# ATP-Citrate Lyase as a Target for Hypolipidemic Intervention. Design and Synthesis of 2-Substituted Butanedioic Acids as Novel, Potent Inhibitors of the Enzyme

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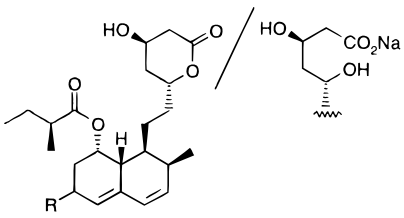
ATP-citrate lyase is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues. Inhibitors of the enzyme represent a potentially novel class of hypolipidemic agent, which are anticipated to have combined hypocholesterolemic and hypotriglyceridemic properties. A series of 2-substituted butanedioic acids have been designed and synthesized as inhibitors of the enzyme. The best compounds, **58**, **68**, **71**, **74** have reversible $K_i$'s in the 1−3 $\mu$M range against the isolated rat enzyme. As representative of this compound class, **58**, has been shown to exert its inhibitory action through a mainly competitive mechanism with respect to citrate and a noncompetitive one with respect to CoA. None of the inhibitors were able to inhibit cholesterol and/or fatty acid synthesis in HepG2 cells. This has been attributed to the adverse physicochemical properties of the molecules leading to a lack of cell penetration. Despite this, a lead structural class of compound has been identified with the potential for modification into potent, cell-penetrant, and efficacious inhibitors of ATP-citrate lyase.

## Introduction

There is increased expert opinion, coupled with ever growing evidence, to support the view that, in man, elevated plasma cholesterol concentrations in combination with increased plasma triglyceride levels, represents a very significant high risk for cardiovascular disease.

For example, in the PROCAM study, 25% of all observed myocardial infarct events occurred in subjects in which the ratio of low density lipoprotein (LDL)/high density lipoprotein (HDL) cholesterol was >5, and where triglyceride concentrations exceeded 200 mg/dL.[1] The Helsinki heart study has reported that the risk of coronary heart disease is tripled for subjects possessing an LDL/HDL ratio >5 and triglycerides >2 mmol/L.[2]

A major advance in therapy for the treatment of hypercholesterolemia has come with the discovery and commercial development of clinically effective inhibitors (e.g. compactin, lovastatin, pravastatin **1a**−**c**) of HMG-CoA reductase, a key regulatory enzyme in mammalian cholesterol biosynthesis.[3] The fibrates, e.g. lopid (gemfibrozil, **2**), have been long known as agents that are effective in lowering plasma triglyceride concentrations.[4] However, no marketed drugs or therapeutic strategies exist which have the ability to decrease effectively both plasma cholesterol and triglyceride levels. The combination of an HMG-CoA reductase inhibitor with a fibrate is not recommended due to increased risk for myopathies,[5] although this combination may be an option with the more modern fibrates. Thus, a compound which could combine the hypocholesterolemic properties of an HMG-CoA reductase inhibitor with the hypotriglyceridemic effects of a fibrate, would have high potential as a novel class of hypolipidemic agent for reducing the mortality and morbidity associated with coronary heart disease. Such an agent would be anticipated to be the drug of choice for the treatment of patients with combined type IIb hyperlipidemia.




**1** a: compactin; R = H (δ-lactone)

b: lovastatin; R = α-Me (δ-lactone)

c: pravastatin; R = β-OH (hydroxyacid)

In this context, we initiated a research program with the objective of discovering, and evaluating the potential of, inhibitors of ATP-citrate lyase (ACL; EC 4.1.3.8) as a novel and therapeutically beneficial class of lipid lowering agent, capable of combining the desired hypocholesterolemic and hypotriglyceridemic properties in the same molecule. ACL is a cytosolic enzyme found in a large variety of animal tissues, and its activity is particularly high in lipogenic tissues such as the liver.[6] It is positioned upstream of HMG-CoA reductase in the mammalian cholesterol biosynthetic pathway and supplies acetyl units for both cholesterol and fatty acid synthesis.[7] It was this feature which attracted us to select the enzyme as a target for hypolipidemic intervention.

Inhibition of ACL would be anticipated to (1) decrease plasma LDL cholesterol, in a similar manner to HMG-

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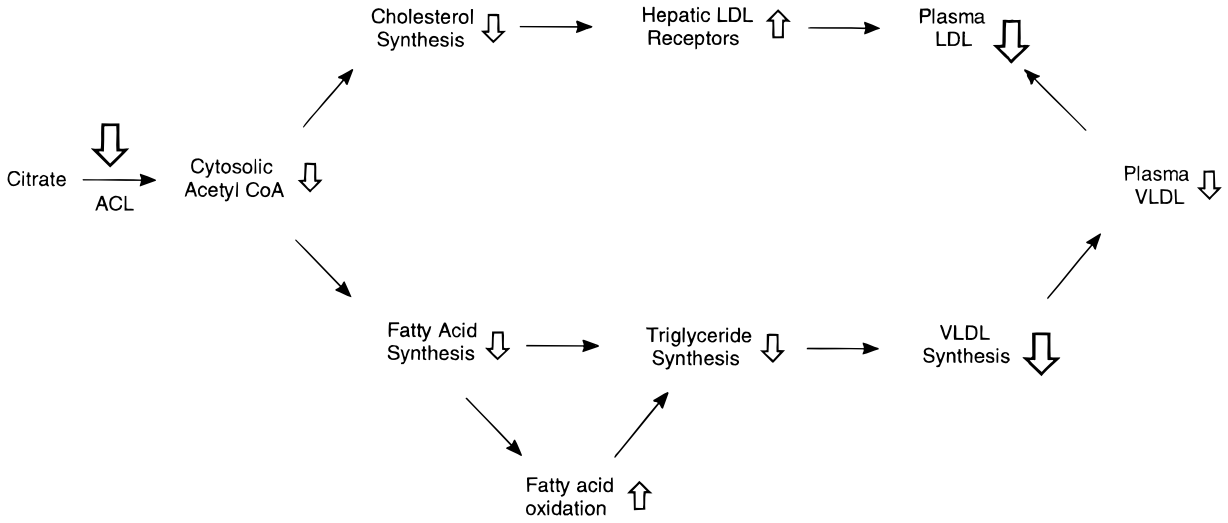
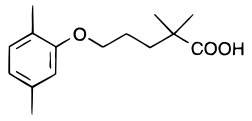
**Scheme 1.** Schematic Outlining the Anticipated Effects of Inhibiting ATP-Citrate Lyase

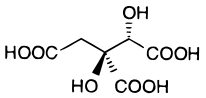



CoA reductase inhibitors, by decreasing cholesterol synthesis and up-regulating LDL receptor activity,[8] and (2) decrease plasma triglycerides as a consequence of a reduction in the synthesis of fatty acids, leading to an ultimate lowering of plasma VLDL. As a precursor for LDL, a reduction in VLDL may also contribute further to plasma LDL lowering. Cytosolic levels of malonyl CoA are also expected to decrease after inhibition of ACL. As malonyl CoA is known to regulate the entry of acyl moieties into the mitochondrial $\beta$-oxidation spiral (at the level of carnitine-palmitoyl transferase I), inhibition of ACL is expected to channel hepatic free fatty acids into $\beta$-oxidation, thereby further decreasing the pool of fatty acids available for triglyceride and VLDL synthesis, in a similar manner to that shown for Ro 22-0654[9] (Scheme 1).

Some evidence to support the view that inhibition of ACL should lead to the desired pharmacological profile can be drawn from studies on (−)-hydroxycitrate (**3**).[10] This citrate analogue is a naturally occurring, potent ($K_i = 0.15\ \mu$M, rat ACL) inhibitor of the enzyme.[11] We have demonstrated inhibition of cholesterol synthesis in HepG2 cells by **3**, albeit at relatively high concentrations ($IC_{50} = 0.1-0.5$ mM) compared to its $K_i$. We have




**2**: gemfibrozil




**3**: (-)-hydroxycitrate

also shown that **3** leads to an increase in HMG-CoA reductase activity and up-regulation of the LDL receptor activity in these cells, analogous to mevinolin.[12] Although literature reports describe attenuation of lipogenesis and cholesterogenesis by **3** in rodents,[13] the compound has many shortcomings as a tool which can be used to challenge the hypothesis cited above: It is poorly cell penetrant, has low oral bioavailability, and stimulates acetyl CoA carboxylase, an enzyme upstream in the cholesterol biosynthesis pathway.[14] Thus, in order to challenge and fully validate the hypothesis presented above in appropriate animal models, we sought an effective, orally active inhibitor of the ATP-citrate lyase enzyme.

ACL catalyses a reversible retro-Claisen reaction utilizing ATP, citrate (**4**), and CoASH as substrates to yield ADP, inorganic phosphate ($P_i$), oxaloacetate (**5**), and acetyl CoA (**6**), by means of the recently revised mechanism depicted in Scheme 2.[15] This differs from the originally accepted mechanism for catalysis, which invoked the necessity for an active site thiol nucleophile to allow formation of a covalently bound citryl-enzyme adduct (**9**) as an intermediary step between **7** and **8.**[7c] In earlier papers we have described our attempts to inhibit the enzyme through the rational design of citrate-based active-site and mechanism-based inhibitors, which were designed to interact with this active-site thiol nucleophile.[16] We have rationalized their lack of success in light of the revised mechanism for catalysis. We have also described our attempts to design potential tight-binding inhibitors of the enzyme.[17] In this paper, we now wish to describe our strategies to design and synthesize a series of 2-substituted butanedioic acids e.g. **58**, **68**, **71**, **74** as novel, potent inhibitors of ATP-citrate lyase.

## Design Strategy

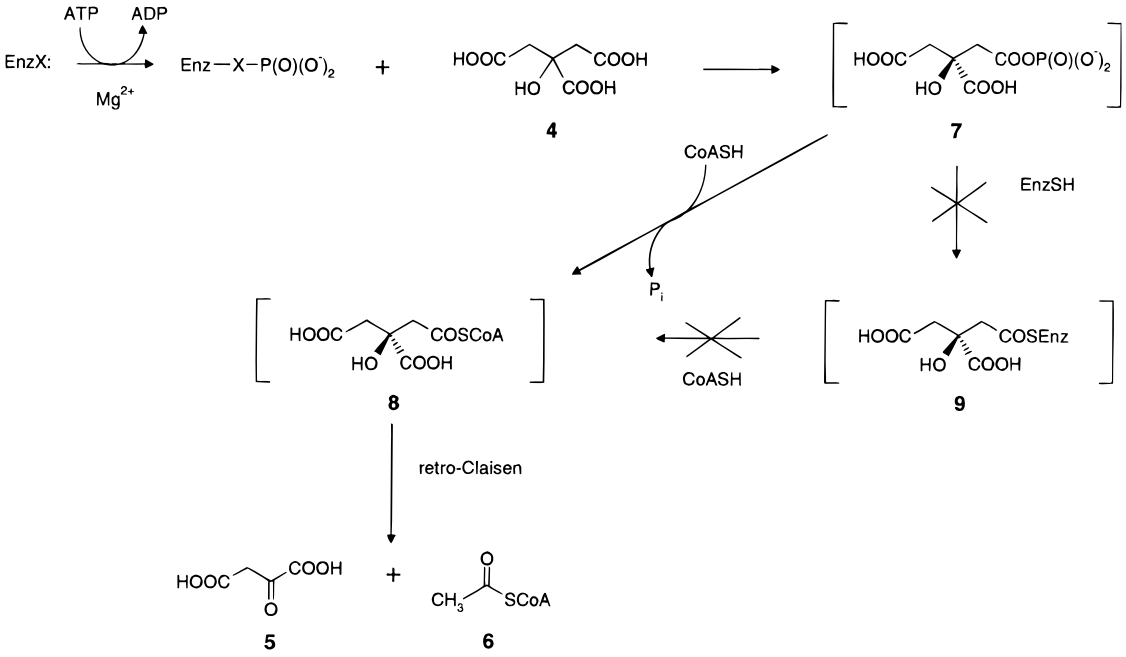
Following on from our attempts to design tight-binding, mechanism-based, and active-site directed inhibitors of ACL, we decided to pursue a third strategy based upon a multisubstrate analogue approach to design novel inhibitors. The revised catalytic mechanism of ACL suggests that analogues of either citryl phosphate (**7**) or citryl CoA (**8**) would fit this category. We considered citryl CoA to be a better target, since it binds moderately well to the enzyme ($K_m = 28\ \mu$M; *cf.* $K_m = 100\ \mu$M for citrate)[18] and provides ample scope for modification. Additionally, we were aware of the MEDICA series of compounds, represented by MEDICA 16 (**10**), as a potential starting point for the design of



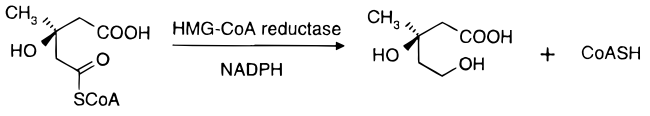

**10**: MEDICA 16

such compounds.[19] The MEDICA compounds are simple $\alpha,\omega$-dicarboxylic acids, possessing hypolipidemic activity through a combination of several mechanisms of action. MEDICA 16 is reported to be a citrate competitive inhibitor of ACL with a $K_i$ of 16 $\mu$M.[19a] During the course of this research effort, we have also established that it is a competitive inhibitor with respect to CoA ($K_i = 3\ \mu$M). We considered that a possible explanation

**Scheme 2.** Revised Mechanism of ATP-Citrate Lyase Catalysis




**Scheme 3.** Role of HMG-CoA Reductase




**11** **12**

for its modest ACL inhibitory activity might be that it is able to mimic the enzyme bound citryl CoA adduct. For such a mimickry, one carboxylic acid group could be associated with the citrate binding site while the lipophilic alkyl chain and other carboxylic acid group may associate with the CoA binding domain. On the basis of this, we rationalized that more potent ACL inhibitors could be designed by incorporating more "citrate-like" features into these molecules, coupled with modifications that would more effectively interact with the CoA binding site. As a means of identifying structural modifications that would more effectively associate with the CoA binding site, we considered the possibility that there may be structural similarities between the CoA sites of ACL and HMG-CoA reductase. The latter enzyme converts HMG-CoA (**11**) to mevalonic acid (**12**), with the concomitant release of CoA in the process (Scheme 3).[20] A study reported in the literature at the time we initiated this research indicated that the decalin moiety of HMG-CoA reductase inhibitors, such as compactin and lovastatin (**1a**, **1b**), plays the role of a hydrophobic anchor in the binding of these compounds to the enzyme active site.[21] This is shown to occur in a region normally occupied by CoA. It was further suggested that HMG-CoA is principally bound by a 3-hydroxy-3-methylglutaryl (HMG) binding region and a hydrophilic adenine binding domain. The area in the binding site of the enzyme that links these two domains is hypothesized to be rather hydrophobic in nature and to not well accommodate the phosphopantothenic acid chain of CoA (Figure 1).[21] After the enzyme-mediated reduction has taken place, it is suggested that the CoA product is bound only in the hydrophilic region and repelled in the hydrophobic domain. It is postulated that the lack of complementarity in this region would

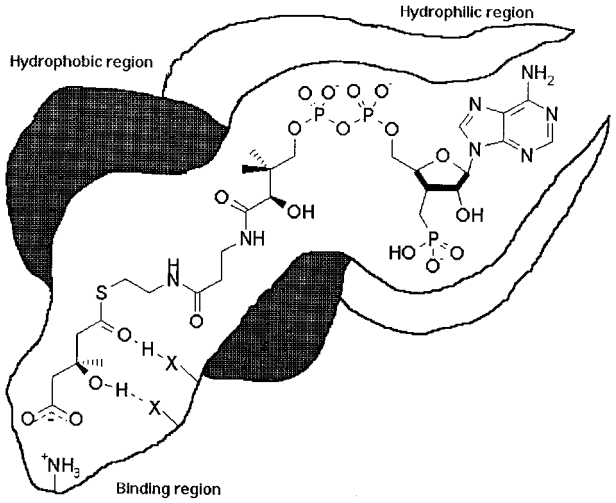
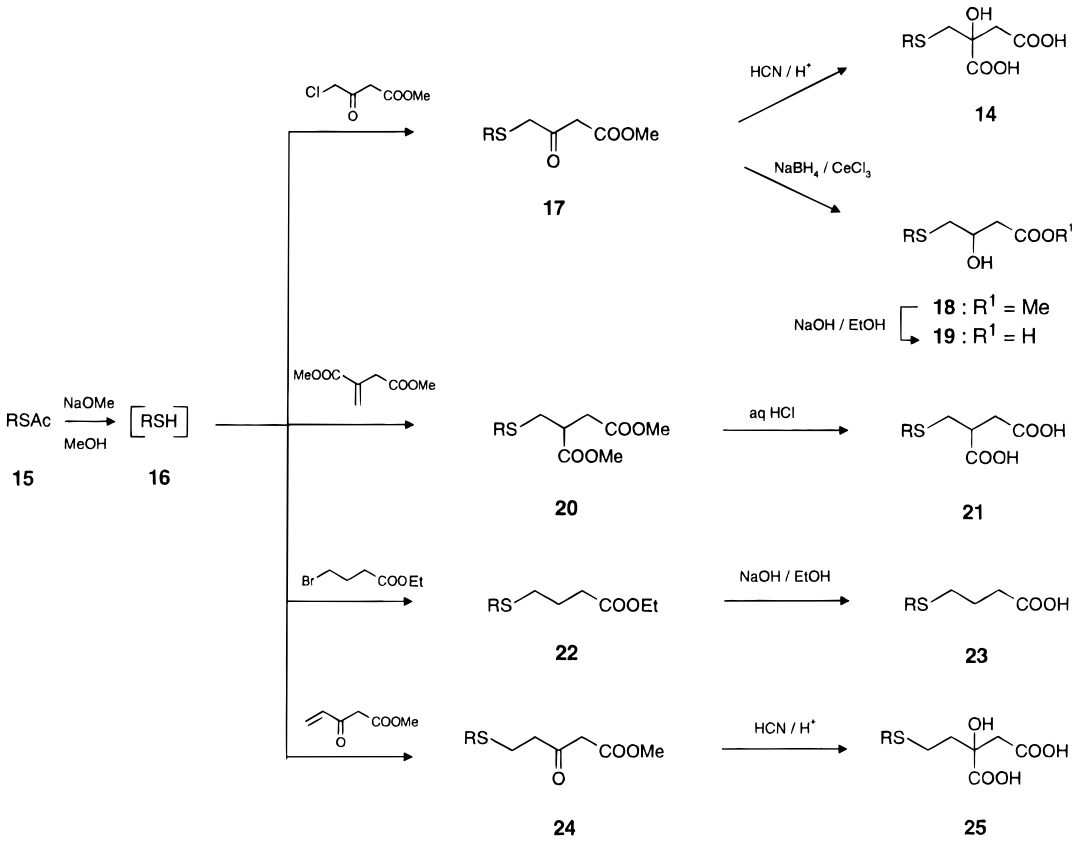


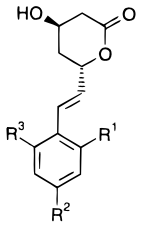

**Figure 1.** Hypothetical model of HMG-CoA reductase binding site, reproduced from reference 21.

assist the expulsion of the product from the active site and facilitate turnover. We speculated that a similar scenario may be possible for ACL, where the final stage of the reaction mechanism relies upon the release of a similar product, acetyl CoA, from the enzyme bound citryl CoA adduct. A large number of inhibitors of HMG CoA reductase are now known in which the hexahydronaphthalene group of **1** has been replaced by a variety of synthetic lipophilic moieties.[22] Compounds which we were aware of at the time we initiated this work included **13a**—**c**.[23] In these, the decalin ring system has been replaced by lipophilic (benzyloxy)-phenyl and biphenyl moieties to give derivatives which can show potencies *ca.* three-fold that of compactin. These compounds can be considered as possessing a mevalonate-like "head" linked to a hydrophobic anchor. By analogy, our initial model for the design of ACL bisubstrate inhibitors, which might make use of a similar hydrophobic pocket, was based upon a similar concept of linking a citrate-like "head" to a lipophilic group. We decided to investigate compounds of this type in a class in which one carboxylic acid group of citric acid was replaced by a lipophilic moiety. In the first

**Chart 1.** Syntheses of the S-Linked Compounds of Tables 1−6




targets identified for synthesis (**14**), we utilized a sulfur heteroatom to link a citrate-like "head" to a chain bearing a terminal 2,4-dichlorophenyl ring, to represent the lipophilic aryl group in **13a** and **13b**.




| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **13** a | Cl | Cl | 4-$FC_6H_4$ |
| **13** b | " | " | 4-$FC_6H_4CH_2O$ |
| **13** c | $CH_3$ | $CH_3$ | 4-$FC_6H_4$ |

## Chemistry

**Synthesis of Final Compounds.** General synthetic methods for the preparation of the S-linked compounds of Tables 1−6 are shown in Chart 1. 2-[(Aralkylthio)-methyl]-2-hydroxybutanedioic acids of general structure **14** were usually prepared from the $\beta$-keto ester intermediate **17** via formation of the cyanohydrin and subsequent acid hydrolysis. Our original method used cHCl/KCN in ether to generate the cyanohydrin,[24] but we later modified this step to employ KCN in aqueous potassium dihydrogen phosphate. The $\beta$-keto esters were prepared by the reaction of the thiol **16**, generated *in situ* from the more stable thioacetates **15**, with methyl 4-chloroacetoacetate. Reduction of the $\beta$-keto ester **17** with $NaBH_4/CeCl_3$, followed by base hydrolysis of the ester groups of **18**, gave the descarboxy analogues **19** (e.g. compound **69**). The deshydroxy derivatives of general structure **21** (e.g. compound **68**) were obtained from the Michael addition of *in situ*-generated thiol to dimethyl itaconate, followed by acid hydrolysis. A similar procedure using methyl 3-oxo-4-pentenoate as the Michael acceptor, followed by elaboration of the intermediate **24** to the hydroxybutanedioic acid in the usual way, gave the chain S-displaced analogue of general structure **25**, typified by **73**. The simple monoacids **23** (e.g. compound **70**) were prepared by reaction of the thiol intermediate with ethyl 4-bromobutanoate, followed by hydrolysis.

The analogous carbon-linked compounds of general structure **29** (e.g. **71** and **72**) were synthesized by alkylation of the dianion of methyl acetoacetate (NaH/$^n$BuLi in THF) with the appropriate aralkyl bromide **27**, followed by subsequent elaboration of the generated keto ester **28** by the usual method described above (Chart 2). The keto compounds **33** were derived from the dihydroisoxazole **31** by treatment with Raney nickel in aqueous boric acid, followed by base hydrolysis. **31** was prepared from the alcohol intermediate **26** in four steps via Swern oxidation, oxime (**30**) formation with hydroxylamine, *in situ* generation of the corresponding nitrile oxide and its subsequent [3 + 2] cycloaddition with dimethyl itaconate (Chart 2).[25]

The diethyl ester of **58** (**111**) and the dimethyl ester of **68** (**113**) (Table 7) were obtained either as intermediates or synthesized by acid-catalyzed esterification of the diacid with the appropriate alcohol. The diacetoxymethyl ester of **58** (**112**) was prepared from reaction of the diacid with bromomethyl acetate using potassium carbonate and 18-crown-6 in DMF.

**Synthesis of Intermediates.** Methods for preparation of the alcohol, bromide, and thioacetate intermediates required for the synthetic schemes depicted in Charts 1 and 2 were dependent upon the functionality to be introduced in the aryl group and chain. For the compounds in Tables 1−3, 5, and 6, where the functionality adjacent to the aryl ring is a methylene group, these intermediates were prepared by the methods shown in Chart 3. The thioacetates of general structure

**Chart 2.** General Syntheses of the Compounds Not Containing a Thioether Linkage in Table 3

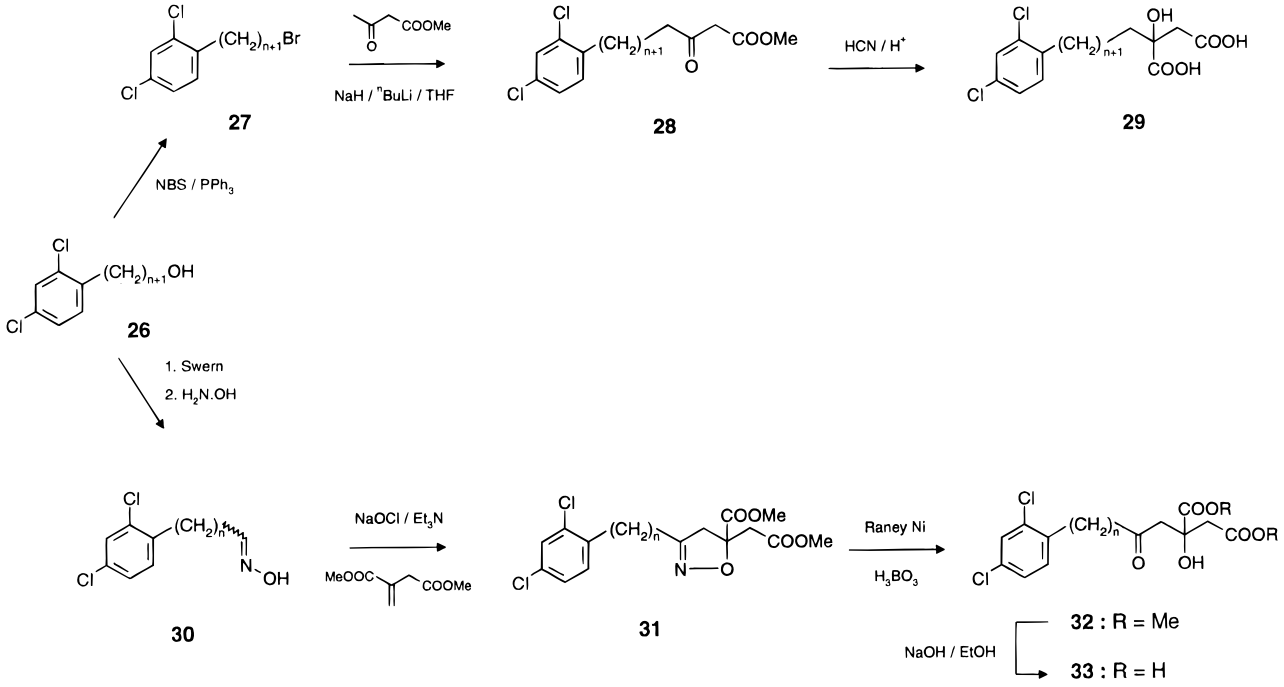
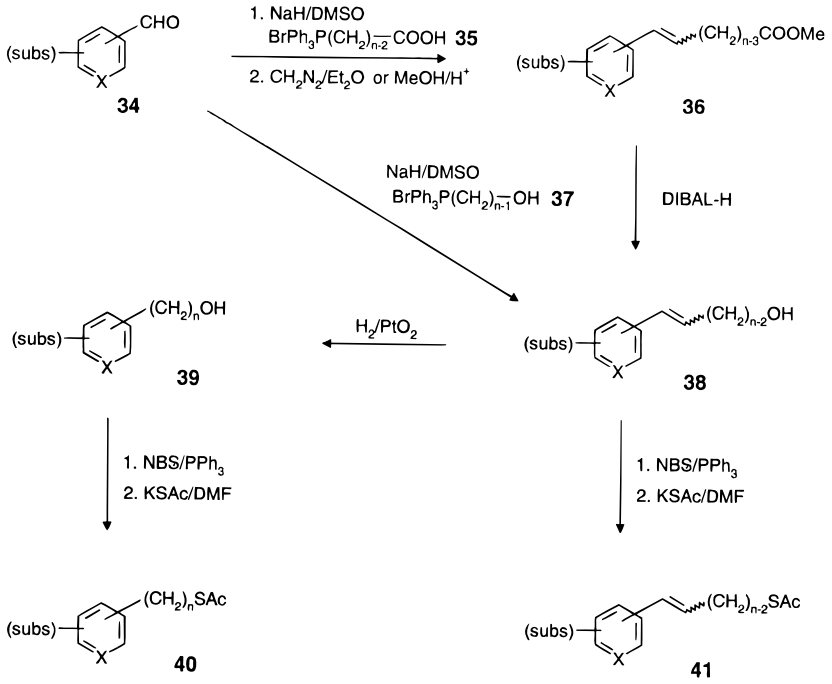
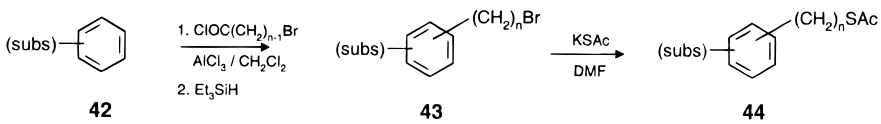



**Chart 3.** Synthesis of the Intermediates Required for the Preparation of the Compounds of Tables 1, 2, 3, 5, and 6

(i) Method A

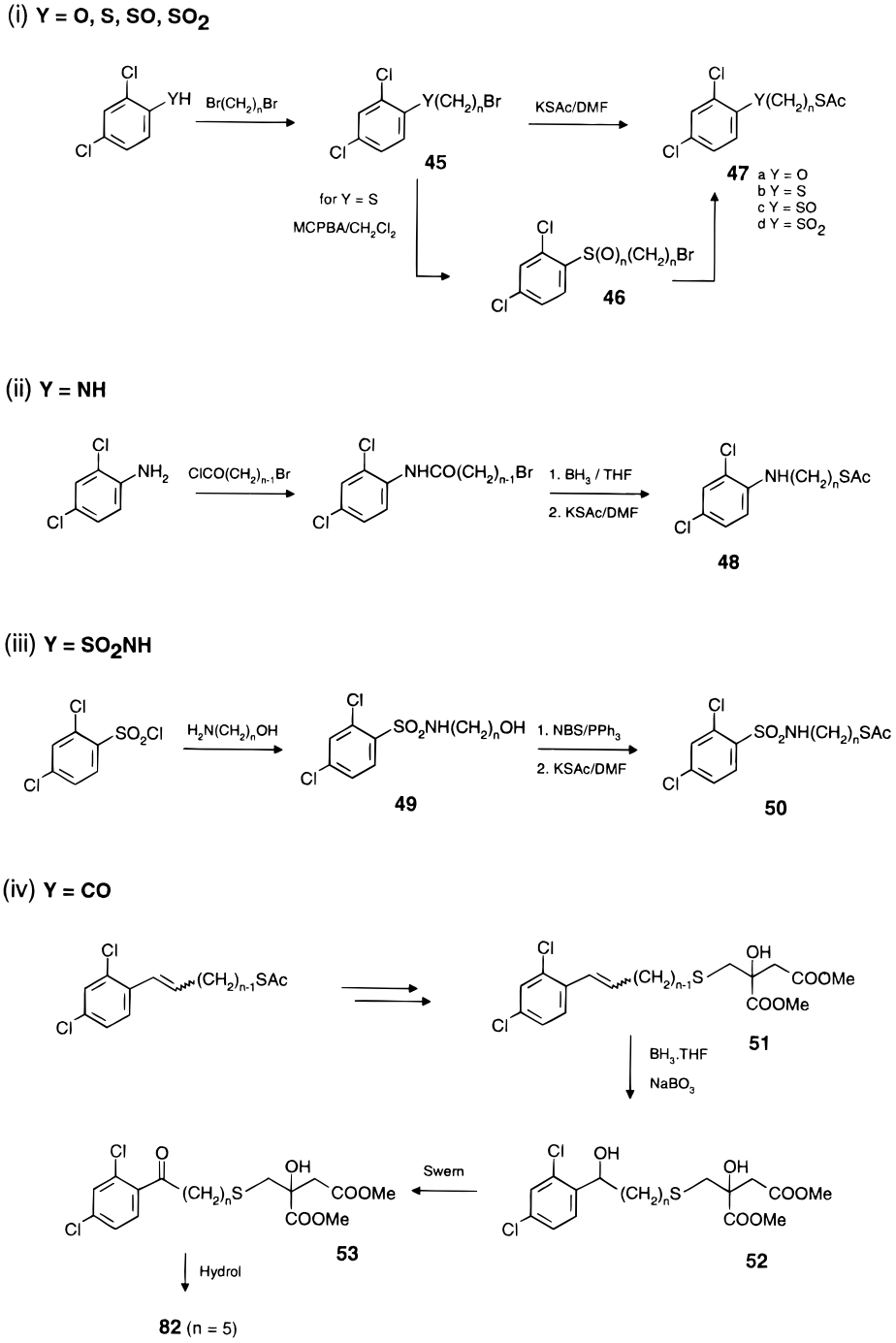



(ii) Method B (for Y = $CH_2$, subs = 2,4-di-Me, 4-F, 4-Cl, 4-Br, 4-OMe)




**40** were generated from the reaction of the $\omega$-arylbromoalkanes with potassium thioacetate in DMF. The latter were generally obtained from the appropriate $\omega$-arylalkanols **39** using NBS/$PPh_3$ in $CH_2Cl_2$. For certain compounds (**106**, **64**, **93**, **94**, **95**), the bromide intermediates **43** were synthesized directly by Friedel–Crafts acylation of the substituted benzene **42** with the appropriate $\omega$-bromoalkanoyl halide, followed by reduction of the keto group with triethylsilane (Chart 3, method B).[26] The alcohols **39** were prepared by stan-

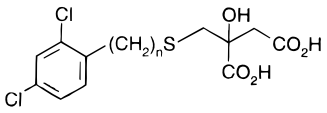
**Chart 4.** Syntheses of the Intermediates Required To Prepare the Compounds of Table 4




dard methodology which was governed by the substituents and the chain length. Generally, the aryl group was introduced from the appropriately substituted benzaldehyde **34** using Wittig chemistry to generate intermediates of the desired chain length (method A). These would normally contain a terminal carboxylic acid or an alcohol group, depending upon the nature of the phosphonium salts **35** or **37** available. Those bearing a terminal carboxylic acid group were converted to their corresponding methyl esters **36** ($CH_2N_2/Et_2O$ or $CH_3OH/cH_2SO_4$) to ease purification and reduced to the alcohol **38** with DIBAL-H in $CH_2Cl_2$. The olefinic bond was then hydrogenated using either Pd/C or $PtO_2$. The latter catalyst was found to be necessary for the halophenyl compounds to prevent dehalogenation. When the desired $\omega$-hydroxy phosphonium salt **37** was not available commercially, this was prepared from triphenylphosphine and the $\alpha,\omega$-bromo alcohol in refluxing toluene.

Compounds containing a functionality other than a methylene group adjacent to the aryl ring (Table 4) were also prepared from an appropriate thioacetate intermediate by an identical procedure to that described above. The *cis* and *trans* olefins **76** and **77** were prepared as described above from the separated isomers of **36** ($n$ = 6). The latter were obtained by preparative HPLC on silica gel using an ether/petroleum ether gradient. Thioacetate intermediates **47a**−**d**, **48, 50**, required for the synthesis of the other compound classes of Table 4, were prepared by the general standard methods depicted in Chart 4. An exception was the analogue containing a keto group adjacent to the aryl ring **53**. This compound was prepared via conversion of the olefin

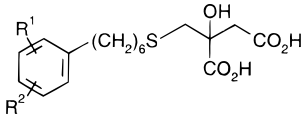
**Table 1.** Effect of Chain Length in 3-[[[(2,4-Dichlorophenyl)-alkyl]thio]methyl]-3-hydroxybutan-1,4-dioic Acids on ACL Inhibitory Activity



| no. | n | $K_i$ ($\mu$M) | analyses |
|---|---|---|---|
| 54 | 1 | 410 ± 9[a] | C, H, Cl, S |
| 55 | 2 | 480 ± 10 | C, H, S |
| 56 | 3 | 390 ± 28 | C,[b] H, S |
| 57 | 5 | 6.8 ± 0.14 | C, H, S |
| 58 | 6 | 3.3 ± 0.18 | C, H |
| 59 | 7 | 7.2 ± 0.36 | C, H, Cl, S |
| 60 | 9 | 300 ± 1.1 | C, H |

[a] Demonstrates cooperative kinetics. [b] C calcd 45.79%, found 46.58%.

**Table 2.** Effect of Chlorine Substitution in 3-[[(Phenylhexyl)-thio]methyl]-3-hydroxybutan-1,4-dioic Acids on ACL Inhibitory Activity



| no. | R1 | R2 | $K_i$ ($\mu$M) | analyses |
|---|---|---|---|---|
| 61 | H | H | 710 ± 50 | C, H |
| 62 | 2-Cl | H | 69 ± 4 | C, H |
| 63 | 3-Cl | H | 150 ± 35 | C, H, Cl, S |
| 64 | 4-Cl | H | 71 ± 4 | C, H, Cl, S |
| 65 | 2-Cl | 3-Cl | 7.3 ± 1 | C, H, Cl, S |
| 58 | 2-Cl | 4-Cl | 3.3 ± 0.18 | see Table 1 |
| 66 | 3-Cl | 4-Cl | 110 ± 15 | C, H, Cl, S |
| 67 | 2-Cl | 6-Cl | 76 ± 11 | C, H, Cl, S |

**51** to the alcohol **52** ($BH_3$, $NaBO_3$), followed by Swern oxidation and hydrolysis.

## Results and Discussion

Activities of the compounds in Tables 1−6 are presented as reversible $K_i$'s for inhibition of purified rat enzyme unless indicated otherwise. During the course of our research program, a method for production of recombinant human ACL was developed. As a consequence, inhibitory data on a few compounds is available only against the latter form of the enzyme. This is indicated where appropriate in the tables. Our most potent inhibitors had almost identical $K_i$'s against both rat and human recombinant forms.

The consequences of varying the length of the chain ($n$) linking the aromatic group to the "citrate-head" is demonstrated with the compounds in Table 1. We were particularly encouraged to discover that **58** ($n = 6$, $K_i = 3.3$ $\mu$M) was quite a potent inhibitor of the enzyme. Its lower and higher homologues **57** and **59** showed small reductions in potency, while a *ca*. 100-fold loss in activity was observed at the other shorter and longer chain lengths examined. Removal of the two chlorine atoms in **61** resulted in a 2 orders of magnitude loss of activity (Table 2). Further examination of the effects of chlorine substitution on activity are also shown in Table 2. Mono chlorine substitution is preferred in either the 2 (**62**) or 4 (**64**) positions and both equally improve activity by 1 order of magnitude over the unsubstituted parent **61**. The 3-chloro isomer **63** is *ca*. half as potent as either of these. A second chlorine gives rise to a further *ca*. 30-fold increase in activity only in the right combination, i.e. 2,4 (**58**) or 2,3 (**65**) disubstituted. The 2-fold increase in potency of the 2,4 analogue over its 2,3 isomer reflects the difference seen with 3 chloro relative to the other monochloro compounds. Neither the 3,4 (**66**) nor the 2,6 (**67**) dichloro derivatives show any improvements over the monochloro analogues.

Encouraged by the diversity of activity associated with the changes so far examined, we embarked upon a program of synthesis to identify structural features of the molecule which were important for enzyme inhibitory activity, with the ultimate goal to improve potency further. For this analysis, we examined changes to the molecule in 3 areas: (i) the "citrate head", (ii) the linking chain, and (iii) the aryl ring.

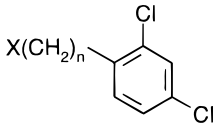
Only the results of our inital modifications to the "citrate head" will be discussed in this manuscript. These are summarized in Table 3. Some more elaborate changes that were undertaken are better discussed in the context of a subsequent paper in this series. Removal of the tertiary hydroxyl group in **68** gave no significant change in ACL inhibitory activity. In contrast, compounds lacking the tertiary carboxylic acid group were much less active (**69**, **70**). These results fit our proposition that compounds of this class are binding to the citrate site of the enzyme and require the dicarboxyate functionality to allow binding as the magnesium chelate, in a similar manner to citrate itself.[7b,27] Further evidence for this postulate arises from data on another compound (**74**) discussed below.

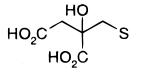
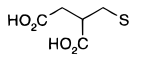
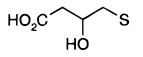
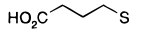
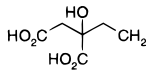
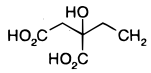
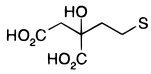
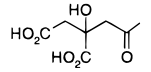
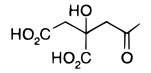
Relocation of the sulfur atom in **58** one atom closer to the aryl ring in **73** gave a compound of equal potency, as did its replacement by a methylene group in **71**. This suggests no critical role for the heteroatom other than being part of a spacer group. Other heteroatom replacements for sulfur (e.g. NR, O) were not examined in this series based on detrimental effects observed in a simpler, related class of compound (data not shown). By a similar analogy, no advantages from oxidation to the corresponding sulfoxide or sulfone was anticipated, and consequently was not examined here.

Replacement of the sulfur by a carbonyl group in **74** gave a modest 3-fold increase in potency ($K_i = 1.2$ $\mu$M). The critical nature of the chain length is reflected more so in this analogue, where activity is reduced 25-fold in the lower homologue **75**.

An interesting observation is that the keto compound **74** can act as a substrate for ACL. When used as an inhibitor of the enzyme, the maximal inhibition of oxaloacetate **5** formation was approximately 85%. Normally inhibition approaching 100% is observed as the inhibitor concentration becomes saturating. In the absence of citrate, a compound **74**-dependent rate of oxaloacetate formation could be observed. This correlated with the apparent incomplete inhibition at saturating inhibitor concentrations. Omission of all the usual substrates for the assay (citrate, ATP, CoA) still resulted in the turnover of **74** to produce oxaloacetate, but only in the presence of $Mg^{2+}$. The maximal rate of turnover was found to be 13% of that for citrate. These results strongly imply that the "citrate head" of the inhibitor occupies the citrate binding pocket sufficiently well to allow an active site base to remove the hydroxyl proton in the first phase of a retro-Claisen cleavage to **5**. The need for $Mg^{2+}$ is consistent with this deduction and would be expected based upon its normal require-

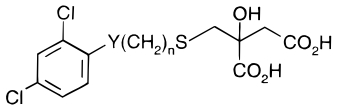
**Table 3.** Some "Citrate-Head" Modifications to **58**



| # | X | n | $K_I$ (μM) | Analyses |
|---|---|---|---|---|
| 58 |  | 6 | 3.3 ± 0.18 | see table 1 |
| 68 |  | 6 | 2.6 ± 0.26 | C, ~~H~~ |
| 69 |  | 6 | 88 ± 9 | C, H, Cl |
| 70 |  | 6 | 25 ± 4 | C, H, Cl |
| 71 |  | 6 | 2.9 ± 0.1 | C, H |
| 72 |  | 5 | 10.5 ± 0.4 | C, H |
| 73 |  | 5 | 2.9 ± 0.1 | C, H |
| 74 |  | 6 | 1.2 ± 0.04 | C, H |
| 75 |  | 5 | 25 ± 0.4 | -[a] |

[a] Microanalysis not obtained, structure consistent by NMR.

**Table 4.** Phenyl Link Modifications



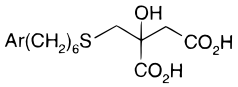
| no. | Y | n | $K_i$ (μM) | analyses |
|---|---|---|---|---|
| 58 | $CH_2$ | 5 | 3.3 ± 0.18 | see Table 1 |
| 76 | *Z*-CH=CH | 4 | 7.4 ± 0.3 | C, H |
| 77 | *E*-CH=CH | 4 | 33 ± 1.4 | C, H |
| 78 | O | 5 | 67 ± 7 | C, H |
| 79 | S | 5 | 10 ± 2.5 | C, H |
| 80 | NH | 5 | 12 ± 0.5 | C, H, N |
| **81**[a] | SO | 5 | 131 ± 21 | C, H[b] |
| 82 | CO | 5 | 130 ± 25 | C, H |
| 83 | $SO_2$ | 5 | 64 ± 7 | C, H |
| 84 | -$SO_2$NH- | 4 | 175 ± 25 | C, H, N |
| 85 | -$SO_2$N($CH_3$)- | 4 | 178 ± 16 | C, H, N |

[a] Compound lacks the tertiary OH group in the "head". This change is assumed to have little effect on activity based upon the equivalent activities of compounds **58** and **68** (Table 3). [b] Analysis for 0.57 M acetone.

ment in the citrate cleavage reaction. In the latter case, citrate binds to the enzyme as its Mg-chelate.[27]

The majority of other linkage modifications that were examined in compounds related to **58** concentrated on changes to the group (Y) linking the alkyl chain directly to the aryl ring (Table 4). A *cis* olefinic group in **76** was preferred to a *trans* in **77,** but neither was preferred over methylene. None of the modifications in Table 4, taking into account matching atom number chain length, were advantageous over a simple methylene in this position. Most noticable was that polar links, e.g. sulfonamido **84**, were particularly detrimental to activity.

**Table 5.** 3-[[(pyridylhexyl)thio]methyl]-3-hydroxybutan-1,4-dioic Acids

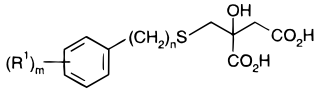


| no. | Ar | $K_i$ (μM) | analyses |
|---|---|---|---|
| 61 | Ph | 710 ± 50 (320 ± 60[a]) | see Table 2 |
| 86 | 2-pyridyl | 30% Inhibn @ 1 mM[a] | C, H, N |
| 87 | 3-pyridyl | 35% Inhibn @ 1 mM[a] | C, H, N |
| 88 | 4-pyridyl | 20% Inhibn @ 1 mM[a] | C, H, N |
| 89 | 3,5-diCl-4-pyridyl | 121 ± 8[a] | C, H, N |

[a] Assayed using hrACL.

Our largest synthetic effort in attempts to significantly improve the potency of **58** was concentrated on aryl ring substitution. Simple replacement by a less lipophilic pyridyl ring reduced activity considerably, even when chlorines were introduced (Table 5).[28] This result was consistent with our inital hypothesis of the aryl ring binding to a lipophilic region of the enzyme. The substituents introduced into the phenyl ring to replace the two chlorine atoms of **58** are summarized in Table 6. More polar/less lipophilic substituents than Cl in the *para* position reduced activity (e.g. compounds **95**, **96**, **97**, **99**). Comparison of 2,4-dichloro (**58**), 2,4-dimethyl (**106**), and 2,4-difluoro (**107**) compounds suggested that substituents that were both lipophilic and

**Table 6.** Effect of Aryl Ring Substitution in 3-[[(phenylhexyl)thio]methyl]-3-hydroxybutane-1,4-dioic Acids on ACL Inhibitory Activity




| # | $(R^1)_m$ | n | rat $K_i$ (μM) | Analyses |
|---|---|---|---|---|
| 58 | 2,4-Cl | 6 | 3.3 ± 0.18 | see table 1 |
| 90 | H | 5 | 490 ± 21 | C, H |
| 61 | H | 6 | 710 ± 50 | see table 2 |
| 91 | 4-Ph | 5 | 226 ± 34 | C, H |
| 92 | 4-Ph | 6 | 38 ± 1.4 | C, H |
| 93 | 4-F | 6 | 120 ± 14 | C, H |
| 94 | 4-Br | 6 | 31 ± 7 | C, H |
| 95 | 4-OMe | 6 | 280 ± 79 | C, H |
| 96 | 4-$SO_2NH_2$ | 6 | 500 ± 25 | C[a], H, N |
| 97 | 4-OH | 6 | 420 ± 100 | C, H |
| 98 | 4-$NO_2$ | 6 | 340 ± 80 | C, H, N |
| 99 | 4-$CO_2H$ | 6 | 420 ± 60 | C, H |
| 100 | 2-$NO_2$ | 6 | 75 ± 11 | C, H, N |
| 101 | 2-Ph | 5 | 21 ± 0.9 | C, H |
| 102 | 2-Ph | 6 | 18 ± 1.3 | C, H |
| 103 | 2-Ph, 4,6-diCl | 6 | 10 ± 1.8 | C, H, Cl[b] |
| 104 | 2-$CH_2$Ph | 6 | 94 ± 0.1 | C, H |
| 105 | 2-$NO_2$,4-Cl | 6 | 26 ± 2.6[c] | C, H, N |
| 106 | 2,4-Me | 6 | 160 ± 13 | C, H |
| 107 | 2,4-F | 6 | 270 ± 87 | C, H |
| 108 | 3,4- | 6 | 93 ± 5 | C, H |
| 109 | 2,3- | 6 | 63 ± 0.9 | C, H |
| 110 | 3,4- | 3 | 300 ± 45[c] | C, H |

[a] C: calcd 48.67%; found 49.79%. [b] Analysis for 0.28 M hexane. [c] Assayed using hr ACL.

electron withdrawing conferred good ACL inhibitory activity. In the *ortho* position, a phenyl group in **102** improved activity 40-fold over the unsubstituted parent and was better than chlorine in this respect. However, further introduction of chlorines in the 4 and 6 positions of **103**, the substitution pattern present in the lipophilic portion of the potent synthetic HMG-CoA reductase inhibitor **13a**, did not give rise to the anticipated increase in potency.[29] The presence of a second *ortho* substituent appears to be detrimental to activity and is consistent with the observation of a lack of any increase in potency for the 2,6-dichloro compound **67** over its 2-chloro analogue **62**.

The 2 substituted nitro compound **100** is more potent than its 4 isomer **98**, despite both compounds having the same lipophilicity. This may suggest a stronger preference for an electron-withdrawing component to this substituent in the 2 position over the 4 position. The 2-nitro-4-chloro compound **105** showed a further modest increase in activity over 2-nitro alone.

However, despite investigating a wide range of changes (Table 6), the best phenyl ring sustitution pattern remained that of 2,4-dichloro. The best inhibitors of ATP-citrate lyase from this series are the S-linked butanedioic acids **58** and **68**, $K_i$'s 3.3 $\mu$M and 2.9 $\mu$M repectively, the all methylene butanedioic acid **71**, $K_i$ = 3 $\mu$M, and the keto butanedioic acid **74**, $K_i$ = 1 $\mu$M.

**Enzyme Kinetic Analysis of 58.** A more detailed analysis of the inhibitory profile of **58** was carried out as representative of the butanedioic acid class of ACL inhibitor. Competition experiments between **58** and both citrate and CoA show that **58** is a mixed type inhibitor with respect to citrate and a noncompetitive inhibitor with respect to CoA. This is illustrated by the Lineweaver−Burke plots in Figures 2a (*vs* citrate) and 2b (*vs* CoA). Although **58** is definitely a mixed type inhibitor with respect to citrate (the probability of this not being the case is $1 \times 10^{-5}$ as judged by the F-test) it is clear that **58** exerts its inhibitory action through a mainly competitive mechanism. The competitive component of $K_i$ is 1 order of magnitude smaller than that for the uncompetitive component. Inhibitors of this structural type are thus kinetically different to the

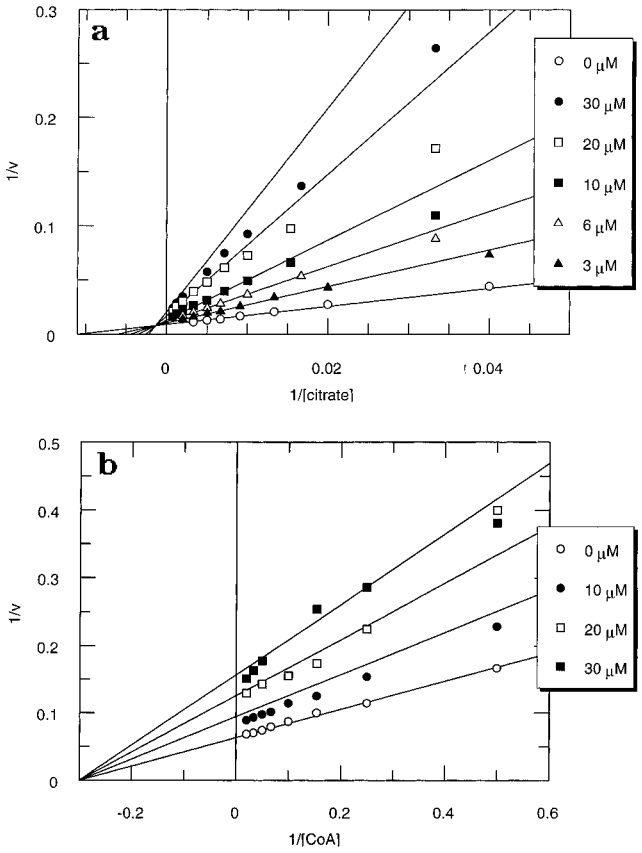



**Figure 2.** (a) Lineweaver−Burke plot demonstrating largely competitive kinetics of compound **58** versus citrate. (b) Lineweaver−Burke plot demonstrating noncompetitive kinetics of compound **58** versus CoA.

profile demonstrated by the MEDICA type of compound, discussed earlier, which we used as our starting point in this work.

The enzyme kinetic analysis of compounds such as **58** and **74** is consistent with them binding to the citrate site through the butanedioic acid moiety. Additional affinity appears to be imparted by their ability to interact with an adjacently located hydrophobic domain, rather that through an interaction with the CoA binding site. Although our initial design strategy made use of the hypothesis that there may be some similarity between the CoA binding domains of HMG-CoA reductase and ACL, the SAR requirements of the lipophilic group in the HMG-CoA reductase inhibitors and our ACL inhibitors show marked differences. For example, the 2,4-dimethylphenyl compound **13c** has similar HMG-CoA reductase inhibitory activity to its 2,4-dichloro analogue **13a**, whereas there is *ca.* 2 orders of magnitude difference in ACL inhibitory activity between compounds **106** and **58**. An optimal group for HMG-CoA reductase inhibitors is 2,4-dichloro-6-phenyl. The same substituent is not particularly well accomodated in ACL inhibitors. In fact two ortho substituents in the phenyl ring are detrimental to activity.

The divergance of lipophilic group SAR between the two enzymes is not surprising. The kinetic analysis of the ACL inhibitors strongly suggests that the aryl group is not binding to the CoA domain, but to some alternative hydrophobic site on the enzyme. Thus, although we started with the concept of designing inhibitors to mimic the bisubstrate intermediate citryl CoA, we have identified inhibitors which do not now fall into that descriptive class. The kinetic profile of our inhibitors should be physiologically advantageous over ones which are competitive with respect to CoA. However, it could also be argued that a more effective inhibitor would be one which was also non- or uncompetitive with repect to citrate.

**HepG2 Cell Assay.** Selected compounds that showed inhibitory activity against isolated ACL enzyme were assayed further for their ability to inhibit both cholesterol and fatty acid synthesis in a cell based assay. For this, we chose to use the well established human hepatoma cell line, HepG2. For this assay, we found it necessary to determine inhibition by measuring the incorporation of $^3H_2O$ into cholesterol and fatty acid synthesis.[12] Cellular ATP levels were also measured in order to determine whether or not any reduction in cholesterol or fatty acid synthesis was due to a toxic effect of the test compound. An initial assay, which measured the incorporation of $^{14}C$ label from [1,5-$^{14}C$-] citrate, gave false positives due to the ability of compounds such as **58** to inhibit uptake of $^{14}C$-citrate into the cell. The results of compound screening are shown in Table 7. Hydroxycitrate, despite its low $K_i$ (0.15 $\mu$M) on the isolated enzyme, is a relatively poor inhibitor of cholesterol synthesis in the HepG2 cell, $IC_{50} = 0.25$ mM. Unfortunately, none of our inhibitors were able to reduce cholesterol or fatty acid synthesis after a 2.5 h exposure. The reduction in cholesterol (86%) and fatty acid (84%) synthesis for **57**, at 1 mM, was associated with a significant reduction (16%) of cellular ATP levels and is likely to be due to a toxic effect.

We considered that the poor efficacy of the inhibitors in HepG2 cells was likely to be a consequence of ineffective or limited cell penetration. The compounds are dicarboxylic acids which will be ionized at neutral pH and consequently are likely to have difficulty in passing through cellular membranes. In an attempt to overcome this, we prepared and evaluated the diethyl (**111**) and diacetoxymethyl (**112**) esters of **58** and the dimethyl ester (**113**) of **68** as potential prodrugs of the diacid inhibitors (Table 7). However, none of these derivatives showed any significant inhibition of cholesterol and/or fatty acid synthesis at the concentrations tested without also having a toxic effect on the cells.

## Summary and Conclusions

The strategy used for the design and identification of a number of reasonably potent inhibitors of ATP-citrate lyase has been described. Inhibition of this enzyme *in vivo* is anticipated to result in an agent which will have combined hypocholesterolemic and hypotriglyceridemic properties. Inhibitor design was based upon the concept of linking a lipophilic group to a "citrate-like" head. The best compounds are butanedioic acid derivatives substituted in the 2 position with an appropriate length (8 atom) spacer group to a 2,4-dichlorophenyl group. These have reversible $K_i$'s in the 1−3 $\mu$M range. However, in a cell based assay (HepG2) none of these inhibitors showed efficacy in reducing cholesterol or fatty acid synthesis. This has been attributed to the adverse physicochemical properties of the molecules leading to a lack of cell penetration. A number of potential ester prodrugs similarly proved either nonefficacious or toxic to the cells. Despite this, a baseline for the design of more potent, cell penetrant ACL inhibitors has been established. Once achieved this should allow the hypothesis to be challenged that

**Table 7.** Compounds Assayed in HepG2 Cells

| no. | concn ($\mu$M) | cholesterol synth % control | fatty acid synth % control | ATP levels % control | analyses |
|---|---|---|---|---|---|
| 3 | 100 | 110 ± 19 | 87 ± 8 | 100 ± 3 | C, H[a] |
| | 300 | 40 ± 6 | 79 ± 11 | 103 ± 3 | |
| | 1000 | 15 ± 9 | 57 ± 4 | 104 ± 5 | |
| 58 | 1000 | 116 ± 10 | 107 ± 15 | 100 ± 2 | see Table 1 |
| 57 | 20 | 104 ± 8 | 96 ± 6 | 102 ± 3 | see Table 1 |
| | 1000 | 14 ± 2 | 16 ± 3 | 84 ± 3 | |
| 74 | 10 | 108 ± 4 | 98 ± 18 | 102±3 | see Table 3 |
| | 1000 | 102 ± 7 | 102 ± 5 | 107±3 | |
| 111 | 10 | n/d | n/d | 91 ± 3 | C, H, Cl, S |
| | 30 | n/d | n/d | 86 ± 2 | |
| | 100 | 3 ± 1 | 6 ± 0 | 81 ± 1 | |
| 112 | 100 | 98 ± 4 | 100 ± 8 | 111 ± 6 | C, H |
| | 1000 | 13 ± 6 | 16 ± 7 | 60 ± 7 | |
| 113 | 10 | 78 ± 6 | 105 ± 12 | 96 ± 3 | C, H |
| | 30 | n/d | n/d | 91 ± 2 | |

[a] Microanalysis performed on the $\gamma$-lactone form; lactone hydrolyzed to **3** prior to use.

inhibition of ACL in an appropriate animal model *in vivo* will result in a decrease in both plasma cholesterol and plasma triglyceride levels.

Our subsequent approaches to the modification of these inhibitors leading to the successful identification of compounds which demonstrate hypolipidemic activity *in vivo* and which validate the hypothesis cited in this communication will be reported in due course.

## Experimental Section

**General Procedures.** Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. Elemental analyses were measured in the Analytical Science Department of SmithKline Beecham, The Frythe, and are within 0.4% of theoretical values unless otherwise indicated. $^1$H NMR spectra were determined on Brucker AM250 or AM 360 spectrometers using tetramethylsilane (TMS) as the internal standard. IR spectra were recorded on a Perkin-Elmer Model 298 instrument. Mass spectra were recorded on a VG-70-250-SEQ instrument. All structural assignments were consistent with NMR, IR, and mass spectra.

**(−)-Hydroxycitrate (3).** Garcinia lactone,[10a] isolated from the fruit rind of *Garcinia cambogia*,[30] was dissolved in water and 3.3 equiv of aqueous sodium hydroxide added. After 10 min, aqueous HCl solution was carefully added to pH 7 and the solution made up to the desired standard concentration.

**Typical Procedure for the Synthesis of 2-[($\omega$-arylalkylthio)methyl]-2-hydroxybutane-1,4-dioic Acids (Tables 1, 2, 5, 6): (±)-2-[[[6-(2,4-Dichlorophenyl)hexyl]thio]-methyl]-2-hydroxybutanedioic Acid (58). (i) Methyl 6-(2,4-Dichlorophenyl)-5-hexenoate (36, X = CH, *n* = 6, subs = 2,4-diCl).** Sodium hydride (60% dispersion in oil, 2.36 g, 58.9 mmol) was washed with petroleum ether 40−60 °C and then heated to 80 °C in DMSO (30 mL) under argon until gas evolution ceased. The solution was cooled in an ice bath, and a solution of (4-carboxybutyl)triphenylphosphonium bromide (12.7 g, 28.6 mmol) in DMSO (60 mL) was added. The solution was stirred for 30 min at room temperature and then cooled in an ice bath. A solution of 2,4-dichlorobenzaldehyde (5.00 g, 28.6 mmol) in DMSO (10 mL) was added, and the mixture was stirred at room temperature for 1 h and then poured into aqueous HCl and extracted with ether. The extracts were washed with water, saturated aqueous NaCl, and dried ($MgSO_4$), and then the solvent was removed under reduced pressure.

Diazomethane was generated from Diazald (12.3 g, 57.2 mmol), carbitol (10 mL), and 60% aqueous KOH (25 mL) in ether (70 mL) and passed in a stream of ether-saturated nitrogen through a solution of the crude acid in 10% methanol/ether (40 mL). When all the acid had reacted (TLC), excess diazomethane was destroyed with acetic acid, and then the solution was poured into aqueous $NaHCO_3$ and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$), and then the solvent was removed under reduced pressure. The residual yellow solid was fractionated on silica gel (20−30% ether/petroleum ether 40−60 °C) to give the title compound (6.37 g, 80%) as an oil, comprising a mixture of *E* and *Z* isomers.

**Separation of the (*E*) and (*Z*) Isomers of Methyl 6-(2,4-Dichlorophenyl)-5-hexenoate.** The product from a repeat preparation of **36** (X = CH, *n* = 6, subs = 2,4-diCl) using 10.0g (57.2 mmol) of 2,4-dichlorobenzaldehyde and 25.4g (57.2 mmol) of (4-carboxybutyl)triphenylphosphonium bromide gave a solid which was fractionated on silica gel (20−30% ether/petroleum ether 40−60 °C), to give the mixture of *E* and *Z* isomers. Further chromatography on a Jobin Yvon Chromatospac Prep HPLC system, using an ether/petroleum ether 40−60 °C gradient, gave the *Z* isomer of the product (3.72 g, 24%) and the *E* isomer (7.33 g, 47%), contaminated with *ca*. 10% of the *Z* isomer.

**(ii) 6-(2,4-Dichlorophenyl)-5-hexen-1-ol (38, X = CH, *n* = 6, subs = 2,4-diCl).** Di-isobutylaluminium hydride (1.0 M in dichloromethane, 51.3 mL, 51.3 mmol) was injected into a stirred solution of methyl 6-(2,4-dichlorophenyl)-5-hexenoate (6.37 g, 23.3 mmol) in dichloromethane (40 mL) at −78 °C under argon. After 5 min, the solution was warmed to 0 °C, stirred for 0.5 h, then cooled again to −78 °C. Water (17 mL) was injected slowly, while allowing the mixture to warm to room temperature. When solid had precipitated, ethyl acetate (50 mL) was added and then excess $NaHCO_3$, and the mixture was stirred vigorously for 15 min. The solids were filtered off through hyflo, the solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel (30−70% ether/petroleum ether 40−60 °C) to give the title compound (4.85 g, 85%) as an oil, comprising a mixture of *E* and *Z* isomers.

**(iii) 6-(2,4-Dichlorophenyl)-1-hexanol (39, X = CH, *n* = 6, subs = 2,4-diCl).** A solution of the mixture of *E* and *Z* isomers of 6-(2,4-dichlorophenyl)-5-hexen-1-ol (4.85 g, 19.8 mmol) in methanol (40 mL) was shaken under hydrogen (50 psi) with platinum oxide (253 mg, 1.11 mmol, added in portions) until no starting material could be detected by NMR spectroscopy. The catalyst was filtered off through hyflo, and the solvent was removed under reduced pressure. The residue was dissolved in ether, and the solution was filtered through a pad of silica gel. The solvent was again removed under reduced pressure to give the title compound (4.70 g, 96%) as an oil.

**(iv) 1-Bromo-6-(2,4-dichlorophenyl)hexane (43, *n* = 6, subs = 2,4-diCl).** *N*-Bromosuccinimide (4.27 g, 24.0 mmol) was added in portions to a stirred solution of 6-(2,4-dichlorophenyl)-1-hexanol (5.38 g, 21.8 mmol) and triphenylphosphine (6.29 g, 24.0 mmol) in dichloromethane (60 mL) at 0 °C, and the resulting solution was stirred for 5min at 0 °C and 20 min at room temperature. The solvent was removed under reduced pressure, and the residue was triturated with 10% ether/petroleum ether 40−60 °C. The extracts were filtered through a pad of silica gel. The solvent was removed from the filtrate under reduced pressure to give the title compound (6.61 g, 98%) as an oil.

**(v) *S*-[6-(2,4-Dichlorophenyl)hexyl]thioacetate (40, X = CH, *n* = 6, subs = 2,4-diCl).** Potassium thioacetate (1.47 g, 12.9 mmol), dried at 60 °C under high vacuum for 1 h, was added to a stirred solution of 1-bromo-6-(2,4-dichlorophenyl)-hexane (2.00 g, 6.45 mmol) in dry DMF (20 mL) containing powdered 4 Å molecular sieves. The mixture was stirred for 2 h under argon and then poured into cold aqueous HCl and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (5−15% ether/petroleum ether 40−60 °C) to give the title compound (1.95 g, 99%) as an oil.

**(vi) Methyl 4-[[6-(2,4-Dichlorophenyl)hexyl]thio]-3-oxobutanoate (17, R = 2,4-diClC$_6$H$_3$(CH$_2$)$_6$).** Sodium methoxide in methanol (7.40 mL of a 0.470 M solution, 3.48 mmol) was injected into a stirred solution of *S*-[6-(2,4-dichlorophenyl)-hexyl]thioacetate (1.01 g, 3.31 mmol) in methanol (10 mL) under argon. After 10 min, methyl 4-chloroacetoacetate (344 $\mu$L, 2.98 mmol) was injected. The mixture was stirred at room temperature for 18 h and then poured into aqueous HCl and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was fractionated on silica gel (10−40% ether/petroleum ether 40−60 °C) to give the title compound (1.14 g, 91%) as an oil.

**(vii) (±)-2-[[[6-(2,4-Dichlorophenyl)hexyl]thio]methyl]-2-hydroxybutanedioic Acid (58).** A solution of $KH_2PO_4$ (9.99 g, 73.4 mmol) in water (60 mL) was injected over 10 min into a stirred mixture of KCN (4.78 g, 73.4 mmol), methyl 4-[[6-(2,4-dichlorophenyl)hexyl]thio]-3-oxobutanoate (2.77 g, 7.34 mmol), and ether (25 mL) at 0 °C initially. The flask was connected to a chloros/NaOH trap system to remove any HCN evolved. The mixture was stirred vigorously for 18 h and then the pH adjusted to 4 with concentrated aqueous HCl. The ether layer was removed by pipette and the aqueous layer extracted with ether twice more. The solvent was removed from the extracts under reduced pressure and the residual oil heated at reflux in 25% aqueous HCl for 3.5 h. After cooling, the mixture was extracted with ether. The extracts were washed with 1 M aqueous NaOH twice. The aqueous phase was washed with ether and then reacidified with 5 M aqueous HCl. The mixture was extracted with ether once again, and the extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude product recrystallized (ether/petroleum ether 40−60 °C) to give the title compound (2.28 g, 76%) as a white solid, mp 88−89 °C. $^1$H NMR (DMSO, 250 MHz) $\delta$ 7.55 (m, 1H, Ar), 7.36 (m, 2H, Ar), 5.10 (br s, 1H, OH), 2.81−2.50 (m, 8H, ArC*H*$_2$, SC*H*$_2$CH$_2$, SC*H*$_2$C(OH), C*H*$_2$CO$_2$H), 1.60−1.20 (m, 8H, SCH$_2$(C*H*$_2$)$_4$). Anal. $C_{17}H_{22}Cl_2O_5S$ (C, H).

**Aldehydes.** Most of the aldehydes required for the syntheses of the compounds in Tables 1−3, 5, and 6 were commercially available. Otherwise they were readily prepared by Swern oxidation of the corresponding benzyl or pyridylmethyl alcohols. 2-Phenyl-4,6-dichlorobenzaldehyde was synthesized by the published procedure.[23c]

**Alternative Syntheses of Intermediate Bromides (43) (Chart 3, method B).** Bromides **43,** where subs = 2,4-diMe, 4-F, 4-Cl, 4-Br, 4-OMe; *n* = 6, were prepared from the substituted benzene and 6-bromohexanoyl halide, by the acylation/reduction method of Jaxa-Chamiec et al.[26] This procedure was modified for the 4-halo derivatives whereby the intermediate ketone was first isolated and then subsequently reduced with $Et_3SiH$ in TFA.

**6-(2-Nitrophenyl)hexyl Bromide and 6-(4-Nitrophenyl)hexyl Bromide** (**43**, *n* = 6, subs = 2 and 4 $NO_2$, respectively). Nitronium tetrafluoroborate (1.58 g, 12 mmol) was added to a solution of 6-phenylhexyl bromide[26] (4.82 g, 20 mmol) in dry $CH_2Cl_2$ (20 mL) at 0 °C and stirred for 2 h. Water was added, the mixture was extracted with ether, and the combined extracts were dried over $MgSO_4$. The residue on evaporation in vacuo was chromatographed (silica, 1:1 pentane/$CH_2Cl_2$) to separate unreacted starting material from the 2-nitro (0.7 g) and 4-nitro (1.03 g) isomers.

**Syntheses of (ω-Hydroxyalkyl)triphenylphosphonium Bromides (37):** (8-Hydroxyoctyl)triphenylphosphonium Bromide (37, *n* = 9). A solution of 8-bromooctanol (4.77 g, 22.8 mmol) and triphenylphosphine (11.97 g, 45.6 mmol) in toluene (15 mL) was heated under reflux for 48 h. On cooling, a gum separated and the toluene was decanted off. This was washed several times with toluene and the solvent decanted off. The residue was triturated under ether to give **37** (*n* = 9) (9.03 g, 19.1 mmol, 84%).

**9-(2,4-Dichlorophenyl)-8-nonen-1-ol** (**38**, **X = CH, *n* = 9, subs = 2,4-diCl).** To a mixture of (**37**, *n* = 9) (9 g, 19.1 mmol) in dry THF (135 mL), cooled to −78 °C, under argon, was added 2.5 M $^n$BuLi in hexanes (15.25 mL, 38.2 mmol), and the mixture allowed to warm to 0 °C. The resulting red solution was stirred at 0 °C for 30 min and recooled to −78 °C. 2,4-Dichlorobenzaldehyde (3.84 g, 22 mmol) in THF (90 mL) was then added dropwise and the mixture then allowed to warm to room temperature. After a further 15 min, the solution was poured into 1 M aqueous HCl and extracted with ether (3 × 150 mL). The combined extracts were washed with water (2x) and brine (2x), dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed (silica gel, 50% ether/petroleum ether 40−60 °C) to give **38** (X = CH, *n* = 9, subs = 2,4-diCl) (2.21 g, 7.7 mmol, 40%) as a colorless oil.

**Syntheses of 76 and 77.** The separated *E* and *Z* isomers of **36** (X = CH, *n* = 6, subs = 2,4-diCl) were reduced to their respective alcohols with DIBAL-H and converted to **76** and **77** by the general procedures described above. **76**: $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.39 (m, 1H, Ar), 7.19 (m, 2H, Ar), 6.43 (d, 1H, *J* = 11.5 Hz, ArC*H*), 5.77 (dt, 1H, *J* = 11.5, 7.5 Hz, ArCH=C*H*), 3.07−2.75 (m, 4H, SC*H*$_2$C(OH), C*H*$_2$CO$_2$H), 2.60 (m, 2H, SC*H*$_2$CH$_2$), 2.17 (m, 2H, CH=CHC*H*$_2$), 1.65−1.40 (m, 4H, SCH$_2$(C*H*$_2$)$_2$). Anal. $C_{17}H_{20}Cl_2O_5S$ (C, H). **77**: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.41 (d, 1H, *J* = 10.6 Hz, Ar), 7.34 (d, 1H, *J* = 2.7 Hz, Ar), 7.17 (dd, 1H, *J* = 10.6, 2.7 Hz, Ar), 6.67 (d, 1H, *J* = 15.4 Hz, ArC*H*), 6.16 (dt, 1H, *J* = 15.4, 7.3 Hz, ArCH=C*H*), 3.10−2.80 (m, 4H, SC*H*$_2$C(OH), C*H*$_2$CO$_2$H), 2.67 (m, 2H, SC*H*$_2$CH$_2$), 2.25 (m, 2H, CH=CHC*H*$_2$), 1.70−1.45 (m, 4H, SCH$_2$(C*H*$_2$)$_2$). Anal. $C_{17}H_{20}Cl_2O_5S$ (C, H).

**(±)-Dimethyl 2-[[[6-(2,4-Dichlorophenyl)hexyl]thio]-methyl]butanedioate (113).** To a stirred solution of *S*-[6-(2,4-dichlorophenyl)hexyl]thioacetate (**40**, X = CH, *n* = 6, subs = 2,4-diCl) (3 g, 9.83 mmol) in MeOH (5 mL), under an argon atmosphere, was added a 0.2 M solution of sodium methoxide in MeOH (51.6 mL, 10.32 mmol). This was stirred at room temperature for 1 h and then a solution of dimethyl itaconate (1.56 g, 9.83 mmol) in MeOH (3 mL) added. This was stirred at room temperature for 18 h, diluted with 0.5 M HCl solution, and extracted with ether (×3). The extracts were washed with water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue chromatographed on silica gel (30% ether/petroleum ether 40−60 °C) to give **113** (3.14 g,76%) as an oil. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (m, 1H, Ar), 7.14 (m, 2H, Ar), 3.72 (s, 3H, OMe), 3.69 (s, 3H, OMe), 3.06 (m, 1H, C*H*CO2Me), 2.88 (dd, 1H, *J* = 14.0, 5.9 Hz, SC*H*$_2$CH(CO$_2$Me) or C*H*$_2$CO$_2$Me), 2.80−2.60 (m, 5H, ArC*H*$_2$, SC*H*$_2$CH(CO$_2$Me), C*H*$_2$CO$_2$Me), 2.51 (m, 2H, SC*H*$_2$-CH$_2$), 1.65−1.30 (m, 8H, ArCH$_2$(C*H*$_2$)$_4$). Anal. $C_{19}H_{26}Cl_2O_4S$ (C, H).

**(±)-2-[[[6-(2,4-Dichlorophenyl)hexyl]thio]methyl]-butanedioic Acid (68). 113** (0.78 g, 1.84 mmol) was stirred at reflux in 25% HCl solution for 18 h. This was extracted with ether (×3), and the extracts were washed with water and brine and then dried over $MgSO_4$. Concentration in vacuo gave **68** (0.71 g, 98%) as an oil. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (d, 1H, *J* = 1.8 Hz, Ar), 7.14 (m, 2H, Ar), 3.05−2.50 (m, 9H, ArC*H*$_2$, SC*H*$_2$, C*H*$_2$CO$_2$H, C*H*CO$_2$H), 1.65−1.30 (m, 8H, ArCH$_2$(C*H*$_2$)$_4$). Anal. $C_{17}H_{22}Cl_2O_4S$ (C, H).

**3-Hydroxy-4-[[6-(2,4-dichlorophenyl)hexyl]thio]butyric Acid (69). (i) Methyl 3-Hydroxy-4-[[6-(2,4-dichlorophenyl)hexyl]thio]butyrate** (**18**, R = 2,4-diClC$_6$H$_3$(CH$_2$)$_6$). $NaBH_4$ (64 mg, 1.7 mmol) was added to a solution of (**17**, R = 2,4-diClC$_6$H$_3$(CH$_2$)$_6$) (0.64 g, 1.7 mmol) and $CeCl_3 \cdot 7H_2O$ (632 mg, 1.7 mmol) in MeOH (10 mL) at 0 °C and then stirred at this temperature for 1 h. 1 M HCl solution (10 mL) was added and this extracted with ether (3×). The combined extracts were washed with water and then brine and dried over $MgSO_4$. Concentration followed by flash column chromatography (silica, 30−50% ether/petroleum ether 40−60 °C) gave the title compound (0.54 g, 1.42 mmol, 84%).

**(ii) 3-Hydroxy-4-[[(2,4-dichlorophenyl)hexyl]thio]butyric Acid (69). 18** (R = 2,4-di$ClC_6H_3(CH_2)_6$) (0.4 g, 1.05 mmol) was stirred in a solution of NaOH (84 mg, 2.1 mmol) in absolute ethanol (1 mL) and water (1 mL) at room temperature for 30 min. The pH was adjusted to *ca.* 1 with 1 M HCl and extracted with $CH_2Cl_2$ (3×). The combined extracts were washed with water and brine and then dried over $MgSO_4$. Concentration gave the title compound as a clear oil (0.363 g, 0.99 mmol, 94%). $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (m, 1H, Ar), 7.14 (m, 2H, Ar), 4.12 (m, 1H, C*H*(OH)), 2.80−2.50 (m, 8H, $CH_2CO_2H$, $CH_2CH(OH)$, $SCH_2CH_2$, $ArCH_2$), 1.70−1.30 (m, 8H, $ArCH_2(CH_2)_4$). Anal. $C_{16}H_{22}Cl_2O_3S$ (C, H, Cl).

**Synthesis of 4-[[6-(2,4-Dichlorophenyl)hexyl]thio]butyric acid (70). (i) Methyl 4-[6-(2,4-Dichlorophenyl)-hexyl]thio]butyrate (22, R = 2,4-di$ClC_6H_3(CH_2)_6$).** To a solution of **40** (X = CH, *n* = 6, subs = 2,4-diCl) (800 mg, 2.62 mmol) in MeOH (10 mL), under argon, was added a freshly prepared (from Na and MeOH) 0.2 M solution of NaOMe in MeOH (14.4 mL, 2.88 mmol). This was stirred at room temperature for 30 min and then ethyl 4-bromobutyrate (0.413 mL, 2.88 mmol) added and the mixture stirred for a further 18 h. 0.5 M HCl was added and this was then extracted with ether (3×), and the combined extracts were washed with water and brine and dried over $MgSO_4$. Flash chromatography (30% ether/petroleum ether 40−60 °C) gave the title compound (0.83 g, 2.28 mmol, 87%).

**(ii) 4-[[6-(2,4-Dichlorophenyl)hexyl]thio]butyric Acid (70).** Hydrolysis of **22** (R = 2,4-di$ClC_6H_3(CH_2)_6$) with NaOH in $EtOH/H_2O$, as described for **69**, gave **70** as a white solid (0.72 g, 2.06 mmol, 95%), mp 48−49 °C. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (d, 1H, *J* = 1.7 Hz, Ar), 7.14 (m, 2H, Ar), 2.71−2.47 (m, 8H, $ArCH_2$, $SCH_2$, $CH_2CO_2H$), 1.91 (m, 2H, $CH_2CH_2CO_2H$), 1.65−1.30 (m, 8H, $ArCH_2(CH_2)_4$). Anal. $C_{16}H_{22}Cl_2O_2S$ (C, H, Cl).

**Syntheses of Compounds 71 and 72 (Chart 2): (±)-3-Carboxy-10-(2,4-dichlorophenyl)-3-hydroxydecanoic Acid (72). (i) Methyl 10-(2,4-Dichlorophenyl)-3-oxodecanoate (28, *n* = 6).** Methyl acetoacetate (696 $\mu$L, 6.45 mmol) was injected into a stirred slurry of NaH (284 mg of a 60% oil dispersion, 7.10 mmol), washed free of oil with petroleum ether 40−60 °C, in THF (8 mL) at 0 °C under argon. The mixture was stirred at room temperature for 30 min and cooled back to 0 °C, and *n*-butyllithium (2.84 mL, 2.5 M in hexanes, 7.10 mmol) was injected dropwise. The resulting solution was stirred at room temperature for 15 min and again cooled to 0 °C. A solution of 1-bromo-6-(2,4-dichlorophenyl)hexane (**43**, *n* = 6, subs = 2,4-diCl) (2.00 g, 6.45 mmol) in THF (5 mL) was added by cannula, and the mixture was stirred for 2 h at room temperature and then poured into cold aqueous HCl and extracted with ether. The extracts were washed with water, saturated aqueous NaCl, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue chromatographed on silica gel (10−40% ether/petroleum ether 40−60 °C) to give the title compound (1.20 g, 54%) as an oil.

**(ii) (±)-3-Carboxy-10-(2,4-dichlorophenyl)-3-hydroxydecanoic Acid (72).** The procedure described for **58** was carried out, using methyl 10-(2,4-dichlorophenyl)-3-oxodecanoate (**28**, *n* = 6) in place of methyl 4-[[6-(2,4-dichlorophenyl)hexyl]thio]-3-oxobutanoate to give the title compound (84%) as a white solid, mp 92−94 °C (ether/petroleum ether 40−60 °C). $^1$H NMR (DMSO, 250 MHz) $\delta$ 12.4 (br s, 2H, $CO_2H$), 7.54 (m, 1H, Ar), 7.35 (m, 2H, Ar), 4.77 (br s, 1H, OH), 2.71 (d, 1H, *J* = 15.4 Hz, $CH_2CO_2H$), 2.65 (m, 2H, $ArCH_2$), 2.44 (d, 1H, *J* = 15.4 Hz, $CH_2CO_2H$), 1.66−1.05 (m, 12H, $ArCH_2(CH_2)_6$). Anal. $C_{17}H_{22}Cl_2O_5$ (C, H).

Compound **71** was synthesized by an analogous procedure from the higher homologue of **43** (*n* = 7). $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.33 (m, 1H, Ar), 7.13 (m, 2H, Ar), 3.02 (d, 1H, *J* = 17.1 Hz, $CH_2CO_2H$), 2.77 (d, 1H, *J* = 17.1 Hz, $CH_2CO_2H$), 2.66 (m, 2H, $ArCH_2$), 1.80−1.20 (m, 14H, $ArCH_2(CH_2)_7$). Anal. $C_{18}H_{24}Cl_2O_5$ (C, H).

**Synthesis of 74 and 75 (Chart 2). (i) 7-(2,4-Dichlorophenyl)-1-heptanol (26, *n* = 6).** This was prepared by an analogous procedure to that described above for the lower homologue (**39**, X = CH, *n* = 6, subs = 2,4-diCl).

**(ii) 7-(2,4-Dichlorophenyl)heptanaldoxime (30, *n* = 6).** DMSO (15.2 mL, 214 mmol) was added slowly to a stirred solution of oxalyl chloride (9.35 mL, 107 mmol) in dichloromethane (150 mL) at −78 °C under argon. After 5 min, a solution of 7-(2,4-dichlorophenyl)-1-heptanol (20.0 g, 76.6 mmol) in dichloromethane (100 mL) was added by cannula. After stirring 0.5h at −78 °C, triethylamine (47 mL, 337 mmol) was injected. The mixture was stirred 5 min, allowed to warm to room temperature, and then poured into 1 M aqueous $NaHSO_4$. The product was extracted with ether. The extracts were washed with water and saturated aqueous NaCl, and then the solvent was removed under vacuum. A solution of the crude aldehyde product in ether (120 mL) was added to a stirred suspension of hydroxylamine hydrochloride (17.0 g, 245 mmol) in water (10 mL) at 0 °C, followed by aqueous $Na_2CO_3$ (2.7 M, 50 mL, 135 mmol). The mixture was stirred at room temperature for 2.5 h, poured into water, and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under vacuum and the residue purified by chromatography on silica gel (30−50% ether/petroleum ether 40−60 °C) to give the title compound (17.3 g, 82%) as a mixture of *E* and *Z* isomers.

**(iii) (±)-5-(Carbomethoxymethyl)-3-[6-(2,4-dichlorophenyl)hexyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole (31, *n* = 6).** Aqueous NaOCl (2.0 M, 340 mL, 680 mmol) and triethylamine (2.5 mL, 18.0 mmol) were added in 4 portions separately over 40 h to a stirred solution of 7-(2,4-dichlorophenyl)heptanaldoxime (17.3 g, 63.1 mmol) and dimethyl itaconate (23.0 g, 145 mmol) in dichloromethane (200 mL). The mixture was stirred vigorously at room temperature over this period and then poured into water and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under vacuum and the residue purified by chromatography on silica gel (30−60% ether/petroleum ether 40−60 °C) to give the title compound (19.5 g, 72%) as an oil.

**(iv) (±)-Methyl 11-(2,4-Dichlorophenyl)-3-hydroxy-3-(methoxycarbonyl)-5-oxo-undecanoate (32, *n* = 6).** A solution of (±)-5-(carbomethoxymethyl)-3-[6-(2,4-dichlorophenyl)hexyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole (19.5 g, 45.3 mmol) and boric acid (8.39 g, 136 mmol) in methanol was shaken with Raney nickel (50% slurry in water, 8 g) under hydrogen (50 psi) at room temperature for 2 h. The catalyst was removed by filtration, and most of the solvent was removed under vacuum. The mixture was diluted with water and extracted with ethyl acetate. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under vacuum, and the residue was purified by chromatography on silica gel (50−80% ether/petroleum ether 40−60 °C) to give the title compound (16.7 g, 85%) as an oil.

**(v) (±)-11-(2,4-Dichlorophenyl)-3-hydroxy-3-carboxy-5-oxoundecanoic acid (74).** A solution of NaOH (602 mg, 15.1 mmol) in water (22 mL) was added slowly to a stirred solution of diester (**32**, *n* = 6) (1.62 g, 3.74 mmol) in ethanol (23 mL) at 0 °C. The solution was stirred 5 min at 0 °C and then 1 h at room temperature. After recooling to 0 °C, the pH was adjusted to 2 with 1 M aqueous HCl, and the mixture was extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue recrystallized (ether/petroleum ether 40−60 °C) to give the title compound (1.05 g, 69%) as a white solid, mp 91−93 °C. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.33 (m, 1H, Ar), 7.13 (m, 2H, Ar), 3.08−2.78 (m, 4H, $COCH_2C(OH)$, $CH_2CO_2H$), 2.65 (m, 2H, $ArCH_2$), 2.44 (m, 2H, $COCH_2CH_2$), 1.60−1.20 (m, 8H, $ArCH_2(CH_2)_4$). Anal. $C_{18}H_{22}Cl_2O_6$ (C, H).

**Synthesis of (±)-3-Carboxy-5-[5-(2,4-dichlorophenyl)-pentyl]thio]-3-hydroxypentanoic Acid (73).** *S*-[5-(2,4-Dichlorophenyl)pentyl] thioacetate (**40**, X = CH, *n* = 5, subs = 2,4-diCl) was prepared by the method described for (**40**, X = CH, *n* = 6, subs = 2,4-diCl) above. To this in MeOH (5 mL) was injected a solution of NaOMe in MeOH (0.47M, 7.65 mL, 3.60 mmol) at room temperature. After 5 min, a solution of methyl 3-oxo-4-pentenoate[31] (440 mg, 3.43 mmol) in methanol (5 mL) was added by cannula and stirring continued for 2 h. The mixture was partitioned between aqueous HCl and ether. The ether extracts were washed with water and saturated

aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue fractionated on silica gel (20−50% ether/petroleum ether 40−60 °C) to give methyl 5-[[5-(2,4-dichlorophenyl)pentyl]thio]-3-oxopentanoate (**24**, R = 2,4-di$ClC_6H_3(CH_2)_5$) (1.10 g, 85%) as a colorless oil. The latter compound was converted to **73** by the general procedures described above. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (d, 1H, $J$ = 1.8 Hz, Ar), 7.14 (m, 2H, Ar), 3.03 (d, 1H, $J$ = 16.9 Hz, C$H_2$-$CO_2$H), 2.79 (d, 1H, $J$ = 16.9 Hz, C$H_2CO_2$H), 2.71−2.43 (m, 6H, ArC$H_2$, SC$H_2$), 2.11−1.95 (m, 2H, $CH_2CH_2$C(OH)), 1.67−1.37 (m, 6H, $ArCH_2(CH_2)_3$). Anal. $C_{17}H_{22}Cl_2O_5S$ (C, H).

**Syntheses of Intermediates for the Preparation of Compounds of Table 4 (Chart 4). *S*-[5-(2,4-Dichlorophenoxy)pentyl] thioacetate (47a**, $n$ = 5). Potassium carbonate (5.00 g, 36.2 mmol) and potassium iodide (2−3 crystals) were added to a solution of 2,4-dichlorophenol (5 g, 31 mmol) and 1,5-dibromopentane (21.39 g, 93 mmol) in acetone (50 mL), and the mixture was heated at reflux for 1 h and then cooled. The solids were filtered off, the solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water. The aqueous layer was washed further with EtOAc. The combined organic extracts were washed with $NaHCO_3$ solution, water, and brine and dried over $MgSO_4$. Concentration in vacuo, followed by flash chromatography (silica, 0−10% ether/petroleum ether 40−60 °C gradient) gave 5-(2,4-dichlorophenoxy)pentyl bromide (**45a**, $n$ = 5) (8.5 g, 27.2 mmol, 88%). The bromide was converted to the corresponding title thioacetate by the standard procedure described above.

***S*-[5-[(2,4-Dichlorophenyl)thio]pentyl] thioacetate (47b**, ***n* = 5).** Potassium carbonate (5.00 g, 36.2 mmol) and potassium iodide (46 mg, 0.28 mmol) were added to a solution of 2,4-dichlorobenzenethiol (4.96 g, 27.7 mmol) and 1,5-dibromopentane (19.3 g, 84.3 mmol) in acetone (50 mL), and the mixture was heated at reflux for 1 h and then cooled. The solids were filtered off, and solvent was removed from the filtrate under reduced pressure. The residue was fractionated on silica gel (0−5% ether/petroleum ether 40−60 °C). The solid product was triturated with petroleum ether 40−60 °C to remove traces of 1,5-dibromopentane leaving 5-bromopentyl (2,4-dichlorophenyl sulfide (**45b,** $n$ = 5) (2.58 g, 28%) as a solid, mp 59−62 °C. The bromide was converted to the corresponding title thioacetate by the standard procedure described above.

***S*-[5-(2,4-Dichlorobenzenesulfinyl)pentyl] thioacetate (47c**, ***n* = 5).** To a solution of 5-bromopentyl 2,4-dichlorophenyl sulfide (**45b,** $n$ = 5) (1.38 g, 4.2 mmol) in $CH_2Cl_2$ (50 mL), at −78 °C, was added 55% *m*-chloroperbenzoic acid (1.32 g, 4.2 mmol), and the solution was stirred for a further 1 h at this temperature and then left in the body of a freezer for 18 h. An aqueous solution of $Na_2SO_3$/$NaHCO_3$ was added, the organic layer separated, and the aqueous solution extracted further with $CH_2Cl_2$. The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and flash chromatographed (silica, 80% ether/petroleum ether 40−60 °C) to give 5-(2,4-dichlorobenzenesulfinyl)pentyl bromide (**46,** $n$ = 5, m = 1) (1.05 g, 3.05 mmol, 72%). The bromide was converted to the corresponding title thioacetate by the standard procedure described above.

***S*-[[5-(2,4-Dichlorobenzenesulfonyl]pentyl]thioacetate (47d**, ***n* = 5).** This compound was obtained by an analogous procedure to that used for **47c** ($n$ = 5), but using 2 equiv of MCPBA at 0 °C, followed by stirring at room temperature for 3 h, for the oxidation step.

***S*-[5-(2,4-Dichlorophenyl)amino]pentyl] thioacetate (48**, ***n* = 5).** 5-Bromopentanoyl chloride (6.16 g, 30.9 mmol) was added slowly to a stirred solution of 2,4-dichloroaniline (5.00 g, 30.9 mmol) and 4-(dimethylamino)pyridine (0.75 g, 6.14 mmol) in pyridine (40 mL) at 0 °C. The mixture was stirred at room temperature for 18 h and then poured into aqueous HCl and extracted with ether. The extracts were washed with aqueous HCl, water, and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue fractionated on silica gel (30−50% ether/petroleum ether 40−60 °C) to give 5-bromo-*N*-(2,4-dichlorophenyl)pentanamide (1.28 g, 13%) as an oil. The latter (1.14 g, 3.51 mmol) in THF (45 mL) was added by cannula to a stirred solution of borane in THF (1 M, 8.51 mL, 8.51 mmol) at 0 °C under argon. The solution was heated at reflux for 3 h and cooled, and 1 M aqueous HCl (8.5 mL) was added. The resulting mixture was heated while allowing the THF to distill off. The residual aqueous phase was then cooled, diluted with water, and extracted with ether. The extracts were washed with water and dried ($MgSO_4$), and the solvent was removed under reduced pressure to leave crude 1-bromo-5-(2,4-dichloroanilino)pentane (1.11 g), sufficiently pure for the next step. This bromide was converted to the corresponding title thioacetate by the standard procedure described above.

***S*-[[4-(2,4-Dichlorobenzenesulfonyl)amino]butyl] thioacetate (50**, ***n* = 4).** To a solution of 2,4-dichlorobenzenesulfonyl chloride (4 g, 16.3 mmol) in pyridine (14 mL) at 0 °C was slowly added a solution of 4-aminobutan-1-ol (1.82 g, 20.36 mmol) in pyridine (6 mL). The solution turned orange-red in color and was stirred at room temperature for 36 h. Water was added, the pH adjusted to 7 with aqueous HCl, and this extracted with ether (3×). The combined ether extracts were washed with 1 M HCl (2×), water, and brine and dried over $MgSO_4$. The residue on evaporation was flash chromatographed (silica, 70−100% ether/petroleum ether 40−60 °C) to give 4-[(2,4-dichlorobenzenesulfonyl)amino]butan-1-ol (**49**, $n$ = 4) (1.29 g, 4.33 mmol, 27%). This alcohol was converted to the corresponding thioacetate by the general procedures described above.

**Synthesis of (±)-2-[[[6-(2,4-dichlorophenyl)-6-oxohexyl]thio]methyl]-2-hydroxybutanedioic Acid (82). (i) (±)-(*E*)-Dimethyl 2-[[[6-(2,4-Dichlorophenyl)hex-5-enyl]thio]-methyl]-2-hydroxybutanedioate (51**, ***n* = 5).** Diazomethane, generated from diazald (5.36 g, 25.0 mmol) and 60% KOH (24 mL) in ether/carbitol (1:1, 48 mL), was bubbled in an ether-saturated nitrogen stream into a solution of **77** (2.55 g, 6.26 mmol) in 10% methanol/ether (30 mL) at room temperature. When the reaction solution turned yellow, the flow was stopped and the excess reagent quenched by the addition of acetic acid. The solvent was removed under reduced pressure and the residue fractionated on silica gel (40−60% ether/petroleum ether 40−60 °C) to give the title compound (2.36 g, 87%) as an oil.

**(ii) (±)-Dimethyl 2-[[[6-(2,4-dichlorophenyl)-6-hydroxyhexyl]thio]methyl]-2-hydroxybutanedioate (52**, ***n* = 5).** A solution of borane in THF (1 M, 3.37 mL, 3.37 mmol) was injected in four portions over 4 h into a stirred solution of **51** ($n$ = 5) (1.00 g, 2.30 mmol) in THF (2 mL) at 0 °C under argon. Thirty minutes after the final addition, water (3 mL) was added and the mixture stirred for five minutes. Sodium perborate (708 mg, 4.60 mmol) was added and stirring continued for 1.5 h. The mixture was diluted with water and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue fractionated on silica gel (50−80% ether/petroleum ether 40−60 °C) to give the title compound (459 mg, 44%, colorless oil) as a mixture of diastereomers.

**(iii) (±)-2-[[[6-(2,4-Dichlorophenyl)-6-oxohexyl]thio]-methyl]-2-hydroxybutanedioic Acid (82).** DMSO (0.161 mL, 2.26 mmol) was injected dropwise into a stirred solution of oxalyl chloride (0.099 mL, 1.13 mmol) in $CH_2Cl_2$ (3 mL) at −78 °C under argon. After 5 min, a solution of **52** ($n$ = 5) (427 mg, 0.942 mmol) in $CH_2Cl_2$ (2 mL) was added by cannula over 5 min and the mixture stirred 0.5 h. Triethylamine (0.495 mL, 3.55 mmol) was injected. The mixture was stirred while warming to room temperature and then poured into 1 M aqueous $NaHSO_4$ and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue fractionated on silica gel (40−70% ether/petroleum ether 40−60 °C) to give **53** ($n$ = 5) (326 mg, 77%) as a colorless oil.. This (311 mg, 0.689 mmol) was hydrolyzed using NaOH in aqueous MeOH, as described above, to give **82** (291 mg, 100%) as a gummy solid. $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.43 (m, 2H, Ar), 7.31 (dd, 1H, $J$ = 8.3 Hz, 1.9 Hz, Ar), 3.08−2.82 (m, 6H, COC$H_2$, SC$H_2$C(OH), C$H_2CO_2$H), 2.64 (m, 2H, SC$H_2CH_2$), 1.75−1.35 (m, 6H, $SCH_2(CH_2)_3$). Anal. $C_{17}H_{20}Cl_2O_6S$ (C, H).

**(±)-Diethyl 2-[[[6-(2,4-dichlorophenyl)hexyl]thio]-methyl]butanedioate (111).** To a solution of **68** (250 mg,

0.64 mmol) in absolute ethanol (4 mL) was added *p*-toluenesulfonic acid (12 mg, 0.064 mmol) and the solution stirred at reflux for 18 h. The solvent was removed under reduced pressure and the residue chromatographed on silica gel (50−100% ether/petroleum ether 40−60 °C) to give the title compound (223 mg, 78%). Anal. $C_{21}H_{30}Cl_2O_4S$ (C, H). $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 7.34 (s, 1H, Ar), 7.14 (m, 2H, Ar), 4.28 (q, 2H, $J$ = 7.1 Hz, $CO_2CH_2$), 4.14 (q, 2H, $J$ = 7.1 Hz, $CO_2CH_2$), 3.93 (s, 1H, O*H*), 2.97−2.57 (m, 8H, $ArCH_2$, $CH_2SCH_2$, $CH_2CO_2$), 1.57−1.21 (m, 14H, $ArCH_2(CH_2)_4$, 2 × $CH_3$).

**(±)-2-[[[6-(2,4-Dichlorophenyl)hexyl]thio]methyl]-2-hydroxybutanedioic Acid, Diacetoxymethyl Ester (112).** Bromomethyl acetate (0.62 mL, 6.4 mmol) was added by syringe to a stirring mixture of **58** (0.26 g, 6.4 mmol), potassium carbonate (0.18 g, 1.27 mmol), 18-crown-6 (0.05 g), and powdered 4A sieves in dry DMF (7 mL) under argon and then stirred at room temperature for 5 days. The reaction mixture was poured into 1 M aqueous HCl and extracted with ether (3×). The combined ether extracts were washed with water (3×) and brine (1×), dried ($MgSO_4$), and concentrated under reduced pressure. The residual oil was chromatographed on silica gel (40% ether/petroleum ether 40−60 °C) to yield title compound (0.21 g, 60%). Anal. $C_{23}H_{30}Cl_2O_9S$ (C, H). $^1$H NMR ($CDCl_3$, 250 MHz) $\delta$ 7.34 (s, 1H, Ar), 7.18−7.19 (m, 2H, Ar), 5.83−5.79 (m, 2H, $CO_2CH_2OCOCH_3$), 5.75−5.68 (m, 2H, $CH_2CO_2CH_2OCOCH_3$), 3.75 (s, 1H, O*H*), 3.01−2.77 (m, 4H, $CH_2C(OH)(CO_2CH_2)CH_2$), 2.70−2.58 (m, 4H, $SCH_2(CH_2)_4CH_2$), 2.13, 2.12 (2s, 6H, 2 × $OCOCH_3$), 1.64−1.56 (m, 4H, $SCH_2CH_2(CH_2)_2CH_2$), 1.38−1.35 (m, 4H, $S(CH_2)_2(CH_2)_2$).

**Enzyme Assays and Analysis of Kinetic Data.** Rat ACL and recombinant human ACL were purified as described previously.[15,32] ACL activity was measured by the maleate dehydrogenase catalyzed reduction of oxaloacetate by NADH. Briefly, ACL was added to buffer containing Tris (100 mM), pH = 8.0, $MgCl_2$ (10 mM), KCl (10 mM), dithiothreitol (10 mM), ATP (250 mM), NADH (35 mM), and maleate dehydrogenase. For initial $K_i$ determinations CoA was present at 200 $\mu$M and citrate was added to 100 $\mu$M ($K_{m\ (citrate)}$ = 100 $\mu$M) to start the reaction. Rates were measured over 20 min by monitoring the decrease in absorbance at 340 nm. To routinely determine $K_i$ values, data was fitted to the equation $v = V_{max}/(2 + [I]/K_i)$ using Grafit 3.0.[33] This assumes that compounds were purely citrate competitive. For substrate competition experiments data was initially fitted to the general equation $v = V_{max}([S]/K_m)/(1 + [S]/K_m + [I]/K_{ei} + [S][I]/K_mK_{esi})$. The significance of either $K_{ei}$ or $K_{esi}$ was then determined by curve fitting in the absence of one of these parameters and applying an F test to the result again using Grafit 3.0.

**Measurement of Effect of Compounds on Cholesterol and Fatty Acid Synthesis in HepG2 Cells.** HepG2 cells were cultured in 24-well cell culture plates in DMEM (Dulbecco's Modified Eagle's Medium) containing Hepes (20 mM), bicarbonate (10 mM), glutamine (2 mM), and fetal calf serum (10% w/v). Once the cells had grown to between 80% and 90% confluence, the medium was replaced by DMEM without the addition of fetal calf serum and the cells incubated overnight. The cells were then incubated for 2.5 h after addition of either vehicle or test compound to the final desired concentration. The rates of cholesterol and fatty acid synthesis were then measured by the addition of $^3H_2O$, to a specific radioactivity of 71 $\mu$Ci/ mmol, for the final 90 min of the incubation. Incubations were terminated and the rates of cholesterol and fatty acid synthesis determined from the amounts of $^3$H incorporated into cellular cholesterol and fatty acids essentially as described previously.[12]

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