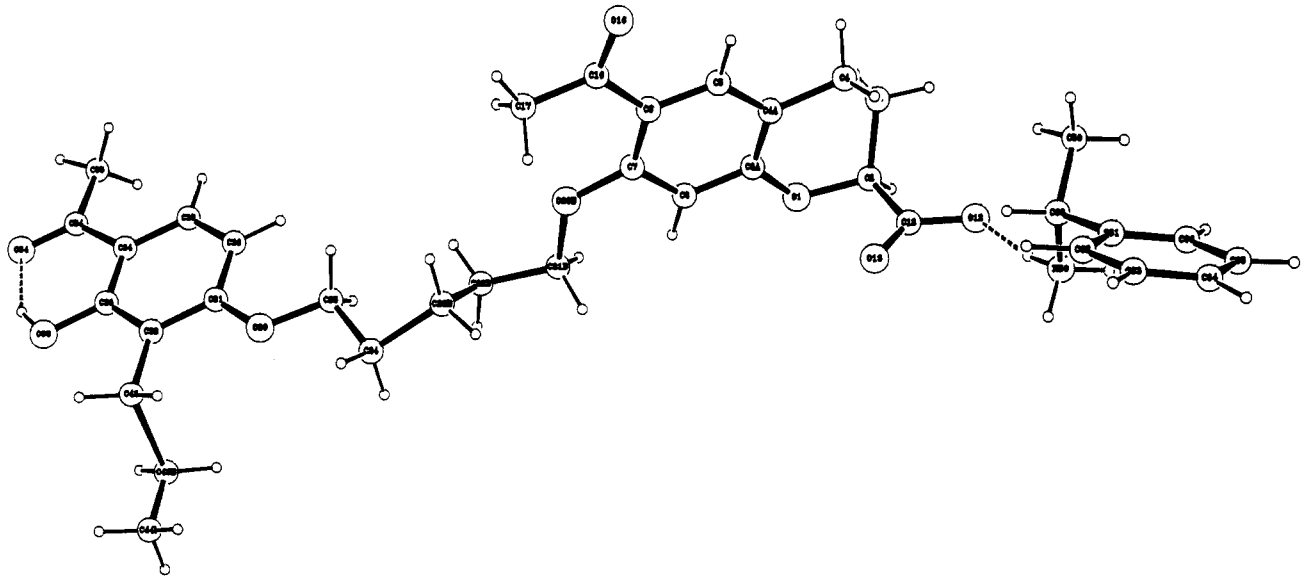
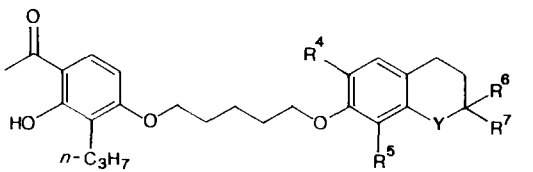


Figure 3. A perspective drawing of the minor rotamer of *S*-7 (*R*)-α-methylbenzylamine salt. The dashed bonds indicate hydrogen bonding.

miscellaneous structures delineated in Table V, all exhibit binding activity with the exception of benzofuran **61**.

In discussing the in vivo LT antagonist properties of this series, we will focus on the aerosol data since our goal was the development of a drug administered via this route. In doing so, we acknowledge certain caveats associated with aerosol drug delivery, including difficulties in controlling dosage and standardization of droplet size, factors which

Table IV. Data for 3,4-Dihydro-2*H*-1-benzopyran-2-carboxylic Acids and Related Compounds: Modification of Dihydrobenzopyran Moiety



compd	R^4	R^5	R^6	R^7	Y	method of synthesis	starting material	% yield[a]	mp, °C	formula[b]	% inhibn of LTD_4 binding at 10 μM (IC_{50}, μM)	% inhibn of LTD_4-induced bronchoconstriction[m] intravenous, 10 mg/kg (ID_{50}, mg/kg)	% inhibn of LTD_4-induced bronchoconstriction[m] aerosol, 1% sol. (IC_{50}, %)
86	H	H	H	CO_2H	O	F	**24**	35	124–126.5[d]	$C_{26}H_{32}O_7$	11	0 ± 0	50 ± 2 (1)*
87	H	CH_3CO	H	CO_2H	O	F	**41**	19	134–136[e]	$C_{28}H_{34}O_8$	72 (5)	19 ± 17	1 ± 10
88	CH_3CO	n-C_3H_7	H	CO_2H	O	F	**39**	36	oil	$C_{31}H_{40}O_8$	NT[c]	NT[c]	44 ± 7
89	CH_3CO	H	H	$(CH_2)_2CO_2H$	O	F	**45**	40	126.5–129[e]	$C_{30}H_{38}O_8$	94 (2)	86 ± 7 (5)*	31 ± 6
90	CH_3CO	H	H	$(CH_2)_4CO_2H$	O	F	**46**	20	116–120.5[e]	$C_{32}H_{42}O_8$	47	10 ± 3	NT[c]
91	CH_3CO	H	H	1-*H*-tetrazol-5-yl	O	K	**104**	95	137–139[f]	$C_{28}H_{34}N_4O_6$	60	0 ± 1[l]	NT[c]
92	CH_3CO	H	H	$CONHSO_2C_6H_5$	O	L	**7**	40	A.S.[g]	$C_{34}H_{39}NO_9S$	87 (2)	26 ± 7	53 ± 4 (1)*
93	Cl	H	H	CO_2H	O	G	**47**	27	111–120[e]	$C_{26}H_{31}ClO_7$	45	16 ± 2	0 ± 2
94	Br	H	H	CO_2H	O	G	**48**	22	68–72[h]	$C_{26}H_{31}BrO_7$	57	13 ± 11	0 ± 6
95	NO_2	H	H	CO_2H	O	G	**49**	73	148–149.5[i]	$C_{26}H_{31}NO_9$	48	18 ± 17	0 ± 7
96	C_2H_5CO	H	H	CO_2H	O	G	**42**	46	75–85[j]	$C_{29}H_{36}O_8$	52	7 ± 14	60 ± 7 (0.3)*
97	CH_3CO	H	CH_3	CO_2H	O	G	**43**	56	114–117[e]	$C_{29}H_{36}O_8$	64 (6)	50 ± 3 (10)*	85 ± 5 (0.2)*
98	CH_3CO	H	n-C_3H_7	CO_2H	O	G	**44**	64	oil	$C_{31}H_{40}O_8$[k]	70 (2)	56 ± 14 (8)*	51 ± 5 (0.9)*
99	CH_3CO	H	H	CO_2H	CH_2	G	**52**	64	154–156[e]	$C_{29}H_{36}O_7$	65 (7)	51 ± 9 (10)*	23 ± 6

[a] Overall yield of pure (^{1}H NMR, IR, UV, MS, TLC), recrystallized or chromatographed product. [b] Unless otherwise noted, C, H analyses were within ±0.4% of the calculated values. [c] Not tested. [d] Recrystallized from ethyl acetate. [e] Recrystallized from acetonitrile. [f] Recrystallized from ethanol. [g] Recrystallized from dichloromethane–ether-petroleum ether to give an amorphous solid exhibiting a broad melting range. [h] Recrystallized from aqueous acetone. [i] Recrystallized from ethyl acetate–hexanes. [j] Triturated with hexanes. [k] Compound did not provide satisfactory microanalytical data; however, the sodium salt, obtained by neutralization of the acid with 0.1 N NaOH in methanol, reverse phase column chromatography on C-18 silica gel, and freeze-drying, was obtained as a colorless, hydrated solid. Anal. Calcd for $C_{31}H_{39}O_8Na\cdot1.5H_2O$: C, 63.13; H, 7.18; Na, 3.89. Found: C, 63.15; H, 6.83; Na, 3.82. This sodium salt was used for pharmacological testing. [l] Tested at 1 mg/kg. [m] An asterisk distinguishes values statistically significant using unpaired t test ($p < 0.05$).

Table V. Miscellaneous LTD_4 Antagonists

compd	% inhibn of LTD_4 binding at 10 μM (IC_{50}, μM)	% inhibn of LTD_4-induced bronchoconstriction[c] intravenous, 10 mg/kg (ID_{50}, mg/kg)	% inhibn of LTD_4-induced bronchoconstriction[c] aerosol, 1% sol. (IC_{50}, %)
6	88 (3)	NT[a]	NT[b]
56	65 (5)	20 ± 5	46 ± 10 (1)*
61	12	25 ± 4	88 ± 2 (0.04)*
72	81 (4)	32 ± 6	83 ± 3 (0.08)*
1 (FPL 55712)	68 (4)	92 ± 2 (2)*	64 ± 5 (0.5)*

[a] Not tested vs LTD_4—exhibited 99 ± 1% inhibition vs LTE_4. [b] Not tested vs LTD_4—exhibited 53 ± 2% inhibition vs LTE_4. [c] An asterisk distinguishes values statistically significant using unpaired t test ($p < 0.05$).

Table VI. Comparison of in Vitro and in Vivo (Guinea Pig) Antagonism of LT-Mediated Events by 1 and 7

	7	1	p^a
trachea (pA_2)[b]	6.6 ± 0.3	6.0 ± 0.4	NS
trachea (slope)	1.1 ± 0.2	1.1 ± 0.3	NS
ileum (IC_{50}, μM)[c]	0.06 ± 0.01	0.05 ± 0.01	NS
LTD_4 binding (IC_{50}, μM)	4 ± 2	4 ± 3	NS
bronchoconstriction (intravenous)			
LTC_4 (ID_{50}, mg/kg)	3 ± 1	6 ± 2	NS
LTD_4 (ID_{50}, mg/kg)	5 ± 2	2 ± 0.3	NS
LTD_4 (duration, min)[d]	40 ± 5[e]	6 ± 0.2[e]	*
LTE_4 (ID_{50}, mg/kg)	1 ± 0.7	1 ± 0.3	NS
LTE_4 (duration, min)[d]	40 ± 5[e]	6 ± 0.4[e]	*
PAF	inact[f]	inact[f]	
histamine	inact[f]	inact[f]	
bronchoconstriction (aerosol)			
LTC_4 (IC_{50}, %)	0.012 ± 0.009	inact[h]	*
LTD_4 (IC_{50}, %)	0.007 ± 0.006	0.5 ± 0.4	*
LTD_4 (duration, min)[d]	150 ± 14[g]	60 ± 4[g]	*
LTE_4 (IC_{50}, %)	0.06 ± 0.05	0.8 ± 0.5	*
LTE_4 (duration, min)[d]	90 ± 5[g]	50 ± 20[g]	*
PAF	inact[h]	inact[h]	
histamine	inact[h]	inact[h]	
LTB_4 (IC_{50}, %)	0.002 ± 0.001	inact[h]	
arachidonic acid (IC_{50}, %)	0.5 ± 0.2	inact[h]	
antigen (IC_{50}, %)	0.027 ± 0.02	0.012 ± 0.01	NS
antigen (duration, min)[d]	250 ± 20[g]	270 ± 30[g]	NS

[a] Significance of 7 vs 1 using unpaired t test; an asterisk indicates $p < 0.05$ response. [b] LTD_4 challenge. [c] SRS-A challenge. [d] Duration of action defined as the time for inhibition to decrease to 40%. [e] Compounds were administered at a dose of 10 mg/kg iv. [f] Inact = no significant inhibition at a dose of 10 mg/kg iv. [g] Compounds were administered at a concentration of 1%, for 5 min, by aerosol. [h] Inact = no significant inhibition at a concentration of 1% by aerosol.

can adversely affect and even obscure SARs and conclusions regarding mode of action. Nonetheless, we note that the compounds in this series, active via the aerosol route, exhibited dose dependency (relative to concentration of drug in the nebulizer) and, while these compounds may possess other modes of action, we have demonstrated that LT antagonism plays a major role, at least, in the mode of action of 7 (see the discussion below). In this context, it is apparent that, of the 10 analogues exhibiting the greatest potency upon aerosol administration (including 1), none is superior or even equivalent to 7. These include **85** (differing from 7 by an isosteric replacement of CH_2 for O in the linking unit), benzofuran analogue **61**, which is second in aerosol potency to 7, and **86** (the desacetyl analogue), all of which lack significant activity at 10 μM in the binding assay. Other aerosol-active compounds are acyl sulfonamide **92**, propionyl homologue **96**, "ring–opened" analogue **56**, and spiro-fused acid **72**. It is interesting to note that the only members of this series exhibiting a profile similar to 1 and 7, that is, showing activity in the binding assay and in vivo via *both* routes of administration are the 2-alkylated analogues **97** and **98**.

Racemic acid 7 was further evaluated in various in vitro and in vivo model systems designed to assess antagonism of LT-mediated phenomena. As indicated above, for in vivo evaluations, emphasis was placed on the aerosol route of administration in order to deliver the highest concentration of drug to the lung with minimal side effects and because this class of LT antagonists, in our hands, failed to exhibit significant oral activity. These results are summarized in Table VI, in which similar data generated for the prototypal LT antagonist 1[6] is presented for comparison purposes. The in vitro assays studied the ability of 1 and 7 to inhibit LTD_4-induced contractions of guinea pig tracheal smooth muscle, to prevent contractions of guinea pig ileum induced by biochemically generated SRS-A, and to inhibit binding of [^{3}H]LTD_4 to receptor sites in homogenized guinea pig lung membranes. In guinea pig tracheal smooth muscle, comparison of the pA_2 values showed 7 to be 4 times more potent than 1 as an antagonist of LTD_4-induced contractions (pA_2 values of 6.6 and 6.0, respectively). The slopes of the Schild plots for the compounds did not differ from 1, indicating competitive antagonism. It should be reiterated that, in the binding assay, both antagonists were substantially less potent than unlabeled LTD_4.

In vivo, acids 1 and 7 were compared in a guinea pig bronchoconstriction model against various mediators, by the intravenous and aerosol routes of administration. Both compounds were inactive vs platelet activating factor (PAF) and histamine-induced bronchoconstriction when evaluated at a 1% concentration of aerosolized drug. When administered intravenously, the drugs exhibited comparable potencies in antagonizing the effects of LTC_4, LTD_4, and LTE_4; however, the duration of action of 7 was significantly greater than that of 1. Upon aerosol administration, acid 7 was found to be 70-fold and 13-fold more potent than 1 vs LTD_4 and LTE_4, respectively. In addition, the durations of action of 7 against these agonists were again much greater than those exhibited by 1. Whereas aerosolized 7 exhibited substantial potency vs LTC_4, 1 was essentially inactive, upon aerosol administration, in inhibiting bronchoconstriction induced by this mediator. At this point, we had demonstrated that, as an aerosol drug, acid 7 appeared to be a peptido LT antagonist with potencies and durations of action much superior to the prototype 1.

It was surprising and interesting to discover that 7 (but not 1) was very potent in inhibiting LTB_4-induced bronchoconstriction. This activity suggests another potential attribute of this acid involving inhibition of the inflammatory component of diseases such as asthma, in which the chemotactic properties of LTB_4 are thought to play a major role. We believe this property of 7 does not result from antagonism of a secondary, thromboxane-mediated bronchospasm, thromboxane synthase inhibition, or cyclooxygenase inhibition since 7 was only a weak inhibitor of arachidonic acid induced bronchoconstrictions (arachidonic acid is thought to act through generation of thromboxane A_2).[31]

We were pleased to find that aerosolized 7 blocked bronchospasm caused by antigen-induced endogenous generation of LTs in sensitized guinea pigs pretreated with indomethacin, pyrilamine, and propranolol.[32a] The potency of 7 in this model was nearly equivalent to that which it exhibited in inhibiting bronchoconstrictions induced by exogenous LTs. Surprisingly, 1 was equipotent with 7 in this model, most likely a result of other modes of pharmacological action exerted by the former compound. Previously, we have described the mediator release inhibitory activity of 1 in vitro.[32b] This property may contribute to its activity in the antigen-induced guinea pig model.

In an effort to determine whether modes of action other than LT antagonism could be contributing to the activity profile observed with acid 7, we investigated the ability of this compound to inhibit ionophore-induced arachidonic acid metabolism in rat peritoneal macrophages. The results are presented in Table VII and reveal that 7 does, in fact, exhibit the ability to suppress arachidonic acid metabolism. As an inhibitor of thromboxane synthase its potency is 20-fold less than that of the standard dazoxi-

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(32) (a) Anderson, W. H.; O'Donnell, M.; Simko, B. A.; Welton, A. F. *Br. J. Pharmacol.* **1983**, *78*, 67. (b) Welton, A. F.; Hope, W. C.; Tobias, L. D.; Hamilton, J. G. *Biochem. Pharmacol.* **1981**, *30*, 1378.

Table VII. Inhibition of Ionophore-Induced Arachidonic Acid Metabolism in Rat Peritoneal Macrophages

compd	inhibn of product formation: IC_{50}, μM		
	TXB_2	PGE_2	LTB_4
7	6	7	4
dazoxiben	0.3	10	inact[a]
indomethacin	0.1	0.1	60
Takeda AA 861	2	5	0.2

[a] Inact = no significant inhibition at a concentration of 100 μM.

Table VIII. A Comparison of Various LT Antagonists

compd	% inhibn of LTD_4 binding at 10 μM (IC_{50}, μM)	% inhibn of LTD_4-induced bronchoconstriction[b]	
		intravenous, 10 mg/kg (ID_{50}, mg/kg)	aerosol, 1% sol. (IC_{50}, %)
7	70 (4)	93 ± 3 (5)*	89 ± 1 (0.007)*
1	68 (4)	92 ± 2 (2)*	64 ± 5 (0.5)*
114	36	77 ± 4 (5)*	62 ± 8 (0.4)*
115	0	73 ± 15 (6)*	70 ± 9 (0.7)*
116	0	72 ± 11 (6)*	NT[a]
117	94 (1)	49 ± 12	NT[a]
118	24	29 ± 10	39 ± 2
119	81 (8)	20 ± 17	58 ± 8 (0.7)*
121	98 (0.001)	100 ± 0 (0.03)*	99 ± 1 (0.006)*
122	74 (6)	61 ± 10 (4)*	NT[a]

[a] NT = not tested. [b] An asterisk distinguishes values statistically significant using unpaired t test ($p < 0.05$).

ben,[33] as a cyclooxygenase inhibitor it is 70-fold less potent than the standard indomethacin, and as a lipoxygenase inhibitor it is 20-fold less potent than the standard AA 861.[34] Thus, in comparison with these reference standards, **7** is a weak inhibitor of arachidonic acid metabolism. Moreover, it is 20–100-fold less active as an inhibitor of arachidonic acid metabolism in this cellular preparation than at antagonizing SRS-A- and LTD_4-induced effects in bioassay systems. We believe such potency comparisons between whole-cell and bioassay systems are more relevant than comparisons between a whole-cell system and a cell-free assay. For this reason, we do not believe that effects on arachidonic acid metabolism contribute significantly to the ability of **7** to antagonize LTD_4-induced events in bioassays or in vivo.

We also evaluated **7** for its ability to inhibit beef heart cyclic nucleotide phosphodiesterase (PDE)[35a] since other hydroxyacetophenone LT antagonists including **1** and **114** have been reported to inhibit PDE from various sources.[35b] Acids **7** and **1** exhibited comparable potency as inhibitors of this PDE (IC_{50}s of 36 and 40 μM, respectively) and were more potent than the standards theophylline (IC_{50} = 500 μM) and methylisobutylxanthine (IC_{50} = 100 μM), which are known bronchodilators. On the basis of this data, we cannot exclude the possibility that bronchodilation resulting from PDE inhibition may contribute to the pharmacological profile of **7** as well as other members of this series.

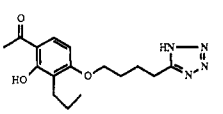
Having demonstrated that **7** was superior to the standard LT antagonist **1**, we were interested in comparing its

Table IX. In Vitro and in Vivo (Guinea Pig) Antagonism of LT-Mediated Events by the Enantiomers of **7**

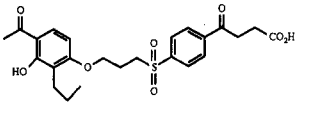
	R-7	*S*-7	p^a
trachea (pA_2)[b]	6.1 ± 0.2	6.2 ± 0.2	NS
trachea (slope)	1.2 ± 0.1	0.9 ± 0.2	NS
ileum (IC_{50}, μM)[c]	0.10 ± 0	0.06 ± 0	*
LTD_4 binding (IC_{50}, μM)	8 ± 5	5 ± 3	NS
bronchoconstriction (intravenous: ID_{50}, mg/kg)			
LTC_4	6 ± 3	4 ± 2	NS
LTD_4	4 ± 2	3 ± 1	NS
bronchoconstriction (aerosol: IC_{50}, %)			
LTC_4	1.0 ± 0.8	0.07 ± 0.06	*
LTD_4	0.03 ± 0.02	0.002 ± 0.001	*

[a] Significance of *R*-7 vs *S*-7 using unpaired t test; an asterisk indicates $p < 0.05$ response. [b] LTD_4 challenge. [c] SRS-A challenge.

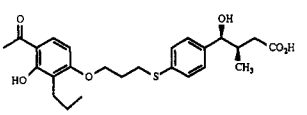
properties to those of other antagonists, including certain compounds reported to be in clinical evaluation or destined for such evaluation. In this regard, we selected representatives of three classes of LT antagonists: (1) other members of the hydroxyacetophenone class (**114**,[23] **115**,[36] **116**,[11] **117**[37]), (2) leukotriene analogues (**118**,[38] **119**[38]), and



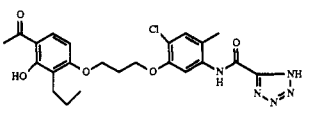
114; LY 171883



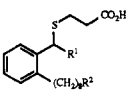
115; L 648051



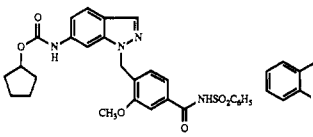
116; L 649923 (racemic)



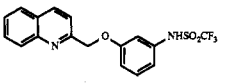
117; GCP 35-949B



118; R^1= $S(CH_2)_2CO_2H$; R^2= n-Bu (SK&F 102081)
119; R^1= $S(CH_2)_2CO_2H$; R^2= C_6H_5 (SK&F 102922)
120; R^1= $CH(OH)CO_2H$; R^2= C_6H_5 (SK&F 104353) (2S,3R)



121 (ICI 198615)



122 (Wy 48252)

(3) miscellaneous structures (**121**,[24] **122**[39]). These compounds were evaluated in the LTD_4 binding assay and in

(33) Randall, M. J.; Parry, M. J.; Hawkeswood, E.; Cross, P. E.; Dickinson, R. P. *Thromb. Res.* **1981**, *23*, 145.

(34) (a) Ashida, Y.; Saijo, T.; Kuriki, H.; Makino, H.; Terao, S.; Maki, Y. *Prostaglandins* **1983**, *26*, 955. (b) Shiraishi, M.; Terao, S. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1591.

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(38) Perchonock, C. D.; McCarthy, M. E.; Erhard, K. F.; Gleason, J. G.; Wasserman, M. A.; Muccitelli, R. M.; DeVan, J. F.; Tucker, S. S.; Vickery, L. M.; Kirchner, T.; Weichman, B. M.; Mong, S.; Crooke, S. T.; Newton, J. F. *J. Med. Chem.* **1985**, *28*, 1145.

(39) (a) Hand, J. M.; Musser, J. H.; Kreft, A. F.; Schwalm, S.; Englebach, I.; Auen, M.; Skowronek, M.; Chang, J. Y. *Pharmacologist* **1987**, *29*, 174. (b) Hand, J. M.; Auen, M. A.; Englebach, I. M.; Chang, J. *Amer. Rev. Resp. Dis.* **1988**, *137 suppl.*, 26. (c) Chang, J.; Borgeat, P.; Schleimer, R. P.; Musser, J. H.; Marshall, L. A.; Hand, J. M. *Eur. J. Pharmacol.* **1988**, *148*, 131.

the guinea pig LTD_4-induced bronchoconstriction model (iv, aerosol routes). The results are presented in Table VIII. All of the hydroxyacetophenone-type compounds exhibit relatively weak ability to compete for LTD_4 binding sites. In fact, the only substance tested having substantial potency in this assay other than the agonist itself is acyl sulfonamide **121**. In the in vivo model, acid **7** is equal or superior to all of the other antagonists when tested by the intravenous route, except for **121**, which exhibits exceptional potency. Upon aerosol administration, **7** and **121** are essentially equivalent in potency and far superior to the other compounds. Thus, **7** appears to compare quite favorably with structurally diverse antagonists, three of which (**114**,[40] **115**,[41] and **116**[42]) have undergone clinical trials in humans.

At the outset of our work, one of our reasons for investigating the chroman series was to secure LT antagonists of the hydroxyacetophenone class chiral in the region of the carboxylic acid function. Thus a comparison of the properties of the enantiomers of **7** was of great interest. The results are presented in Table IX. In both in vitro assays, the antipodes were similar although the *S* enantiomer exhibits a small but statistically significant potency enhancement in the guinea pig ileum model. The binding results suggest a lack of stereospecificity in the interaction of these antagonists with the LTD_4 receptor in guinea pig lung and are consistent with the in vivo bronchoconstriction data when the drugs were administered intravenously, in which case similar potencies were observed. During the course of our work, a group at Merck reported that the enantiomers of acid **116** exhibited quite similar properties both in vitro and in vivo as LTD_4 antagonists.[11] In contrast to these results, significant differences in potency vs LTC_4 and LTD_4 were observed when the antipodes of **7** were administered by the aerosol route. By inhalation, the *S* enantiomer was approximately 15-fold more potent than the *R* antipode vs both LTC_4 and LTD_4, suggesting modest stereoselectivity in some aspect of the pharmacological activity observed by this route of administration. In view of the binding data, this preference may be related to the pharmacodynamics of the enantiomers rather than drug–receptor interactions. The inconsistencies we have observed between the in vivo data obtained by the two routes of administration are not understood and leave the question of stereochemical requirements for the binding of antagonists of this class to the LT receptor unanswered. It should be noted that among LTD_4 antagonists, the design of which was based upon the agonist structure, acid **120** has been reported to exhibit 36-fold greater affinity for the receptor than its enantiomer.[43]

In summary, we have prepared a representative number of chroman acids of generic structure **5** and examined their ability to block the actions of LTD_4 in vitro and in vivo. Our results do not demonstrate a strong correlation between the [^{3}H]LTD_4 binding and in vivo data in this series of compounds. Possible explanations for the lack of correlation in the structure–function relationships include either interactions of members of this series (and, possibly, hydroxyacetophenone-type antagonists in general) with different populations of LTD_4 receptors or multiple modes of pharmacological action in addition to LTD_4-receptor antagonism. From this study has emerged Ro 23-3544 (**7**), which is the most potent analogue when administered by the aerosol route and appears to represent optimization of the structural factors required for activity by this route. The reasons for its enhanced aerosol potency relative to closely related substances remain obscure but may include pharmacokinetic phenomena. Both enantiomers of **7** are active as LT antagonists with the *S* antipode exhibiting somewhat greater potency when administered as an aerosol. In addition, **7** was found to inhibit bronchoconstriction induced by LTB_4, suggesting potential utility of this compound in modulating the inflammatory component of asthma. In view of its interesting pharmacological profile, acid **7** was selected for clinical evaluation.

Experimental Section

General Information. All of the reactions described below, except hydrogenations were carried out under an atmosphere of argon. The "usual workup" conditions involve three extractions with the indicated solvent, washing the combined organic extracts with water and saturated brine, drying the organic solution over anhydrous magnesium sulfate, suction filtration, and concentration of the filtrate under water aspirator pressure using a rotary evaporator. The residue was then dried to constant weight under high vacuum. Column chromatography was performed with EM silica gel 60 (0.063–0.2 mm). Thin-layer chromatography was employed to monitor reactions and determine product purity and was performed with EM silica gel 60 F-254 precoated plates. Hexanes–ether, toluene–ethyl acetate, dichloromethane–ethyl acetate, or toluene–ethyl acetate–acetic acid mixtures were generally used as the mobile phases. Spots were detected with UV light and phosphomolybdic acid–ceric sulfate sprays followed by heating. HPLC was performed with a Waters 500A instrument on silica gel columns. The IR, NMR, UV, and mass spectral data obtained for all compounds were consistent with the assigned structures. ^{1}H NMR spectra (100 or 200 MHz) were obtained in $CDCl_3$ solution. Chemical shifts are reported relative to tetramethylsilane as an internal standard. Optical rotations were measured as 1% solutions in chloroform. LC analyses of the diastereomeric esters were carried out with a 15 cm × 4.6 mm i.d. column of ES Ind. silica gel (3 μm) with 25% THF in heptane or 10% ethyl acetate in dichloromethane as the mobile phase. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. LTC_4, LTD_4, and LTE_4 were synthesized as previously described.[2c,d] FPL 55712 (**1**) was synthesized as described previously.[6] LTB_4 was synthesized by a combination of the methods of Maehr[44] and Rokach.[45] Compounds **114**,[23] **118**,[38] **119**,[38] and **121**[24c] were prepared as reported. Takeda AA 861 [2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone] was prepared as described.[34b] Compounds **115** and **116** were supplied by Dr. J. Rokach, Merck Frosst Canada, Inc.; compound **117** was supplied by Dr. A. Beck, Ciba-Geigy Ltd.; and compound **122** was supplied by Dr. J. M. Hand, Wyeth Laboratories.

***rac*-3,4-Dihydro-2,5,7,8-tetramethyl-6-(2-propenyloxy)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (9).** A 365-mg (7.6 mmol) portion of 50% sodium hydride–mineral oil dispersion was washed with hexanes and suspended in 32 mL of anhydrous DMF. To the stirred slurry was added 1 g (3.78 mmol) of phenol **8**.[13] After being stirred at room temperature for 45 min, the mixture was treated dropwise, with 3.2 mL (37 mmol) of allyl bromide. Stirring was continued for 90 h at room temperature, at which point 3 mL of methanol was added. The mixture was poured into water and worked up with ether in the usual manner, giving 1.8 g of an amber oil. This material was purified by column chromatography and then HPLC (19:1 hexanes–ethyl acetate). There was obtained 0.772 g (67.2%) of ester ether **9** as a pale-yellow oil. This material was evaporatively distilled (bp 105–107

(40) *Drugs Future* **1987**, *12*, 812 and references cited therein.
(41) Barnes, N. Conference on the Biology of the Leukotrienes; The New York Academy of Sciences; June 28–July 1, 1987; Abstr. No. 34.
(42) Barnes, N.; Piper, P. J.; Costello, J. *J. Allergy Clin. Immunol.* **1987**, *79*, 816.
(43) See ref. 8. These workers suggest that the observed receptor-antagonist activity of the less potent enantiomer may result from incomplete resolution (i.e. the presence of ca 2% of the more potent antipode).
(44) Maehr, H.; Perrotta, A.; Smallheer, J. *J. Org. Chem.* **1988**, *53*, 832.
(45) Zamboni, R.; Rokach, J. *Tetrahdron Lett.* **1982**, *23*, 2631.

°C, bath temperature, 0.2 mm), giving an oil, which crystallized on standing to a solid, mp 68–70 °C. Anal. ($C_{18}H_{24}O_4$) C, H.

***rac*-3,4-Dihydro-2,5,7,8-tetramethyl-6-(3-hydroxypropoxy)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (10).** To a solution of 1.8 g (5.92 mmol) of olefin **9** in 36 mL of anhydrous THF was added 1.98 mL (21 mmol) of borane–dimethyl sulfide with stirring and ice-bath cooling. The reaction mixture was stirred for 1.5 h at 0–5 °C and then decomposed by the dropwise addition of 9 mL of water. After being stirred at 0–5 °C for 10 min, the mixture was treated dropwise with 6.84 mL of 3 N NaOH followed by 2.2 mL of 30% hydrogen peroxide. The mixture was stirred at 5–10 °C for 1 h and then acidified with 7 mL of 3 N HCl. Workup with ether in the usual manner gave 2 g of an oil, which was purified by HPLC (1:1 hexanes–ethyl acetate). There was obtained 1.22 g (64%) of hydroxy ester **10** as a colorless oil. Anal. ($C_{18}H_{26}O_5$) C, H.

***rac*-3,4-Dihydro-2,5,7,8-tetramethyl-6-(3-iodopropoxy)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (11).** To a solution of 0.3 g (0.9 mmol) of hydroxy ester **10** in 5 mL of anhydrous methylene chloride and 0.33 mL of triethylamine was added 0.17 mL (2.2 mmol) of methanesulfonyl chloride. The mixture was stirred at room temperature for 45 min and then treated with 1 N sulfuric acid and worked up with methylene chloride in the usual manner. There was obtained 0.461 g of methanesulfonate as an oil, which was used without further purification. This material, 595 mg (3 mmol) of NaI, and 5 mL of acetone was stirred at room temperature for 43.5 h and then treated with water and worked up with ether in the usual manner (the ether extracts were additionally washed with dilute $NaHSO_3$ solution). The oily product was chromatographed on 20 g of silica gel. Elution with 19:1 and 9:1 hexanes–ethyl acetate afforded 0.306 g (79%) of iodo ester **11** as a colorless oil. In another experiment, the product prepared in this way crystallized to a solid, mp 73–75.5 °C.

***rac*-6-[3-(4-Acetyl-3-hydroxy-2-propylphenoxy)propoxy]-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (6 Methyl Ester).** A mixture of 0.137 g (0.7 mmol) of **12**,[6] 0.195 g (1.4 mmol) of anhydrous K_2CO_3, and 3 mL of anhydrous DMF was stirred for 1 h at room temperature. A solution of the iodide **11** from the preceding experiment (0.306 g, 0.7 mmol) in 5 mL of DMF was added and the mixture was stirred for 30 min at room temperature and at 60 °C for 31 h. After being cooled, the reaction mixture was treated with 1 N HCl and worked up with ether in the usual manner. The oily product was chromatographed on 20 g of silica gel. Elution with 4:1 hexanes–ethyl acetate gave 0.291 g (82.5%) of **6** methyl ester as a pale-yellow oil. In another experiment, material prepared in this way crystallized and afforded a colorless solid, mp 121–123 °C, after recrystallization from ethanol.

***rac*-6-[3-(4-Acetyl-3-hydroxy-2-propylphenoxy)propoxy]-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylic Acid (6).** A solution of 0.246 g (0.49 mmol) of the ester from the preceding experiment, and 0.227 g (5.67 mmol) of NaOH in 8 mL of methanol and 2 mL of water was stirred at room temperature for 1.5 h and then treated with 1 N HCl and worked up with ether in the usual manner. The crude product was redissolved in ether and the solution was washed with dilute $NaHCO_3$ solution. The aqueous, alkaline phase was acidified with 2 N HCl and worked up with ether in the usual manner, giving 0.161 g (67.3%) of acid as a yellow oil, which crystallized on standing. Recrystallization from benzene–hexanes gave **6** as a colorless solid, mp 137–139 °C. Anal. ($C_{28}H_{36}O_7$) C, H.

***rac*-3,4-Dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (24).** A 500-mL pressure bottle was charged with 23.6 g (0.10 mol) of chromone ester **23**,[6] 4 g of 10% palladium on charcoal, and 250 mL of glacial acetic acid. The mixture was shaken at room temperature, under 50 lb of hydrogen pressure, on a Paar apparatus, for 17 h. At the end of this time, the hydrogen pressure was readjusted to 50 lb and the mixture was shaken for an additional 24 h. The catalyst was removed by suction filtration on a Celite pad and the filter cake was washed with ethyl acetate. The filtrate and washes were combined and concentrated in vacuo. The residue was dissolved in ethyl acetate and the solution was washed twice with saturated $NaHCO_3$ solution. Completion of the usual workup gave 21.5 g of a colorless solid, which was recrystallized from carbon tetrachloride. After standing overnight at 0 °C, the slurry was filtered with suction, giving 16.3 g (73.4%) of pure chroman ester **24** as a colorless solid, after drying under high vacuum. The analytical specimen was obtained from another experiment by recrystallization from hexanes–ethyl acetate, mp 81–82.5 °C. Anal. ($C_{12}H_{14}O_4$) C, H.

***rac*-3,4-Dihydro-7-hydroxy-8-propyl-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (27).** With use of the procedure of the preceding experiment, chromone ester **26**[6] was reduced to chroman ester **27** in 43.6% yield, obtained as a viscous amber oil.

***rac*-6-Acetyl-3,4-dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (40) (Method A).** A solution of 27.9 g (0.125 mol) of chromanol ester **24** in 276 mL of pyridine was stirred with ice-bath cooling while 276 mL of acetic anhydride was added dropwise. After being stirred at room temperature for 24 h, the resulting solution was concentrated under water-aspirator pressure. The residual oil was dissolved in ethyl acetate and the solution was washed with water, 1 N HCl, water, saturated $NaHCO_3$ solution, and brine. Completion of the usual workup gave 35.3 g of a yellow, oily product. This material was chromatographed on 750 g of silica gel. Elution with 4:1 hexanes–ethyl acetate gave 26.1 g (79.0%) of pure *O*-acetate followed by 5 g (15.2%) of slightly less pure product. A mixture of the pure material (26.1 g, 0.0987 mol), 260 mL of glacial acetic acid, 0.6 mL of acetic anhydride, and 26 mL of boron trifluoride etherate was stirred and refluxed for 18.5 h. The reaction mixture was cooled and concentrated under high vacuum, giving a yellow, semisolid residue, which was treated with 1 L of water. The mixture was stirred as solid $NaHCO_3$ was cautiously added until the acid had largely dissolved. The resulting pink solution was extracted with ethyl acetate (organic extract discarded) and acidified to pH 1 with 3 N HCl, giving rise to a yellow precipitate. Usual workup with 1:1 ether–THF gave 21 g of an orange-yellow solid. This acid was treated with 400 mL of methanol and 1.5 g of *p*-toluenesulfonic acid monohydrate and the mixture was stirred and refluxed for 20 h, then cooled to room temperature whereupon a solid precipitated. Concentration in vacuo left a solid, which was dissolved in ethyl acetate. The solution was washed with saturated $NaHCO_3$ solution and workup was completed in the usual manner, giving 21.7 g of a solid mixture of **40** and **41**. Recrystallization from carbon tetrachloride (with Darco treatment) afforded 15.6 g (63.1%) of pure **40** as a colorless solid, mp 130–132 °C. These isomers could also be separated by HPLC by which means the minor 8-acetyl isomer **41** was isolated in pure form.

***rac*-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (7) (Method G).** A mixture of 15.6 g (0.062 mol) of hydroxy keto ester **40**, 26.36 g (0.191 mol) of anhydrous K_2CO_3, 2.6 g (9.85 mmol) of 18-crown-6, 68 mL (114.8 g, 0.5 mol) of 1,5-dibromopentane, and 440 mL of acetonitrile was stirred and refluxed for 22 h. The reaction mixture was cooled, diluted with ether, and washed with water and saturated brine. Completion of the usual workup gave 127 g of a yellow oil, which was chromatographed on 750 g of silica gel. Elution with 19:1 toluene–ethyl acetate gave 20.2 g (81.6%) of bromo ester **100** as a yellow oil. The analytical specimen was obtained from another experiment. Anal. ($C_{18}H_{23}BrO_5$) C, H.

This bromide (20.2 g, 0.05 mol), 11.0 g (0.057 mol) of **12**, 25.4 g (0.184 mol) of anhydrous K_2CO_3, 436 mL of dry acetone, and 218 mL of dry DMF were stirred and refluxed for 5.5 h. The reaction mixture was cooled and diluted with ethyl acetate, and the organic phase was washed with water and saturated brine. Completion of the usual workup gave 28.3 g of an amber oil. This material was purified by HPLC (1:1 hexanes–ethyl acetate), giving 24.83 g (96.8%) of pure desired ester as an oil, which crystallized on standing. The analytical specimen of **7** methyl ester was obtained from another experiment as a low melting, colorless solid upon recrystallization from acetonitrile. Anal. ($C_{29}H_{36}O_8$) C, H.

A mixture of 2.6 g (5.07 mmol) of this methyl ester and 4.1 g (97.6 mmol) of $LiOH \cdot H_2O$ in 60 mL of 1:1 THF–water was stirred at room temperature for 6 h and then diluted with water and acidified to pH 1 with 2 N HCl. Workup with ethyl acetate in the usual manner gave 2.5 g of acid. Two recrystallizations from hexanes–ethyl acetate gave **7** as a colorless solid (1.8 g, 72%; see Table II). This material was homogeneous on TLC analysis (6.5:2.5:1 toluene–ethyl acetate–acetic acid). In some of the ex-

amples given in Table IV prepared with this basic procedure (**93**, **95**, **96**), the 18-crown-6 was omitted in the first alkylation. The saponifications could be carried out equally well with NaOH or KOH in aqueous methanol.

Method F. A mixture of 2.1 g (7.95 mmol) of ester **38**, 5.4 g (39.1 mmol) of anhydrous K_2CO_3, 10 mL (73.4 mmol) of 1,5-dibromopentane, 60 mL of dry acetone, and 30 mL of DMF was stirred and refluxed for 27 h. The resulting mixture was cooled, diluted with ether, poured into water, and worked up with ether in the usual manner, giving 16.3 g of a yellow liquid. This material was chromatographed on 100 g of silica gel. Elution with 19:1 toluene–ethyl acetate gave 2.7 g (82.2%) of **100** ethyl ester as a yellow oil, which was used without further purification. The alkylation of **12** with this bromo ester and subsequent saponification were carried out as described in method G (61.4% overall yield).

***rac*-3,4-Dihydro-7-(phenylmethoxy)-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (25).** A mixture of 4.44 g (0.02 mol) of phenol ester **24**, 5.75 mL (0.05 mol) of benzyl chloride, 6.9 g (0.05 mol) of anhydrous K_2CO_3, and 20 mL of DMF was stirred at room temperature for 65 h. The resulting mixture was diluted with ether and filtered with suction. The solids were washed with ether. The filtrate and washes were combined, washed with water and brine, and processed in the usual manner. The oily residue was chromatographed on 100 g of silica gel. Elution with 49:1 toluene–ethyl acetate gave 5.4 g (86.5%) of ether ester **25** as a colorless oil.

***rac*-3,4-Dihydro-7-(phenylmethoxy)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (28).** A solution of 5.4 g (17.3 mmol) of ethyl ester **25** from the preceding experiment, in 25 mL of methanol, was treated with 20 mL (60 mmol) of 3 N aqueous NaOH. The mixture was stirred and refluxed for 15 min, giving a solution, which, after cooling, was diluted with water and extracted twice with ether (ether extracts discarded). The aqueous alkaline solution was acidified with 6 N HCl and the precipitate was filtered with suction, washed with water, and dried under high vacuum, giving 4.7 g of the acid as an off-white solid. A solution of 4.1 g (14.4 mmol) of this acid and 0.18 g of *p*-toluenesulfonic acid monohydrate in 72 mL of methanol was stirred and refluxed for 5.5 h and then cooled and concentrated in vacuo. The residue was dissolved in ether and the solution was washed with saturated $NaHCO_3$ solution and workup was completed in the usual manner, giving ester **28** (4.25 g, 94.6%) as a pale-yellow oil. Anal. ($C_{18}H_{18}O_4$) C, H.

***rac*-3,4-Dihydro-2-methyl-7-(phenylmethoxy)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (29).** To a solution of 3.7 g (12.4 mmol) of ester **28** in 76 mL of anhydrous THF, cooled to −72 °C (dry ice–acetone bath) was added dropwise, with stirring, 14.9 mL (14.9 mmol) of 1 M lithium bis(trimethylsilyl)amide in THF. After stirring at −72 °C for 1.25 h, the solution was treated, dropwise, with a solution of 8 mL (128 mmol) of methyl iodide in 14 mL of anhydrous THF. The reaction mixture was stirred overnight, allowing the temperature to rise gradually to room temperature, and then was treated with 10 mL of methanol, poured into water, and worked up with ethyl acetate in the usual manner. The oily product was purified by HPLC (9:1 hexanes–ethyl acetate), giving 1.8 g (46.5%) of ester **29** as a colorless oil. In addition, 1.1 g (29.7%) of the starting ester was recovered. The analytical specimen of **29** was obtained from another experiment. Anal. ($C_{19}H_{20}O_4$) C, H. In another experiment, in which lithium diisopropylamide was employed as the base, pure **29** was obtained in 84.2% yield.

***rac*-3,4-Dihydro-7-(phenylmethoxy)-2-(2-propenyl)-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (30).** The alkylation was carried out as in the preceding experiment except that lithium diisopropylamide was used instead of lithium bis(trimethylsilyl)amide and allyl bromide was substituted for methyl iodide. In this manner, 0.579 g (1.94 mmol) of **28** afforded 0.475 g (72.4%) of **30** as a colorless oil, purified by column chromatography. Anal. ($C_{21}H_{22}O_4$) C, H.

***rac*-3,4-Dihydro-2-methyl-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (32).** A mixture of 1.8 g (5.7 mmol) of ether ester **29** (pretreated with a small amount of Raney nickel slurry in ethyl acetate to remove poisons) and 0.5 g of 10% palladium on charcoal, in 250 mL of ethyl acetate, was stirred in an atmosphere of hydrogen, at room temperature, until gas uptake ceased (152 mL H_2; 144 mL theoretical uptake). The catalyst was removed by suction filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on 50 g of silica gel. Elution with 2:1 hexanes–ethyl acetate gave 1.24 g (98.0%) of phenol ester **32** as a colorless solid, mp 100–103 °C. Anal. ($C_{12}H_{14}O_4$) C, H.

***rac*-3,4-Dihydro-7-hydroxy-2-propyl-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (31).** With use of the procedure of the preceding experiment, 2.19 g (6.47 mmol) of **30** gave 1.43 g (88.8%) of phenol ester **31** as a colorless oil. Anal. ($C_{14}H_{18}O_4$) C, H.

***rac*-3,4-Dihydro-7-(phenylmethoxy)-2*H*-1-benzopyran-2-carboxaldehyde (33).** To a solution of 6.9 g (22 mmol) of ester **25** in 70 mL of toluene cooled to −78 °C was added dropwise, with stirring, 15.6 mL (11.3 mmol) of 25% diisobutylaluminum hydride solution in toluene. The reaction mixture was stirred at −78 °C for 2 h whereupon 2.5 mL of methanol was cautiously added followed by ice and 2 N HCl. Workup with ethyl acetate in the usual manner gave an oily product, which was chromatographed on 150 g of silica gel. Elution with 9:1 toluene–ethyl acetate gave 5.6 g (70%) of aldehyde **33** as a pale-yellow oil.

***rac*-3,4-Dihydro-7-(phenylmethoxy)-2*H*-1-benzopyran-2-propenoic Acid Ethyl Ester (34).** A solution of 2.7 g (10 mmol) of aldehyde **33** and 3.7 g (10.6 mmol) of (carbethoxymethylene)triphenylphosphorane in 30 mL of toluene was stirred for 1.5 h at 100 °C. After being cooled, the solution was applied to 50 g of silica gel. Elution with toluene afforded 3.0 g (88.8%) of ester **34** (mixture of *E* and *Z* isomers) as a pale-yellow oil.

***rac*-5-[3,4-Dihydro-7-(phenylmethoxy)-2*H*-1-benzopyran-2-yl]-2,4-pentadienoic Acid Ethyl Ester (35).** A solution of 2.7 g (10 mmol) of aldehyde **33** and 4.4 g (12 mmol) of ethyl 4-(triphenylphosphoranylidene)-2-butenoate[46] in 30 mL of toluene was stirred at 100 °C for 3.5 h. The cooled solution was applied to 100 g of silica gel. Elution with toluene and 19:1 toluene–ethyl acetate gave 1.1 g (30%) of ester **35** (mixture of *E* and *Z* isomers) as a yellow oil.

***rac*-3,4-Dihydro-7-hydroxy-2*H*-1-benzopyran-2-propanoic Acid Ethyl Ester (36).** Hydrogenation of 3 g (8.9 mmol) of ester **34** over 0.3 g of 10% palladium on carbon, in 35 mL of ethyl acetate, at atmospheric pressure and room temperature gave 2.4 g (100+%) of oily ester **36** after filtration of the catalyst and solvent evaporation. This material was used without further purification.

***rac*-3,4-Dihydro-7-hydroxy-2*H*-1-benzopyran-2-pentanoic Acid Ethyl Ester (37).** Hydrogenation of 1.1 g (3.02 mmol) of dienoic ester **35** over 0.95 g of 10% palladium on carbon, in 15 mL of ethyl acetate, at atmospheric pressure and room temperature gave 0.9 g (100%) of pentanoate **37** as a pale-yellow oil, which was used without further purification.

***rac*-6-Acetyl-3,4-dihydro-2-methyl-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (43) (Method B).** A solution of 1.15 g (5.17 mmol) of phenol ester **32**, 0.05 mL of acetic anhydride, and 1.1 mL of boron trifluoride etherate in 11 mL of acetic acid was stirred at 120 °C for 21 h. The reaction mixture was cooled and poured into water. Workup with ethyl acetate in the usual manner gave 1.2 g of brown, solid acid. This material was dissolved in 60 mL of methanol containing 0.5 g of *p*-toluenesulfonic acid monohydrate and the solution was stirred and refluxed for 19 h and then concentrated in vacuo. The residue was chromatographed on 50 g of silica gel. Elution with 4:1 and 2:1 hexanes–ethyl acetate gave 0.699 g (51%) of **43** as a colorless solid, which was used without further purification. The 8-acetyl isomer was isolated from fractions eluted with 9:1 hexanes–ethyl acetate. In the case of the homologue **42**, the desired product could be isolated in pure form by direct crystallization from the esterification mixture.

***rac*-6-Chloro-3,4-dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (47) (Method C).** A mixture of 11.1 g (50 mmol) of ester **24**, 7.35 g (55 mmol) of *N*-chlorosuccinimide, and 50 mL of carbon tetrachloride was stirred and refluxed for 2.25 h. After being cooled, the dark-red reaction mixture was diluted with ether and the solution was decanted from insoluble materials and washed with water, saturated

(46) Howe, R. K. *J. Am. Chem. Soc.* **1971**, *93*, 3457.

$NaHCO_3$ solution, and brine. Completion of the usual workup gave 11.7 g of a red semisolid. This material was dissolved in 100 mL of methanol and 150 mL of 2 N NaOH was added. The mixture was refluxed for 15 min, and then kept at room temperature for 45 min whereupon most of the methanol was removed in vacuo. Water was added to the residue, which was extracted twice with ether (ether extracts discarded). The aqueous alkaline solution was acidified with 6 N HCl and worked up as usual with ether. The residue was dissolved in 250 mL of methanol and 0.5 g of *p*-toluenesulfonic acid monohydrate was added. The solution was stirred at reflux for 4 h and overnight at room temperature before being concentrated in vacuo. The residue was dissolved in ether and the solution was washed with saturated $NaHCO_3$. Completion of the usual workup gave 10.35 g of red solid, which was chromatographed on 100 g of silica gel. Elution with 19:1 toluene–ethyl acetate gave 5.6 g of mixtures followed by 2.47 g of chloro ester **47** as a yellow solid, which was used without further purification.

***rac*-6-Bromo-3,4-dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (48) (Method D).** A mixture of 5 g (22.5 mmol) of ester **24**, 4.21 g (23.7 mmol) of *N*-bromosuccinimide, and 50 mL of carbon tetrachloride was stirred and refluxed for 2.25 h. The reaction mixture was cooled, treated with water, and worked up with carbon tetrachloride in the usual manner (the organic extracts were additionally washed with saturated $NaHCO_3$ solution). The residue was chromatographed on 250 g of silica gel. Elution with 9:1 toluene–ethyl acetate afforded 2.25 g of bromo ester **48** as a pink solid. A sample was recrystallized from carbon tetrachloride, giving the analytical specimen.

***rac*-3,4-Dihydro-7-hydroxy-6-nitro-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (49) (Method E).** To a stirred solution of 6.66 g (30 mmol) of ester **24** in 240 mL of glacial acetic acid was added 2 mL (32 mmol) of concentrated nitric acid, dropwise. A deep-red solution formed and a mild exotherm was noted. The mixture was stirred at room temperature for 16 h and then poured into 1 L of ice–water. Workup with dichloromethane in the usual manner gave 7.72 g of a black, semisolid residue. This material was chromatographed on 200 g of silica gel. Elution with 9:1 hexanes–ethyl acetate gave 4.5 g (56.2%) of pure nitro ester **49** as a light-yellow solid. The analytical specimen was obtained by recrystallization of a sample from ethanol.

***rac*-6-Acetyl-1,2,3,4-tetrahydro-7-hydroxy-2-naphthalenecarboxylic Acid (51).** The phenol acid **50**[17] (3.9 g, 20.3 mmol) was subjected to the Fries rearrangement conditions as described above in method B; the esterification step was omitted. The crude acid was dissolved in saturated $NaHCO_3$ solution and the aqueous phase was extracted with ethyl acetate (organic extracts discarded), filtered through Celite, and acidified with 1 N HCl. Workup with ethyl acetate in the usual manner gave a semisolid residue, which was triturated with hexanes. The solid was dried under high vacuum, giving 3.6 g (75.7%) of **51** as a brown solid. The analytical specimen was obtained by recrystallization of sample from acetonitrile as a yellow solid, mp 166–171 °C. Anal. ($C_{13}H_{14}O_4$) C, H.

***rac*-6-Acetyl-1,2,3,4-tetrahydro-7-hydroxy-2-naphthalenecarboxylic Acid Methyl Ester (52).** Esterification of acid **51** with methanol–*p*-toluenesulfonic acid as described above gave ester **52** as a colorless solid, mp 102–105 °C, after purification by column chromatography (eluted with 7:1 toluene–ether) and recrystallization from carbon tetrachloride–hexanes. Anal. ($C_{14}H_{16}O_4$) C, H.

[4-Acetyl-3-[(5-bromopentyl)oxy]phenoxy]acetic Acid Methyl Ester (55). Alkylation of **54**[47] with 1,5-dibromopentane was carried out by using the procedure of method G above. Bromo ester **55** was obtained in 80.9% yield as a colorless solid, mp 47–54 °C, purified by HPLC (19:1 toluene–ethyl acetate). Anal. ($C_{16}H_{21}BrO_5$) C, H, Br.

[4-Acetyl-3-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)-pentyl]oxy]phenoxy]acetic Acid Methyl Ester (56 Methyl Ester). Alkylation of **12** with **55** was carried out by using the procedure described in method G above, giving the methyl ester of **56** in 84.5% yield, as a solid, mp 57–61 °C, purified by HPLC (2:1 hexanes–ethyl acetate). Anal. ($C_{27}H_{34}O_8$) C, H.

[4-Acetyl-3-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)-pentyl]oxy]phenoxy]acetic Acid (56). The methyl ester of **56** was saponified with LiOH in THF–water by using the procedure described above in method G. Acid **56** was obtained in 75.2% yield as a colorless solid, mp 135.5–138 °C, after recrystallization from acetonitrile. Anal. ($C_{26}H_{32}O_8$) C, H.

2,3-Dihydro-6-hydroxybenzofuran-2,2-dicarboxylic Acid Diethyl Ester (58). The hydroxy diester **57**[18] (27.2 g, 70.5 mmol) was hydrogenated over 5.4 g of 10% palladium on carbon, in 900 mL of glacial acetic acid containing 18 drops of concentrated sulfuric acid, at room temperature and 3 atm of H_2 until hydrogen uptake was complete. The catalyst was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in ethyl acetate and the solution was washed with saturated $NaHCO_3$ and water and workup was completed in the usual manner, giving 19.8 g of a yellow oil. This material was purified by HPLC (3:1 hexanes–ethyl acetate), affording 16.9 g (85.6%) of diester **58** as a colorless solid. The analytical specimen was obtained from a separate experiment by recrystallization from carbon tetrachloride, giving colorless solid, mp 79.5–81.5 °C. Anal. ($C_{14}H_{16}O_6$) C, H.

***rac*-5-Acetyl-2,3-dihydro-6-hydroxybenzofuran-2-carboxylic Acid Methyl Ester (59).** A solution of 15 g (53.5 mmol) of diester **58**, 155 mL of glacial acetic acid, 2.4 mL of acetic anhydride, and 15 mL of boron trifluoride etherate was stirred and heated at 120 °C for 19 h. The reaction mixture was cooled, poured into ice–water, and worked up with ethyl acetate in the usual manner, giving 9.6 g of a tan solid. This acid and 11 mL of boron trifluoride etherate, in 220 mL of methanol, was stirred and refluxed for 17 h. After being cooled, the reaction mixture was poured into cold water and worked up in the usual manner with ethyl acetate (the organic extracts were additionally washed with saturated $NaHCO_3$ solution), giving 10 g of a solid. This material was chromatographed on 600 g of silica gel. Elution with 1:1 hexanes–ethyl acetate afforded 9.85 g (80%) of **59** as a pale-yellow solid, mp 124–127.5 °C. The analytical specimen was obtained from a separate experiment by recrystallization from carbon tetrachloride giving a pale-yellow solid, mp 126–127.5 °C. Anal. ($C_{12}H_{12}O_5$) C, H.

***rac*-5-Acetyl-6-[(5-bromopentyl)oxy]-2,3-dihydrobenzofuran-2-carboxylic Acid Methyl Ester (60).** With use of the procedure described above in method G, **59** was alkylated with 1,5-dibromopentane, giving bromo ester **60**, in 67.6% yield, as a colorless solid, mp 105–107.5 °C, purified by HPLC (19:1 toluene–ethyl acetate). Anal. ($C_{17}H_{21}BrO_5$) C, H.

***rac*-5-Acetyl-6-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-2,3-dihydrobenzofuran-2-carboxylic Acid Methyl Ester (61 Methyl Ester).** With use of the procedure described above in method G, **12** was alkylated with bromo ester **60**, giving the methyl ester of **61**, in 78.8% yield, as a colorless solid, mp 95–97.5 °C, purified by HPLC (2:1 hexanes–ethyl acetate). Recrystallization of a sample from carbon tetrachloride gave a material with mp 98.5–100 °C. Anal. ($C_{28}H_{34}O_8$) C, H.

***rac*-5-Acetyl-6-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-2,3-dihydrobenzofuran-2-carboxylic Acid (61).** A solution of 2.15 g (4.31 mmol) of the methyl ester from the preceding experiment and 2.15 g (38.4 mmol) of KOH in 80 mL of methanol was stirred for 3 h at room temperature and then concentrated in vacuo. The residue was dissolved in water, acidified with 1 N HCl, and the mixture was worked up with ether in the usual manner. The residue was recrystallized from acetonitrile, giving 1.56 g (74.8%) of acid **61** as a colorless solid, mp 116–119.5 °C. The analytical specimen exhibited mp 117.5–119.5 °C. Anal. ($C_{27}H_{32}O_8$) C, H.

7-(6-Hydroxy-1-hexynyl)-4-oxo-4*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (63). A mixture of 5.85 g (19.7 mmol) of bromochromone **62**,[21] 160 mg of anhydrous CuI, 2.56 g (26.2 mmol) of 5-hexyn-1-ol, 297 mg (0.423 mmol) of bis(triphenylphosphine)palladium(II) chloride, and 80 mL of anhydrous triethylamine was stirred and refluxed for 2 h. The resulting black, dense slurry was cooled, diluted with toluene, and filtered with suction. The solids were washed thoroughly with toluene, and then the filtrate and washes were combined and concentrated in vacuo. The residual dark oil was filtered through a plug of silica gel in ethyl acetate. The filtrate containing the desired product

(47) Jpn. Kokai Tokkyo Koho 58 88340, Dainippon Ink and Chemicals Inc.; *Chem. Abstr.* **1983**, *99*, 122052.

was concentrated in vacuo and the residue was purified by HPLC (1:1 hexanes–ethyl acetate), giving 4.2 g (67.9%) of **63** as an oil, which crystallized on standing, mp 54–58 °C.

***rac*-7-[6-(Acetyloxy)hexyl]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (64).** A 2-g (6.4 mmol) sample of acetylenic chromone **63** was hydrogenated over 0.48 g of 10% palladium on carbon, in 80 mL of glacial acetic acid containing 3 drops of concentrated sulfuric acid, at room temperature and 42 psi for 18 h. The catalyst was filtered with suction and the filtrate was concentrated in vacuo. The residue was dissolved in ethyl acetate and the solution was washed with saturated $NaHCO_3$ solution and water. Completion of the usual workup gave 2.1 g of a pale-yellow oil. This material was chromatographed on 100 g of silica gel. Elution with 4:1 hexanes–ethyl acetate afforded 1.8 g (81.2%) of pure acetate **64** as a colorless oil. Anal. ($C_{20}H_{28}O_5$) C, H.

***rac*-6-Acetyl-7-[6-(acetyloxy)hexyl]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid Ethyl Ester (65).** To a solution of 1 g (2.87 mmol) of diester **64** and 0.41 mL (5.74 mmol) of acetyl chloride, in 50 mL of dry dichloromethane, was added 1.58 g (11.7 mmol) of anhydrous aluminum chloride in one portion, with stirring and ice-bath cooling. The resulting solution was stirred at 0–5 °C for 2 h and then treated with ice. Workup with dichloromethane in the usual manner gave an oil, which was chromatographed on silica gel. Elution with 2:1 hexanes–ethyl acetate gave 1.05 g (93.8%) of keto diester **65** as a colorless oil. Anal. ($C_{22}H_{30}O_6$) C, H.

***rac*-6-Acetyl-7-(6-hydroxyhexyl)-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid Methyl Ester (66).** A solution of 3.5 g (8.97 mmol) of diester **65** and 0.4 g of *p*-toluenesulfonic acid monohydrate in 50 mL of methanol was stirred and refluxed for 20.5 h and then concentrated in vacuo. The residue was dissolved in ether and the solution was washed with saturated $NaHCO_3$ solution and water. Completion of the usual workup gave an oil, which was purified by HPLC (1:1 hexanes–ethyl acetate). There was obtained 2.6 g (86.7%) of hydroxy ester **66** as an oil.

***rac*-6-Acetyl-7-[6-(4-acetyl-3-hydroxy-2-propylphenoxy)-hexyl]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (85) (Method J).** A solution of 2.6 g (7.78 mmol) of hydroxy ester **66**, 3.24 mL (23.4 mmol) of triethylamine, and 1.18 mL (15.3 mmol) of methanesulfonyl chloride in 60 mL of dichloromethane was stirred with ice-bath cooling for 2.5 h. The reaction mixture was poured into cold 1 N sulfuric acid and worked up with dichloromethane in the usual manner, giving 3.4 g of mesylate **67** as a yellow oil, which was used without further purification. This mesylate was used to alkylate 1.59 g (8.24 mmol) of **12** by using the procedure described above (method G). The crude product was purified by HPLC. There was obtained 3.0 g (75.6%) of **85** methyl ester as an oil, which crystallized on standing. Anal. ($C_{30}H_{38}O_7$) C, H. This material was saponified with KOH in methanol as described above for the preparation of **61** giving 2.14 g (73.5%) of acid **85** after recrystallization from acetonitrile.

***rac*-3,4-Dihydro-7-hydroxy-4-oxospiro[2*H*-1-benzopyran-2,1′-cyclohexane]-4′-carboxylic Acid Ethyl Ester (68).** A mixture of 4.56 g (30 mmol) of 2′,4′-dihydroxyacetophenone (**53**), 6.8 g (40 mmol) of ethyl 4-oxocyclohexanecarboxylate,[22] 0.7 mL (8.4 mmol) of pyrrolidine, and 30 mL of toluene was stirred at room temperature for 1 h and then at reflux, with water removal with a Dean–Stark trap, for 5 h. The reaction mixture was cooled and treated with 3 N HCl and water. Workup with ether in the usual manner gave 9 g of a viscous, orange-brown oil. This material was chromatographed on 100 g of silica gel. Elution with 9:1 toluene–ethyl acetate afforded 4.68 g (51.3%) of chromanone **68** as a viscous, yellow oil. The ^1H NMR spectrum of this material revealed it to be a mixture of epimers.

***rac*-3,4-Dihydro-7-hydroxyspiro[2*H*-1-benzopyran-2,1′-cyclohexane]-4′-carboxylic Acid Ethyl Ester (69).** A solution of 4 g (13.1 mmol) of chromanone **68** in 20 mL of 1,2-dimethoxyethane was stirred with ice-bath cooling while 3.1 mL of boron trifluoride etherate was added dropwise followed by 1.3 mL of borane–dimethyl sulfide complex, also dropwise. The resulting solution was stirred with ice-bath cooling for 4 h and then decomposed by the cautious addition of 1 N HCl. The mixture was poured into water and worked up with ether in the usual manner (the ether extracts were also washed with saturated $NaHCO_3$ solution), giving 3.67 g of a yellow, viscous oil. This material was chromatographed on 50 g of silica gel. Elution with 4:1 hexanes–ethyl acetate afforded 2.42 g (63.7%) of chroman **69** as a colorless oil. The ^1H NMR spectrum revealed this material to be a mixture of epimers.

***rac*-6-Acetyl-3,4-dihydro-7-hydroxyspiro[2*H*-1-benzopyran-2,1′-cyclohexane]-4′-carboxylic Acid Methyl Ester (70).** A 3.01-g (10.38 mmol) sample of chroman **69** was treated under the Fries rearrangement conditions described above in method B. The crude acid was isolated by suction filtration, after quenching the acetic acid reaction mixture into water, and esterified with methanol as described above. The crude ester was chromatographed on 50 g of silica gel. Elution with 1:1 hexanes–ether gave 2.06 g of a yellow solid. Recrystallization from acetonitrile afforded 1.34 g of **70** as a pale-yellow solid, mp 131–132.5 °C. The ^1H NMR spectrum of this material revealed it to be a single epimer. Anal. ($C_{18}H_{22}O_5$) C, H.

***rac*-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydrospiro-[2*H*-1-benzopyran-2,1′-cyclohexane]-4′-carboxylic Acid Methyl Ester (71).** Alkylation of hydroxyacetophenone **70** (1.2 g, 3.77 mmol) with 1,5-dibromopentane (4.1 mL, 30.1 mmol) was carried out as described above (method G, crown ether omitted), giving 1.64 g (93.1%) of bromo ester **71** as a pale-yellow oil purified by chromatography (4:1 hexanes–ethyl acetate).

***rac*-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydrospiro[2*H*-1-benzopyran-2,1′-cyclohexane]-4′-carboxylic Acid (72).** Alkylation of **12** (0.775 g, 3.99 mmol) with bromide **71** (1.64 g, 3.51 mmol) was carried out as described above (method G), giving 1.81 g (88.8%) of the methyl ester of **72**, as a viscous, yellow oil, purified by column chromatography (1:1 hexanes–ether). This material crystallized on standing and was saponified with 7.5 mL of 1 N NaOH in 12.5 mL of methanol as described above. The crude acid was recrystallized twice from acetonitrile, giving 1.03 g (58.3%) of acid **72** as a tan solid, mp 138–140 °C. Anal. ($C_{33}H_{42}O_8$) C, H.

***rac*-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (101).** This material was obtained by saponification of ester **100** (25 g, 62.6 mmol) with NaOH (3 g) in 180 mL of water and sufficient methanol to effect solution. The mixture was stirred at 35 °C for 5 h. Acidification gave the acid in two crops: 13.5 g, mp 153–55 °C and 5.7 g, mp 151–52 °C, (79.7% total yield). The analytical specimen was obtained as a colorless solid, mp 158–160 °C, by recrystallization from ethanol. Anal. ($C_{17}H_{21}BrO_5$) C, H.

***rac*-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxamide (102).** A solution of 2 g (5.19 mmol) of acid **101** and 1 g (6.17 mmol) of 1,1′-carbonyldiimidazole in 50 mL of dry THF was stirred at room temperature for 2 h whereupon 50 mL of concentrated NH_4OH was added and stirring was continued overnight. The resulting precipitate was filtered with suction, washed with cold water, and dried, giving 1.2 g (60.2%) of amide **102**, mp 147–149 °C. More product could be obtained by adding water to the mother liquor. The analytical specimen was obtained by recrystallization from ethyl acetate as a colorless solid, mp 157–158 °C. Anal. ($C_{17}H_{22}BrNO_4$) C, H, N.

***rac*-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carbonitrile (103).** A solution of 5.68 g (14.8 mmol) of bromo amide **102** and 3 mL of pyridine in 50 mL of dry dioxane was stirred with ice-bath cooling while 7.2 mL of trifluoroacetic anhydride was added dropwise. The resulting mixture was stirred with cooling for 0.5 h and at room temperature for 4 h before being poured into ice–water. After being stirred for 0.5 h, the mixture was filtered and the solid was washed with water and dried. There was obtained 5 g (92.3%) of bromo nitrile **103**, mp 63–65 °C. A sample was recrystallized from aqueous ethanol, giving a colorless solid, mp 68–69 °C. Anal. ($C_{17}H_{20}BrNO_3$) C, H, N.

***rac*-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carbonitrile (104).** Alkylation of **12** (3.0 g, 15.5 mmol) with bromo nitrile **103** (12.0 mmol) was carried out as described above. There was obtained 4 g (69.5%) of **104** as a solid. A sample was recrystallized from ethanol, giving colorless solid, mp 98–100 °C. Anal. ($C_{28}H_{33}NO_6$) C, H, N.

***rac*-1-[7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)-pentyl]oxy]-3,4-dihydro-2-(1*H*-tetrazol-5-yl)-2*H*-1-benzo-**

pyran-6-yl]ethanone (91) (Method K). A mixture of 1.44 g (3 mmol) of nitrile **104**, 0.25 g (3.8 mmol) of sodium azide, 0.2 g (3.8 mmol) of ammonium chloride, and 15 mL of dry DMF was stirred at 120 °C for 5 h and then cooled and poured into water. The mixture was filtered with suction and the solid was washed with water and dried, giving 1.5 g (95.5%) of tetrazole **91**. The analytical specimen was obtained by recrystallization from ethanol.

***rac*-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-*N*-(phenylsulfonyl)-2*H*-1-benzopyran-2-carboxamide (92) (Method L).** A mixture of 3 g (6 mmol) of acid **7**, 0.96 g (6 mmol) of benzenesulfonamide, 0.96 g (7.8 mmol) of 4-(dimethylamino)pyridine, 1.08 g (6 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 5 g of 4A molecular sieves, and 60 mL of dry dichloromethane was stirred at room temperature for 3.5 days. The reaction mixture was filtered and the filtrate was washed with 1 N HCl, water, and brine, and the workup was completed in the usual manner. The residue was purified by HPLC (10:1 dichloromethane–acetone) and recrystallization from dichloromethane–ether–petroleum ether, giving 1.55 g (40.5%) of acyl sulfonamide **92** as an amorphous, colorless solid.

***rac*-6-Acetyl-7-[[5-(4-acetyl-3,6-dihydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (75) (Method H).** To a mixture of the sodium salt from 26.1 mmol of acid **7** and 100 mL of pyridine was added a solution of 6 g (92.1 mmol) of KOH in 100 mL of water. The resulting mixture was stirred at room temperature while a solution of 10.5 g (38.8 mmol) of potassium persulfate in 250 mL of water was added dropwise, over a 5-h period. After being stirred at room temperature for 18 h, the dark solution was treated with 10 g (96.1 mmol) of $NaHSO_3$ followed by the slow addition of 250 mL of concentrated HCl. The black, gummy mixture was stirred in a 70 °C oil bath for 2 h and then cooled and worked up with dichloromethane in the usual manner (some insoluble, black gum was separated). There was obtained 11.63 g of a brown foam, which was dissolved in 150 mL of methanol containing 0.3 g of *p*-toluenesulfonic acid monohydrate. The solution was stirred at reflux for 2 h, at room temperature for 15 h, and refluxed again for 3 h before being concentrated in vacuo. The residue was dissolved in dichloromethane and the solution was washed with brine and treated with Norit A. Completion of the usual work up gave a red-black, viscous oil, which was chromatographed on 200 g of silica gel. Elution with 9:1 and 4:1 toluene–ether gave 4.17 g (31.2%) of the methyl ester of starting acid **7** followed by 4.49 g (32.6%) of the methyl ester of **75** which crystallized. A total of 8.33 g of **75** methyl ester prepared in this way was triturated with ether. The solid was filtered with suction, washed with ether, and dried under high vacuum, giving 7.5 g of a yellow solid, mp 87–93 °C. Anal. ($C_{29}H_{36}O_9$) C, H. A mixture of 6.3 g (11.93 mmol) of this ester, 60 mL of methanol, and 60 mL of 1 N NaOH was stirred and refluxed for 2.5 h. The resulting dark red-brown solution was cooled, diluted with water, acidified with 3 N HCl, and worked up with ether in the usual manner, giving 6.33 g of a tan solid. This material was combined with 1.05 g of similarly prepared crude acid and recrystallized from acetonitrile. There was obtained 6.73 g of pure acid **75** as a tan solid. This compound retained acetonitrile tenaciously.

1-[2-Hydroxy-4-[[2-(2-hydroxyethyl)phenyl]methoxy]-3-propylphenyl]ethanone (17). A 39.6-g (0.295 mol) sample of isochroman was saturated with gaseous HBr and kept at room temperature for 17 h. The dark brown mixture was dissolved in ether and worked up in the usual manner, giving 48.0 g of an oil, which was chromatographed on silica gel. Elution with 4:1 hexanes–ether gave 40.2 g (63.5%) of 2-(bromomethyl)benzeneethanol. A solution of 15.55 g (72 mmol) of this material, 14.5 g (0.173 mol) of dihydropyran, and 0.45 g of *p*-toluenesulfonic acid monohydrate in 250 mL of ether was stirred for 4 h at room temperature and then washed with saturated $NaHCO_3$ solution. Completion of the usual workup and purification of the crude product by silica gel chromatography (eluting with 1:1 hexanes–ether) afforded 17.0 g (78.6%) of the desired THP ether. A mixture of 8.5 g (28.4 mmol) of this bromo ether, 6.5 g (33.5 mmol) of dihydroxyacetophenone **12**, 16 g (115 mmol) of anhydrous K_2CO_3, 2 g of 18-crown-6, and 250 mL of acetonitrile was stirred and refluxed for 17 h. The resulting mixture was cooled, diluted with ether, and worked up in the usual manner. The residue was chromatographed on silica gel, giving 10.5 g (90%) of **16** eluted with 4:1 hexanes–ether. A mixture of 5.2 g (12.5 mmol) of this material, 25 mL of 2 N HCl, and 50 mL of THF was stirred at room temperature for 20 h. Workup with ether in the usual manner and purification of the crude product by chromatography on silica gel (eluting with ether) afforded 3.95 g of alcohol **17**. Recrystallization from ethyl acetate–ether–hexanes gave 3.28 g (79.5%) of colorless solid, mp 122–124 °C. Anal. ($C_{20}H_{24}O_4$) C, H.

***rac*-6-Acetyl-7-[2-[2-[(4-acetyl-3-hydroxy-2-propylphenoxy)methyl]phenyl]ethoxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (82) (Method I).** To a stirred, cooled (ice bath) mixture of 3.28 g (10 mmol) of **17**, 4.7 mL (33.6 mmol) of triethylamine, and 75 mL of dichloromethane was slowly added 2.3 mL (29 mmol) of methanesulfonyl chloride. The reaction mixture was stirred for 3 h at room temperature and then poured into saturated $NaHCO_3$ solution. Workup with dichloromethane in the usual manner gave 5.5 g of crude mesylate. Recrystallization from ethyl acetate–ether–hexanes afforded 3.7 g (94.0%) of mesylate **18**. A mixture of 2.03 g (5 mmol) of this mesylate, 1.25 g (5 mmol) of ester **40**, 3 g (21.7 mmol) of anhydrous K_2CO_3, 80 mL of acetone, and 40 mL of DMF was stirred and refluxed for 17 h. The reaction mixture was diluted with ether and worked up in the usual manner. The crude product was chromatographed on silica gel. Elution with ether afforded the desired product, which was recrystallized from ethyl acetate–ether–hexanes, giving 0.85 g (30.2%) of **82** methyl ester as a colorless solid, mp 98.5–100.5 °C. A mixture of 0.78 g (1.4 mmol) of this ester, 5 mL of 10% aqueous LiOH, and 15 mL of THF was stirred at room temperature for 2 h and then poured into 2 N HCl. Workup with ether in the usual manner gave a solid, which was recrystallized from ethyl acetate–ether–hexanes, affording 0.65 g (84%) of acid **82** as a pale-yellow solid.

1-[4-[[3-(Bromomethyl)phenyl]methoxy]-2-hydroxy-3-propylphenyl]ethanone (19). A mixture of 2.64 g (10 mmol) of 1,3-bis(bromomethyl)benzene, 1.82 g (9.4 mmol) of **12**, 6.9 g of anhydrous K_2CO_3, and 50 mL of acetonitrile was stirred and refluxed for 2 h. The reaction mixture was cooled and filtered, and the solids were washed with ether. The filtrate and washes were combined, washed with water, and processed in the usual manner, giving 3.15 g of crude product. Chromatography on silica gel afforded 1 g (26.6%) of bromide **19**. Recrystallization of a sample from ethyl acetate–ether–hexanes gave a colorless solid, mp 100–102 °C. Anal. ($C_{19}H_{21}BrO_3$) C, H, Br.

***rac*-6-Acetyl-7-[[3-[(4-acetyl-3-hydroxy-2-propylphenoxy)methyl]phenyl]methoxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (83).** Alkylation of 0.665 g (2.66 mmol) of ester **40** with 1 g (2.6 mmol) of bromide **19** was carried out as described above in method G [K_2CO_3 (1.38 g), 18-crown-6 (80 mg), acetonitrile (22 mL)]. The crude product was purified by chromatography on silica gel. Elution with 4:1 ether–hexanes gave 0.71 g of product, which was recrystallized from ethyl acetate–ether–hexanes. There was obtained 0.535 g (36.2%) of **83** methyl ester as a colorless solid, mp 120–122 °C. Anal. ($C_{32}H_{34}O_8$) C, H. Saponification of 0.3 g (0.55 mmol) of this ester with LiOH as described above gave 0.242 g (82.6%) of acid **83** as a colorless solid purified by recrystallization from ethyl acetate–ether–hexanes.

1-[2-Hydroxy-4-[(5-hydroxy-3,3-dimethylpentyl)oxy]-3-propylphenyl]ethanone (21). A mixture of 3.7 g (28 mmol) of 3,3-dimethyl-1,5-pentanediol,[48] 1.95 mL (21.4 mmol) of dihydropyran, 0.2 g of *p*-toluenesulfonic acid monohydrate, and 30 mL of THF was stirred for 18 h at room temperature and then worked up with saturated $NaHCO_3$ solution and ethyl acetate in the usual manner. The residue was chromatographed on silica gel, giving 1.4 g (20%) of the mono-THP ether as a pale-yellow oil. A mixture of this hydroxy ether (6.48 mmol) and 1.4 mL of triethylamine in 20 mL of dichloromethane was stirred with ice-bath cooling while 0.55 mL (2.11 mmol) of methanesulfonyl chloride was added. The reaction mixture was stirred at room temperature for 1 h and then worked up with saturated $NaHCO_3$

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solution and ethyl acetate in the usual manner, giving 1.8 g (94.5%) of the mesylate. A mixture of this mesylate (6.12 mmol), 1.25 g (6.44 mmol) of **12**, 4.2 g (30.4 mmol) of K_2CO_3, 0.2 g of 18-crown-6, and 35 mL of acetonitrile was stirred and refluxed for 18 h. After being cooled, the reaction mixture was diluted with acetonitrile and filtered with suction. The filtrate was concentrated in vacuo, giving 2.7 g of a brown oil. Chromatography of this material on silica gel afforded 1.4 g (58.2%) of ether **20** as a pale-yellow oil eluted with 4:1 hexanes–ethyl acetate. A mixture of this THP ether (3.57 mmol) and 0.5 mL of 2 N HCl in 20 mL of methanol was stirred at room temperature for 4 h. Workup with saturated $NaHCO_3$ solution and ethyl acetate in the usual manner gave 1.2 g of product, which was chromatographed on silica gel. Elution with 1:1 hexanes–ethyl acetate gave 0.9 g (82%) of alcohol **21** as a pale-yellow oil. Anal. ($C_{18}H_{28}O_4$) C, H.

***rac*-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)-3,3-dimethylpentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (84).** With use of the procedure described above for the preparation of **18**, 0.9 g (2.9 mmol) of the alcohol **21** from the preceding experiment was converted into the mesylate **22** (1.2 g), which was used without purification. Alkylation of ester **40** (0.8 g, 3.2 mmol) with this mesylate was carried out by using the procedure described above for the preparation of **83**. The methyl ester of **84** was obtained in 59.5% yield, as a pale-yellow oil, after chromatography on silica gel (eluted with 1:1 hexanes–ethyl acetate). Saponification of 0.549 g (1.02 mmol) of this ester with LiOH as described above afforded 0.532 g of crude, oily acid, which was chromatographed on silica gel. Elution with 4:1 ethyl acetate–methanol gave acid **84** (0.28 g, 52.2%) as a gum.

[2*R*,*S*-[2-(1*R,2*R**)]]-6-Acetyl-3,4-dihydro-7-hydroxy-2*H*-1-benzopyran-2-carboxylic Acid 2-Hydroxy-1-methylpropyl Ester (106).** To a solution of 8.3 g (31.4 mmol) of ester **38** in 80 mL of THF was added a solution of 4.5 g of LiOH monohydrate in 80 mL of water. After being stirred for 4 h at room temperature, approximately 50 mL of solvent was removed in vacuo and water and hexanes were added to the residue. The organic phase was separated and discarded and the aqueous phase was acidified with 2 N HCl. Workup with ether in the usual manner gave 7.5 g (100%) of acid **105**. The analytical specimen was obtained from another experiment as a colorless solid, mp 242.5–244.5 °C (recrystallized from acetonitrile). Anal. ($C_{12}H_{12}O_5$) C, H. A solution of this acid in 220 mL of dichloromethane was added to a mixture of 7.2 g (80 mmol) of D-(−)-2,3-butanediol, 6.8 g (33 mmol) of *N*,*N*′-dicyclohexylcarbodiimide, and 0.38 g of 4-(dimethylamino)pyridine in 100 mL of dichloromethane. The slurry was stirred at room temperature for 4 h at which point the solids were removed by suction filtration. The filtrate was washed with water, 5% acetic acid, water, and brine and then processed in the usual manner, giving 14 g of a yellow, oily residue. This material was purified by HPLC (2:1 toluene–ethyl acetate). The early fractions afforded 3.5 g (35.5%) of ester **106** enriched in the less polar 2*S* epimer (^{1}H NMR). The later fractions furnished 2.9 g (29.4%) of ester **106** enriched in the more polar 2*R* epimer (^{1}H NMR).

[2*S*-[2β(1*R,2*R**)]]-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid 2-Hydroxy-1-methylpropyl Ester (108).** A mixture of the ester **106** enriched in the less polar 2*S* epimer from the preceding experiment (3.5 g, 11.4 mmol), 20.2 g (88 mmol) of 1,5-dibromopentane, and 7.2 g (52 mmol) of anhydrous K_2CO_3 in 100 mL of acetone and 50 mL of DMF was stirred and refluxed for 18 h. The resulting slurry was cooled, treated with water and brine, and worked up with ethyl acetate in the usual manner. Column chromatography of the residue on silica gel gave 3.1 g (59.6%) of the ether ester mixture **107** enriched in the 2*S* epimer (eluted with 3:1 toluene–ethyl acetate). Purification of this material by HPLC (2:1 toluene–ethyl acetate; recycling) afforded 2.1 g (40.3%) of the less polar 2*S* epimer as a yellow oil, which crystallized. Recrystallization from ether gave **108** as a colorless solid: mp 94.5–98 °C; LC purity 98.6%; $[\alpha]^{25}_D$ −7.99°; ^{1}H NMR δ 1.27, 1.13 ppm (2 d, 6, J = 6.5 Hz, CH_3CHO). Anal. ($C_{21}H_{29}BrO_6$) C, H.

[2*R*-[2α(1*R,2*R**)]]-6-Acetyl-7-[(5-bromopentyl)oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid 2-Hydroxy-1-methylpropyl Ester (109).** With use of the procedure of the preceding experiment, 2.9 g (9.44 mmol) of ester **106** enriched in the 2*R* epimer was converted into **109** in 24% yield: mp 80–83 °C (from ether); LC purity 99.4%; $[\alpha]^{25}_D$ −21.49°; ^{1}H NMR δ 1.26, 1.20 ppm (2 d, 6, J = 6 Hz, CH_3CHO). Anal. ($C_{21}H_{29}BrO_6$) C, H. The structure of **109** was determined by a single-crystal X-ray analysis. The crystal data are summarized in Table X. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately 0.04 × 0.06 × 0.25 mm; the data were corrected for absorption. Of the 1496 independent reflections for $\theta < 57°$, 1286 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[49] and was refined by full-matrix least squares. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices are R = 0.053 and R_w = 0.055 for the 1286 observed reflections. The major peaks from the final difference map, none of which are greater than ±0.6 e A^{-3}, are near the bromine atom.

Table X. Crystal Data for **109**

formula	$C_{21}H_{29}BrO_6$
formula weight	457.36
crystal system	triclinic
space group	$P\bar{1}$
a	5.805 (1) Å
b	9.670 (2) Å
c	10.075 (2) Å
α	79.20 (1)°
β	84.64 (1)°
γ	88.93 (2)°
Z	1
d_{calcd}	1.373 g cm^{-3}
μ(Cu Kα)	30.7 cm^{-1}

[2*S*-[2β(1*R,2*R**)]]-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid 2-Hydroxy-1-methylpropyl Ester (110).** A mixture of 199.8 mg (0.437 mmol) of the 2*S*-bromo ester **108**, 90.8 mg (0.468 mmol) of **12**, and 0.3 g (2.17 mmol) of anhydrous K_2CO_3, in 5 mL of dry acetone and 2.5 mL of dry DMF, was stirred and refluxed for 2 h. The reaction mixture was cooled, treated with water and ethyl acetate, and worked up with ethyl acetate in the usual manner. The residue was purified by column chromatography on silica gel. The desired product was recrystallized from ethyl acetate–hexanes, giving 106 mg (42.6%) of **110**, as a colorless solid: LC purity 98.2%; mp 124–127.5 °C; $[\alpha]^{25}_D$ −7.25°; ^{1}H NMR δ 1.27, 1.14 ppm (2 d, 6, J = 6.5 Hz, CH_3CHO). Anal. ($C_{32}H_{42}O_9$) C, H.

[2*R*-[2α(1*R,2*R**)]]-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid 2-Hydroxy-1-methylpropyl Ester (111).** With use of the procedure of the preceding experiment, **12** was alkylated with the 2*R*-bromo ester **109**, giving **111**, in 50.8% yield, as a colorless solid: mp 100–105 °C; $[\alpha]^{25}_D$ −15.75°; LC purity 96.1%; ^{1}H NMR δ 1.26, 1.20 ppm (2 d, 6, J = 7 Hz, CH_3CHO). Anal. ($C_{32}H_{42}O_9$) C, H.

***(S*)-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (*S*-7).** A mixture of 405.6 mg (0.712 mmol) of ester **110** and 0.6 g (14.3 mmol) of LiOH monohydrate in 10 mL of 1:1 water–THF was stirred at room temperature for 2.5 h. After being acidified with 2 N HCl, the mixture was worked up with ethyl acetate in the usual manner, giving 0.4 g of a pale-yellow oil, which crystallized on standing. Recrystallization from ether–hexanes afforded 186 mg (52.5%) of acid *S*-7 as a colorless solid: mp 94–97 °C; LC purity of the methyl ester, 95% determined with a Pirkle column.[26]

***(R*)-6-Acetyl-7-[[5-(4-acetyl-3-hydroxy-2-propylphenoxy)pentyl]oxy]-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic Acid (*R*-7).** With use of the procedure of the preceding experiment, ester 111 was saponified, in 42.3% yield, to acid *R*-7, as a colorless solid: mp 102.5–108 °C; LC purity of the methyl

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Table XI. Crystal Data for *S*-7 (*R*)-α-Methylbenzylamine Salt

formula	$C_{28}H_{34}O_8 \cdot C_8H_{11}N$
formula weight	619.76
temperature	110 (1) K
crystal system	orthorhombic
space group	$P2_12_12_1$
a	6.900 (4) Å
b	16.506 (4) Å
c	29.112 (9) Å
Z	4
d_{calcd}	1.241 g cm^{-3}
μ(Cu Kα)	6.7 cm^{-1}

ester, 99.1%, determined with a Pirkle column.[26]

Optical Resolution of Racemic 7. A 10.5 g (21 mmol) sample of *rac*-**7** was dissolved in 80 mL of warm 3A ethanol and 1.4 mL (10.86 mmol) of (*S*)-(−)-α-methylbenzylamine ([α]$^{25}_D$ −40.0° (neat)) was added in one portion. The solution was cooled to room temperature and 45 mL of anhydrous ether was added followed by a seed crystal of the pure *R*-acid *S*-amine salt. The mixture was kept at room temperature overnight. The precipitate was filtered with suction and washed with 3A ethanol and ether and then dried under high vacuum, giving 4.08 g of an off-white solid, mp 165–169 °C (with shrinking from 148 °C). The mother liquor and washes were saved for isolation of the *S*-acid as described below. The salt was recrystallized twice from 35–40 parts of 3A ethanol, giving 2.15 g (33%) of pure *R*-acid *S*-amine salt as pale-yellow needles, mp 168–171 °C (with some shrinking from 165 °C). A small sample of this material was dried overnight at 50–60 °C/high vacuum to provide an anlytical specimen, [α]$^{25}_D$ −24.20°. Anal. ($C_{28}H_{34}O_8 \cdot C_8H_{11}N$) C, H, N. A 2.05-g (3.31 mmol) sample of this *R*-acid *S*-amine salt was vigorously shaken with ether and 10 mL of 1.5 N HCl. When the salt had completely dissolved, the ether layer was washed with 1.5 N HCl and the aqueous acidic layers were combined and extracted with ether. Processing the combined ether extracts in the usual manner gave 1.62 g of an almost colorless foam. This material was recrystallized from 7 mL of acetonitrile. Seeding with a crystal of the *R*-acid led to the formation of a dense slurry, which was kept overnight at room temperature. There was obtained 1.28 g (77.6%) of *R*-**7** as an off-white solid: mp 98.5–100 °C; [α]$^{25}_D$ +4.78°. Anal. ($C_{28}H_{34}O_8$) C, H. LC analysis of the methyl ester of this acid on a covalent (*R*)-phenylglycine Pirkle[26] column revealed an enantiomeric purity of 100% (t_R 53.7 min). This methyl ester (**113**) exhibited [α]$^{25}_D$ −6.52°.

The mother liquor and washes from the first precipitation of the *R*-acid *S*-amine salt above were combined and concentrated in vacuo. The residue was treated with ethyl acetate and 1 N HCl and the mixture was shaken. The aqueous layer was extracted twice more with ethyl acetate. The ethyl acetate extracts were combined, washed twice with 1 N HCl, and processed in the usual manner, giving 7.2 g of acid **7** enriched in the *S* enantiomer. This material (14.25 mmol) was dissolved in 70 mL of warm 3A ethanol, and 1.8 mL (13.98 mmol) of (*R*)-(+)-α-methylbenzylamine ([α]$^{25}_D$ +40.0° (neat)) was added in one portion. The solution was seeded with *S*-acid *R*-amine salt and kept at room temperature overnight. The precipitate was recrystallized twice from 35–40 parts of 3A ethanol. There was obtained 2.9 g (45.2%) of *S*-acid *R*-amine salt as pale-yellow needles, mp 171–172.5 °C (with shrinking from 166 °C). A sample of this material was dried at 50–60 °C/high vacuum to provide an analytical specimen, [α]$^{25}_D$ +23.66°. Anal. ($C_{28}H_{34}O_8 \cdot C_8H_{11}N$) C, H, N. The structure of this salt was determined by a single-crystal X-ray analysis. The crystal data are summarized in Table XI. The intensity data were measured on an Enraf-Nonius CAD4 diffractometer (graphite-monochromated Cu Kα radiation, θ–2θ scans). The crystal used for data collection, approximately 0.06 × 0.13 × 0.29 mm, was cooled with a nitrogen gas stream to 110 ± 1 K. The data were not corrected for absorption. Of the 2836 independent reflections for $\theta < 60°$, 1610 were considered to be observed [$I > 2.0\sigma(I)$]. The structure was solved by a multiple-solution procedure[50] and was refined by full-matrix least squares. In the final refinment, the non-hydrogen atoms were refined anisotropically, except for the disordered atoms O20A, C21A, C22A, C23A, C43A, and C44A in the major rotamer and O20B, C21B, C22B, C23B, C43B, and C44B in the minor rotamer, which were refined isotropically. The occupancies of the disordered atoms in the major rotamer were adjusted to 0.65, and those in the minor rotamer were adjusted to 0.35, in order to obtain approximately equal isotropic thermal factors for corresponding atoms between the two rotamers. The hydrogen atoms were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices are $R = 0.068$ and $R_w = 0.062$ for the 1610 observed reflections. The final difference map has no peaks greater than 0.2 eA^{-3}.

A 2.8-g (4.52 mmol) portion of this *S*-acid *R*-amine salt was processed as described above, giving 2.22 g of *S*-**7** as a foam. This material was recrystallized from 10 mL of acetonitrile, seeding with the *S*-acid. There was obtained 1.7 g (75.5%) of *S*-acid as an off-white solid: mp 108–111 °C; [α]$^{25}_D$ −4.50°. Anal. ($C_{28}H_{34}O_8$) C, H. LC analysis of the methyl ester of this acid on the Pirkle column[26] revealed an enantiomeric purity of 99.1% (t_R 51.6 min). Methyl ester **112** exhibited [α]$^{25}_D$ +6.65°.

Esterification of the Acid Enantiomers. Samples of 62 mg (0.1 mmol) of the amine salts or 50 mg (0.1 mmol) of the acids were treated with 0.35 mL of 0.75 M boron trifluoride etherate in methanol. With the acids, slight warming was required to effect solution. The solution was stirred at room temperature for 6 h during which time the methyl ester frequently precipitated, giving a dense slurry. The mixture was diluted with ether and the ether solution was washed with water, 1 N HCl (only when the amine salt was employed), water, saturated aqueous $NaHCO_3$, and brine and then the workup was completed in the usual manner. The yield was in the range of 45–52 mg of colorless solid ester, which was homogeneous on TLC analysis (1:1 hexane–ethyl acetate; R_f ~0.5).

Pharmacological Techniques. LTD_4 Receptor Binding Assay. (a) Preparation of Membrane Homogenate. Previously described procedures were used.[51] Male albino guinea pigs (Hartley strain, 400–500-g body weight) were sacrificed by decapitation. The lungs were removed, frozen in liquid nitrogen, and stored at −70 °C until use. The frozen tissue (5 g) was thawed, minced into small pieces, and rinsed in phosphate-buffered saline. The tissue was placed in 40 mL of homogenization buffer containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.5), and the following protease inhibitors: soybean trypsin inhibitor (5 μg/mL), bacitracin (100 μg/mL), benzamidine (10^{-3} M), and phenylmethanesulfonyl fluoride (10^{-4} M). The protease inhibitors were included to inhibit proteolysis during the processes of homogenization and centrifugation. The tissue was then homogenized at 0–4 °C with a Brinkman PT-20 Polytron for a total of 1 min (10-s pulses at a setting of 6). The homogenate was centrifuged (1000*g* for 10 min) to remove tissue clumps, unbroken cells, and nuclei. The supernatant was recentrifuged at 30000*g* for 30 min to yield pellets which were referred to as crude membrane fractions. This fraction was then resuspended in the incubation buffer (10 mM PIPES buffer, pH 7.5, 50 mM NaCl), homogenized in a Teflon homogenizer, and recentrifuged at 30000*g* for 30 min. The pellets were finally resuspended in the incubation buffer with a Teflon homogenizer, at a concentration of 10–20 mg/mL of protein in the suspension. The concentrations of proteins were determined using the Bio-Rad reaction kit.

(b) Receptor–Ligand Binding Assay. Optimum assay conditions were determined with an assay mixture containing Tyrode solution, 0.1% bovine serum albumin (BSA), 1 mM glycine, 1 mM cysteine, 3.5 nM [^{3}H]-LTD_4, and the membrane preparation (100–200 μg protein), in a final volume of 250 μL. The incubation was carried out at 20 °C for 30 min. At 20 °C, binding increased linearly with protein concentration, reached equilibrium in 20 min, was saturable, and reversible upon additions of unlabeled LTD_4. Separation of bound from free [^{3}H]-LTD_4 was performed by rapid filtration on GF/C glass-fiber filters and washing with 2–4-mL aliquots of Tyrode solutions containing 0.1% BSA. Radioactivity remaining on the filters was measured in 10

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mL of Aquasol. Specific binding was defined as that displaced by 10^{-6} M unlabeled LTD_4 and was 95% of the total binding. Compounds were initially evaluated in triplicate determinations, at a concentration of 10 μM, and percent specific inhibition was determined. Those compounds which specifically inhibited LTD_4 binding greater than 50% were reevaluated (in triplicate determinations) at three or more concentrations, which caused significant inhibitory effects between 10% and 90%. IC_{50} values were determined by linear regression, from plots of specific binding vs concentration. The correlation coefficient of the regression line of each compound was always greater than 0.95.

In Vitro Guinea Pig Ileum Bioassay. The effects of compounds on SRS-A-induced contraction of guinea pig ileal smooth muscle were evaluated by techniques previously described.[9]

In Vitro Guinea Pig Trachea Bioassay. The effects of compounds on LTD_4-induced contraction of guinea pig tracheal smooth muscle were evaluated by techniques previously described.[14b]

In Vitro Rat Peritoneal Macrophage Assay. The rat peritoneal macrophage assay measures the ability of a test compound to influence the release of arachidonic acid (AA) from phospholipid stores in the plasma membrane and the subsequent metabolism of AA through the cyclooxygenase and lipoxygenase pathways to the final products secreted by the cells: LTB_4, PGE_2, and TXB_2 (stable form of TXA_2). The amounts of these products are then measured by radioimmunoassay. Macrophages were obtained from rats by peritoneal lavage with phosphate-buffered saline minus Ca^{2+} and Mg^{2+} (PBS). Cells were washed three times with PBS and resuspended in Delbecco's modified eagle medium containing L-glutamine and D-glucose (Gilco Laboratories) and supplemented with 10% fetal calf serum. Cells were counted on a Coulter ZBI cell counter and then resuspended to a concentration of 4×10^6 cells/mL. Cell suspension (3 mL) was added to plastic culture dishes (3 cm), and the cells were allowed to adhere to the dishes for 90 min at 37 °C. Dishes were washed three times with PBS to remove nonadherent cells. Radiolabeled arachidonic acid ([^{14}C]AA, approximately 54 μCi/mmol) was added to the cells (1 μCi/dish) and allowed to incorporate for 90 min. The cell layer was again washed three times with PBS to remove unincorporated [^{14}C]AA. Cells were incubated with test compounds or the solution used to dissolve the test compounds (control) for 30 min at 37 °C and the cells were then stimulated with calcium ionophore A 23187 (0.5 μM) for 20 min. The extracellular fluid was removed and ^{14}C radioactivity released into this fluid from AA metabolism was measured by liquid-scintillation spectroscopy. The amounts of LTB_4, PGE_2, and TXB_2 were measured in the extracellular fluid by radioimmunoassay with specific antisera. Test compounds were initially evaluated in triplicate determinations, at a concentration of 30 μM, and the percent inhibition of the maximum effect produced in the presence of A23187 was calculated. Those compounds which significantly inhibited ionophore-induced arachidonic acid metabolism greater than 50% were reevaluated at three or more concentrations and IC_{50} values were determined from plots of inhibition vs concentration.

In Vitro Phosphodiesterase Assay. The effects of compounds on beef heart cyclic nucleotide phosphodiesterase (Sigma) were evaluated by techniques previously described.[35a]

In Vivo Bronchoconstriction Models. The in vivo ability of compounds to inhibit LTD_4-induced bronchoconstriction in guinea pigs was assessed by the intravenous and aerosol routes of administration. The intravenous technique utilized male guinea pigs (Hartley strain, Charles River) weighing 400–600 g. Animals were anesthetized with urethane (2 g/kg) intraperitoneally and a polyethylene cannula was inserted into the jugular vein for intravenous drug administration. Tracheal pressure (centimeters of water) was recorded from a Statham pressure transducer (P 32 AA). Since previous work[52] had demonstrated a potentiating effect of intravenous propranolol (0.1 mg/kg) on bronchoconstriction induced with synthetic LTs, propranolol was administered 5 min prior to challenge with LT. Two minutes later, spontaneous breathing was arrested with succinylcholine chloride (1.2 mg/kg) administered intravenously, and the animals were ventilated with a Harvard Model 680 small-animal respirator set at 40 breaths/min and 4.0 cm^3 stroke volume. Control vehicle or test drug was administered through the cannula into the jugular vein 30 s before the animals were challenged with a maximum constrictory dose of LTD_4 (25 μg/kg) given intravenously. Compounds were initially evaluated at a dose of 10 mg/kg intravenously, and the change in tracheal pressure was averaged for three control and five drug-treated animals. Those compounds which significantly inhibited LTD_4-induced bronchoconstriction greater than 50% were reevaluated at three or more doses which caused significant inhibitory effects between 10% and 90%. The median inhibitory doses (ID_{50} values) were determined from linear regression calculated by log dose–response curves. The correlation coefficient for the regression line of each antagonist was always greater than 0.95. Statistical analyses were performed by using Student's unpaired t test, with a probability value $p < 0.05$ regarded as significant.

The aerosol technique[53] utilized male guinea pigs anesthetized and surgically prepared as described above. Propranolol (0.1 mg/kg) was administered intravenously 5 min prior to aerosol exposure. Spontaneously breathing animals were exposed for a 5-min period to an aerosol prepared from an aqueous solution of test drug neutralized with NaOH or to distilled water. A Monaghan Model 670 ultrasonic nebulizer was used to administer varying concentrations (% w/v) of all compounds by inhalation. Aqueous solutions were freshly prepared and introduced into the chamber of the nebulizer. The output of the nebulizer was made available to the animal by directing a bias flow of aerosol through a Y-tube connected to the tracheal cannula. At the end of the 5-min exposure period, spontaneous breathing was arrested with succinylcholine (1.2 mg/kg, iv), and the animals were ventilated with a Harvard Model 680 small-animal respirator set at 40 breaths/min and 4.0 cm^3 stroke volume. The animals were challenged with a maximum constrictory dose of LTD_4 (50 μg/kg) administered intravenously 30 s after the end of the 5-min exposure to aerosolized drug. Compounds were initially evaluated at a concentration of 1% in the nebulizer and the change in tracheal pressure was averaged for three control and five drug-treated animals. Those compounds which significantly inhibited LTD_4-induced bronchoconstriction greater than 50% were reevaluated at three or more concentrations which caused significant inhibitory effects between 10% and 90%. The median inhibitory concentrations (IC_{50} values) were determined as described above. Statistical analyses were performed by using Student's unpaired t test, with a probability value $p < 0.05$ regarded as significant.

The effects on LTC_4 (50 μg/kg, iv), LTE_4 (50 μg/kg, iv), LTB_4 (400 μg/kg, iv), PAF (10 μg/kg, iv), arachidonic acid (500 μg/kg, iv), and histamine (50 μg/kg, iv) induced bronchoconstriction were evaluated by techniques similar to those described for LTD_4. The in vivo model previously described by Anderson et al.[32a] was employed to measure the inhibition of antigen-induced, endogenous, LT-mediated bronchoconstriction.

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Supplementary Material Available: Tables of final atomic positional parameters, atomic thermal parameters, and bond distances and angles for compound **109** and the (*R*)-α-methylbenzylamine salt of *S*-7 (12 pages). Ordering information is given on any current masthead page.

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