A RATIONAL APPROACH TO THE DESIGN OF

A LEUKOTRIENE ANTAGONIST

A thesis presented by

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ABSTRACT

Leukotrienes are newly discovered metabolites of arachidonic acid believed to play a mediatory role in the pathogenesis of asthma. It was the intention, therefore to develop a leukotriene antagonist which may be clinically beneficial in this disease state.

The known leukotrienes LTC₄, LTD₄, and LTE₄ were synthesised and studied in vitro on a range of animal tissues, which indicated the existence of three discrete leukotriene receptors. Synthesis of leukotriene analogues aimed at simplifying the agonist pharmacophore, resulted in the identification of a highly potent agonist of greater structural simplicity than the natural leukotrienes. From this starting point, using concepts of molecular rigidity and pharmacophoric group displacement, a compound was developed which antagonised the effects of the leukotrienes on the three receptor systems.

This antagonist, bearing such a broad profile of activity, constitutes a major breakthrough in the field of leukotriene research.
Acknowledgements

I wish to thank Dr. W.A. Taylor (Research Director) and the directors of Miles Laboratories Limited for allowing me to undertake the studies presented in this thesis. I would also like to thank Dr. T.S. Abram, my internal supervisor, and Professor J.A. Elvidge, my external supervisor, for their help and guidance during this work. My thanks are also due to Jim Bloxsidge for the 90 MHz $^1$H nmr spectra and to all my colleagues at Miles Laboratories for helpful discussions during the course of this project. Finally, my very sincere thanks to Mrs Mandy Raynham for her excellent work in typing this thesis.
The work discussed in this thesis forms part of a group research project undertaken at Miles Laboratories. There is therefore material reported herein which is the work of others in the group. It was necessary to include this work so that a clear understanding of the authors thoughts and designs in the area may be given.

The following indicates the synthetic and pharmacological contributions reported in this thesis.

**Synthesis**

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</tr>
<tr>
<td>A-20, A-21, A-22</td>
<td>M.A. Jennings</td>
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<tr>
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<td>T.S. Abram P. Norman M.A. Jennings S.R. Tudhope</td>
</tr>
<tr>
<td>FPL 55712</td>
<td>S.R. Tudhope</td>
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I wish to dedicate this thesis to my parents and to Chris.
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Abbreviations used in this text

A    Analogue
Ac   Acetyl
AMP  Adenosine monophosphate
ANT  Antagonist
br   Broad
c    Concentrated
d    Doublet
ether Diethyl ether
EtOAc Ethyl acetate
FA   Full agonist
FAB  Fast atom bombardment
h    Hours(s)
Hist. Histamine
HMPA Hexamethylphosphoramide
HPETE Hydroperoxyeicosatetraenoic acid
HPLC High pressure liquid chromatography
I    Inactive
LT   Leukotriene
LTA₄ 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid
LTA₄-Me Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoate
LTB₄ 5(S),12(R)-Dihydroxy-6(Z),8(E),10(E),14(Z)-eicosatetraenoic acid
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<td>PA</td>
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<td>Phosphate buffered saline</td>
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<td>WA</td>
<td>Weak agonist</td>
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INTRODUCTION
In 1938 Feldberg and Kellaway demonstrated the release of a substance from dog and monkey lung, in response to stimulation by cobra venom, which caused a delayed and slow contraction of the guinea-pig jejunum. Two years later Kellaway and Trethewie described the release of a similar substance upon specific antigen challenge by guinea-pig heart-lung preparations and termed this SRS (slow-reacting substance). Brocklehurst subsequently showed that a slow-reacting substance released during anaphylaxis of guinea-pig, rabbit, monkey and human lung, termed SRS-A (slow-reacting substance of anaphylaxis) was unaffected by the histamine antagonist mepyramine and so began the speculation of an involvement of SRS-A in human asthma.

SRS-A could only be produced in very small quantities from biological systems which for many years precluded detailed structural analysis. Furthermore the biologically derived material was crude and it wasn't until improved chromatographic techniques became available that purified SRS-A could at last be studied. Bach and Brashler demonstrated that increased SRS production was obtained from peritoneal cells challenged in vitro and in vivo with the calcium ionophore A23187 which was further enhanced by the presence of certain mercaptans, especially cysteine. With the improved purification techniques described by Morris, Taylor et al in 1978, utilising recently invented
high pressure liquid chromatographic (HPLC) systems, the door was finally open to discovering the identity of the elusive SRS.

Early studies on crude SRS had suggested that it was an unsaturated acidic lipid containing sulphur possibly as a sulphate ester. In 1977 Bach and Jakschik independently reported evidence that SRS was a metabolite of arachidonic acid, although they disagreed as to whether it was a product of the lipoxygenase or cyclooxygenase pathway. Although the addition of mercaptans to peritoneal cell incubations had been shown to markedly improve the yield of SRS it was uncertain whether the mercaptans merely stimulated SRS release or were actually incorporated into the molecule. Radiolabelling studies were inconclusive, probably due to the minute quantity of material being studied. Bach and Brashler, however, studying the stimulation of SRS release by a wide range of mercaptans found that only mercapto carboxylic acids were effective. Furthermore the SRS-type products appeared to be non-identical and they thus proposed the incorporation of mercaptans into SRS by the trapping of a labile intermediate.

In 1979 Borgeat and Samuelsson detected an unstable intermediate in the transformation of arachidonic acid into dihydroxy acids in leukocytes. The dihydroxy acids were characterised by HPLC and Mass spectrometry as 5,6-dihydroxeyicosatetraenoic acid and
5,12-dihydroxyeicosatetraenoic acid and they thus proposed that the unstable intermediate was the epoxide 5,6-oxido-7,9,11,14-eicosatetraenoic acid. In the same year Samuelsson et al.\textsuperscript{16} characterised a cysteinyl derivative of 5,6-oxido-7,9,11,14-eicosatetraenoic acid as the SRS produced by murine mastocytoma cells stimulated with A23187 in the presence of cysteine.

Samuelsson et al.\textsuperscript{17} introduced the term leukotriene to describe the newly discovered family of arachidonic acid metabolites containing the unstable epoxide intermediate (Leukotriene A), its enzymatic hydrolysis product 5,12-dihydroxyeicosatetraenoic acid (Leukotriene B) and the thiopeptidyl derivative (Leukotriene C). It soon became apparent, however, that SRS's from different sources were slightly different from one another, varying in the peptide substituent attached to C-6\textsuperscript{18,19,20,21}. An elegant synthesis by Corey et al.\textsuperscript{22,23} confirmed the structures of the natural products as those resulting from addition of either glutathione, cysteinylglycine or cysteine to C-6 of leukotriene A. These are now known as leukotrienes C\textsubscript{4}, D\textsubscript{4} and E\textsubscript{4} respectively\textsuperscript{24} (Fig. 1). Thus SRS, and indeed SRS-A\textsuperscript{25}, are mixtures of the potent spasmogens leukotriene C\textsubscript{4} (LTC\textsubscript{4}), leukotriene D\textsubscript{4} (LTD\textsubscript{4}) and leukotriene E\textsubscript{4} (LTE\textsubscript{4}); the relative proportions of each varying from one source to another, depending on the local enzyme population.
It is now known that glutathione S-transferase converts leukotriene \( \Lambda_4 \) (LTA\(_4\)) into LTC\(_4\) which is metabolised to LTD\(_4\) by a \( \gamma \)-glutamyltranspeptidase\(^{18,26}\). LTD\(_4\) is further metabolised by an aminopeptidase to LTE\(_4\)\(^{24}\) which was shown in the rat\(^{27}\) to be further metabolised to N-acetyl LTE\(_4\). A recent study in man\(^{28}\), however, has shown that LTE\(_4\) is the end metabolite of LTC\(_4\), being predominantly excreted in the urine.

The term leukotriene was derived from leukocytes, the cells in which the family was first identified and a common structural feature, the conjugated triene; the subscript denoting the total number of double bonds in the molecule\(^{24}\).

Leukotrienes are produced from a number of inflammatory cells including leukocytes, eosinophils, macrophages and mast cells (see review\(^{29}\)) by a variety of stimuli including immunological challenge and calcium ionophore stimulation. However, as the majority of cell types contain the precursor to leukotriene formation, arachidonic acid, as a component of their cell membranes, it is tempting to speculate that most mammalian cells have the capacity to produce leukotrienes.

**BIOLOGICAL ACTIVITY OF LEUKOTRIENES**

The sulphidopeptidyl leukotrienes (LTC\(_4\), LTD\(_4\) and LTE\(_4\)) are potent contractile agents on many smooth muscle tissues in a variety of species\(^{30,31,32}\).
In general they have 100-1000 times the potency of histamine on a molar basis, are vasoconstricting and, furthermore, increase vascular permeability giving rise to oedema.

Leukotriene B₄ is a potent chemotactant, attracting neutrophils which can subsequently cause major tissue damage. Thus the family of leukotrienes could play important mediatory roles in the clinical diseases associated with asthma, allergy and inflammation.

The association of leukotrienes with the human asthmatic state began with the discovery of SRS-A in specifically challenged human asthmatic lung effluents. Subsequent studies with synthetic peptido leukotrienes have shown that they preferentially constrict the peripheral airways unlike the prostaglandins which selectively act on the larger passages. This may explain the inability of prostaglandin synthesis inhibitors such as indomethacin to be effective in asthma therapy (where peripheral airways constriction is a major problem) and enhances the belief that leukotriene antagonists or synthesis inhibitors may be clinically beneficial.

The leukotrienes have been shown to contract smooth muscle tissue via a direct and an indirect mechanism. In guinea-pig lung parenchyma leukotrienes cause the release of thromboxane A₂ which enhances the direct effect of leukotriene-induced contraction.
Leukotriene B₄, a non-peptido leukotriene, contracts guinea-pig lung parenchyma solely via thromboxane A₂ release, having no direct action itself. For this reason the general use of the word 'leukotriene' in this report shall henceforth be limited to the sulphidopeptidyl leukotrienes LTC₄, LTD₄ and LTE₄, as it is their direct mode of action on smooth muscle tissue that is of major interest herein.

The reported biological profiles of the leukotrienes on various tissues have been somewhat conflicting over the years. This is probably due to a combination of four factors: The chemical instability of leukotrienes giving purity and quantitation problems; metabolic instability; indirect effects caused by LT-induced mediator release and last but not least, 'biological variability'. The first problem may be overcome by using freshly synthesised material temporarily stored under an inert atmosphere at very low temperature, thawing immediately prior to use as repeated thawing and freezing has been shown to be detrimental to activity. Secondly, enzyme inhibitors added to the tissue baths remove the possibility of leukotriene metabolism during the course of an experiment and thirdly the use of cyclooxygenase inhibitors such as indomethacin or meclofenamic acid inhibit prostaglandin and thromboxane production. Although biological variability is a persistent problem, generation of high 'n' values and adoption of the above precautions should minimise many errors.
The SRS-A antagonist FPL 55712\textsuperscript{45} (Fig. 2) has been known for many years and reported to be a specific leukotriene antagonist\textsuperscript{46}. Its failure to work in vivo though, has been attributed to a very short half-life associated with a rapid uptake mechanism\textsuperscript{47}. Many structural analogues of FPL 55712, however, have also been clinically unsuccessful, suggesting an inherent flaw in its mode of action.

FPL 55712 has been reported to be a competitive antagonist of LTD\textsubscript{4} on both the guinea-pig ileum\textsuperscript{48} and trachea\textsuperscript{43}. However, FPL 55712 fails to antagonise LTC\textsubscript{4} on the trachea\textsuperscript{43} and parenchyma\textsuperscript{49,50} suggesting a heterogeneity of leukotriene receptors in guinea-pig airways. This is not altogether surprising in the light of histamine\textsuperscript{51} and adrenergic\textsuperscript{52} receptor investigations, especially as the leukotrienes are apparently equally ubiquitous.

Fig. 2
Recent clinical studies in man using inhaled leukotrienes have been somewhat conflicting. It is universally agreed though, that leukotrienes cause immediate long-lasting bronchoconstriction (reminiscent of their in vitro activity) with a far greater potency than histamine. Holroyde and others suggested that the leukotrienes were selective for the small airways, which agreed with similar animal experiments although Smith and Barnes found that leukotrienes constricted both the upper and lower airways, in agreement with human in vitro data. Studies with human asthmatics indicated a hyperreactivity to LTD₄ although a similar increased sensitivity was also shown for methacholine. Griffin et al showed hyperreactivity to histamine in human asthmatics but failed to find any such effect with LTD₄. Weiss et al showed that the LTD₄-induced bronchoconstriction in man was unaffected by pretreatment with aspirin, implying that it is a direct leukotriene effect and not mediated by cyclo-oxygenase. In one study FPL 55712 was shown to partly inhibit bronchoconstriction in one of two normal subjects, although a cough response was obtained to inhaled leukotrienes in this subject which has not been substantiated by other workers.

Thus the role of leukotrienes in human asthma remains equivocal and will probably remain so until specific antagonists are
developed. Specific leukotriene antagonists may also assist the clinical classification of respiratory diseases and in particular what today is loosely termed 'asthma'.

STRUCTURE-ACTIVITY RELATIONSHIPS

The structure-activity approach to drug design has been utilised to improve many existing drugs found in nature and to improve drug profiles (potency, side-effects etc) of man made therapeutic agents; for example, the development of lidocaine from cocaine, with optimal local anesthetic properties and isoproterenol from adrenaline, with maximal $\alpha$-adrenergic properties.

The use of structure-activity relationships in the design of specific antagonists from agonists, however, is a relatively new science and has had limited success to date. Black et al successfully used this approach to design Cimetidine, a specific $H_2$-receptor antagonist. Further structure-activity studies in the Glaxo laboratories produced Ranitidine, a highly potent and specific $H_2$-receptor antagonist currently marketed, like Cimetidine, for the treatment of stomach ulcers.

The decision to approach the development of a LT-antagonist from a structure activity study of the agonists was based on the rationale that the known LT-antagonist FPL 55712, and analogues thereof, were ineffective against LTC$_4$ on guinea-pig airway smooth muscle, implying a different structural requirement for
the relevant receptors. Furthermore it was hoped that a systematic analogue approach would indicate differences in receptor systems assisting pharmacological evaluation of the test tissues.

RECEPTOR THEORY

The concept of specific recognition sites for certain molecules or drugs is very old indeed. Titus Lucretius Carus envisaged sensations such as taste to be a result of such a specific interaction in the didactic poem De Rerum Natura circa 50BC. Somewhat nearer to our own time, Langley\textsuperscript{67} used the term "receptive substance" to describe the site where nicotine and curari act; but it was Ehrlich\textsuperscript{68} who coined the term receptor to describe such specific molecular recognition sites.

The early treatment of receptor theory (Clark [1926])\textsuperscript{69} supposed that the magnitude of a pharmacological effect was directly proportional to the fractional receptor occupancy and that the reaction between drug and receptor obeyed Langmuirs adsorption isotherm\textsuperscript{70}. Although this crude mathematical treatment of receptor theory was later improved by Ariens\textsuperscript{71} and Stephenson\textsuperscript{72} to include terms such as efficacy (the capacity of a drug to initiate a response) and the principle of receptor reserve (the ability of a drug to elicit maximal tissue response without total receptor occupancy), it gave little or no insight into the mechanism of receptor activation.
The receptor theories of Clark, Ariens and Stephenson were based on receptor occupancy and were further developed and unified by Furchgott. Paton, however, proposed the rate theory of receptor activation which postulated that the biological response was proportional to the rate at which the drug associates with the receptor and not the number or proportion of receptors occupied.

Thus, under the rate theory, a full agonist was a compound that combined and dissociated rapidly from the receptor so that many 'associations' could be made leading to maximal activity. An antagonist would theoretically dissociate very slowly thus impeding further activation.

Although the rate theory explained why some drugs were agonists and other antagonists, it failed to be consistent with many observed phenomena and was generally considered to be of little use.

Until this time mathematical principles were being applied to receptor-ligand interactions without consideration of events at the molecular level. Belleau then proposed the macromolecular perturbation theory based on the enzyme-substrate induced fit model of Koshland. The macromolecular perturbation theory proposed two types of drug-receptor interactions. The first being that induced by agonists, termed a specific conformational perturbation (SCP) and the second being a non-specific
conformational perturbation (NSCP), induced by antagonists. The SCP was proposed to lead to a biological response whereas the NSCP did not. Partial agonists were explained by being in equilibrium between SCP and NSCP. This finally linked the occupational theory with events at the molecular level and explained in mechanistic terms the previous occupational theory enigmas such as affinity and efficacy.

\[
\begin{align*}
[R] + [L] & \rightleftharpoons K_2 [R] + [L]^* \\
K_1 [RL] & \rightleftharpoons K_2 [R] + [L]^* \\
K_3 [RL]^* & \rightleftharpoons [R] + [L]
\end{align*}
\]

Fig. 3

Fig. 3 represents the macromolecular perturbation theory where a receptor [R] combines with ligand[L]. This can give rise to the antagonistic complex (NSCP) [RL] or the agonistic complex (SCP) [RL]*. The affinity of interaction is given by the kinetic rate constants $K_1$ or $K_3$ and the efficacy of the drug in question by $K_2$.  

A further description of partial agonist effects and tissue dependance is given in a review by Kenakin.

We thus have a working schematic hypothesis explaining previously confused terms such as affinity and efficacy. However, the medicinal chemist requires an understanding of the molecular interactions effecting binding and activation of a receptor.

The binding of ligand to receptor is highly specific; indeed chiral specificity is an often used prerequisite for evidence of a receptor-mediated effect. Thus on the molecular scale a three-dimensional electronic network is 'recognised' by the receptor which probably takes the form of bonding between certain groups on the receptor and on the ligand. Such important sites on the ligand are termed 'pharmacophoric groups' and their active spacial arrangement is termed the receptor 'pharmacophore'.

\[ [R] + [L] \rightarrow [RL]^* \]

Fig. 4
A diagramatic representation of receptor activation is shown in figure 4. In this picture it can be seen that the efficacy term is determined by the energetics of the interaction between ligand and receptor. For an agonist of high efficacy the majority of ligand-receptor complexes will be found in the activated state. This is represented by a conformational change in the active site of the receptor which elicits its messenger role by a simultaneous conformational change at a distinct site on the receptor protein. The receptor may, for example, be coupled to a guanidine nucleotide protein so that receptor activation decreases the intracellular levels of cyclic-AMP and thus causes the cell to contract. An antagonist, by definition, will bind to the ground state of the receptor and not elicit a response.

The aim of 'a rational drug design by analogue approach' is to firstly identify the pharmacophoric groups (shown in fig. 4 by the triangle, square and semicircle) and then to construct a ligand which will bind to the receptor in the ground state ([RL]) and be incapable of activating the receptor i.e. have zero efficacy.

There are three popular approaches to such a design:-

1. Removal of a pharmacophoric group essential to receptor activation and replacing the lost affinity by the introduction of a new group which binds to a neighbouring 'accessory' or 'auxiliary' site.
2. Shortening or lengthening the interatomic distances between pharmacophoric groups to favour binding to the ground state conformation of the receptor.

3. Introducing molecular rigidity in the ligand to favour binding to the ground state receptor and inhibit the conformational change.

This thesis will discuss the application of aspects of the above principles in the design of leukotriene antagonists.
The synthesis and biological evaluation of reference compounds

Introduction

To facilitate a detailed study of leukotrienes and leukotriene analogues it was necessary, firstly, to obtain sufficient quantities of the natural agonists for reference purposes. As only minute quantities could be isolated from biological sources and the cost of synthetic material (when available) prohibitive, it was apparent that a workable synthetic route had to be found.

The structural complexity of leukotrienes posed many synthetic problems, notwithstanding two chiral centres and four double bonds in leukotriene $A_4$ giving a possible sixty four geometrical and optical isomers. A number of elegant syntheses of leukotriene $A_4$ have been reported since Corey first prepared racemic LTC$_4$\textsuperscript{89}. These mostly utilised the chiral epoxide intermediate 1 (fig. 5), constructing the remainder of the molecule by a variety of Wittig extensions.

\[
\text{Fig. 5}
\]
\(^1\) has been prepared from a number of chiral building blocks, but mainly sugars such as D-ribose, L-arabinose and 2-deoxy-D-ribose. An alternative method of introducing a chiral epoxide is by Sharpless epoxidation of an allylic alcohol which has also proved effective in the syntheses of \(^9_1,9_2\).

Of the synthetic routes reported, that outlined by Rokach to yield the epoxyaldehyde \(^1\) appeared the most promising, and with a few modifications was largely adopted. The remainder of the molecule was constructed by Wittig extension as described by Ernest to give enantiomerically pure leukotriene \(A_4\) (Fig 6).

FPL 55712 was required as a reference antagonist to characterise the biological test systems. That is, to discover whether or not the leukotriene-induced contractions of various tissues were antagonised by this compound. FPL 55712 was prepared in multigram quantity by the method of Appleton \(et\ al\) \(^9_4\) (Fig. 8).

**Leukotriene Synthesis**

2-Deoxy-D-ribose was extended via Wittig reaction with methoxycarbonylmethylenetriphenylphosphorane to give methyl 5(S),6(R),7-trihydroxyhept-2(E)-enoate (II). The methyl ester was preferred as it afforded a crystalline solid compared with a gummy oil for the reported ethyl ester prepared via an analogous reaction with ethoxycarbonylmethylenetriphenylphosphorane.
Fig. 6
Triphenylphosphine oxide was removed by aqueous extraction of the product, and recrystallisation from dichloromethane/ether removed the C-glycoside by-product which is produced by intramolecular Michael addition under the reaction conditions.

Catalytic hydrogenation of (II) was effected over 10% palladium on carbon in ethyl acetate to afford the crystalline product (III), methyl 5(S),6(R),7-trihydroxyheptanoate. Tosylation in pyridine at -10°C selectively yielded the primary tosylate (IV) which was again purified by recrystallisation. The tosylate was prepared in preference to the reported mesitylene sulphonyl derivative because it was found to cyclise more readily in the following step. Thus treatment of (IV) with potassium carbonate in methanol afforded the terminal epoxide (V) which rearranged on prolonged base treatment to yield methyl 7-hydroxy-5(S),6(S)-oxidoheptanoate (VI) as a colourless oil.

Collins oxidation of (VI) gave the key intermediate methyl 7-oxo-5(S),6(R)-oxidoheptanoate (I), which condensed with 4-oxobut-2(E)-enylidenetriphenylphosphorane (prepared by the method of Berenguer et al.\textsuperscript{95,96}) to give a mixture of the 7-cis and trans forms of methyl 5(S),6(S)-oxido-11-oxo-7,9(E)-undecadienoate. Iodine-induced isomerisation afforded the 'all-trans' configuration, (VII), as a crystalline solid.

3(Z)-Nonenyltriphenylphosphonium tosylate was prepared by the method of Ernest\textsuperscript{93} and the resulting ylide reacted with (VII) in
the presence of HMPA to yield exclusively the desired cis-configuration of methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoate, LTA$_4$-Me (VIII).

LTC$_4$, LTD$_4$ and LTE$_4$ were prepared by addition of protected glutathione, cysteinylglycine and cysteine respectively to LTA$_4$-Me in basic methanol followed by deprotection (Fig. 7). The reaction selectively takes place at the activated C-6 position to afford the desired 5(S),6(R) stereochemistry of the product.

A novel UV method of following the reaction between LTA$_4$-Me and thiol was developed. LTA$_4$-Me exhibits a $\lambda$ max at 280nm as do the 6-thioether derivatives (LTC$_4$, LTD$_4$, LTE$_4$); treatment of LTA$_4$-Me with dilute mineral acid, however, instantaneously produces a mixture of methyl dihydroxyeicosatetraenoates which have a $\lambda$ max at 270nm. Thus the reaction of LTA$_4$-Me with thiol is deemed to be complete when acidification of a UV sample does not affect the recorded spectrum maxima.

Hydrolysis of the ester functions plus removal of the trifluoroacetyl group was conducted in a saturated solution of lithium hydroxide in 50% aqueous tetrahydrofuran following the reaction progress by RP HPLC. When complete saponification had been achieved the product was de-salted on a Reverse Phase (RP) plug and stored in 20% ethanol-phosphate buffered saline (PBS) pH7.2 under argon at -70°C.
Fig. 7

\[ X_0 = \text{LTC}_4 \quad Y_0 = \text{LTD}_4 \quad Z_0 = \text{LTE}_4 \]
Fig. 8
Resacetophenone (XV) was converted into 4-O-allylresacetophenone (XVI) by the action of allyl bromide in acetone in the presence of potassium carbonate. The regiospecificity of this O-alkylation, shown by the absence of 2-O-allylresacetophenone in the product, indicates substantial intramolecular chelation between the acetyl and ortho-hydroxyl groups (fig 9); thus reducing the acidity of the ortho-hydroxyl group.

4-O- Allylresacetophenone (XVI) underwent Claisen rearrangement at elevated temperature to give 3-allylresacetophenone (XVII) with some decomposition. The product was purified by sublimation in high yield, a technique which gave excellent results on large scale preparations and a product of high purity.

The Claisen rearrangement appeared to occur regiospecifically as no 5-allylresacetophenone was observed in the product. The proposed mechanism requires the presence of a formal double bond between the sites of migration, which favours the observed
product due to the chelation \textit{vide supra} between neighbouring acetyl and hydroxyl groups (Fig. 10).

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\includegraphics[width=1cm]{fig10a.png}};
\node (B) at (2,0) {\includegraphics[width=1cm]{fig10b.png}};
\node (C) at (4,0) {\includegraphics[width=1cm]{fig10c.png}};
\draw[->] (A) -- (B);
\draw[->] (B) -- (C);
\end{tikzpicture}
\end{center}

\textbf{Fig. 10}

Catalytic hydrogenation of 3-allylresacetophenone (XVII) was effected in ethanol under a slight pressure of hydrogen with 10\% palladium on carbon catalyst. Stopping the reaction after the absorption of one equivalent of molecular hydrogen gave 3-propylresacetophenone (XVIII) in good yield. It was noticed, however, that if the reaction was allowed to continue after the theoretical uptake of hydrogen, absorption continued at a slightly slower rate. Complete hydrogenation of a sample of (XVII) resulted in reduction of the aromatic nucleus and the carbonyl group. The unexpected ease of this reduction clearly illustrated the reduced aromaticity of ortho-hydroxyacetophenones.

Condensation of the phenol (XVIII) with epichlorohydrin in basic ethanol afforded the epoxide (XIX) in good yield. Claisen condensation of (XVIII) with diethyl oxalate under the action of a strong base gave the intermediate $\alpha,\gamma$-diketo ester (XXIII) (Fig. 11), which cyclised smoothly in acidic ethanol to give the chromone (XX).
Nucleophilic addition of the chromone (XX) to the epoxide (XIX), promoted by N-benzyltrimethylammonium hydroxide in refluxing dimethylformamide gave the ethyl ester of FPL 55712 (XXI) which was saponified by the action of sodium hydrogen carbonate in refluxing ethanol to give the sodium salt FPL 55712 (XXII).

![Chemical Structure](image)

**XXIII**

**Fig. 11**
Pharmacological evaluation of reference compounds

Introduction

The isolated guinea-pig ileum has been the tissue of choice for the detection of leukotrienes since the discovery of SRS. This tissue is highly sensitive to leukotrienes, the effects of which are reportedly antagonised by the leukotriene antagonist FPL 55712. The guinea-pig lung parenchymal preparation has also been shown to be leukotriene sensitive but FPL 55712 fails to antagonise the LTC₄-induced responses in this tissue. The differential effects of FPL 55712 in these two systems suggested a heterogeneity of leukotriene receptors and for this reason they were adopted as contrasting tissue preparations for the evaluation of leukotriene and leukotriene-analogue activities.

Following the disclosure by Krell of a homogeneous FPL 55712-insensitive receptor population on ferret trachea, a study by Cuthbert resulted in the inclusion of the ferret spleen preparation in the bank of test systems for leukotriene analogue evaluation. The ferret spleen was adopted in preference to the reported tracheal preparation as it exhibited an identical biological profile but with greater consistency.

Reported herein are the studies of Gardiner et al. using the reference leukotrienes C₄, D₄ and E₄ and FPL 55712 to give preliminary receptor classification which is later enhanced.
by the leukotriene analogue studies described in Chapters 2 and 3 of this thesis.

**Materials**

The following drugs were used:— histamine acid phosphate, noradrenaline and indomethacin (Sigma), L-cysteine and L-serine (SAS Pharmaceuticals), boric acid and sagatal (BDH Chemicals Ltd), LTC\(_4\), LTD\(_4\), LTE\(_4\) and FPL 55712 (synthesised as described earlier).

Indomethacin (3 x 10\(^{-2}\)M) was dissolved in absolute ethanol and further dilutions prepared using phosphate buffered saline pH 7.2. All other test drugs were dissolved in phosphate buffered saline pH 7.2 and added to the tissue bath in a volume not exceeding 100 µl. The concentration of each drug refers to the final bath concentration.

Tyrodes solution consisted of the following ANALAR grade substances: NaCl, 137 mM; KCl, 2.7 mM; CaCl, 2.4 mM; MgCl, 2.1 mM; Na\(_2\)HPO\(_4\), 0.5 mM; NaHCO\(_3\) 11.9 mM; D-glucose, 9.2 mM. This Tyrodes solution routinely contained 3 x 10\(^{-6}\)M indomethacin. Experiments in which enzyme inhibition was studied involved the inclusion to Tyrodes solution of L-cysteine (10 mM), L-serine (45 mM) and boric acid (45 mM).\(^{43}\)
Methods

Guinea-Pig Ileum

Male Dunkin Hartley guinea-pigs (400-450 g) were sacrificed by cervical dislocation and the abdomen opened. The ileum was removed and 3 cm segments cut and suspended in 10 ml tissue bath containing Tyrodes solution and indomethacin (3 x 10^{-6} M) at 37°C aerated with carbogen (95 % O_2, 5 % CO_2). The tissues were attached to Harvard isotonic transducers with a load of 1 g and washed at 10 min intervals until equilibrium was attained. Histamine (10^{-4} M and 3 x 10^{-4} M) was added cumulatively to achieve maximal tissue contraction. The leukotrienes were then separately evaluated using a cumulative dosing procedure and expressed as a percentage of the maximal histamine response.

Guinea-pig Lung

Male Dunkin Hartley guinea-pigs (400-450 g) were sacrificed by cervical dislocation and the thorax opened. The lungs were removed and after discarding the peripheral layer, 3 cm longitudinal sections were cut and suspended in 10 ml tissue baths containing Tyrodes solution and indomethacin (3 x 10^{-6} M) at 37°C aerated with carbogen. L-Cysteine and serine-borate complex were routinely added to Tyrodes solution for this test system. The tissues were attached to Hugo Sachs isotonic transducers with a load of 0.5 g and washed at 30 min intervals.
until equilibrium was attained. Histamine (10^{-4} M and 3 \times 10^{-4} M) was added cumulatively to achieve maximal tissue contraction. The leukotrienes were then separately evaluated using a cumulative dosing procedure and expressed as a percentage of the maximal histamine response.

**Ferret Spleen**

Adult male ferrets (Froxfield Animal Supplies) weighing 1090 ± 65 g were sacrificed by an intraperitoneal injection of Sagatal (10 mg.kg\(^{-1}\)). The spleen (10.4 ± 1.5 g) was removed, placed in Tyrodes solution and cut into longitudinal strips (30 mm x 5 mm; 212 ± 15 mg).

The strips were mounted in 10 ml tissue baths containing Tyrodes solution at 37°C aerated with carbogen. Indomethacin (3 \times 10^{-6} M) was routinely added to the Tyrodes solution. The tissues were attached to isometric (Statham UC2) transducers under an initial tension of 1g and allowed to equilibrate over 1 h whereupon a maximal contraction (5.1 ± 0.25 mg tension per mg wet tissue weight) was induced by noradrenaline (10^{-5} M). Upon returning to their original baseline tone a single cumulative dose–response curve for the test compound was constructed per tissue and the results normalised as a percentage of the maximal contraction induced by noradrenaline.
Statistical Analysis

EC$_{50}$ (concentration of an agonist producing 50% of its maximal response) were calculated using a least squares regression analysis using data from the 'straight line' portion of the dose-response curves. Antagonism was quantified using Schild analysis$^{102}$ and expressed as a pA$_2$ value. When the slope of the Schild plot was significantly different from unity or for single dose antagonism studies, the antagonism was express as pK$_B$ determined using the formula:

$$K_B = (\text{Antagonist concentration}) \times (\text{dose ratio}-1)^{-1}$$
RESULTS

1) Guinea-pig ileum

In contrast to histamine-induced contractions of the guinea-pig ileum, leukotriene C₄-, D₄-, E₄- induced contractions were slower in onset and took longer to reach their maximal effect (Fig. 12). This is consistent with the results described for crude SRS which was discovered on this tissue and hence the acquired terminology. It is also apparent from Fig. 12. however, that the LTC₄⁻ induced contractions are even slower to reach their maximum than LTD₄ or LTE₄ which had similar profiles. Furthermore, the contraction induced by LTC₄ was maintained until the bath fluid was changed whereas LTD₄ and LTE₄ induced responses were transient, returning to baseline after the maximal response had been achieved.

The dose-response curves of LTC₄, D₄ and E₄ are shown in Fig. 13. The results are expressed as a percentage of the maximal response attained by LTD₄. It can be seen that LTC₄ achieved a greater maximal contraction than LTD₄ although the EC₅₀'s are similar [LTC₄ EC₅₀ = (4.6 ± 0.8) x 10⁻⁹ M; LTD₄ EC₅₀ = (3.2 ± 1.2) x 10⁻⁹ M] LTE₄, however, is less effective and less potent [LTE₄ EC₅₀ = (1.0 ± 2.2) x 10⁻⁸ M] than LTC₄ or LTD₄ in this tissue.
Characteristic Leukotriene-Induced Contractions in Guinea-Pig Ileum

Fig. 12
Leukotriene Responses on Guinea-Pig Ileum

Fig. 13

Effect of Enzyme Inhibitors on LTC₄ Responses on Guinea-Pig Ileum

Fig. 14
Effect of FPL55712 on LTD₄ Responses on Guinea-Pig Ileum

![Graph showing the effect of FPL55712 on LTD₄ responses on Guinea-Pig Ileum.](image)

Fig. 15

Effect of FPL55712 on LTC₄ Responses on Guinea-Pig Ileum

![Graph showing the effect of FPL55712 on LTC₄ responses on Guinea-Pig Ileum.](image)

Fig. 16
Effect of FPL55712 on LTE\textsubscript{4} Responses on Guinea-Pig Ileum

**Fig. 17**

**Effect of LTE\textsubscript{4} on LTD\textsubscript{4} Responses on Guinea-Pig Ileum**

**Fig. 18**
Effect of LTE4 on LTC4 Responses on Guinea-Pig Ileum

Concentration (M) vs. % Maximum LTC4 Response

KEY
- LTC4
- LTC4 + LTE4 10^{-7} M
- LTC4 + LTE4 10^{-8} M
- LTC4 + LTE4 10^{-9} M

Fig. 19
The presence of the enzyme inhibitors L-cysteine and serine borate did not significantly affect the contractions induced by LTC$_4$ on this tissue (Fig. 14) indicating that there is little or no metabolism occurring under the experimental conditions. LTD$_4$ was evaluated in a similar manner and again no significant difference in its maximal activity occurred (data not shown).

In the light of these results and the problem of spontaneous activity induced by the enzyme inhibitors in this tissue, the following experiments were performed in the absence of enzyme inhibitors.

FPL 55712 was found to be a competitive antagonist of LTD$_4$, giving a Schild plot with slope of unity and a pA$_2$ value of 6.9 (Fig. 15). Although FPL 55712 antagonised the effects of LTC$_4$ on this tissue, it was of a non-competitive nature making calculation of a pA$_2$ value invalid (Fig. 16). FPL 55712 was also shown to be a good antagonist of LTE$_4$ (Fig. 17) although a pA$_2$ value could not be calculated from this experiment. The dose response curves of LTE$_4$ were shifted to a similar degree by a given concentration of FPL 55712 as those of LTD$_4$; LTC$_4$ is apparently less effected by the same concentration.

As LTE$_4$ had a significantly lower maximal efficacy than either LTC$_4$ or LTD$_4$ it was possible to test for partial agonist activity of LTE$_4$ against LTC$_4$ and LTD$_4$. The results shown in Figs. 18 and 19 clearly indicate that LTE$_4$ was a partial agonist of LTD$_4$ and
LTC\textsubscript{4} although the antagonism of LTD\textsubscript{4} appears greater than that of LTC\textsubscript{4} for the lower concentration of LTE\textsubscript{4}. LTE\textsubscript{4} had no effect on the histamine dose-response curve at the same concentration (data not shown).

ii) Guinea-pig Lung

Leukotriene C\textsubscript{4}- and D\textsubscript{4}-induced contraction of guinea-pig lung parenchymal strip preparations were much slower than that induced by histamine, taking approximately 20-25 minutes to reach maximal effect (Fig. 20). The contractions were long-lasting and difficult to wash out. Although the EC\textsubscript{50}'s of LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4} were similar [LTC\textsubscript{4} EC\textsubscript{50} = (4.2 ± 1.5)x 10\textsuperscript{-8} M; LTD\textsubscript{4} EC\textsubscript{50} = (4.6 ± 2.4)x 10\textsuperscript{-8} M; LTE\textsubscript{4} EC\textsubscript{50} = (2.7 ± 2.7)x 10\textsuperscript{-8} M], the maximal attainable contractions differed. LTC\textsubscript{4} was more efficacious than LTD\textsubscript{4} which, in turn, was more efficacious than LTE\textsubscript{4} (Fig. 21).

The lower efficacy of LTD\textsubscript{4} and LTE\textsubscript{4} made it possible to test for partial agonism at the LTC\textsubscript{4} receptor. Fig. 22 and Fig. 23 show the effect of a 10\textsuperscript{-5} M dose of LTD\textsubscript{4} and LTE\textsubscript{4} respectively on the LTC\textsubscript{4} dose-response curve. The higher doses of LTC\textsubscript{4} are apparently antagonised by both LTD\textsubscript{4} and LTE\textsubscript{4}, indicative of partial agonist activity.
Characteristic Leukotriene-Induced Contractions in Guinea-Pig Lung

Fig. 20
Leukotriene Responses on Guinea-Pig Lung

**Fig. 21**

Effect of LTD₄ on LTC₄ Responses on Guinea-Pig Lung

**Fig. 22**

- LTC₄
- LTD₄
- LTE₄

**KEY**

- LTC₄
- LTD₄
- LTE₄

Concentration (M)
Effect of LTE$_4$ on LTC$_4$ Responses on Guinea-Pig Lung

**Effect of LTE$_4$ on LTC$_4$ Responses on Guinea-Pig Lung**

**Effect of FPL 55712 on LTC$_4$ Responses on Guinea-Pig Lung**
Effect of FPL 55712 on LTD₄ Responses on Guinea-Pig Lung

**KEY**

- ○ = LTD₄
- ● = LTD₄ + FPL 10⁻⁸ M

% Maximum LTD₄ Response

[Graph showing concentration-response relationship with data points and fitted curves for LTD₄ and LTD₄ + FPL 10⁻⁸ M]
FPL 55712 failed to antagonise either the LTC$_4^-$ or the LTD$_4^-$-induced contractions in this tissue (Fig. 24,25) but potentiated the higher doses of LTD$_4^-$-induced contraction.

### iii) Ferret Spleen

LTC$_4^-$ and LTD$_4^-$ are potent contractants of this tissue, capable of achieving >80% of the maximal noradrenaline response (Fig. 26). The leukotriene-induced contractions, however, were slower in onset than those induced by noradrenaline, taking approximately 4 minutes to reach a maximum compared with 30 seconds - 1 minute for the adrenergic receptor agonist. LTC$_4^-$ and LTD$_4^-$ have similar maximal efficacies on this tissue with EC$_{50}$'s of $(2.8 \pm 0.7) \times 10^{-7}$ M and $(3.7 \pm 0.7) \times 10^{-7}$ M respectively compared with noradrenalines EC$_{50}$ of $(5.3 \pm 0.5) \times 10^{-7}$ M. LTE$_4^-$, however, was a poor agonist achieving only 20% of the maximal contraction of LTD$_4^-$ at $10^{-5}$ M (Fig. 27).

The presence of enzyme inhibitors did not significantly affect the dose-response curves of either LTC$_4^-$ or LTD$_4^-$, implying a lack of metabolism in this system (Fig. 28). Furthermore, FPL 55712 was without effect on either the LTC$_4^-$ or LTD$_4^-$-induced contractions of this tissue (Fig. 29,30).

LTE$_4^-$, however, was found to be a partial agonist in this tissue, antagonising the effects of both LTC$_4^-$ and LTD$_4^-$ in the presence or absence of enzyme inhibitors (Figs 31,32). It did not affect the
Characteristic Leukotriene-Induced Contractions in Ferret Spleen

Fig. 26
Leukotriene Responses on Ferret Spleen

**KEY**
- ♦ = LTC₄
- ○ = LTD₄
- △ = LTE₄

Effect of Enzyme Inhibitors on LTC₄ Responses on Ferret Spleen

**KEY**
- ♦ = LTC₄
- ♦ = LTC₄ + Enzyme Inhibitors

Fig. 27

Fig. 28
Effect of LTE₄ on LTD₄ Responses on Ferret Spleen

**KEY**

- ○ = LTD₄
- ● = LTD₄ + LTE₄ 10⁻⁵ M

**Fig. 31**

Effect of LTE₄ on LTC₄ Responses on Ferret Spleen

**KEY**

- ◊ = LTC₄
- • = LTC₄ + LTE₄ 10⁻⁵ M

**Fig. 32**
responses induced by noradrenaline. The dissociation constant 
\( k_p \) for \( \text{LTE}_4 \) at \( 10^{-5} \text{M} \) was determined using the Schild regression 
technique for partial agonists and was found to be \( 2.5 \times 10^{-6} \) for 
\( \text{LTD}_4 \) and \( 1.03 \times 10^{-6} \) for \( \text{LTC}_4 \).

**DISCUSSION**

The rank order of potency on guinea-pig ileum was found to be 
\( \text{LTC}_4 > \text{LTD}_4 > \text{LTE}_4 \). The lower efficacy of \( \text{LTE}_4 \) enabled it to be 
tested as a partial agonist against \( \text{LTC}_4 \) and \( \text{LTD}_4 \) and indeed it 
was found to antagonise the responses to both full agonists. 
This would suggest that the leukotrienes \( \text{C}_4, \text{D}_4 \) and \( \text{E}_4 \) are acting 
at the same receptor, which is further substantiated by the 
observed antagonism by FPL 55712 of the three leukotrienes.

The differential tissue response time observed between 
\( \text{LTC}_4 \)-induced contractions and \( \text{LTD}_4 \) or \( \text{LTE}_4 \)-induced contractions 
could be excused by membrane transport effects. That is, if the 
receptor is intracellular and the leukotrienes must translate the 
cell membrane by a passive diffusion process, it is conceivable 
that the most polar leukotriene \( \text{LTC}_4 \) is transported more slowly 
through the lipid bilayer, resulting in a slower response time. 
This would also explain the difficulty in washing out the 
response to \( \text{LTC}_4 \) as the reverse diffusion process would be 
equally as slow. It does not, however, explain why the \( \text{LTD}_4 \) or 
\( \text{LTE}_4 \)-induced responses should be transient whereas those to \( \text{LTC}_4 \) 
are maintained. Furthermore, a single receptor theory does not
explain the inconsistency of FPL antagonism in this tissue, being apparently a competitive antagonist of LTD$_4$ but a non-competitive antagonist of LTC$_4$. The partial antagonism by LTE$_4$ of LTC$_4$ also appeared more competitive than that against LTD$_4$, which again is inconsistent with a single receptor mechanism but is not a strong enough discrepancy to evoke multiple receptor mechanisms in this tissue.

The guinea-pig lung parenchymal preparation showed the same rank order of potency as on the ileum viz LTC$_4$ > LTD$_4$ > LTE$_4$. The differential efficacy again allowed the least efficacious compounds to be tested as partial agonists. LTD$_4$ and LTE$_4$ were both found to antagonise the contractions induced by LTC$_4$ in the presence of enzyme inhibitors which suggested a homogenous receptor population akin to the guinea-pig ileum system. However, in contrast to the ileum, the LTC$_4$ and LTD$_4$ induced contractions in the parenchymal preparation were not antagonised by the reference antagonist FPL 55712, which implies a distinct receptor type on the guinea-pig lung parenchyma.

In contrast to the previous systems, the ferret spleen showed a rank order of potency LTC$_4$ = LTD$_4$ > LTE$_4$. LTE$_4$ again behaved as a partial agonist, antagonising contractions induced by both LTC$_4$ and LTD$_4$ on this tissue. In similarity to the guinea-pig lung, FPL 55712 failed to antagonise the leukotriene-induced contractions of this tissue. These results clearly indicate a homogenous population of receptors on the ferret spleen system.
The ferret spleen receptor system is similar to that in the guinea-pig lung since both systems are FPL 55712 resistant, although the rank order of potencies for the leukotrienes suggests that the receptors are non-identical. If the receptors in guinea-pig lung and ferret spleen were identical then comparing EC₅₀'s one would expect the lung strip to have a greater receptor reserve (LTC₄ EC₅₀ LUNG = (4.2 ± 1.5) x 10⁻⁸ M; EC₅₀ SPLEEN = (2.8 ± 0.7) x 10⁻⁷ M). This is consistent with the partial agonist LTE₄ having greater efficacy in the lung compared with the spleen, but it is inconsistent with the partial agonist LTD₄ on lung exhibiting full agonist effects on a system with a lower receptor reserve ie ferret spleen.

Thus it is proposed that the guinea-pig ileum test system contains a homogenous population of receptors characterised by a rank order of potency LTC₄ > LTD₄ > LTE₄ and is antagonised by FPL 55712. This shall be termed the LT₁ receptor. Furthermore, the guinea-pig lung test system contains a distinct homogenous receptor population termed LT₂, characterised by a rank order of potency LTC₄ > LTD₄ > LTE₄ and is unaffected by the antagonist FPL 55712. Finally, the ferret spleen preparation contains a third type of receptor, LT₃, characterised by a rank order of potency LTC₄ = LTD₄ >> LTE₄ and is also unaffected by FPL 55712.
CHAPTER 2
Characterisation of the agonist pharmacophoric groups

INTRODUCTION

Due to the structural complexity of leukotrienes a clear indication of the ligand pharmacophore would only result from the synthesis of a considerable number of carefully designed analogues. In the first instance the design and synthesis of analogues was limited to structures which could be constructed relatively simply from the synthetic fragments used in the synthesis of leukotrienes (Chapter 1). Furthermore, it was decided to critically focus attention on the functionally less complicated leukotrienes LTD₄ and LTE₄.

A "Peptide Analogues"

The first approach was to investigate the pharmacophoric nature of the peptide region of leukotrienes. Such analogues were prepared by reacting LTA₄-methyl ester with a thiol followed by hydrolysis of protecting groups in a manner analogous to the synthesis of natural leukotrienes. This gave a series of compounds differing from the parent molecule only in the nature of the substituent attached to the sulphur at C-6. The analogues in this series can be likened to LTE₄ and are shown alongside the parent in Figure. 33.
Fig. 33
SYNTHESIS

Thus Analogue 1 (A-1) was prepared by the reaction of methane thiol with LTA₄-Me in basic media followed by subsequent hydrolysis of the C-1 ester.

Cysteamine hydrochloride was protected as the trifluoroacetyl derivative prior to reaction with LTA₄-Me. Deprotection in the usual manner afforded A-2. Similarly A-3 and A-4 were prepared from methyl 3-mercaptopropionate and 2-mercaptoethanol respectively. 1-mercaptobutan-3-one was prepared from the addition of hydrogen sulphide to methylvinylketone by the method of Ross¹⁰³ and subsequently reacted with LTA₄-Me to yield A-5. In all cases the resulting analogues were purified by a combination of reverse-phase preparative thin-layer chromatography (RP-PTLC) and reverse-phase high pressure liquid chromatography (RP-HPLC).

RESULTS

The biological activities of analogues A-1 - A-5 are given in Figure 34 and the dose-response curves are included at the end of the chapter. The results are given as means of n = 2-4 experiments and for this reason statistical analysis is not possible.
KEY

FA = Full agonist; Analogue capable of eliciting maximal, or near-maximal tissue contraction compared to LTD$_4$ or LTC$_4$.

WA = Weak agonist; Analogue which does not elicit >50% LTD$_4$ maximal contraction at 10$^{-5}$M concentration, and is devoid of antagonistic activity.

PA = Partial agonist; Analogue which effects less than maximal tissue contraction and antagonises reference agonist responses.

ANT = Antagonist.

I = Inactive

Note: Unless otherwise stated, LTD$_4$ was used as the reference agonist (control)
<table>
<thead>
<tr>
<th>Analogue</th>
<th>R</th>
<th>Guinea-pig Ileum</th>
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* LTC₄ Control

Fig. 34
i) Guinea-pig ileum

With comparison to LTE\textsubscript{4}, it can be seen that removal of the carboxyl group (A-2) and further removal of the aminomethyl group (A-1) result in virtually complete loss of agonistic activity on this tissue. Furthermore, as A-1 and A-2 in combination with LTD\textsubscript{4} or LTC\textsubscript{4} do not affect the agonist responses, it can be assumed that they have little or no affinity for the leukotriene receptors in this system.

This implies that the carboxyl group of LTE\textsubscript{4} is a pharmacophoric group, essential for the affinity and activity of the molecule. This is further substantiated by the effect of A-3 on the guinea-pig ileum. A-3 differs from LTE\textsubscript{4} only in that it lacks the $\alpha$-amino functionality; however, it is a highly potent agonist, more so than LTE\textsubscript{4} itself and it also achieved maximal tissue contraction relative to LTD\textsubscript{4}.

Taken together these results imply a redundancy of the $\alpha$-amino group of LTE\textsubscript{4} in the agonistic pharmacophore and the necessity of the $\alpha$-carboxyl group.

Replacing the propionic acid substituent of A-3 with an ethyl alcohol residue (A-4) results in a 10-fold loss of activity compared with a 100-fold loss of activity for the carbonyl derivative A-5. This could imply that the nature of the pharmacophoric interaction is hydrogen bonding with hydrogen.
donation from the ligand. If this were so then a carboxyl group would interact best in its unionised form which would be favoured by a higher pKa. Indeed this is seen to be the case comparing LTE₄ (pKa = 2.1) and A-3 (pKa = 4.5). Removal of the amino functionality & to a carboxyl group raises the pKa of the acid thus increasing the proportion of unionised carboxylate groups and favours interaction by hydrogen bonding rather than by coulombic effects. As A-3 was indeed observed to be more potent and efficacious than LTE₄ this could well be the mode of interaction of the carboxyl pharmacophoric group. Furthermore, comparing the aminoethyl derivative, A-2 with the hydroxyethyl analogue, A-4, it is apparent that the stronger hydrogen bonding alcohol confers considerably greater activity to the molecule, supporting the proposed hydrogen bonding interaction of this site with the receptor.

ii) Guinea-pig lung

This system appears to be extremely accommodating for this series of analogues; with the possible exception of A-2 they were all full agonists of similar potency. It would seem that the nature of the substituent attached to the sulphur is not crucial to activity although an acidic function (e.g. A-3) may be slightly favoured. The general trend of results, however, is similar to that on the ileum; indeed the rank order of potencies is the same. A-3 > LTE₄ > A-4 > A-5 > A-1 > A-2.
iii) Ferret spleen

This preparation is in direct contrast to that of the guinea-pig lung strip; all analogues were inactive with the exception of A-3. As LTE₄ itself was a partial agonist in this system it is not surprising that A-3 showed a similar profile of activity. Whereas in the guinea-pig ileum the acidic analogue A-3 exhibited the greatest activity, it appears that the carboxyl group is essential for activity on the ferret spleen.
Another approach was to investigate the pharmacophoric nature of the lipophilic 'tail' region of LTD$_4$. For this purpose a number of LTA$_4$-Me analogues were prepared by Wittig condensation of alkyl ylides with either the 7-C aldehyde (1) or the 11-C intermediate (VII). The resulting LTA$_4$-Me analogues were condensed with protected cysteinyl glycine and saponified to give the tail-modified analogues of LTD$_4$ shown in Fig. 35.

![Fig. 35](image-url)
SYNTHESIS

Thus n-dodecyltriphenylphosphorane, prepared in the usual manner at -78°C, condensed with the 7-C aldehyde (1) to yield methyl 5(S),6(S)-oxido-7(Z)-nonadecenoate (XXIV) with exclusively cis double bond geometry. Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-heptadecatrienoate (XXV) and methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-tridecatrienoate (XXVI) were similarly prepared by condensation of the ylides from n-hexyltriphenylphosphorane and ethyltriphenylphosphorane with the 11-C intermediate (VII) respectively (Fig. 36).

XXIV

XXV

XXVI

Fig. 36
The lack of extended conjugation in epoxide (XXIV) compared with the natural triene unit of LTA₄ confers considerably greater stability to the molecule. Indeed, as a result of the forcing conditions required to effect opening of the epoxide, a mixture of isomers resulting from Sn2 attack at both the C-6 and C-5 positions was obtained (A-6 and A-7 respectively). Epoxides (XXV) and (XXVI), however, reacted smoothly with the thiol as expected, to give the corresponding addition products A-8 and A-9 upon deprotection.
RESULTS

The functional activity of analogues A-6 to A-9 are summarised in Figure 37 and the dose-response curves included at the end of the chapter.

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<th>Analogue</th>
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<th>Ferret Spleen</th>
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<td>Lung</td>
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<td>A-9</td>
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* LTC₄ Control

![Fig. 37](image)

1) Guinea-pig ileum

The guinea-pig ileum system showed remarkable tolerance to reduction of the 9-, 11- and 14- double bonds as indicated by the
virtual equipotency of A-6 to the parent LTD4. Even more surprising was the good agonistic activity displayed by the structural isomer A-7 implying an equally efficacious receptor occupancy. However, retention of the conjugated triene moiety and shortening the tail length resulted in a dramatic loss of activity for the C17 analogue A-8 and virtual inactivity for the C13 analogue A-9.

These results imply that the guinea-pig ileum system requires a substantial hydrophobic moiety although a high degree of unsaturation is not required in the agonist pharmacophore.

ii) Guinea-pig lung

The guinea-pig lung strip was slightly more sensitive to the loss of unsaturation than the ileum, as shown by the hexahydro-analogue A-6. The necessity of the correct substitution and orientation was furthermore displayed by the weak activity of A-7, the structural isomer of A-6. Shortening of the tail, however, with retention of the triene system retained good activity in the guinea-pig lung strip at C17 length (shown by A-8) with some decline in activity at C13 length (A-9).

Thus in contrast to the ileum the guinea-pig lung strip system is tolerant to tail shortening and tail saturation but requires the thiopeptidyl substitution at C-6 for good agonistic activity.
iii) **Ferret spleen**

The ferret spleen was comparable to the guinea-pig lung strip results for the compounds A-6 and A-7. These analogues display the tolerance of the ferret spleen to loss of unsaturation in the tail but the dependance for activity on the correct substitution pattern.

C  "Alkanoyl Analogues"

It was demonstrated in the first section of this chapter that a pharmacophoric carboxyl group was present in the peptide region of leukotrienes. To further evaluate the nature of this pharmacophoric group in the various receptor systems and to achieve a better understanding of the receptors' requirements in this region, it was decided to synthesise a homologous series of analogues to determine the optimal position for the carboxyl function within the receptor pharmacophore.

**SYNTHESIS**

Thus, methyl mercaptoacetate, methyl 3-mercaptopropionate (previously described), methyl 4-mercaptobutanoate, methyl 5-mercaptopentanoate and methyl 7-mercaptoheptanoate were reacted with LTA$_4$-Me in the usual manner and subsequently deprotected to afford the analogues A-10, A-3, A-11, A-12 and A-13 respectively (Fig. 38). The biological results are summarised in Fig. 39 and the dose-response curves included at the end of the chapter.
Fig. 38

Analogue 10

Analogue 3

Analogue 11

Analogue 12

Analogue 13
### RESULTS

<table>
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<tr>
<th>Analogue</th>
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* LTC4 Control

**Fig. 39**

i) **Guinea-pig ileum**

On the guinea-pig ileum good agonism is observed for the short (n = 1-4) alkanoyl analogues with apparent loss of activity when n = 6 (A-13). Interestingly, A-11 only achieved approximately 60% of the maximal contraction induced by LTD4 on this tissue and at a concentration of 10^{-6} M significantly antagonised both LTD4 and LTC4 induced contractions, which is similar to the results observed with LTE4 on this tissue.
Thus, the agonistic pharmacophore on the guinea-pig ileum accepts a range of structural variations in this region. However, when \( n = 3 \) (A-11), the ligand begins to induce binding to the ground state or antagonistic conformation of the receptor as well as the agonistic state, which gives rise to the observed partial agonistic effect. This indicates that there is a narrow window of antagonistic activity in this range of analogues and perhaps by slight structural modification it may be possible to optimise this effect.

ii) Guinea-pig lung

This series of analogues also displayed good agonistic activity on the guinea-pig lung parenchymal preparation, with perhaps slight loss of activity at \( n = 2 \) and \( n = 6 \). This depression of agonism may be likened to that for \( n = 3 \) and \( n = 6 \) on the guinea-pig ileum, although the apparent sensitivity of the lung to agonistic effects may mask any slight antagonistic activity which would otherwise be observed.

iii) Ferret spleen

The ferret spleen preparation shows a remarkable profile of activity for this series of analogues. A-10 (\( n = 1 \)) is a full agonist of equal potency to LTD\(_4\). A-3 and A-11 (\( n = 2 \) and 3 respectively) behave, like LTE\(_4\), as antagonists and when \( n = 4 \) (A-12) the antagonistic activity weakens again and some agonistic
activity returns to the molecule. A-13 (n=6) is inactive in this system.

Thus the ferret spleen follows the same trend as the guinea-pig ileum (and possibly the guinea-pig lung strip) with agonistic activity peaking at n = 1 and 4, sandwiching a region of antagonistic activity. The marked difference, of course, is the ease of antagonism on the ferret spleen.

DISCUSSION

It has been shown that the guinea-pig ileum agonist pharmacophore requires a proton-donating group, especially a protonated carboxyl, in the peptide region of the analogue. Ideally the carboxyl group should be separated from the sulphur atom by one or two methylene groups; partial agonism being observed when the separation is increased to three methylene groups. The hydrophobic tail may be considerably saturated with retention of good agonistic activity, however, the natural length must be maintained.

The guinea-pig lung strip agonist pharmacophore defies analysis. It appears that the peptide moiety of leukotrienes is not necessary for full agonistic activity and furthermore that the hydrophobic tail may be considerably saturated or shortened with little effect on activity.
The ferret spleen agonist pharmacophore is more clearly defined, requiring either the natural LTD\textsubscript{4} or LTC\textsubscript{4} peptide for full agonistic effect or the thioacetyl derivative, as in A-10. For the natural peptide of LTD\textsubscript{4}, the tail accepted considerable saturation without dramatic loss in activity, again implying a redundancy of the 9-, 11- and 14- double bonds. In contrast to the previously described systems, the ferret spleen is readily antagonised, indeed the difference between the full agonist A-10 on this system and the antagonist A-3 is merely one methylene group.

Thus although the three test systems behave differently to one another, the underlying trends are similar. It is apparent that the agonist pharmacophore may be simplified to the thioacetyl analogue A-10, which suggests that the pharmacophoric interaction between the peptide carboxyl and the receptor is quite close to the sulphur atom in the agonistic conformation of the receptor. Fig. 40 shows a possible conformation of the peptide of LTD\textsubscript{4} complying with the structural restrictions imposed by A-10. A proposed mechanism of receptor activation, therefore, could be the attraction of a distant group in the receptor site by hydrogen bonding to the hydroxyl group leading to the formation of the agonist complex (Fig. 41).
The polar group in the receptor responsible for co-ordination with the carboxyl group of the ligand may be, for example, the hydroxyl of a serine residue. To make this residue more reactive to hydrogen bonding it could be enclosed in a lipophilic 'pocket' making 'solvation' by the ligand an energetically favourable process.
This model would also serve to explain how, by extending the carboxyl group by one or two methylene units, a region of antagonistic activity is obtained. It can be seen that binding to the ground state receptor could easily be achieved by A-11, for example, as was observed in the ferret spleen test system (Fig. 42); or equally, a different conformation may be adopted, leading to agonistic activity as was observed in the guinea-pig lung test system (Fig. 43).

A combination of these two conformations would lead to partial agonist activity, as was observed on the guinea-pig ileum.

This model, therefore, goes a long way towards explaining the observed results for the analogues encountered in this chapter. The following chapter will test the model by using it as a basis for the design of leukotriene antagonists.
DOSE-RESPONSE CURVES

KEY

○ = LTD₄ Control

● = LTD₄ in presence of 10⁻⁵ M test drug

◊ = LTC₄ Control

♦ = LTC₄ in presence of 10⁻⁵ M test drug

※ = Test drug alone
Analogue 1 on Guinea-Pig Ileum

Analogue 1 on Guinea-Pig Lung
Analogue 3 on Ferret Spleen

% Maximum LTD4 Response

Concentration (M)

10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}

Analogue 4 on Guinea-Pig Ileum

% Maximum LTD4 Response

Concentration (M)

10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
Analogue 4 on Guinea-Pig Lung

% Maximum LTD$_4$ Response

Concentration (M)

10$^{-10}$, 10$^{-9}$, 10$^{-8}$, 10$^{-7}$, 10$^{-6}$, 10$^{-5}$

Analogue 4 on Ferret Spleen

% Maximum LTD$_4$ Response

Concentration (M)

10$^{-9}$, 10$^{-8}$, 10$^{-7}$, 10$^{-6}$, 10$^{-5}$
Analogue 5 on Guinea-Pig Ileum

Analogue 5 on Guinea-Pig Lung
Analogue 6 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)

10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

Analogue 6 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)

10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵
Analogue 7 on Guinea-Pig Ileum

% Maximum LTC4 Response

Concentration (M)

10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}

Analogue 7 on Guinea-Pig Lung

% Maximum LTD4 Response

Concentration (M)

10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
Analogue 7 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)

10⁻¹₀ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

Analogue 8 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)

10⁻¹₀ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵
Analogue 8 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)

10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

Analogue 9 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)

10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵
Analogue 9 on Guinea-Pig Lung

% Maximum LTD4 Response

Concentration (M)

10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}

Analogue 10 on Guinea-Pig Ileum

% Maximum LTD4 Response

Concentration (M)

10^{-12} 10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
Maximun LTC4 Response

Analogue 11 on Guinea-Pig Ileum

Concentration (M)

% Maximum LTD4 Response

Concentration (M)
Analogue 11 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)

Analogue 11 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)
Analogue 12 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)

10⁻¹² 10⁻¹¹ 10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

Analogue 12 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)

10⁻¹² 10⁻¹¹ 10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵
Analogue 12 on Ferret Spleen

Analogue 13 on Guinea-Pig Ileum
Analogue 13 on Guinea-Pig Lung

% Maximum LTD$_4$ Response

Concentration (M)

Analogue 13 on Ferret Spleen

% Maximum LTD$_4$ Response

Concentration (M)
INTRODUCTION

The model of leukotriene receptor activation proposed in Chapter 2 involved the attraction of a hydrophilic moiety buried in a lipophilic pocket. It was discovered that simple \( \omega \)-thioalkylcarboxylic acids display good antagonistic activity in the ferret spleen test system yet retain potent agonism in the guinea-pig ileum and lung preparations. In an attempt to retain receptor affinity and induce binding solely to the ground state receptor it was decided to introduce alkyl substituents \( \omega \) to the carboxylic acid which may interact with the hydrophobic pocket and achieve the desired affect. A second approach was to restrict the conformation of the acidic 'peptide' substituent by incorporating the carboxylic acid on a thiophenyl leukotriene derivative. In this way the position of the carboxyl group is limited to a distinct region of space with regards to the sulphur atom and it may be possible to restrict binding to the ground state receptors.

A ALKYLATION STUDIES

Analogues A-10 and A-3 were chosen as the basis for the alkylation studies because of their good affinities on all three biological test systems. It was decided to initially
restrict the study to methylation $\alpha$ to the carboxyl groups, 
giving a series of six compounds as shown in Fig. 44.

SYNTHESIS

The analogues were prepared in the usual manner of reacting 
the thiol with LTA$_4$-Me followed by deprotection. The thiols 
were either commercially available or were prepared from the 
corresponding halides by treatment with sodium 
hydrogensulphide. Where the halides were not available they 
were prepared from the corresponding alcohols by treatment 
with thionyl chloride. Esterification of the free acids 
prior to addition to LTA$_4$-Me was effected by treatment with 
diazomethane. The diastereomers A-14 and A-15 were 
separated at the protected stage but their absolute 
structures were not assigned. The biological results are 
summarised in Figure 45 and the dose-response curves 
included at the end of the chapter.
Fig. 44

RESULTS

<table>
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<td>Lung</td>
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* LTC₄ Control
i) Guinea-pig ileum

On the guinea-pig ileum system, one of the monomethyl derivatives, A-15, retained the full agonist potency of its parent, A-10. The epimer, A-14, however, was at least an order of magnitude less potent and the dimethyl derivative, A-16, also displayed diminished agonistic activity. The methyl analogues of A-3 showed a similar profile of activity, with the S isomer, A-18, retaining considerably more agonistic activity than the R isomer, A-17. The dimethyl derivative A-19 was similarly less effective than the S-monomethyl analogue, A-18.

It can be seen from the dose-response curve of A-17 on guinea-pig ileum that the R-monomethyl derivative may have been exhibiting a degree of antagonism here, although the effect is masked by the residual agonistic activity of the molecule.

ii) Guinea-pig lung

Similar trends were observed for the analogues on this tissue as on the guinea-pig ileum. That is, the dimethyl analogues A-16 and A-19 were less potent than the monomethyl derivatives and for the A-3 type analogues A-17 and A-18, the S isomer, A-18 retained the greatest agonistic activity. Unfortunately no hint of antagonistic activity was observed here.
iii) Ferret spleen

On this tissue, antagonistic activity was observed for the whole series of 'methyl' analogues. Although the 'A-10' series retained some agonistic behaviour, good antagonism was observed with one of the mono-methyl isomers, A-15. Interestingly, this is the same isomer that retained full agonistic potency on the ileum test system. The epimer, A-14, and the dimethyl analogue A-16, were an order of magnitude weaker antagonists which also reflected their agonistic profile on the guinea-pig ileum. The introduction of R and S methyl groups on A-3 was tolerated by the ferret spleen, although no improvement in antagonistic activity was observed. The dimethyl derivative A-19, however, was a considerably weaker antagonist than either of the monomethyl derivatives A-17, A-18 or the parent, A-3.
The second approach of structural manipulation to confer antagonistic properties on leukotriene analogues was to restrain the carboxyl group by the incorporation of an aromatic ring in the 'peptide' moiety. The analogues synthesised are shown in Figure 45 and the biological results in Figure 46.

SYNTHESIS

Ortho, meta and para-methyl mercaptobenzoates were reacted with LTA$_4$-methyl ester followed by deprotection in the usual manner to afford analogues A-20, A-21 and A-22 respectively. The thiophenols were prepared by the method of Wiley, from the corresponding aminobenzoic acids by diazotisation and elimination with ethyl potassium xanthate. Hydrolysis of the xanthate esters yielded the thiols, the free carboxyl groups being esterified prior to reaction with LTA$_4$-Me.

![Analogue 20](image)

Analogue 20

![Analogue 21](image)

Analogue 21

![Analogue 22](image)

Analogue 22

Fig. 45
**RESULTS**

![Chemical Structure](image)

<table>
<thead>
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<th>Analogue</th>
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* LTC4 Control

Fig 46
i) Guinea-pig ileum

On the guinea-pig ileum this series of analogues show a remarkable range of activity from good full agonism to antagonism. The receptor requirements are clearly defined as is shown by the difference between the meta-analogue A-21, being a good full agonist, and the para-analogue A-22, which is a potent antagonist.

ii) Guinea-pig lung

A similar trend is observed in the guinea-pig lung as on the ileum, with the meta-substituted analogue A-21 displaying the greatest agonism and the para-substituted analogue A-22 being an antagonist.

iii) Ferret spleen

The ferret spleen shows some weak agonism for the ortho analogue A-20 but the meta- and para- analogues A-21 and A-22 respectively are good antagonists.

DISCUSSION

The alkyl-substituted series of analogues, A-14 - A-18, showed little of the desired increase in antagonistic activity. In the ferret spleen partial agonism was observed for the methyl series
of A-10 and in the guinea-pig ileum the R-monomethyl derivative, A-17 showed weak partial agonism. With these exceptions the series generally reflected the activity of the parent molecules with one of the monomethyl optical isomers showing greater activity than the other and the dimethyl derivatives having the weakest activity. Although the desired profile of activity was not observed with this series it is interesting to note that the alkyl substituents were generally tolerated by the receptors implying that the initial premise was not completely wrong.

The mercaptobenzoic acid series of analogues, A-20 - A-22, possessed a wide range of activities from good full agonism to strong antagonism in the three test systems.

The ortho- and meta-analogues, A-20 and A-21, show a profile of activity reminiscent of the alkanoyl analogue A-3, being agonists on the guinea-pig ileum and lung, and displaying antagonistic activity on the ferret spleen. The para-analogue, A-22, however, has the desired spectrum of activity, being an antagonist in all three systems.

These results fit nicely into the proposed model of leukotriene receptor activation discussed in Chapter 2. Fig. 47 shows a comparison of the 'peptide' region of A-20 and A-21 with A-3; it is not too difficult to imagine these compounds showing similar profiles of activity. The para-analogue, A-22, was shown to be an antagonist in all three systems; being devoid of agonistic
activity, it must bind solely to the ground state of the receptors. The proposed model is consistent with the observed phenomena as shown in Fig. 48.

\[ \text{Fig. 47} \]

\[ \text{Fig. 48} \]
It is remarkable that such a small structural change such as para-substitution compared to meta-substitution resulted in completely opposite biological effects in guinea-pig tissues. This clearly demonstrates the strict structural requirements of the receptor in this region of the pharmacophore and serves to further reinforce the belief that the observed phenomena are indeed mediated by receptor interactions, as physiological agonism-antagonism would tend to be less specific.

The observed difference in effectiveness of A-22 to antagonise LTC₄ induced contractions in the three test systems as shown by the differing pKᵦ values, is further evidence for the receptor types being different. This is consistent with the receptor classification discussed in chapter 1 which identified LT1 receptors on guinea-pig ileum, LT2 receptors in guinea-pig lung and LT3 receptors in the ferret spleen. It can be seen from Fig. 46 that A-22 is approximately an order of magnitude more effective on LT1 than on LT3, and approximately an order of magnitude more effective on LT3 than on the LT2 receptor.
DOSE-RESPONSE CURVES

KEY

○ = LTD₄ Control

● = LTD₄ in presence of 10⁻⁵ M test drug

◊ = LTC₄ Control

◆ = LTC₄ in presence of 10⁻⁵ M test drug

★ = Test drug alone
Analogue 14 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)

Analogue 14 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)
Analogue 16 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)

Analogue 17 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)
Analogue 17 on Guinea-Pig Lung

% Maximum LTD₄ Response

Concentration (M)

10⁻¹² 10⁻¹¹ 10⁻¹⁰ 10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵

Analogue 17 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)

10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵
Analogue 20 on Ferret Spleen

% Maximum LTD₄ Response

Concentration (M)

Analogue 21 on Guinea-Pig Ileum

% Maximum LTD₄ Response

Concentration (M)
Analogue 21 on Guinea-Pig Lung

% Maximum LTD$_4$ Response

Concentration (M)

10$^{-12}$ 10$^{-11}$ 10$^{-10}$ 10$^{-9}$ 10$^{-8}$ 10$^{-7}$ 10$^{-6}$ 10$^{-5}$

Analogue 21 on Ferret Spleen

% Maximum LTD$_4$ Response

Concentration (M)

10$^{-10}$ 10$^{-9}$ 10$^{-8}$ 10$^{-7}$ 10$^{-6}$ 10$^{-5}$
Analogue 22 on Ferret Spleen

% Maximum LTC4 Response

Concentration (M)

10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5}
CONCLUSION
Chapter 1 described the synthesis and biological evaluation of the leukotrienes: the natural agonists of the receptors under discussion. This is a necessary part of drug development as they are required as internal controls for the comparison of agonists and are obviously required to show the action of antagonists. The intermediates synthesised for the preparation of leukotrienes were then utilised in the preparation of leukotriene analogues to probe the receptor agonist and antagonist pharmacophores.

Chapter 2 dealt with the simple modifications of the leukotriene structure aimed at identifying pharmacophoric groups. The most crucial pharmacophoric group was found to be the peptidyl carboxyl group and it was further discovered that the agonist pharmacophore could be simplified to A-10.

This greatly simplified molecule retained all the potency and activity of the natural leukotrienes LTD$_4$ and LTC$_4$ and was the starting point for the development of antagonists.
Chapter 3 details two areas from which the design of antagonists was approached. The introduction of hydrophobic moieties in the peptidyl region was generally tolerated but did not significantly affect the activity profiles. However, when rigidity combined with displacement of a pharmacophoric group was introduced into the molecule the desired profile of activity was achieved. Thus by 'locking' the pharmacophoric carboxyl moiety at a site distinct from the known position of the agonist pharmacophoric binding site it was possible to confer binding solely to the ground state receptor.

\[
\text{ANTAGONIST}
\]

ANALOGUE 22

A leukotriene antagonist has been designed which was effective on three different classes of leukotriene receptors. In comparison with FPL55712, which was only active on one of these receptor systems, it is apparent that this is a major breakthrough in the field of leukotriene research.

Further structure-activity studies are currently progressing in our laboratories in an attempt to improve the binding affinity and increase the stability of A-22, however this is not the subject of this thesis.
Thus in conclusion, it has been shown that by applying receptor theory and concepts of molecular recognition it has been possible to design an antagonist based on the chemical structure of an agonist. It has yet to be shown that a compound with the profile of activity of A-22 is therapeutically beneficial but compounds of this type may play a major role in alleviating the symptoms of asthma and other allergic disorders involving leukotrienes.
EXPERIMENTAL
Experimental Notes

Solvents

Dichloromethane was distilled from phosphorous pentoxide and stored under an inert atmosphere over 3Å molecular sieves.

Hexamethylphosphoramide was dried by stirring with powdered calcium hydride overnight followed by distillation under reduced pressure and stored under an inert atmosphere over 4Å molecular sieves.

Methanol was distilled from sodium methoxide and stored under an inert atmosphere over 3Å molecular sieves.

Pyridine was distilled from potassium hydroxide pellets and stored over 4Å molecular sieves.

Tetrahydrofuran was dried by stirring with powdered calcium hydride overnight followed by distillation and stored under an inert atmosphere over 4Å molecular sieves.

Diethyl ether, ethyl acetate, hexane and water were all distilled.

Chloroform was used as supplied (May & Baker Ltd)
HPLC Solvents

Acetonitrile and water were used as supplied (Rathburn Chem Co Ltd). The pH of solvent mixtures was adjusted by the addition of ammonia to the required pH.

Preparative tlc

RP preparative tlc was performed on Whatman PLKC 18F plates, 1 mm thickness. The product bands were identified under UV light and separated from the plate. The product was removed from the support by washing three times with methanol.

Reagents

Reagents were of the highest commercially available purity from the following suppliers: Aldrich Chem Co, BDH and Lancaster Synthesis.

n-Butyllithium was titrated against diphenylacetic acid in dry THF at room temperature immediately prior to use.

Instrumentation

R.P.HPLC was performed on a Perkin-Elmer series 3B liquid chromatograph. Analytical columns were Waters μBondapak C₁₈, 3.9 mm x 300 mm and used at a flow rate of 1 ml.min⁻¹ unless
otherwise stated. Preparative columns were Du Pont Zorbax C_{18}, 21.2 mm x 250 mm and were used at a flow rate of 15 ml.min^{-1} unless otherwise stated.

Normal phase pHPLC was performed on a Waters preparative LC/System 500-A using a single silica column 50 mm x 300 mm.

Mass spectra were obtained on a VG Analytical ZAB-HF mass spectrometer fitted with an Ion-Tech saddle field gun. Xenon was used as a reactant gas to provide an ion source and a thioglycerol matrix was used to assist ion formation. In practice, thioglycerol was smeared on the probe and then the sample applied in methanol solution. Approximately 20 µg of a leukotriene was required for a good spectrum. The trace was recorded by a UV oscilloscope as the signal half-life was too short for computer analysis, and calibrated by the cluster ions obtained from a glycerol FAB mass spectrum.

Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected.

60 MHz \textsuperscript{1}H nmr spectra were recorded on a Hitachi Perkin-Elmer high resolution nmr spectrometer R-24B.

90 MHz \textsuperscript{1}H nmr and \textsuperscript{13}C nmr spectra were recorded on a Bruker WH90 pulse Fourier transform spectrometer.
Optical rotations were determined on a Perkin-Elmer polarimeter 241.

Short-distance distillation was performed on a Buchi GKR-50 Kugelrohr apparatus

Ultra-violet spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer.

Analytical Methods

CHN analysis was performed by CHN Analysis Ltd.

FAB-mass spectra were recorded by M-Scan Ltd.

De-Salting Method

Waters sep-pak C\textsubscript{18} cartridges were used for the de-salting of leukotrienes and analogues after deprotection by the following method. After removal of organic solvents under reduced pressure the solution containing the leukotriene salt was acidified to pH 1 with 1 M HCl and loaded via a syringe onto a Sep-pak which had been pre-wetted by elution with methanol (2 ml) followed by water (5 ml). The cartridge was then flushed with water (5 ml) and the leukotriene eluted as the free acid with methanol (5 ml).
Preparation of Methyl 7-oxo-5(S),6(R)-oxidoheptanoate

(a) Methyl 5(S),6(R),7-trihydroxyhept-2(E)-enoate (II)

2-Deoxy-D-ribose (135 g, 1 mol) and methoxycarbonylmethylene-triphenylphosphorane (340 g, 1.02 mol) were refluxed together in dry tetrahydrofuran (1 l) for 9 h. The solvent was removed at reduced pressure and the resultant syrup was dissolved in chloroform (1 l). The chloroform solution was thoroughly extracted with water; the aqueous extract was washed with chloroform then concentrated at reduced pressure. The resultant syrup was re-dissolved in chloroform (400 ml) and dried with magnesium sulphate. The solution was filtered and concentrated to give a white gum (199 g, 100 %).

Recrystallisation from dichloromethane/ether gave the title compound as white flakes (84 g, 42 %) m.p. 60-62°C, [α]D = -19.1° (C = 1.0, MeOH). A second crop of crystals may be obtained from the residue.

CHN Analysis: Found: C, 50.24; H, 7.46% Calculated for C8H14O5: C, 50.52; H, 7.42 %

1H nmr (60 MHz, CDCl3): δ = 2.5 m, 2H, 4-CH2; 3.5 m, 2H, 7-CH2OH; 3.54 s, 3H, CO2CH3; 4.1 m, 3H, 3 x OH (exchanges D2O); 5.85 d, 1H, 2-CH, J2,3 = 16Hz; 7.0 dd, 1H, 3-CH, J2,3 = 16Hz, J3,4 = 8Hz.
(b) Methyl 5(S),6(R),7-trihydroxyheptanoate (III)

The heptanoate (II) (50 g, 0.26 mol) was dissolved in dry ethyl acetate (300 ml) together with 10% palladium on charcoal (5 g) and hydrogenated at atmospheric pressure until the theoretical quantity of hydrogen had been absorbed. The suspension was filtered through a pad of Celite and concentrated at reduced pressure to give a colourless oil. Crystallisation from dichloromethane-ether gave the title compound as white flakes, m.p. 45-47°C, $[\alpha]_D = -15°$ (C = 1.0, MeOH) (27 g, 53%).

$^1$H nmr (60 MHz, CDCl$_3$); $\delta = 1.6$ m, 4H, 3-CH$_2$, 4-CH$_2$; 2.4 m, 2H, 2-CH$_2$; 3.62 s, 3H, CO$_2$CH$_3$; 3.7 m, 4H, 5-CHOH, 6-CHOH, 7-CHOH; 4.0 br, 3H, 3 x OH (exchanges D$_2$O).

(c) Methyl 5(S),6(R)-dihydroxy-7-(p-toluenesulphonyloxy)-heptanoate (IV)

The heptanoate (III) (29 g, 0.151 mol) and p-toluenesulphonyl chloride (30 g, 0.157 mol) were dissolved in dry pyridine (380 ml) and the resultant solution stirred for 25 h at -10°C. The solution was then poured into a mixture of ice, hydrochloric acid and dichloromethane (200 ml). During the mixing the temperature was kept below 20°C. The layers were separated and the aqueous phase was extracted three times with dichloromethane.
The combined extracts were washed with brine, dried and concentrated at reduced pressure to give a sticky solid (52 g). Recrystallisation from ether gave the title compound as white needles (24.8 g, 47%) m.p. 59-61°C, $[\alpha]_D = +3.03^\circ$ (C = 1.09, CHCl$_3$).

CHN Analysis: Found: C, 52.29; H, 6.28; S, 8.92 % C$_{15}$H$_{22}$O$_7$S requires: C, 52.00; H, 6.40; S, 9.26 %

$^1$H nmr (60 MHz, CDCl$_3$): $\delta$ = 1.6 m, 4H, 3-CH$_2$, 4-CH$_2$; 2.3 m, 2H, 2-CH$_2$; 2.39 s, 3H, CO$_2$CH$_3$; 3.16 s, 2H, 2 x OH (exchange D$_2$O); 3.60 s, 3H, CO$_2$CH$_3$; 3.6 m, 2H, 5-CH(OH), 6-CH(OH); 4.12 d, 2H, 7-CH$_2$OTs, J$_{6,7} = 4$Hz; 7.26 d, 2H, 2'-H, 6'-H, J$_{2',3'} = 8$Hz; 7.76 d, 2H, 3'-H, 5'-H.

(d) **Methyl 5(S)-hydroxy-6(S),7-oxidoheptanoate** (V)

The tosylate (IV) (31.8 g, 92 mmol) was dissolved in dry methanol (127 ml) and anhydrous potassium carbonate (25.4 g, 0.184 mol) was added. The mixture was stirred for 45 min, then filtered and concentrated at reduced pressure. The residue was partitioned between aqueous ammonium chloride solution and chloroform (150 ml). The aqueous phase was extracted twice more with chloroform. The combined extracts were washed with brine, dried and concentrated to give the title compound as a colourless oil (15.5 g, 97%), $[\alpha]_D = +17.4^\circ$ (C = 1.5, CHCl$_3$).
\[ ^1H \text{nmr} (60 \text{ MHz, CDCl}_3): \delta = 1.7 \text{ m}, 4H, 3-\text{CH}_2, 4-\text{CH}_2; 2.3 \text{ t}, 2H, 2-\text{CH}_2; J_{2,3} = 7 \text{ Hz}; 2.72 \text{ d}, 1H, 7-\text{CH}_2\text{H}_3 \text{, } J_{A,B} = 4 \text{ Hz}; 2.8 \text{ m}, 1H, 6-\text{CH}; 2.90 \text{ dd}, 1H, 7-\text{CH}_2\text{H}_3 \text{, } J_{A,B} = 8 \text{ Hz}, J_{A,B} = 4 \text{ Hz}; 3.60 \text{ s}, 3H, \text{CO}_2\text{CH}_3; 3.6 \text{ m}, 2H, \text{CH(OH)} \text{ (1H exchanges D}_2\text{O)}. \]

(e) **Methyl 7-hydroxy-5(S),6(S)-oxidoheptanoate (VI)**

The 6,7-epoxide (V) (15.5 g, 90 mmol) was dissolved in dry methanol (60 ml) and anhydrous potassium carbonate (12.4 g, 90 mmol) was added. The mixture was stirred at room temperature for 28 h then filtered, concentrated at reduced pressure and partitioned between aqueous ammonium chloride solution and chloroform (100 ml). The aqueous phase was extracted twice more with chloroform. The combined extracts were washed with brine, dried and concentrated to give the title compound as a colourless oil (6.4 g, 82 %), \([\alpha]_D = -30.2^\circ \text{ (C = 1.7, CHCl}_3) \).

\[ ^1H \text{nmr} (60 \text{ MHz, CDCl}_3): \delta = 1.6 \text{ m}, 4H, 3-\text{CH}_2, 4-\text{CH}_2; 2.4 \text{ m}, 2H, 2-\text{CH}_2; 2.8 \text{ m}, 2H, 5-\text{CH}, 6-\text{CH}; 3.6 \text{ m}, 3H, 7-\text{CH}_2\text{OH (1H exchanges D}_2\text{O); 3.62 s, 3H, CO}_2\text{CH}_3. \]

(f) **Methyl 7-oxo-5(S),6(R)-oxidoheptanoate (I)**

The alcohol (VI) (1.65 g, 9 mmol) was dissolved in dry dichloromethane (20 ml) and added to a solution of dipyridinium chromate in dry dichloromethane (330 ml). After 10 min the
mixture was poured onto the top of a column of silica (ca. 200 cm³) and eluted under pressure with dichloromethane (1.5 l) to give the title compound as a yellow oil (1.22 g, 74 %), [α]_D^20 = +68° (C = 1.3, CHCl₃).

This was purified by chromatography on silica with hexane/ethyl acetate (4:1) plus triethylamine (0.5 %) to give a pale yellow oil (1.17 g, 71 %), [α]_D^20 = +94.2° (C = 1.2, CHCl₃).

1H nmr (60 MHz, CDCl₃): δ = 1.7 m, 4H, 3-CH₂, 4-CH₂; 2.4 m, 2H, 2-CH₂; 3.15 dd, 1H, 6-CH, J₅,₆ = 2.0 Hz, J₆,₇ = 6.0 Hz; 3.2 dt, 1H, 5-CH, J₄,₅ = 6.0 Hz; 3.62 s, 3H, CO₂CH₃; 9.01 d, 1H, CHO.

Methyl 5(S),6(S)-oxido-11-oxo-7(E),9(E) undecadienoate (VII)

(a) The aldehyde (I) (1.2 g, 7 mmol) was dissolved in dry dichloromethane (30 ml) and slowly added to a solution of 4-oxobut-2(E)-enyltriphenylphosphorane (VIII) (2.8 g, 8.5 mmol) in dry dichloromethane (30 ml) over 1.5 h. The solution was stirred for a further 2 h at room temperature then concentrated at reduced pressure to give a sticky, dark red solid. This was chromatographed on silica gel with ether/hexane (plus 0.5 % triethylamine) to give as colourless oils the pure 7(Z) isomer (268 mg), [α]_D^20 = -49.0° (C = 1.8, CHCl₃) and mixtures with the (E) isomer (692 mg).
\[^1\text{H nmr (60 MHz, CDCl}_3\text{):} \delta = 1.7 \text{ m, 4H, 3-CH}_2\text{, 4-CH}_2; 2.4 \text{ m, 2H, 2-CH}_2; 3.0 \text{ m, 1H, 5-CH; 3.6 \text{ m, 1H, 6-CH; 3.63 s, 3H, CO}_2\text{CH}_3; 5.55 dd, 1H, 7-CH, J_{6,7} = 8 \text{ Hz, } J_{7,8} = 11 \text{ Hz; 6.15 dd, 1H, 10-CH, } J_{9,10} = 15 \text{ Hz, } J_{10,11} = 8 \text{ Hz; 6.35 dd, 1H, 8-CH, } J_{7,8} = 11 \text{ Hz, } J_{8,9} = 12 \text{ Hz; 7.66 dd, 1H, 9-CH; 9.62 d, 1H, CHO.}\]

b) The combined fractions (960 mg, 4.3 mmol) were dissolved in dry dichloromethane (40 ml) with a trace of iodine (6 mg) and the solution stirred (in sunlight) for 3 h. This was concentrated at reduced pressure to give a yellow solid which was rapidly chromatographed on silica with ether (plus 0.25 % triethylamine) to give a yellow solid (919 mg). Recrystallisation from ether-hexane gave the title compound as yellow needles (716 mg, 75 %), m.p. 59-60°C, [\alpha]_D = -37.0° (C = 1.0, CHCl\textsubscript{3}).

CHN Analysis: Found: C, 63.96; H, 7.06; O, 28.88 % C\textsubscript{12}H\textsubscript{16}O\textsubscript{4}
requires: C, 64.27; H, 7.19; O, 28.54 %.

\[^1\text{H nmr (90 MHz, CDCl}_3\text{):} \delta = 1.73 \text{ m, 4H, 3-CH}_2\text{, 4-CH}_2; 2.40 \text{ t, 2H, 2-CH}_2, J_{2,3} = 6.59 \text{ Hz; 2.92 dt, 1H, 5-CH, } J_{4,5} = 5.56 \text{ Hz, } J_{5,6} = 2.05 \text{ Hz; 3.22 dd, 1H, 6-CH, } J_{6,7} = 7.32 \text{ Hz; 3.68 s, 3H, CO}_2\text{CH}_3; 5.96 dd, 1H, 7-CH, J_{7,8} = 14.93 \text{ Hz; 6.16 dd, 1H, 10-CH, } J_{9,10} = 15.08 \text{ Hz, } J_{10,11} = 7.61 \text{ Hz; 6.63 dd, 1H, 8-CH, } J_{8,9} = 10.83 \text{ Hz; 7.10 dd, 1H, 9-CH; 9.58 d, 1H, CHO.}\]
Preparation of 4-oxo-2(E)-butenylidene triphenylphosphorane (XI)

a) 1-Bromo-4-oxobut-2(E)-ene (IX)

1-Acetoxy-1,3-butadiene (29.5 g, 0.26 mol) was dissolved in hexane (120 ml) with barium carbonate (60 mg) and cooled to -78°C. To this stirred solution was added bromine (43.7 g, 0.28 mol) in carbon tetrachloride (150 ml) dropwise over 75 min. The solution was then allowed to warm to room temperature. Sodium bicarbonate (50 g) and water (220 ml) were added and the mixture shaken mechanically for 3 h in a conical flask fitted with a steam distillation splash head. The phases were separated and the aqueous layer extracted with chloroform. The combined extracts were washed with brine, dried and concentrated to yield a pale yellow oil (50.3 g). This product was immediately vacuum distilled to afford a clear oil (29.4 g, 76%), b.p. 49–52°C/0.8 mBar.

$^1$H nmr (60MHz, CDCl₃): $\delta = 4.05$ d, 2H, CH₂Br, $J_{1,2} = 7$ Hz; 6.20 dd, 1H, 3-CH, $J_{2,3} = 16$ Hz, $J_{3,4} = 7$Hz; 6.90 dt, 1H, 2-CH; 9.55 d, 1H, CHO.

b) 4-Oxobut-2(E)-enyltriphenylphosphonium bromide (X)

1-Bromo-4-oxobut-2(E)-ene (IX) (29.4 g, 0.20 mol) was dissolved in acetone (120 ml) and cooled to 0°C. Triphenylphosphine...
(57.6 g, 0.22 mol) was added to this mechanically stirred solution which was allowed to return to room temperature overnight. The precipitate thus formed was filtered off under an inert atmosphere, washed with acetone (2 x 100 ml) and vacuum dried to afford a cream solid (82.0 g, 100 %) m.p. 180.5-182°C.

c) 4-Oxo-2(E)-butenylidenetriphenylphosphorane (XI)

4-Oxobut-2(E)-enyltriphenylphosphonium bromide (X) (82.0 g, 0.20 mol) was added to dichloromethane (600 ml) and sodium hydroxide solution (300 ml; 1 M) in a separating funnel. The mixture was shaken for 5 min and then separated. The aqueous phase was further extracted with dichloromethane (400 ml). The combined extracts were washed with brine, dried and filtered. Diethyl ether (3 l) was added to this solution and cooled to -20°C overnight. The title compound was collected as dark red crystalline solid (38.6 g, 58 %; m.p. 160-165°C), washed with diethyl ether, dried and stored under argon in amber bottles at 0°C.

Preparation of 3(Z)-nonenyltriphenylphosphonium tosylate (XIV)

(a) 3(Z)-Nonen-1-ol (XII)

3-Nonyn-1-ol (10.5 g, 75 mmol) dissolved in dry, distilled methanol (100 ml) containing 5 % Pd/BaSO₄ catalyst (400 mg) poisoned with quinoline (400 μl) was stirred vigorously under an
atmosphere of hydrogen until the theoretical quantity of hydrogen (1.6 l) had been absorbed and hydrogen uptake had ceased. The reaction mixture was filtered through Celite and concentrated to yield 3(Z)-nonen-1-ol (10.7 g, quantitative yield) as a yellow oil.

\[ ^1H \text{ nmr (60MHz, CDCl}_3 \text{): } \delta = 0.90 \text{ t, 3H, 9'-CH}_3, J_{8',9'} = 6 \text{ Hz; 1.3 m, 6H, 6'-CH}_2, 7'-CH}_2, 8'-CH}_2; 2.3 m, 5H, 2'-CH}_2, 5'-CH}_2, \text{OH (1H exchanges D}_2O); 3.6 t, 2H, CH}_2OH, J_{1',2'} = 6.5 \text{ Hz; 5.45 m, 2H, 3'-CH, 4'-CH.} \]

(b) 3(Z)-Nonenyl-p-toluenesulphonate (XIII)

3-Nonen-1-ol (XI) (10.6 g, 75 mmol) dissolved in dry, distilled pyridine (50 ml) was cooled to 0°C under argon. Recrystallized p-toluenesulphonyl chloride (17.1 g, 90 mmol, 1.2 equivalents) was added and the reaction stirred overnight (approximately 16 h) at 0°C before quenching on crushed ice (100 g). The product was extracted twice with ether (200 ml), washed with brine (200 ml), dried and concentrated. The crude product was eluted through a short silica pad with dichloromethane to give a pale yellow oil (19.4 g, 88 % yield).

\[ ^1H \text{ nmr (60MHz, CDCl}_3 \text{): } \delta = 0.87, \text{ t, 3H, 9'}-CH}_3, J_{8',9'} = 6 \text{ Hz; 1.25 m, 6H, 6'-CH}_2, 7'-CH}_2, 8'-CH}_2; 1.90 m, 2H, 2'-CH}_2; 2.3 m, 5H, 5'-CH}_2, 4'-CH}_3; 3.98 t, 2H, 1'-CH}_2, J_{1',2'} = 6.8 \text{ Hz; 5.32 m, 2H, 3'-CH, 4'-CH; 7.53 dd.} \]
(c) 3(Z)-Nonenyltriphenylphosphonium tosylate (XIV)

(XII) (19.3 g, 65 mmol) and recrystallized triphenylphosphine (17.0 g, 65 mmol, 1 equivalent) were heated under gentle reflux at 90°C in acetonitrile (25 ml) for 48 h. The solvent was removed under reduced pressure and the product recrystallized from dichloromethane/diethyl ether (100 ml/150 ml) to give 3(Z)-nonenyltriphenylphosphonium tosylate (24.0 g, 66 % yield) as white needles m.p. 77-80°C.

CHN Analysis: Found: C, 71.45; H, 6.92; N, 0.00; Cl, 1.96; S, 5.52; P, 5.33 % Calculated for C_{34}H_{39}O_3PS: C, 71.56; H, 6.92; N, 0.00; Cl, 2.16; S, 5.59; P, 5.40 %

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoate (LTA₄-Me) (VIII)

The phosphonium tosylate (XIII) (3.59 g, 6.43 mmol) was dissolved in dry tetrahydrofuran (100 ml) and cooled to -20°C under argon. n-Butyllithium solution (5.89 mmol) in hexane was added dropwise and stirred for 5 min at -20°C before cooling to -78°C. After a further 10 min hexamethylphosphoramide (12.2 ml; 12 equivalents) in tetrahydrofuran (12.2 ml) was slowly added and stirred for 15 min before addition of the aldehyde (VII) (1.2 g, 5.36 mmol) in tetrahydrofuran (5 ml). The reaction mixture was stirred at -78°C for 30 min and then quenched with methanol (1 ml),
allowing the reaction temperature to slowly rise to room temperature (1 h).

The product was partitioned between diethyl ether and water, washed well with further water and brine, dried over magnesium sulphate and concentrated. The crude product was purified by rapid column chromatography on silica (previously deactivated by standing for 3 h in the eluent), eluting with hexane/diethyl ether/triethylamine (88 : 10 : 2; 250 ml) under argon pressure. The eluent, upon concentration, yielding the desired LTA₄-Me (1.80 g, 100 % yield).

\[ \lambda_{\text{max}} \text{ (cyclohexane): } 271, 280.8 \text{ (} \varepsilon = 50,000 \text{), } 292.6 \text{ nm.} \]

\[ ^1H \text{ nmr (90 MHz, CDCl}_3\text{)}: \delta = 0.89 \text{ t, } 3H, 20-\text{CH}_3 \text{, } J_{19,20} = 6 \text{ Hz;} \]
\[ 1.29 \text{ s, } 6H, 17-,18-,19-\text{-CH}_2 \text{; } 1.7 \text{ m, } 4H, 3-,4-\text{-CH}_2 \text{; } 2.06 \text{ m, } 2H, \]
\[ 16-\text{CH}_2 \text{; } 2.38 \text{ t, } 2H, 2-\text{-CH}_2 \text{, } J_{2,3} = 6.7 \text{ Hz;} \]
\[ 2.93 \text{ m, } 3H, 13-\text{-CH}_2 \text{, } 5-\text{H;} \]
\[ 3.13 \text{ dd, } 1H, 6-\text{H, } J_{5,6} = 2 \text{ Hz, } J_{6,7} = 7.9 \text{ Hz;} \]
\[ 3.67 \text{ s, } 3H, \text{ CO}_2\text{-CH}_3 \text{;} \]
\[ 5.4 \text{ m, } 4H, 7-,12-,14-,15-\text{-H;} \]
\[ 6.3 \text{ m, } 4H, 8-,9-,10-,11-\text{-H.} \]

\[ ^13C \text{ nmr (23 MHz, CDCl}_3\text{)}: \delta = 14.1 \text{, } 20-\text{CH}_3 \text{; } 21.3-33.6 \text{, } 8X \text{ CH}_2 \text{; } \]
\[ 51.6 \text{, CO}_2\text{-CH}_3 \text{; } 58.3 \text{, } 5-\text{CH;} \]
\[ 60.5 \text{, } 6-\text{CH;} \]
\[ 126.9-134.6 \text{, } 8X \text{ CH=; } 173.6 \text{, CO}_2\text{-CH}_3. \]
6(R)-S-(Glutathione)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (LTC₄)

Leukotriene A₄ methyl ester (100 mg, 0.3 mmol) was dissolved in methanol/triethylamine (1:1; 1.5 ml) containing dimethyl N-trifluoroacetylglutathionate (195 mg, 0.45 mmol) and stirred at room temperature under an atmosphere of argon for 6 h, by which time acidification of a UV sample indicated complete consumption of LTA₄-Me. The crude product was purified by reverse phase preparative tlc (RP ptlc) (CH₃CN/H₂O 4:1; Rf 0.5–0.7) to yield protected LTC₄ (210 mg, 91% yield; UV (MeOH) λₘₐₓ = 280.9 nm, ε = 50,000). The product was re-purified by reverse phase preparative HPLC (RP-HPLC) (CH₃CN/H₂O 70:30; flow 15 ml.min⁻¹, Tr = 15 min) and subsequently deprotected by the action of lithium hydroxide (100 mg) in tetrahydrofuran/water (1:1; 10 ml) over 2 h. The product was de-salted on a reverse phase plug to yield the title compound in high purity (167 mg, 89% yield; UV (H₂O) λₘₐₓ = 281.8 nm, ε = 40,000). HPLC Analysis: CH₃CN/H₂O/ACOH 40:60:0.1 pH 5.6, flow 1.0 ml.min⁻¹, Tr = 7.0 min, 93% 11-cis isomer, 5% 11-Trans isomer. FAB MS (+ve ion): M + H, 625 au.

6(R)-S-(Cysteinylglycine)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (LTD₄)

Leukotriene A₄ methyl ester (100 mg, 0.3 mmol) was dissolved in methanol/triethylamine (1:1, 2 ml) containing methyl N-trifluoroacetylcysteinylglycinate (210 mg, 0.6 mmol) and
stirred at room temperature under argon for 5 h. By this time, acidification of a UV sample indicated the reaction to be complete so the reaction mixture was quenched by applying it to reverse phase tlc plates and eluting with acetonitrile/water (4:1). The crude product (Rf 0.42–0.69; 170 mg, 90 % yield; UV (MeOH) \( \lambda_{\text{max}} = 280.9 \text{ nm, } \varepsilon = 50,000 \)) was purified by RP HPLC (CH\(_3\)CN/H\(_2\)O 70:30; flow 15 ml.min\(^{-1}\), Tr = 19 min) to give protected LTD\(_4\) in high purity (99 % by HPLC) (120 mg, 64 % yield). Deprotection was effected in a solution of lithium hydroxide (100 mg) in tetrahydrofuran/water (1:1; 10 ml) over 6 h and de-salted on a reverse phase plug to give the title compound in good yield (95 mg, 64 % yield; UV (H\(_2\)O) \( \lambda_{\text{max}} = 281.9 \text{ nm, } \varepsilon = 40,000 \)). HPLC analysis: CH\(_3\)CN/H\(_2\)O/AcOH 50:50:0.1 pH=5.6, flow 1.0 ml.min\(^{-1}\), Tr = 5.3 min. 95 % 11-cis isomer, 5 % 11-Trans isomer. FAB MS (-ve ion): M - H, 495 au.

6(R)-S-(Cysteine)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (LTD\(_4\))

LTD\(_4\)-Me (50 mg, 0.15 mmol) was dissolved in methanol/ triethylamine (1:1, 1 ml) containing methyl N-trifluoroacetylcysteinate (66 mg, 0.3 mmol) and stirred at room temperature under an atmosphere of argon for 2 h. Preparative tlc (RP CH\(_3\)CN/H\(_2\)O 5:1) afforded the crude protected product (Rf 0.35–0.5) (38 mg, 45 % yield; UV (MeOH) \( \lambda_{\text{max}} = 280.8 \text{ nm, } \varepsilon = 50,000 \)) which was further purified by RP HPLC (CH\(_3\)CN/H\(_2\)O 75:25; flow 15 ml.min\(^{-1}\), Tr = 20 min) to give pure protected LTD\(_4\)
(25 mg, 29 % yield). Deprotection was effected with lithium hydroxide (50 mg) in tetrahydrofuran/water (1:1, 5 ml) over 3 h and subsequent de-salting furnished the title compound (19 mg, 29 % yield; UV (H₂O) λ<sub>max</sub> = 280.6 nm, ε = 40,000). HPLC Analysis: CH₃CN/H₂O/H₃PO₄ 45:55:0.1 pH 3.7, flow 1.0 ml.min⁻¹, Tr = 12 min, 95 % 11-cis isomer, 3 % 11-Trans isomer.

Preparation of sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712) (XXII)

(a) 4-O-Allylresacetophenone (XVI)

Resacetophenone (100 g, 0.658 mol) was dissolved in acetone (300 ml) containing allyl bromide (88 g, 1.1 eq) and potassium carbonate (120 g, 1.3 eq) and refluxed with stirring for 6 h. When cool, the reaction mixture was concentrated and partitioned between 1 M hydrochloric acid and ether, the organic layer separated and the aqueous phase re-extracted with further ether. The combined ether extracts were shaken with 4 M sodium hydroxide solution and the resultant sodium salt collected by filtration and washed with ether. The salt was re-converted to the phenol by treatment with 5 M hydrochloric acid and extracted twice with ether. The combined extracts were washed with water, dried (MgSO₄) and concentrated. The crude product was distilled (116-120°C/2.0 mbar) to give a pale yellow oil (108 g, 85 % yield).
1H nmr (60 MHz, d₆ Acetone): δ = 2.50 s, 3H, CH₃; 4.60 d, 2H, CH₂, J = 5 Hz; 5.35 m, 2H, CH=CH₂; 6.00 m, 1H, CH=CH₂; 6.45 m, 2H, 3-H, 5-H; 7.75 d, 1H, 6-H, J₅,₆ = 9 Hz; 12.65 s, 1H, OH.

(b) 3-Allylresacetophenone (XVII)

4-O-Allylresacetophenone (197 g, 1.03 mol) was heated at 210-215°C, with stirring under argon, for 1.5 h. A red solid was obtained upon cooling which was purified by sublimation (140-150°C/1 mbar) to give a pale yellow crystalline solid (180 g, 91 % yield, mp 133°C (lit. 97 132-3°C).

1H nmr (60 MHz, d₆ Acetone): δ = 2.50 s, 3H, CH₃; 3.40 d, 2H, CH₂, J = 6 Hz; 4.95 m, 2H, CH=CH₂; 5.95 m, 1H, CH=CH₂; 6.45 d, 1H, 5-H, J₅,₆ = 9 Hz; 7.55 d, 1H, 6-H; 9.00 br, 1H, 4-OH; 13.00 s, 1H, 2-OH.

(c) 3-Propylresacetophenone (XVIII)

3-Allylresacetophenone (28 g, 0.146 mol) was dissolved in ethanol (250 ml) containing 10 % Pd/C catalyst (2.8 g) and vigorously stirred under a slight pressure of hydrogen. The reaction was stopped when the theoretical absorption of hydrogen had taken place and the rate of absorption had dropped considerably. The solution was filtered through Celite and concentrated to afford a white solid which recrystallised from chloroform to yield white plates (23 g, 81 % yield), m.p. 128.5-9.5°C, (lit. 97 127-8°C).
\(^1\)H nmr (60 MHz, d\(_6\) Acetone) \(\delta = 0.90\) t, 3H, CH\(_2\)-CH\(_3\), J = 7 Hz;
1.55 m, 2H, CH\(_2\)-CH\(_3\); 2.55 s, 3H, C(0)CH\(_3\); 2.60 t, 2H, Ar-CH\(_2\), J = 
7 Hz; 6.40 d, 1H, 5-H, J\(_{5,6}\) = 9 Hz; 7.50 d, 1H, 6-H; 8.90 br, 1H, 
4-OH; 13.15 s, 1H, 2-OH.

(d) \(4'-(2,3\)-Epoxy\)propoxy-3'-propylresacetophenone (XIX)

3-Propylresacetophenone (50 g, 0.258 mol) and epichlorohydrin
(71.6 g, 3 eq) were brought to reflux, with stirring, in ethanol
(75 ml). Dropwise, at reflux, was added a solution of potassium
hydroxide (15.9 g, 1.1 eq) in ethanol (75 ml) and water (1.5 ml).
After a further 2 h of reflux, the reaction mixture was
concentrated and partitioned between ether and water. The ether
layer was separated, dried (MgSO\(_4\)) and concentrated to give a
yellow oil which solidified on standing. The product was
distilled (148-150°C/0.2 mbar) to give a pale yellow solid on
cooling, which recrystallised from 40-60° petrol yielding
off-white crystals (42 g, 65 % yield), mp 57-8°C, (lit\(^94\) 54-5°C).

\(^1\)H nmr (60 MHz, d\(_6\) Acetone) \(\delta = 0.90\) t, 3H, CH\(_2\)-CH\(_3\), J = 7 Hz;
1.55 m, 2H, CH\(_2\)-CH\(_3\); 2.55 s, 3H, C(0)CH\(_3\); 2.75 m, 4H, Ar-CH\(_2\),
Ar-O-CH\(_2\); 3.35 m, 1H, O-CH\(_2\); 4.20 m, 2H, O-CH\(_2\); 6.60 d, 1H, 5-H,
J\(_{5,6}\) = 9 Hz; 7.70 d, 1H, 6-H; 12.85 s, 1H, OH.
Ethyl 7-hydroxy-8-propyl-4-oxo-4H-1-benzopyran-2-carboxylate (XX)

3-Propylresacetophenone (90 g, 0.464 mol) and diethyloxalate (95 ml, 2.2 eq) were dissolved in a mixture of ethanol (110 ml) and ether (110 ml). This was added to a stirred solution of sodium ethoxide (1.86 mol; 4 eq) prepared from sodium (42.7 g) and ethanol (600 ml) and the mixture refluxed under argon for 3 h. When cool, the reaction mixture was poured into an excess of 2 M hydrochloric acid (1200 ml) and extracted with ether. The ether extracts were washed with water, dried (MgSO\(_4\)) and concentrated under reduced pressure to afford the intermediate \(\alpha',\gamma\)-diketo ester (XXIII) as an oil.

The oil was refluxed in ethanol (350 ml) containing c. HCl (6 ml) for 45 min, concentrated to near-dryness and partitioned between ethyl acetate and water. The organic layer was washed with saturated sodium hydrogen carbonate solution, water; dried (MgSO\(_4\)) and concentrated to give a yellow solid. Recrystallisation from ethyl acetate/hexane (2:1) afforded the title compound as yellow powdery crystals (80 g, 62% yield), mp 168-8.5°C (lit\(^94\) 166-7°C).

\(^1\)H nmr (60 MHz, d\(_6\) Acetone): \(\delta = 1.00\) t, 3H, CH\(_2\)-CH\(_2\)-CH\(_3\), \(J = 7\) Hz; 1.40 t, 3H, C(O)CH\(_2\)-CH\(_3\), \(J = 7\) Hz; 1.60 m, 2H, CH\(_2\)-CH\(_2\)-CH\(_3\); 2.90 t, 2H, CH\(_2\)-CH\(_2\)-CH\(_3\); 4.40 q, 2H, C(O)-CH\(_2\)-CH\(_3\); 6.80 s, 1H, 3-H; 7.00 d, 1H, 5-H, \(J_{5,6} = 9\)Hz; 7.80 d, 1H, 6-H; 9.50 br, 1H, OH.
(f) Ethyl 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (XXI)

4'-((2,3-Epoxy)propoxy-3'-propylresacetophenone (15 g, 0.060 mol) and ethyl 7-hydroxy-8-propyl-4-oxo-4H-1-benzopyran-2-carboxylate (15 g, 0.054 mol) were dissolved in dimethylformamide (75 ml) containing N-benzyltrimethylammonium hydroxide (60 μl; 40% in methanol) and refluxed under argon for 2 h. The resulting brown solution was concentrated to a viscous oil (30 g).

The crude product was dissolved in dichloromethane and purified by normal phase preparative HPLC (silica) using diethyl ether as eluent, to give the ester as an off-white solid (9.3 g, 29% yield) m.p. 135-141°C (Lit\textsuperscript{97} 121-3°C).

\[^{1}\text{H} nmr (60 MHz, CDCl}_3; 5 = 0.90 m, 6H, 2X CH\_2-CH\_2-CH\_3; 1.50 m, 7H, 2X CH\_2-CH\_2-CH\_3, C(0)-CH\_2-CH\_3; 2.50 s, 3H, C(0)CH\_3; 2.70 m, 4H, 2X CH\_2-CH\_2-CH\_3; 4.35 m, 8H, C(0)-CH\_2-CH\_3, O-CH\_2-CH(OH)-CH\_2-O; 6.40 d, 1H, 6'H, J\textsubscript{5',6} = 9 Hz; 6.95 m, 2H, 3-H, 5-H; 7.55 d, 1H, 5'-H; 7.95 d, 1H, 6-H, J\textsubscript{5,6} = 9 Hz; 12.65 s, 1H, 3'-OH.

(g) Sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712) (XXII)

The ester (XXI) (9.3 g) was saponified by refluxing for 1 h with
sodium hydrogencarbonate (9.3 g) in 10 % aqueous ethanol (125 ml) and the free acid isolated by dichloromethane extraction of the acidified solution. The acid was neutralised with sodium hydrogencarbonate, twice recrystallised from water and freeze-dried to give the title compound as white crystalline flakes (7.5 g, 82 % yield).

CHN Analysis: Found: C, 60.80; H, 5.77 %. C_{27}H_{29}O_{9}Na. 0.71 H_{2}O requires C, 60.81; H, 5.75 %.

5(S)-Hydroxy-6(R)-methylthio-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-1)

LTA_{4}-Me (50 mg, 0.15 mmol) was dissolved in methanol/triethylamine (1:1; 5 ml) and methane thiol bubbled slowly through the mixture for 8 h. The reaction was shown by HPLC (CH_{3}CN/H_{2}O 90:10 flow 1.0 ml.min^{-1} Tr (LTA_{4}-Me) = 7.9 min, Tr (product) = 7.2 min) to be approximately 80 % complete at this stage so the reaction vessel was sealed and left to stir for a further 16 h to reach completion. The reaction mixture was concentrated under reduced pressure and applied in acetonitrile to two RP ptlc plates, eluting with acetonitrile/water (4:1). The product (Rf 0.21-0.36) was extracted from the stationary phase with methanol (16.8 mg; UV(MeOH) \lambda_{\text{max}} = 279.8 nm, \varepsilon= 50,000) and purified by RP HPLC (CH_{3}CN/H_{2}O 75:25 flow 20 ml.min^{-1} Tr = 23.5 min) to yield the desired methyl 5(S)-hydroxy-6(R)-methylthio-7(E),9(E),11(Z),14(Z)
eicosatetraenoate (11.5 mg by UV determination). The product was deprotected by the action of lithium hydroxide (50 mg) in aqueous tetrahydrofuran (THF/H$_2$O 1:1; 5 ml) over 30 min, (the extent of reaction was followed by RP HPLC CH$_3$CN/H$_2$O/AcOH 80:20:0.1 pH 5.6; Tr (product) = 7.5 min) concentrated under reduced pressure to remove the tetrahydrofuran and acidified in HCl to pH 1.0. De-salting on a reverse-phase plug yielded the title compound. (9 mg, 16 % yield; UV(H$_2$O) $\lambda_{max}$ = 281.3 nm, $\varepsilon$ = 40,000). FAB MS(-ve ion): M - H, 365 au. FAB MS (+ve ion): M + Na, 389 au; M - H + 2Na, 411 au.

N-Trifluoroacetylcysteamine

Cysteamine hydrochloride (11.35 g, 0.1 mol) was stirred in trifluoroacetic acid (50 ml) under argon at room temperature until complete dissolution was obtained (approximately 10 min). The solution was then cooled to 0°C and trifluoroacetic anhydride (18 ml; 1.3 eq) slowly added. The reaction mixture was stirred at 0°C for 30 min then at room temperature for 1 h before being concentrated to dryness. The crude product 22 g; 4.7 g above theoretical) smelled strongly of trifluoroacetic acid so was dissolved in methylene chloride and swirled with alumina. After filtration and concentration this yielded the title compound in 38 % yield (6.5 g).

$^1$H nmr (60 MHz, CDCl$_3$): $\delta$ = 1.5 t, 1H, SH, $J$ = 7 Hz; 2.8 m, 4H, CH$_2$-CH$_2$; 7.8 br, 1H, NH.
5(S)-Hydroxy-6(R)-2'-'aminoethylthio-7(E),9(E),11(Z),14(Z)－
eicosatetraenoic acid (A-2)

LTA₄-Me (50 mg, 0.15 mmol) was dissolved in methanol/
triethylamine (1:1; 5 ml) containing N-trifluoroacetylcysteamine
(260 mg, 1.5 mmol, 10 eq) and stirred at room temperature under
argon for 1 h. The solution was then concentrated to near
dryness, applied to two RP ptlc plates and eluted with
acetonitrile/water (5:1). The product (Rf 0.47) was extracted
with methanol (13.1 mg; UV(MeOH) λ max = 280.5 nm, ε = 50,000) and
purified by preparative RP HPLC (CH₃CN/H₂O/AcOH 75:25:0.1 pH 5.6,
flow 15 ml.min⁻¹; Tr = 26 min). The protected product was
saponified by the action of lithium hydroxide (50 mg) in
tetrahydrofuran/water (1:1, 5 ml), followed by analytical HPLC in
the afore-mentioned solvent system (flow 1 ml.min⁻¹ Tr (product) =
8 min; Tr (protected) = 11 min (broard)). Deprotection was
complete after 2 h and the solution neutralised with 1 M HCl,
de-salted on a reverse-phase plug to yield the title compound.
(4.2 mg, 7 % yield; UV(H₂O) λ max = 280.1 nm, ε = 40,000).

FAB MS (¬ve ion): M - H, 394 au; M + Cl, 430 au. FAB MS (+ve
ion): M + H, 396 au.

5(S)-Hydroxy-6(R)-2'-'carboxyethylthio-7(E),9(E),11(Z),14(Z)－
eicosatetraenoic acid (A-3)

LTA₄-Me (25 mg, 0.075 mmol) was dissolved in methanol/
triethylamine (1:1; 2.5 ml) containing methyl
3-mercaptopropionoate (83 μl, 0.75 mmol) and stirred under argon
for 17 h. The reaction mixture was concentrated, dissolved in
acetonitrile and applied to a RP ptlc plate, eluting with
acetonitrile/water (4:1). The product (Rf 0.27–0.37) was
extracted with methanol to yield the desired methyl
5(S)-hydroxy-6(R)-2'-(methoxycarbonyl)ethylthio-7(E),9(E),11(Z),
14(Z)-eicosatetraenoate (12 mg; UV(MeOH) λ_{max} = 280.3 nm
ε = 50,000). This product was considered pure enough to continue
without preparative HPLC purification and was saponified by the
action of lithium hydroxide (50 mg) in aqueous tetrahydrofuran
(THF/H₂O 1:1, 5 ml) over 30 min (the reaction being followed by
RP HPLC CH₃CN/H₂O/AcOH 80:20:0.1 pH 5.6 flow 1.0 ml.min⁻¹ Tr
(protected) = 11 min; Tr (product) = 5 min). The saponification
mixture was concentrated, acidified to pH 1.0 with 1 M HCl and
de-salted on a reverse-phase plug to yield the title compound.
(9.6 mg, 30 % yield; UV(H₂O) λ_{max} = 280.1 nm, ε = 40,000).

^1^H nmr (protected) (90 MHz, CDCl₃): δ = 0.89 t, 3H, 20-CH₃, J₁₉,₂₀
= 5.5 Hz; 1.28 m, 6H, 17-,18-,19-CH₂; 1.7 m, 4H, 3-,4-CH₂; 2.00
s, 1H, OH; 2.03 m, 2H, 16-CH₂; 2.34 t, 2-CH₂, J₂,₃ = 7 Hz; 2.58
t, 3H, 2'-CH₂, 6-H, J₁,₂' = 5.5 Hz; 2.68 t, 2H, 13-CH₂, J₁₂,₁₃
J₁,₂₀ = 5.2 Hz; 2.94 t, 2H, S-CH₂; 3.42 dd, 1H, 5-H, J₄,₅ = 4.1
Hz, J₅,₆ = 9.1 Hz; 3.66 s, 3H, 1-CO₂CH₃; 3.69 s, 3H, 2'-CO₂CH₃;
5.6 m, 4H, 7-,12-,14-,15-H; 6.28 m, 4H, 8-,9-,10-,11-H.
5(S)-Hydroxy-6(R)-2'-hydroxyethylthio-7(E),9(E),11(Z),14(Z)-
eicosatetraenoic acid (A-4)

LTA\textsubscript{4}-Me (50 mg, 0.15 mmol) was dissolved in methanol/
triethylamine (1:1; 5 ml) containing 2-mercaptoethanol (107 \textmu l,
1.5 mmol) and stirred under argon at room temperature for 2.5 h.
The reaction mixture was concentrated under reduced pressure,
dissolved in acetonitrile and applied to two preparative RP tlc
plates and eluted with acetonitrile/water (4:1). The product was
extracted with acetonitrile to give the methyl ester of the title
compound. (24.8 mg, 40 \% yield; UV(MeOH) \lambda_{\text{max}} = 279.9 nm, \epsilon =
50,000). Saponification was effected with lithium hydroxide (100
mg) in aqueous tetrahydrofuran (THF/H\textsubscript{2}O 1:1, 10 ml) over 30 min,
following the reaction progress by RP HPLC (CH\textsubscript{3}CN/H\textsubscript{2}O/AcOH
70:30:0.1 pH 5.6 flow 1.0 ml.min\textsuperscript{-1} Tr (ester) = 12.5 min, Tr
(product) = 7.0 min). The tetrahydrofuran was removed under
reduced pressure and the aqueous solution acidified with 1 M HCl,
de-salted on a reverse phase plug to yield the title
compound. (22.8 mg, 38 \% yield; UV(H\textsubscript{2}O) \lambda_{\text{max}} = 280.4 nm,
\epsilon = 40,000).

\textit{1-Mercaptobutan-3-one}

Methylvinylketone (7 g, 0.1 mol) was added slowly to a solution
of sodium sulphide (48 g, 0.62 mol) in water (50 ml), maintaining
the temperature below 15°C. After complete addition, the
reaction mixture was stirred for 3 h at ambient temperature
then poured onto crushed ice, neutralised to pH 7.0 with 
c\textsubscript{4}HCl and extracted with ether. The combined ether extracts were 
dried over magnesium sulphate, filtered and concentrated to yield 
the crude product (6.3 g). Short-distance distillation (70°C/5 
mbar) furnished the title compound as a colourless mobile oil. 
(2.6 g, 25 \% yield).

\textsuperscript{1}H nmr (60 MHz, CDCl\textsubscript{3}); \delta = 1.65 t, 1H, SH, J = 8 Hz; 2.15 s, 3H, 
CH\textsubscript{3}; 2.70 m, 4H, CH\textsubscript{2}-CH\textsubscript{2}.

5(S)-Hydroxy-6(R)-3'-oxobutanylthio-7(E),9(E),11(Z),14(Z)-
eicosatetraenoic acid (A-5)

LTA\textsubscript{4}-Me (25 mg, 0.075 mmol) was dissolved in methanol/
triethylamine (1:1; 2.5 ml) containing 1-mercaptobutan-3-one (100
mg, 0.96 mmol, ca 13 eq) and stirred at room temperature under 
argon for 6 h. HPLC (CH\textsubscript{3}CN/H\textsubscript{2}O 90:10) indicated the reaction to 
be approximately 75 \% complete by this time so the solution was 
concentrated, dissolved in acetonitrile and applied to two RP 
ptlc plates, eluting with acetonitrile/water (5:1). The product 
(Rf 0.3 - 0.44) was extracted with methanol (17 mg; UV(MeOH) \lambda\textsubscript{max} = 279.9 nm, \varepsilon = 50,000) and purified by preparative RP HPLC 
(CH\textsubscript{3}CN/H\textsubscript{2}O 70:30 flow 20 ml.min\textsuperscript{-1}. Tr = 22 min) to yield the 
title compound as its methyl ester (8.2 mg by UV determination). 
The ester was hydrolysed by the action of lithium hydroxide (50 
mg) in aqueous tetrahydrofuran (THF/H\textsubscript{2}O 1:1, 5 ml) over 30 min, 
concentrated and acidified with 1 M HCl. De-salting on a
reverse-phase plug yielded the title compound in 19 % overall yield (6 mg; UV(H_2O) \lambda_{max} = 281.0 \text{ nm}, \epsilon = 40,000)

Methyl 5(S),6(S)-oxido-7(Z)-nonadecenoate (XXIV)

n-Dodecyltriphenylphosphonium bromide (0.95 g, 1.86 mmol) was dissolved in dry, distilled tetrahydrofuran (15 ml) and cooled, with stirring under argon, to -78°C. A solution of n-butyllithium in hexane (1.03 ml, 1.49 M; 1.53 mmol) was added dropwise, stirring for 10 min before the addition of HMPA (3.2 ml, 18.4 mmol) in tetrahydrofuran (3.2 ml). After a further 10 min methyl 5(S),6(R)-oxido-7-oxoheptanoate (53 mg, 0.31 mmol) dissolved in tetrahydrofuran (3 ml) was added, continuing stirring at -78°C for 15 min then at 0°C for 15 min before quenching with methanol (65 \mu l). The product was extracted with ether, washing well with water and brine, dried over magnesium sulphate and concentrated to dryness. Purification was effected by eluting rapidly through a short silica column with 2 % triethylamine/hexane to give the title compound as a pale yellow oil in quantitative yield (98.5 mg)

^1H nmr (90 MHz, CDCl3): \delta = 0.88 t, 3H, 19-CH, J_{18,19} = 6 \text{ Hz}; 1.26 s, 20H, 3-CH_2, 10-19-CH_2; 1.75 m, 2H, 4-CH_2; 2.20 q, 2H, 9-CH_2, J_{8,9} = 6.8 \text{ Hz}, J_{9,10} = 6.8 \text{ Hz}; 2.39 t, 2H, 2-CH_2, J_{2,3} = 6.5 \text{ Hz}; 2.82 dt, 1H, 5-CH, J_{4,5} = 6.5 \text{ Hz}, J_{5,6} = 1.5 \text{ Hz}; 3.30 dd, 1H, 6-CH, J_{6,7} = 9 \text{ Hz}; 3.68 s, 3H, CO_2Me; 5.08 t, 1H, 7-CH, J_{7,8} = 11 \text{ Hz}; 5.71 dt, 1H, 8-CH.
6(R)-S-(Cysteinylglycine)-5(S)-hydroxy-7(Z)-nonadecenoic acid and 5(S)-S-(cysteinylglycine) 6(R)-hydroxy-7(Z)-nonadecenoic acid (A-6, A-7)

Methyl 5(S),6(S)-oxido-7(Z)-nonadecenoate (45.4 mg, 0.14 mmol) was dissolved with stirring under argon in methanol/triethylamine 1:1 (5 ml) containing methyl N-trifluoroacetyl cysteinylglycinate (250 mg, 6 eq). The temperature was raised to 40°C and the extent of reaction followed by RP HPLC (MeOH/H2O 90:10 flow 2.0 ml.min⁻¹ detect 200 nm; Tr (epoxide) = 7.6 min, Tr (major product) = 4.0 min; Tr (minor product) = 3.6 min). After 7 h the reaction was approximately 85% complete so was left overnight (21 h in total) to reach completion. The crude reaction product was purified as before by RP ptlc, eluting with CH3CN/H2O 9:1 (Rf 0.5-0.75) and subsequently purified by RP preparative HPLC to separate the isomers (MeOH/H2O 90:10 flow 15 ml.min⁻¹, detect 200 nm, Tr (major) = 10.5 min, Tr (minor) = 7.6 min). The major isomer (25 mg) was identified by ¹H nmr (90 MHz) as that resulting from 6-addition, dimethyl 5(S)-hydroxy-6(R)-S-(N-trifluoroacetyl cysteinylglycinate)-7(Z)-nonadecenoate; and the minor isomer, identified similarly as that resulting from 5-addition, dimethyl 6(R)-hydroxy-5(S)-S-(N-trifluoroacetyl cysteinylglycinate)-7(Z)-nonadecenoate.

The protected products were saponified by the action of lithium hydroxide in aqueous tetrahydrofuran as before (3 h reaction
time) and de-salted to give the title compound (23 mg and 3 mg respectively 38% combined yield).

A-6 (Protected)

$^1$H nmr (90 MHz, CDCl$_3$): $\delta$ = 0.88 t, 3H, 19-CH$_3$; $J_{18,19}$ = 6 Hz; 1.26 s, 18H, [10-19]-CH$_2$; 1.63 quintet, 2H, 3-CH$_2$; $J_{2,3}$ = 7.5 Hz; $J_{3,4}$ = 7.5 Hz; 1.82 m, 3H, 4-CH$_2$; 5-CH(OH); 2.05 m, 2H, 9-CH$_2$; 2.37 t, 2H, 2-CH$_2$; 2.62 m, 1H, 6-CH; 2.74 dd, 1H, S-CH$_A$(H$_B$); $J_{A,B}$ = 14 Hz, $J_{1',2'}$ = 7.9 Hz; 3.08 dd, 1H, 5-CH$_A$(H$_B$); $J_{1',2'}$ = 5.5 Hz; 3.67 s, 3H, 1-CO$_2$CH$_3$; 3.79 s, 3H, CO$_2$CH$_3$ (glycinyl); 3.84 dt, 1H, 5-CH$_2$; $J_{4,5}$ = 4.5 Hz; $J_{5,6}$ = 4.5 Hz; 4.09 d, 2H, HN-CH$_2$-CO$_2$CH$_3$; $J$ = 5.3 Hz; 4.60 q, 1H, HN-CH-CH$_2$; $J$ = 7 Hz; 5.43 dt, 1H, 7-CH, $J_{6,7}$ = 2 Hz, $J_{7,8}$ = 11 Hz; 5.73 dt, 1H, 8-CH; $J_{8,9}$ = 4.4 Hz; 7.07 t, 1H, NH-CH$_2$; $J$ = 4.7 Hz; 7.59 d, 1H, NH-CH; $J$ = 5.7 Hz.

A-7 (Protected)

$^1$H nmr (90 MHz, CDCl$_3$): $\delta$ = 0.88 t, 3H, 19-CH$_3$; $J_{18,19}$ = 6 Hz; 1.26 s, 20H, [10,19]-CH$_2$; 3-CH$_2$; 1.85 m, 2H, 4-CH$_2$; 2.05 m, 2H, 9-CH$_2$; 2.36 t, 2H, 2-CH$_2$; 2.57 m, 1H, 6-CH; 3.01 s, 3H, 5-CH$_2$; 5-CH(OH); 3.49 s, 3H, CO$_2$CH$_3$ (glycinyl); 3.67 s, 3H, 1-CO$_2$CH$_3$; 3.90 m, 1H, 6-CH; 4.08 m, 2H, HN-CH$_2$-CO$_2$CH$_3$; 4.44 m, 1H, NH-CH=CH$_2$; 5.32 t, 1H, 7-CH; $J_{7,8}$ = 11 Hz; 5.71 dt, 1H, 8-CH; 6.78 br, 1H, NH; 7.03 br, 1H, NH.
Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-heptadecatrienoate (XXV)

n-Hexyltriphenylphosphonium bromide (0.88 g, 2.0 mmol) was dissolved in dry, distilled tetrahydrofuran (15 ml) and cooled to −78°C under argon. Freshly titrated n-butyllithium solution in hexane (1.15 ml, 1.49 M soln; 1.7 mmol) was slowly added and the mixture stirred for 10 min at −78°C before the addition of HMPA (3.5 ml, 12 eq) in tetrahydrofuran (3.5 ml). After a further 10 min at −78°C, methyl 5(S),6(S)-oxido-11-oxoundec-7(E),9(E)-dienoate (76.7 mg, 0.34 mmol) in tetrahydrofuran (3 ml) was slowly added, stirred for 15 min at −78°C then raised to 0°C for a further 15 min before quenching with methanol (0.5 ml). The product was extracted with ether, washing with water and brine, dried and concentrated. Purification was effected by rapid elution through a short silica column with hexane/2 % triethylamine to give, upon concentration, the title compound in 37 % yield (37 mg)

λ_{max} 270.2, 279.5, 291.5 nm (cyclohexane); HPLC Analysis (SiO_2) Hexane/EtOAc/TEA 98.5 : 0.5 : 1.0 flow 2.0 ml.min^{−1} Tr = 5.5 min (cis), Tr = 8.2 min (trans) 92 % cis; 8 % trans; no other appreciable impurities.

6(R)-S-(Cysteinylglycine)-5(S)-hydroxy-7(E),9(E),11(Z)-heptadecatrienoic acid (A-8)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-heptadecatrienoate (25 mg;
0.086 mmol) was dissolved in methanol/triethylamine 1:1 (2.5 ml) containing methyl N-trifluoroacetylcycteinylglycinate (100 mg; 0.34 mmol, 4 eq) and stirred under argon at room temperature for 6 h. After this time RP HPLC (CH\textsubscript{3}CN/H\textsubscript{2}O 90:10 flow 1.0 ml.min\textsuperscript{-1}) indicated the reaction to have virtually stopped (75 % conversion Tr (epoxide) = 7.5 min; Tr (product) = 4.9 min). The product was purified by RP ptlc (CH\textsubscript{3}CN/H\textsubscript{2}O 4:1; Rf 0.65-0.80) to yield the desired product (13.7 mg by UV determination; 28 % yield).

Saponification was conducted in tetrahydrofuran-water (1:1; 10 ml) containing lithium hydroxide (100 mg) over 4 h (following the extent of reaction by RP HPLC CH\textsubscript{3}CN/H\textsubscript{2}O/AcOH 60:40:0.1 pH 5.6); which after de-salting, yielded the title compound (4.75 mg; 12 % yield) \( \lambda_{\text{max}} = 280.9 \text{ nm} \).

**Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-tridecatrienoate (XXVI)**

Ethyltriphenylphosphonium bromide (370 mg, 1 mmol) was suspended in dry, distilled tetrahydrofuran (10 ml) at room temperature under argon and a solution of n-butyllithium in hexane (640 \textmu l, 1.40 M; 0.9 mmol) added dropwise. After 15 min the reaction was cooled to -78°C and HMPA (1.9 ml; 11 mmol) in tetrahydrofuran (1.9 ml) added, stirring for 10 min before the addition of methyl 5(S),6(S)-oxido-11-oxoundec-7(E),9(E)-dienoate (100 mg;
0.45 mmol) in tetrahydrofuran (0.5 ml). After a further 15 min
the reaction was quenched with methanol (250 µl), brought to room
temperature and extracted with ether, washed well with water and
brine, dried (MgSO₄) and concentrated. The crude product was
purified by eluting rapidly through a silica column (25 x 100 mm)
with hexane/ether/triethylamine (88:10:2; 400 ml) under argon
pressure and concentrated to give the title compound in 69 %
yield (73 mg).

5(S)-Hydroxy-6(R)-S-(cysteinylglycine)-7(E),9(E),11(Z)-
tridecaatrienoic acid (A-9)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)-tridecatrienoate (73 mg,
0.31 mmol) was dissolved in methanol/triethylamine (1:1; 1 ml)
containing methyl N-trifluororacetylcysteinylglycinate (200 mg;
0.69 mmol, 2.2 eq) and stirred under argon at room temperature
for 3 h. After this time RP HPLC (CH₃CN/H₂O 90:10; flow 1.0
ml.min⁻¹) showed the reaction to be complete (Tr (epoxide) = 3.9
min; Tr (product) = 3.1 min) and the product was semi-purified by
preparative RP ptlc (CH₃CN/H₂O 4:1; Rf 0.7-1.0) to give the title
compound in protected form (118 mg by UV determination; 73 %
yield). A small sample was purified by preparative RP HPLC
(CH₃CN/H₂O 40:60; 20 ml.min⁻¹) to give a pure sample for ¹H nmr and
deprotection.

Saponification of the product (6.5 mg) was conducted in
tetrahydrofuran/water (1:1; 5 ml) containing lithium hydroxide
(50 mg) over 4 h (following the extent of reaction by RP HPLC
$\text{CH}_3\text{CN/H}_2\text{O}/\text{AcOH} 30:70:0.1 \text{ pH 5.6}$); which, after de-salting,
yielded the title compound in 87 % yield (4.3 mg; UV($H_2O) \lambda_{\text{max}} =
277.8 \text{ nm, } \varepsilon = 40,000$) FAB MS (-ve ion): $M - 2H + Na$, 422 au.

A-9 (Protected)

$^1H$ nmr (90 MHz, CDC$_3$): $\delta = 1.61 \text{ m, } 4H, [3,4]-\text{CH}_2$; 1.78 d, 3H,
13-CH$_3$, $J_{12,13} = 6.1 \text{ Hz}$; 2.36 t, 2H, CH$_2$-CH$_2$-CO$_2$CH$_3$, $J_2,3 = 7 \text{ Hz}$;
2.5 m, 1H, 6-H; 2.78 dd, 1H, S-CH$_2$H$_3$, $J_{A,B} = 14 \text{ Hz}$, $J_{A,X} = 7.5$
Hz; 3.04 dd, 1H, S-CH$_2$H$_3$, $J_{A-B} = 6 \text{ Hz}$; 3.57 dt, 1H, 5-H, $J_{4,5} =
9 \text{ Hz}$, $J_{5,6} = 4 \text{ Hz}$; 3.67 s, 3H, 1-CO$_2$CH$_3$; 3.77 s, 3H, CO$_2$CH$_3$
(glycinyl); 4.08 d, 2H, NH-CH$_2$-CO$_2$CH$_3$, $J = 5.3 \text{ Hz}$; 4.64 q, 1H,
CH$_2$-CH-NH, $J = 6.8 \text{ Hz}$; 5.6 m, 2H, 7-H, 12-H; 6.3 m, 4H, [8-11]-H;
7.04 t, 1H, CH$_2$-NH, $J = 4.5 \text{ Hz}$; 7.55 d, 1H, CH-NH, $J = 6.8 \text{ Hz}$.

6(R)-Carboxymethylthio-5(S)-hydroxy-7(Z),9(Z),11(E),14(E)-
eicosatetraenoic acid (A-10)

LTA$_4$-Me (25 mg, 0.075 mmol) was dissolved in methanol/
triethylamine (1:1; 0.5 ml) containing ethyl 2-mercaptoacetate
(90 mg, 0.75 mmol, 10 eq) and stirred under argon at room
temperature for 3 h. After this time acidification of a sample
did not significantly effect the recorded UV spectrum so the
reaction was deemed complete. The reaction mixture was purified
by RP ptlc (Rf 0.18 - 0.50) eluting with $\text{CH}_3\text{CN/H}_2\text{O}$ 4:1 to yield
the desired protected material (31.6 mg by UV; 93 % yield). The
product was shown to contain some material resulting from trans-esterification of the ethyl ester in methanol but with this exception was of high purity and was subsequently deprotected without further purification. The product was deprotected by the action of lithium hydroxide (100 mg) in aqueous tetrahydrofuran (1:1; 10 ml) over 15 min and de-salted on a RP plug to yield the title compound in 69% overall yield (21.2 mg). RP HPLC CH₃CN/H₂O/ACOH 55:45:0.1 pH 5.6 Tr = 8.3 min.

6(R)-(3'-Carboxypropylthio)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z) eicosatetraenoic acid (A-11)

LTA₄-Me (25 mg, 0.075 mmol) was dissolved in methanol/triethylamine (1:1; 1 ml) containing methyl 4-mercaptopropanoate (50 mg, 0.37 mmol) and stirred under argon at ambient temperature for 24 h. The reaction mixture was purified by RP ptlc to afford the protected analogue (26.9 mg; UV(MeOH) \( \lambda_{\text{max}} = 280.4 \text{ nm, } \varepsilon = 50,000 \)). Deprotection was performed using lithium hydroxide (100 mg) in aqueous tetrahydrofuran (1:1; 10 ml) at room temperature over 1.5 h. The product was de-salted on a reverse phase plug to yield the title compound (23.2 mg; UV(MeOH) \( \lambda_{\text{max}} = 280.9 \text{ nm, } \varepsilon = 50,000 \)) shown by RP HPLC (CH₃CN/H₂O/ACOH 60:40; 0.1 pH 5.6) to only be 73% pure (Tr = 12 min). The product was re-purified by preparative HPLC to furnish the title compound (7.9 mg; UV(H₂O) \( \lambda_{\text{max}} = 281.5 \text{ nm, } \varepsilon = 40,000 \)).
6(R)-(4'-Carboxybutylthio)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)
eicosatetraenoic acid (A-12)

LTA₄-Me (25 mg, 0.075 mmol) was dissolved in methanol/
triethylamine (1:1; 1 ml) containing methyl 4-mercaptobutyrate
(41 mg, 0.28 mmol) and stirred under an inert atmosphere for 24
h at room temperature. The crude reaction mixture was purified
by RP ptlc to yield the protected analogue (19.1 mg; UV(MeOH)
λₘₐₓ = 280.6 nm, ε = 50,000). Purification was effected by RP
pHPLC (CH₃CN/H₂O 90:10, Tr = 6.9 min) to yield the title compound
as the dimethyl ester (5.5 mg by UV determination). The product
was deprotected by the action of lithium hydroxide (50 mg) in
aqueous tetrahydrofuran 1:1; 5 ml) over 5 h to yield the title
compound after de-salting (11.8 mg; UV(H₂O) λₘₐₓ = 281.2 nm,
ε = 40,000) RP HPLC (CH₃CN/H₂O/AcOH 60:40:0.1 pH 5.5) indicated
the product to only be 75 % pure Tr = 13.0 min) being
contaminated with 12 % of the 11-trans isomer (Tr = 14.6 min) and
so was re-purified on RP pHPLC to give the pure title compound
(5.2 mg; UV(H₂O) λₘₐₓ = 281.6 nm, ε = 40,000).
LTA₄-Me (25 mg, 0.075 mmol) was dissolved in methanol/triethylamine (1:1; 1 ml) containing methyl 7-mercaptoheptanoate (100 mg; 0.56 mmol) and stirred under argon for 6 days at room temperature. The reaction mixture was then concentrated and purified by RP ptlc, eluting with CH₃CN/H₂O (5:1) to afford the protected analogue (24 mg by UV determination; 67 % yield). The product was purified by RP pHPLC (CH₃CN/H₂O 80:20; tr = 20 min) to give the title compound as its dimethyl ester 5.3 mg by UV determination; λₘₐₓ (MeOH) 280.9 nm. Deprotection was effected by the action of lithium hydroxide (50 mg) in 50 % aqueous tetrahydrofuran (5 ml) over 1.5 h and the product de-salted in the usual manner to afford the title compound (4.9 mg, UV(H₂O) λₘₐₓ 281.5 nm. HPLC RP CH₃CN/H₂O/AcOH 80:20:0.1 pH 5.6 tr = 7.4 min).

(±)-Thiolactic acid (67 µl, 0.753 mmol, 10 eq) was treated with excess diazomethane in ether, concentrated under reduced pressure and dissolved in methanol/triethylamine 1:1 (0.5 ml). This solution was added to LTA₄-Me (25 mg; 0.0753 mmol) and stirred
for 2 h at room temperature after which time acidification of a UV sample indicated complete reaction. Purification by RP ptlc (CH₃CN/H₂O 4:1; Rf 0.18 - 0.44) yielded a mixture of diastereomers (27.6 mg; 81 % combined yield) which were separable by HPLC (RP CH₃CN/H₂O 70:30; flow 1.0 ml.min⁻¹ Tr = 24.5 min Tr = 26.8 min). The purified diastereomers were separately deprotected by the action of lithium hydroxide in aqueous tetrahydrofuran as before, and de-salted to yield analogues A-14 and A-15. HPLC CH₃CN/H₂O/AcOH 60:40:0.1 pH 5.6; A-14 Tr = 6.9 min; A-15 Tr = 8.2 min. FAB MS (-ve ion): M - H, 423 au for both analogues.

Methyl 2-methyl-2-thiopropanoate

To a solution of 2-bromoisobutyric acid (3.34 g, 20 mmol) in water (50 ml) was added sodium hydrogensulphide hydrate (6 g, 106 mmol) and the mixture heated to 100°C for 2 h. The reaction was cooled to ambient temperature, acidified to pH 2, saturated with sodium chloride and extracted with ether (3 x 50 ml). The combined ether extracts were dried (MgSO₄) and evaporated to a small volume. To this solution was added dropwise an ethereal solution of diazomethane until a yellow colour persisted. The solution was purged with argon and evaporated in vacuo to give a pungent oil which was purified by distillation (68-70°C/20 mbar) (0.8 g, 30 % yield).

¹H nmr (60 MHz, CDCl₃): δ = 1.4 s, 6H, C(CH₃)₂; 1.6 s, 1H, 5H; 3.8 s, 3H, CO₂CH₃.
6(R)-[1'-Carboxy-1'-methylethylthio]-5(S)-hydroxy-7(E),9(E),
11(Z),14(Z)-eicosatetraenoic acid (A-16)

LTA₄-Me (14.8 mg, 0.045 mmol) and methyl 2-methyl-2-thio-
propanoate (60 mg, 0.45 mmol; 10 eq) were stirred together at
room temperature under argon in a solution of methanol (300 μl)
and N,N-diethylcyclohexylamine (100 μl). After an 8 h reaction
time the crude product was purified by RP preparative HPLC
(CH₃CN/H₂O 80:20 flow 6 ml.min⁻¹, Tr = 13.2 min) to give the
title compound as its dimethyl ester (5.15 mg, 25 % yield). UV
(MeOH) A max = 280 nm. The product was deprotected with lithium
hydroxide (75 mg) in a solution of methanol (3 ml) and water
(3 ml) over 6.5 h to yield the title compound after de-salting
in the usual fashion (4.81 mg, 25 % yield). UV (MeOH) A max = 281
nm.

Methyl 3-chloro-2(R)-methylpropanoate

Methyl 3-hydroxy-2(S)-methylpropanoate (12.5 g, 105.8 mmol) was
dissolved in dry dichloromethane (20 ml) containing imidazole
(300 mg) and stirred at 0°C under argon. Thionyl chloride
(20 ml, 274 mmol) was added slowly over 10 min and the reaction
mixture maintained at 0°C for a further 1.5 h before raising
the temperature to reflux for 30 min. The title compound was
isolated by distillation (52-54°C/38 mbar) in 84 % yield
(12.1 g). [α]D (C = 1.0, CHCl₃) = +15.3°
$^1$H nmr (60 MHz, CDCl$_3$): $\delta$ = 1.3 d, 3H, CH-CH$_3$, $J$ = 7 Hz; 3.0 tq, 1H, $J_{2,3}$ = 2 Hz; 3.7 d, 2H, 3-CH$_2$; 3.8 s, 3H, CO$_2$CH$_3$.

**Methyl 2(R)-methyl-3-thiopropanoate**

Methyl 3-chloro-2(R)-methylpropanoate was converted by the previously described method into the title compound (bp 70-75°C/26 mbar).

$^1$H nmr (60 MHz, CDCl$_3$): $\delta$ = 1.3 br.d, 3H, CH-CH$_3$; 3.0 m, 4H, 2-CH, 3-CH$_2$SH; 3.7 s, 3H, CO$_2$CH$_3$.

6(R)-[2'(R)-Carboxypropylthio]-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-17)

LTA$_4$-Me (22.5 mg, 0.068 mmol) and methyl 2(R)-methyl-3-thiopropanoate (50 mg, 0.37 mmol; 5 eq) were stirred in a solution of methanol (500 $\mu$l) and N,N-diethylcyclohexylamine (200 $\mu$l) at ambient temperature for 3 h. The product was purified by RP preparative HPLC (CH$_3$CN/H$_2$O 70:30, flow 3.0 ml.min$^{-1}$, Tr = 79 % yield). A sample of the protected material (11.25 mg, 24 $\mu$mol) was saponified in a solution of methanol (2 ml) and water (2 ml) containing lithium hydroxide (50 mg) to give the title compound after de-salting (9.0 mg, 68 % overall yield). UV(MeOH) $\lambda_{max}$ = 280 nm.
Methyl 2(S)-methyl-3-thiopropanoate

Methyl 3-chloro-2(S)-methylpropanoate [α]D (C = 1.07, CHCl₃) = -14.9° (1Hnmr as for methyl 3-chloro-2(R)-methylpropanoate) was prepared from 3-hydroxy-2(R)-methylpropanoic acid as before and subsequently converted into the title compound as previously described (1Hnmr as for the R-isomer).

6(R)-[2'(S)-Carboxypropylthio]-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-18)

6(R)-Me (20 mg, 0.060 mmol) and methyl 2(S)-methyl-3-thiopropanoate (30 mg, 0.22 mmol; 3.7 eq) were dissolved in a solution of methanol (250 µl) containing N,N-diethylcyclohexylamine (125 µl), and stirred under argon at ambient temperature for 3 h. Purification by RP preparative HPLC (CH₃CN/H₂O 70:30, flow 3.0 ml.min⁻¹, Tr = 36 min) yielded the title compound as the dimethyl ester (7.93 mg, 28 % yield). The product was deprotected by the action of lithium hydroxide (50 mg) in aqueous methanol (1:1; 4 ml) over 2 h and de-salted to give the title compound (6.15 mg, 23 % overall yield).

UV(MeOH) λmax = 280 nm.

Methyl 3-mercapto-2,2-dimethylpropanoate

3-Chloropivalic acid (2.7 g, 20 mmol) was dissolved in water
(40 ml) containing sodium hydrogensulphide (6.0 g, 106 mmol) and refluxed for 16 h. The reaction mixture was then cooled, acidified with c. H$_2$SO$_4$, saturated with sodium chloride and extracted with ether (3 x 50 ml). The combined ether extracts were dried over magnesium sulphate and concentrated. Esterification with diazomethane in ether yielded the title compound (2.4 g, 80 % yield) which was purified by distillation (1.5 g, 50 % purified yield).

$^1$H nmr (60 MHz, CDCl$_3$): $\delta$ = 1.3 s, 6H, C(CH$_3$)$_2$; 1.6 t, 1H, SH, J = 4 Hz; 2.4 d, 2H, CH$_2$; 3.8 s, 3H, CO$_2$CH$_3$.

(6(R)-[2'-Carboxy-2'-methylpropylthio]-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-19)

LTA$_4$-Me (15 mg, 0.045 mmol) and methyl 3-mercaptopropanoate (30 mg, 0.20 mmol; 4.4 eq) were dissolved in methanol (400 $\mu$l) containing N,N-diethylcyclohexylamine (100 $\mu$l) and the reaction mixture stirred under argon at ambient temperature for 5 h. The crude product was purified by RP preparative HPLC (CH$_3$CN/H$_2$O 70:30, flow 4.5 ml.min$^{-1}$, Tr = 25 min) to give the title compound as the dimethyl ester (6.5 mg, 32 % yield). Saponification was effected by the action of lithium hydroxide (50 mg) in aqueous methanol (1:1, 4 ml) over 2 h. De-salting in the usual manner furnished the title compound. (6.10 mg, 30 % overall yield). UV(MeOH) $\lambda_{max}$ = 280 nm.
Methyl-2-mercaptobenzoate

2-mercaptobenzoic acid (10 g) was dissolved in methanol (200 ml) and refluxed for 4 h with c. sulphuric acid (20 ml). The solution was partially concentrated of reduced pressure and poured into water. The mixture was extracted with ether (2 x 120 ml) and the combined organic extracts were washed with sodium hydrogen carbonate solution, brine, dried (MgSO₄) and concentrated to give cream crystals.

\[ \text{6(R)-(2'-Carboxyphenylthio)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-20)} \]

\( \text{LTA}_{4}\)-Me (25 mg, 0.075 mmol) was dissolved in tetrahydrofuran/ triethylamine (1:1; 1 ml) containing methyl thiosalicylate (126 mg; 0.75 mmol). Potassium tertiary butoxide (84 mg; 0.75 mmol) was added slowly (over 10 min) and the reaction mixture stirred at room temperature for 16 h. The crude product was purified by RP ptlc followed by RP pHPLC (CH₃CN/H₂O 90:10 tr = 8 min) to yield the title compound as its dimethyl ester (6.2 mg; \( \lambda_{\text{max}} (\text{MeOH}) = 280.8 \text{ nm} \)). Deprotection was effected in 50% aqueous tetrahydrofuran (5 ml) containing lithium hydroxide (50 mg) over 3 h. De-salting in the usual manner afforded the title compound (4.8 mg). FAB MS (-ve mode): M - H, 471 au.
Methyl 3-mercaptobenzoate

3-Aminobenzoic acid (13.7 g, 0.1 mol), sodium nitrite (6.9 g, 0.1 mol) and sodium hydroxide (4.4 g, 0.11 mol) were dissolved in water (80 ml) and slowly added to a solution of 4 M hydrochloric acid (107 ml) at -5°C. The mixture was stirred for 1 h and then a solution of ethyl potassium xanthate (48 g, 0.3 mol) added, the temperature being raised to 70°C for 1 h before cooling and acidifying to precipitate the xanthate ester which was collected.

\[
^1H\text{ nmr (60 MHz, CDCl}_3\text{): } \delta = 3.85 \text{ s, 4H, SH, CH}_3; 7.20 \text{ t, 1H, 5-H, } J_{4,5} = 8 \text{ Hz; 7.40 d, 1H, 4-H; 7.8 d, 1H, 6-H; 7.95 s, 1H, 2-H.}
\]

Hydrolysis was effected by heating on a steam bath for 30 min with 4 M sodium hydroxide solution (50 ml). The solution was cooled and acidified to precipitate the disulphide of the title compound. The product was collected by filtration and refluxed for 24 h with powdered zinc (25 g) in acetic acid (200 ml). The reaction mixture was then filtered and acidified with c. hydrochloric acid (400 ml). The resulting precipitate was washed with water, collected and dried by dessication over silica gel to yield the title compound as a beige powder. mp. 142-145°C (lit. 142-145°C). The acid was esterified in methanol containing c. sulphuric acid as before to yield the title compound.
6(R)-(3'-Carboxyphenylthio)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-21)

LTA₄-Me (25 mg, 0.075 mmol) was dissolved in tetrahydrofuran/triethylamine (1:1; 1 ml) containing methyl 3-mercaptobenzoate (126 mg, 0.75 mmol). Potassium tertiary butoxide (84 mg, 0.75 mmol) was added slowly and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified by RP ptlc and pHPLC (CH₃CN/H₂O 90:10 tr = 13.8 min) to yield the protected analogue (7.8 mg; UV (MeOH) λ_max = 282 nm). Deprotection was effected by the action of lithium hydroxide (50 mg) in 50 % aqueous tetrahydrofuran (5 ml) over 4 h at room temperature to yield, after de-salting, the title compound (7.0 mg; HPLC CH₃CN/H₂O/AcOH 90:10:0.1 pH 5.6 tr = 4.0 min). FAB MS (-ve mode): M - H, 471 au.

Methyl 4-mercaptobenzoate

4-aminobenzoic acid was diazotised and reacted with ethyl potassium xanthate in an analogous manner to that described for methyl 3-mercaptobenzoate. Hydrolysis and cleavage of the disulphide furnished the free acid (mp 219-221°C, Lit²¹ 216-219°C) which was esterified as before to yield the title compound.

¹H nmr (60 MHz, CDCl₃): δ = 3.65 s, 1H, SH; 3.85 s, 3H, CH₃; 7.2 d, 2H, 3,5-H J₂,₃ = 8 Hz; 7.85 d, 2H, 2,6-H.
6(R)-(4'-Carboxyphenylthio)-5(S)-hydroxy-7(E),9(E),11(Z),
14(Z)-eicosatetraenoic acid (A-22)

LTA₄-Me (0.3 mg, 0.90 mmol) was dissolved in tetrahydrofuran/triethylamine (1:1; 3 ml) was stirred under argon at room temperature with methyl 4-mercaptobenzoate (1.5 g, 11 mmol) for 3 h. The crude product was purified by RP ptlc followed by RP pHPLC (CH₃CN/H₂O 80:20) to yield the title compound as its dimethyl ester (tr = 20 min, 132 mg; 29 %). Treatment with lithium hydroxide (126 mg) in tetrahydrofuran/water (1:1; 4 ml) under argon at room temperature for 20 h afforded the title compound (130 mg, 28 % yield; UV(MeOH) λ_max = 278.5 nm. FAB MS (-ve mode): M - H, 471 au.
SPECTRA
$^{1}H$ nmr (60 MHz, CDCl$_3$)

*Methyl 5(S),6(R),7-trihydroxyheptanoate* (III)
$^1$H nmr (60 MHz, CDCl$_3$)

Methyl 5(S),6(R)-dihydroxy-7-$p$-toluenesulphonyloxy-heptanoate (IV)
$^1$H nmr (60 MHz, CDCl$_3$)

Methyl 5(S)-hydroxy-6(S),7-oxidoheptanoate (V)

D$_2$O shake offset
$^1$H nmr (60 MHz, CDCl$_3$)

Methyl 7-hydroxy-5(S),6(S)-oxidoheptanoate (VI)
$^1H$ nmr (60 MHz, CDCl$_3$)

Methyl 7-oxo-5(S),6(R)-oxidoheptanoate (I)
H nmr (60 MHz, CDCl₃)  
Methyl 5(S),6(S)-oxido-11-oxo-7(E),9(E)-undecadienoate (VII)
$^1$H nmr (90 MHz, CDC$_3$)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoate

(LTA$_4$-Me) (XIII)
$^{13}$C nmr (23 MHz, CDCl$_3$)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-eicosatetraenoate

(LTA$_4^{-}$-Me) (XIII)
$^1$H nmr (90 MHz, CDCl$_3$)

Methyl 5(S)-hydroxy-6(R)-(2'-methoxycarboxyethylthio)-7(E),9(E),
11(Z),14(Z)-eicosatetraenoate (A-3 - Prot)
$^1$H nmr (90 MHz, CDCl$_3$)

Methyl 5(S),6(S)-oxido-7(Z)-nonadecenoate (XXIV)
$^1$H nmr (90 MHz, CDCl$_3$)

Dimethyl 5(S)-hydroxy-6(R)-S-(N-trifluoroacetylcysteinyl-glycinate)-
7(Z)-nonadecenoate (A-6 - Prot)
$^1$H nmr (90 MHz, CDCl$_3$)

Dimethyl 6(R)-hydroxy-5(S)-S-(N-trifluoroacetyl cysteinyl-glycinate) - 7(Z)-nonadecenoate (A-7 - Prot)
$^1$H nmr (90 MHz, CDCl$_3$)

Dimethyl 5(S)-hydroxy-6(R)-S-(N-trifluoroacetylcyteinylglycinate)-
7(E),9(E),11(Z)-tridecatrienoate (A-9 -Prot)
UV (Cyclohexane)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z),14(Z)-
eicosatetraenoate (LTA₄-Me) (VIII)
Determination of the extent of reaction between LTA$_4$-Me and methyl N-trifluoroacetylcysteinylglycinate

--- = sample of reaction mixture
--- = Acidified sample

A = After 2 hrs
B = After 4 hrs
C = After 6 hrs
UV (H₂O)

6(R)-S-(Glutathione)-5(S)-hydroxy-7(E),9(E)
11(Z),14(Z)-eicosatetraenoic acid (LTC₄)
UV (H₂O)

6(R)-S-(Cysteinylglycine)-5(S)-hydroxy-7(E),
9(E),11(Z),14(Z)-eicosatetraenoic acid (LTD₄)
5(S)-Hydroxy-6(R)-2'-carboxyethylthio-7(E),9(E)-11(Z),14(Z)-eicosatetraenoic acid (A-3)
UV (Cyclohexane)

Methyl 5(S),6(S)-oxido-7(E),9(E),11(Z)
heptadecatrienoate (XXV)
UV (H₂O)

5(S),Hydroxy-6(R)-S-(Cysteinylglycine)-7(E),
9(E),11(Z)-tridecatetraenoic acid (A-9)
5(S)-Hydroxy-6(R)-methylthio-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-1)
5(S)-Hydroxy-6(R)-methylthio-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid (A-1)
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