# ATP-Citrate Lyase as a Target for Hypolipidemic Intervention. 2. Synthesis and Evaluation of (3*R**,5*S**)-ω-Substituted-3-carboxy-3,5-dihydroxyalkanoic Acids and Their γ-Lactone Prodrugs as Inhibitors of the Enzyme in Vitro and in Vivo

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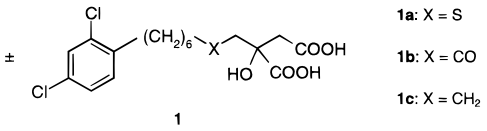
A series of (3*R**,5*S**)-ω-substituted-3-carboxy-3,5-dihydroxyalkanoic acids have been synthesized and evaluated as inhibitors of the recombinant human form of ATP-citrate lyase. The best of these have $K_i$'s in the 200−1000 nM range. As the corresponding thermodynamically favored γ-lactone prodrugs, a number of compounds are able to inhibit cholesterol and fatty acid synthesis in HepG2 cells and reduce plasma triglyceride levels in vivo. The best of these, compound **77**, is able to induce clear hypocholesterolemic and hypotriglyceridaemic responses when administered orally to rat and dog. These results provide evidence to support the hypothesis that compounds which inhibit ATP-citrate lyase have the potential to be a novel class of hypolipidemic agent, which possess combined hypocholesterolemic and hypotriglyceridemic activities.

## Introduction

ATP-citrate lyase (ACL) catalyzes the cleavage of citrate in the cytosol of many tissues and is the principal producer of acetyl CoA units for lipid synthesis.[1] Inhibition of hepatic ACL is expected to decrease both cholesterol and fatty acid synthesis and to precipitate hypotriglyceridemic and hypocholesterolemic responses in animals and human by inhibition of very low-density lipoprotein (VLDL) synthesis and up-regulation of hepatic low-density lipoprotein (LDL) receptors. A more detailed discussion of this concept is presented in earlier communications from these laboratories.[2] Such considerations led us to propose and investigate ACL as a potential target for hypolipidemic intervention. An inhibitor of ACL would be expected to be a novel and therapeutically beneficial class of hypolipidemic agent, which should have advantages over a pure hypocholesterolemic agent, particularly in light of the emerging coimplication of hypertriglyceridemia in coronary heart disease.

The primary goal of our research effort was to identify a potent, orally active compound that would allow us to interrogate the hypothesis that inhibition of ACL in vivo would result in both hypotriglyceridemic and hypocholesterolemic responses. In earlier papers, we have described in detail the mechanism of the enzyme-mediated reaction and the rationale of our unsuccessful efforts toward the design and synthesis of mechanism-based, active-site directed, and tight-binding inhibitors.[2b−e] In the preceding paper, we described our design strategy leading to the identification of a series of 2-substituted butanedioic acids which were reasonably potent inhibitors of the enzyme.[2a] The best of these were compounds **1a**−**c**, which had $K_i$ values against




**1**

both isolated rat and recombinant human forms of the enzyme in the 1−3 μM range. Unfortunately, in a HepG2 cell assay used to investigate effects of compounds on cholesterol and fatty acid synthesis, none of these inhibitors demonstrated any activity. This lack of activity was attributed to a poor or ineffective penetration of the inhibitor into the cells, making the compounds unsuitable for use in vivo. In this paper, we now describe the results of our continued research efforts in this area which has led to the identification of compound **77**, a prodrug of the active hydroxy acid **45**, as a potent, efficacious, and orally active inhibitor of ACL. The pharmacology on **77** is consistent with the hypothesis that inhibition of ACL is a feasible target for hypolipidemic intervention and that this compound, or a related analogue, may have potential for development as a novel hypolipidemic agent.

## Chemistry

**General Preparation of (3*R**,5*S**)-ω-Substituted-3-carboxy-3,5-dihydroxyalkanoic Acid Inhibitors.** Compound **45** (Table 1) and its related analogues of Table 3 were normally prepared by the general route

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**Scheme 1.** General Synthesis of (3*R**,5*S**)-3,5-Dihydroxybutanedioic Acids and Their Corresponding γ-Lactones

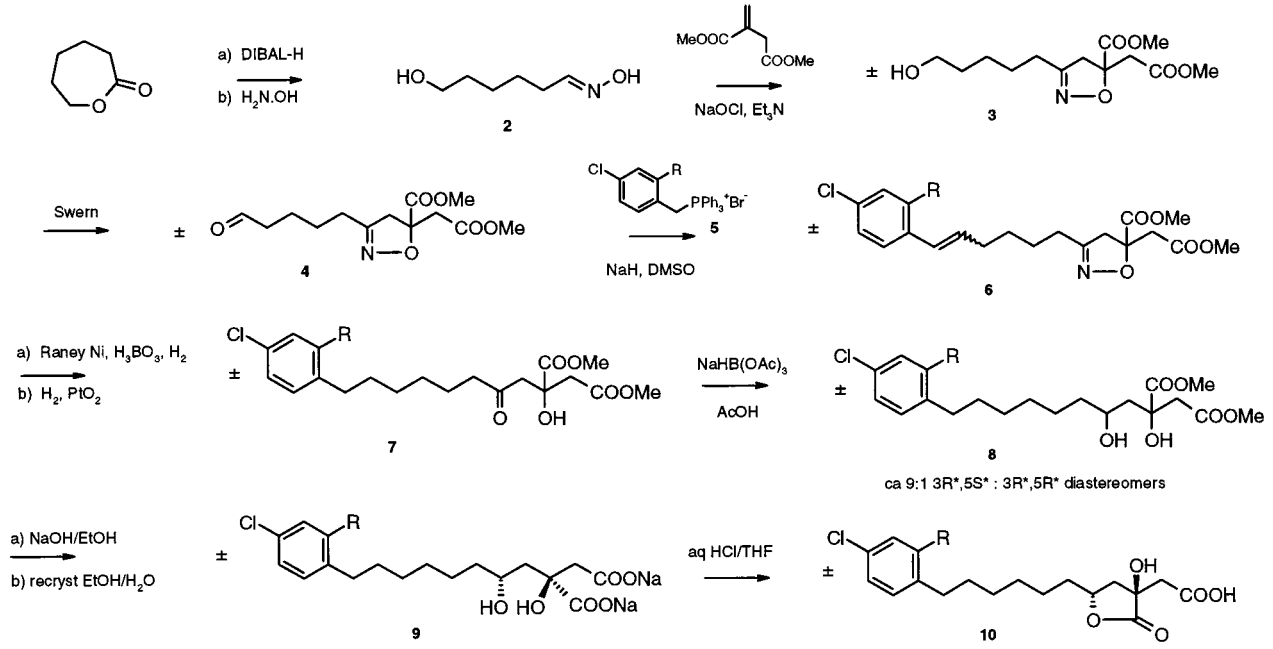
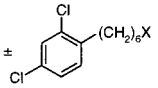
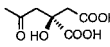
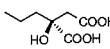
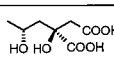
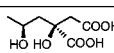
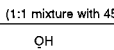
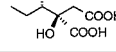
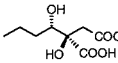
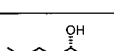



**Table 1.** Effect of Head Hydroxylation

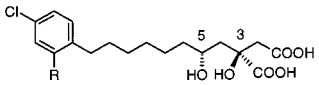


| # | X | $K_i$ (μM) | Analyses[a] |
|---|---|---|---|
| 1b |  | 1.2 ± 0.04 (rat ACL)[b] | |
| 1c |  | 2.9 ± 0.1 (rat ACL)[b] | |
| 45 |  | 1.0 ± 0.1 (rat ACL)<br>1.0 ± 0.1 (hrACL) | C, H |
| 46 |  <br>(1:1 mixture with 45) | 2.0 ± 0.17 | C, H |
| 47 |  | 5.7 ± 0.3 | C, H[c] |
| 48 |  | 18.0 ± 2.0 | C, H[d] |
| 49 |  | 0.62 ± 0.07 | C, H |
| 50 |  | 0.55 ± 0.07 | C, H |

[a] Microanalyses reported for the disodium salts. Most compounds contained varying levels of $H_2O$ of crystallization. [b] See ref 2a. [c] Analysis for free diacid. [d] Microanalysis performed on γ-lactone derivative; lactone hydrolyzed to **48** prior to use.

depicted in Scheme 1. ϵ-Caprolactone was converted to the oxime **2** by treatment with DIBAL-H followed by subsequent reaction of the intermediary hydroxy aldehyde with hydroxylamine. In situ generation of the corresponding nitrile oxide (NaOCl, $Et_3N$) and its subsequent [3 + 2] cycloaddition with dimethyl itaconate[3] afforded the isoxazole intermediate **3**. Swern oxidation generated the aldehyde **4**, a prerequisite compound for reaction with the appropriately substituted phosphonium salt **5** (NaH/DMSO) to give the

**Table 2.** Activities of Resolved Enantiomers of Compounds **45** and **58**



| # | R | stereochemistry | rat ACL $K_i$ (μM) | hr ACL $K_i$ (μM) | Analyses[a] |
|---|---|---|---|---|---|
| (+)-45 | Cl | S-[3R*,5S*] | 0.7 ± 0.07 | 0.88 ± 0.08 | C, H |
| (-)-45 | Cl | R-[3R*,5S*] | 0.7 ± 0.07 | 0.77 ± 0.07 | C, H |
| (+)-58 |  | S-[3R*,5S*] | | 0.58 ± 0.05 | -[b] |
| (-)-58 |  | R-[3R*,5S*] | | 0.34 ± 0.03 | -[b] |

[a] Microanalyses reported for the disodium salts. Most compounds contained varying levels of $H_2O$ of crystallization. [b] Microanalysis not obtained. Purity confirmed by NMR and MECC.

olefin **6**. Hydrogenation in the presence of Raney Ni and boric acid, followed by further hydrogenation of the olefinic bond with platinum oxide, gave the keto compound **7**, without any loss of aryl chlorine substituents. Reduction of this ketone using sodium triacetoxyborohydride in acetic acid gave a ca. 9:1 mixture of the 3*R**,5*S**:3*R**,5*R** diastereomeric diesters **8**. An earlier procedure, producing similar diastereoselectivity, employed the use of $NaBH_4$ in EtOH in the presence of $CeCl_3$, but this suffered from competing ester group reduction. The desired pure 3*R**,5*S** diastereomer of the final hydroxy acid product **9** was obtained following hydrolysis of the diester (NaOH/EtOH) and recrystallization of the resulting disodium salt. The corresponding five-membered ring lactones **10** were prepared as required for secondary screening by heating the hydroxy acids in aqueous HCl or in aqueous HCl/THF. An alternative synthesis of the isoxazole intermediate, proceeding via 2,4-dichloroheptanaldoxime,[2a] was used in earlier preparations of compound **45**.

Methods for preparations of the di- and trihydroxy compounds of Table 1 varied depending upon the position of substitution. The 3*R**,5*R** diastereomer **46**

**Scheme 2.** Syntheses of the Hydroxylated Analogues of Table 1

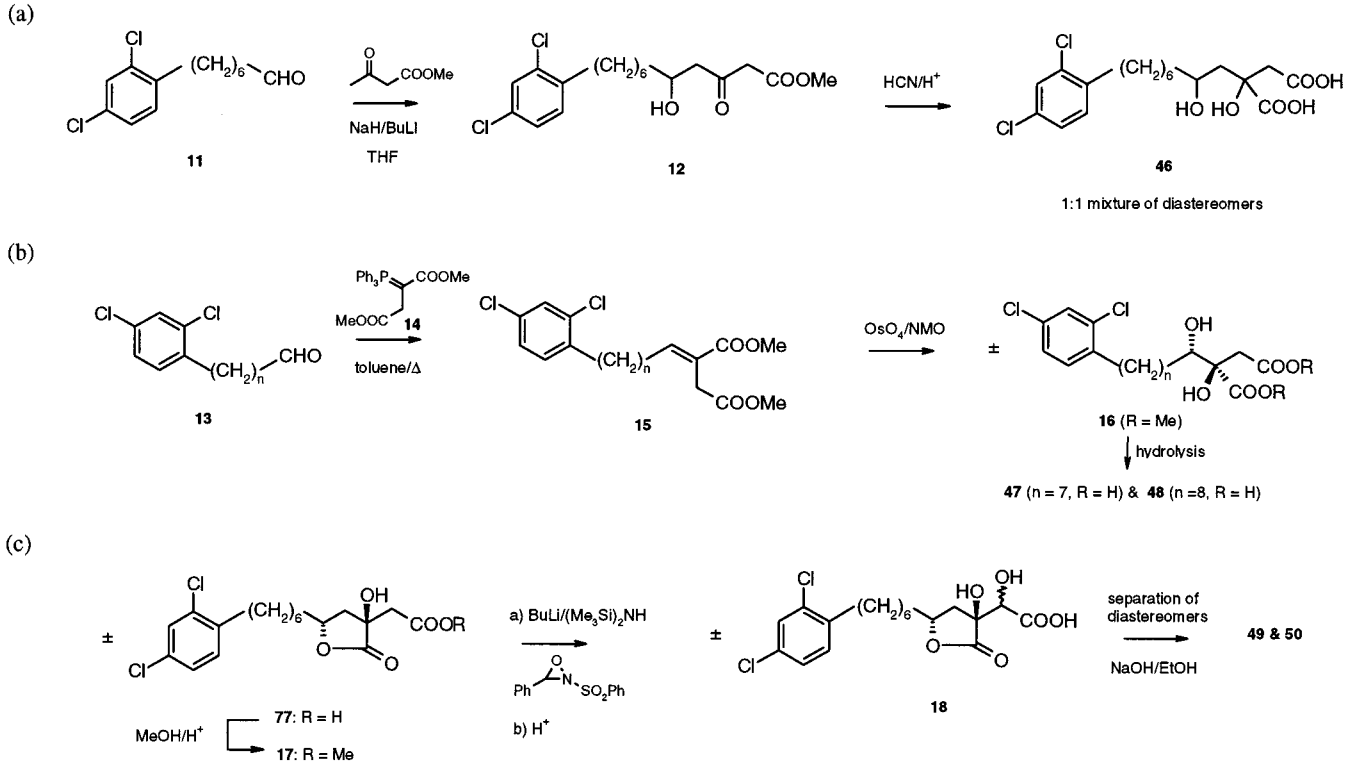
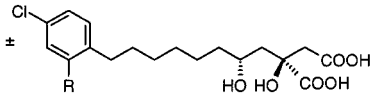



**Table 3.** Effect of Ortho Substitution of the Phenyl Ring



| # | R | $K_i$ (μM) | Analyses[a] |
|---|---|---|---|
| 45 | Cl | 1.0 ± 0.1 | C, H |
| | $R^1$ | | |
| 51 | H | 0.75 ± 0.05 | C, H, Cl |
| 52 | 4-F | 2.7 ± 0.15 | C, H, F[b] |
| 53 | 4-Me | 2.7 ± 0.28 | C, H, Cl |
| 54 | 4-OMe | 5.1 ± 0.61 | C, H |
| 55 | 4-Cl | 5.0 ± 0.53 | C, H, Cl |
| 56 | 2,4-diCl | 21 ± 2.5 | C, H, Cl |
| 57 |  | 3.9 ± 0.25 | C, H, N |
| 58 |  | 0.3 ± 0.02 | C, H, N |
| 59 |  | 67% inhibition @ 100μM | C, H, N |
| 60 | OH | 24% inhibition @ 100μM | C, H[c] |
| 61 | $OCH_3$ | 19 ± 2.2 | C, H |
| 62 | $OCH_2Ph$ | 1.1 ± 0.14 | C, H |

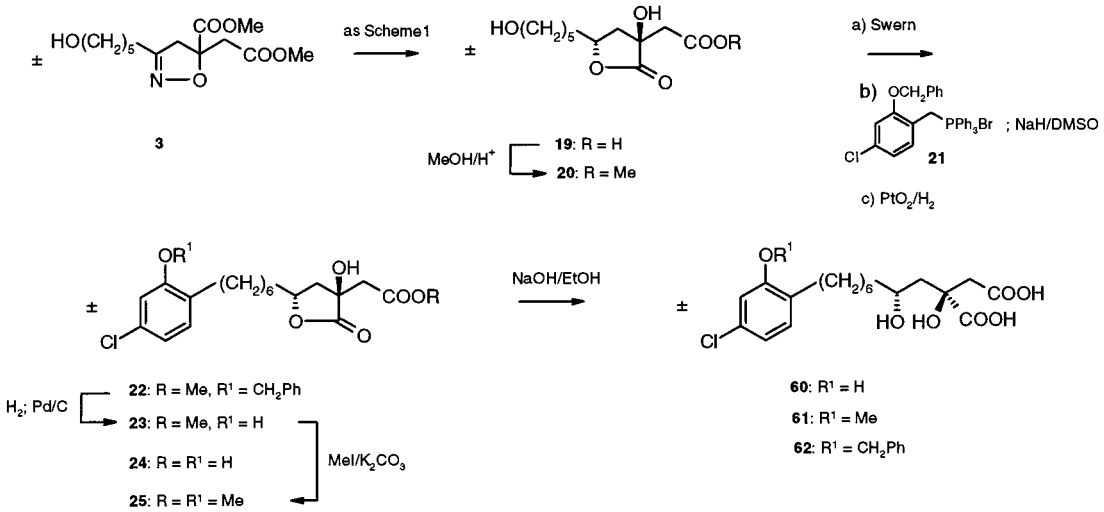
[a] Microanalyses reported for the disodium salts. Most compounds contained varying levels of $H_2O$ of crystallization. [b] Anal. ($C_{24}H_{26}ClFO_6Na_2 \cdot 2.5H_2O$) C, F, H: calcd 5.62, found 4.97%. [c] Microanalysis performed on γ-lactone derivative; lactone hydrolyzed to **60** prior to use.

of compound **45** was prepared as a 1:1 mixture with **45** by the route shown in Scheme 2a. Reaction of the dianion of methyl acetoacetate with the aldehyde **11** gave the keto compound **12** which was converted to the final product via the cyanohydrin as described previously.[2a] The 4-hydroxylated derivative **47**, and its higher homologue **48**, were prepared by the route shown in Scheme 2b. The *E*-olefin **15**, prepared from heating the aldehyde **13**[2a] with the phosphorane **14** in toluene,[4] was dihydroxylated ($OsO_4$/NMO) to yield the desired diacids after hydrolytic workup. These were converted to their respective five-membered ring lactones, as described above, for purification and hydrolyzed back to the diacid prior to enzyme screening. The 2-hydroxy group of compounds **49** and **50** was introduced through the reaction of the anion of the lactone **17** with 2-(benzenesulfonyl)-3-phenyloxaziridine[5] in THF, followed by separation of the resulting mixture of diastereomers and hydrolysis (Scheme 2c).

The ortho O-substituted analogues **60**, **61**, and **62** of Table 3 were prepared by a modification of the standard procedure of Scheme 1. For these, the intermediate isoxazole **3** was first converted to its corresponding lactone **19**, by analogous procedures to those described above, and esterified to give **20**. This was oxidized (Swern) to the aldehyde prior to use and reacted with the phosphonium salt **21** to give **22** (Scheme 3). Simple hydrolysis of **22** gave **62**, while its hydrogenolysis and hydrolysis afforded compound **60**. Methylation of the hydroxy intermediate **23** (MeI/$K_2CO_3$ in DMF) gave the lactone **25** which was hydrolyzed to compound **61**.

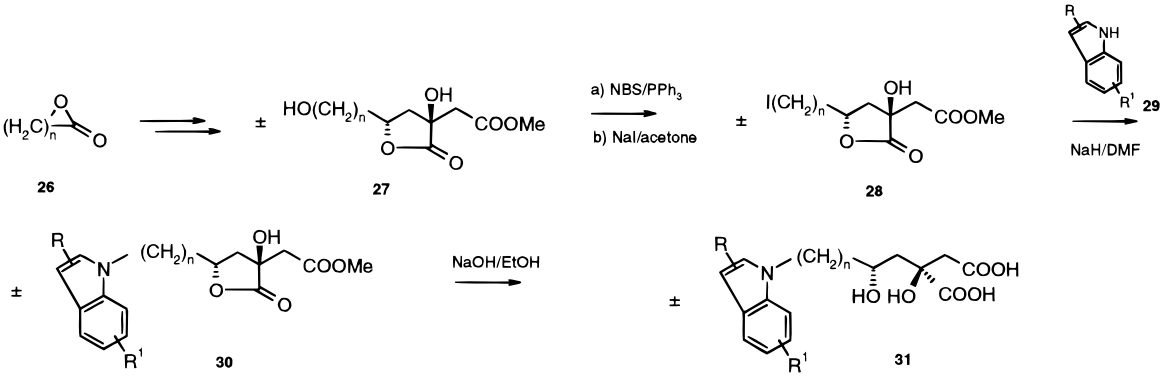
The indole derivatives of Table 4 were prepared via alkylation of the appropriately substituted indole with either the iodolactone **28** using NaH/DMF or the bromoisoxazole **32** using KOH in DMSO (Scheme 4, a and b). An exception was compound **65** for which the indole was alkylated with 7-bromoheptanol to give the alcohol **35**, the latter oxidized under Swern conditions to the aldehyde and this converted to the oxime **36** (Scheme 4c). The standard chemistry from these intermediates, outlined in Scheme 1, was then employed to obtain the

**Scheme 3.** Syntheses of the Ortho-O-Substituted Compounds of Table 3

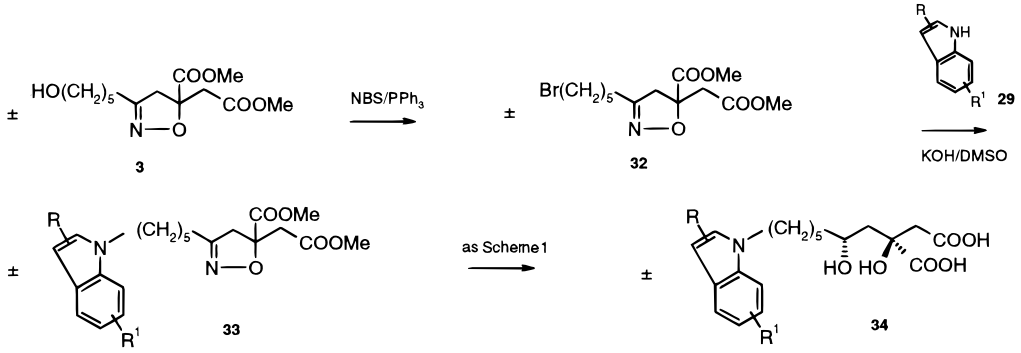



**Scheme 4.** Syntheses of Indole Analogues

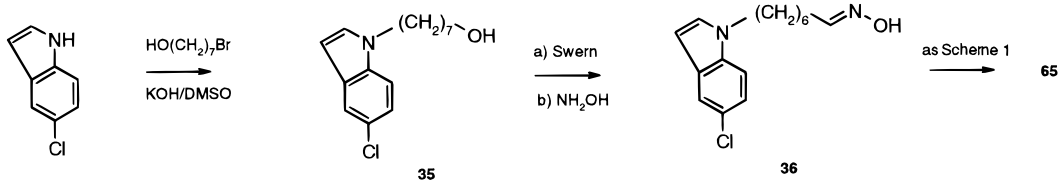
(a) method A




(b) method B

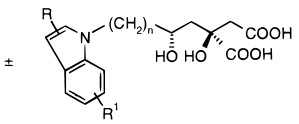



(c) method C




final compounds. The halides, **28** and **32**, were derived from the alcohols **27** and **3**, respectively, by standard procedures. The bromoisoxazole intermediate required for the synthesis of compound **63** was obtained in an identical manner to its $n = 5$ analogue by substituting $\delta$-valerolactone **26** ($n = 4$) for $\epsilon$-caprolactone in the reaction sequence of Scheme 1. An equivalent strategy was used for the synthesis of the carbazole derivatives in Table 5. The fluorene **76** (Table 5) was prepared as a mixture of diastereomers by the route depicted in Scheme 5. For this, 3-chlorofluoren-9-one **37** was reacted with the Grignard reagent derived from 6-benzyloxyhexyl bromide to give **38**, which was hydrogenated (Pd/C in MeOH/aq HCl) to give alcohol **39**. The latter

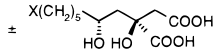
**Table 4.** Indole Derivatives

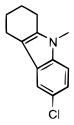
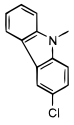
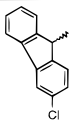


| no. | *n* | R | $R^1$ | $K_i$ ($\mu$M) | method of synthesis[a] | analyses[b] |
|---|---|---|---|---|---|---|
| **63** | 4 | H | 5-Cl | 160 ± 23 | A | C, H, N |
| **64** | 5 | H | 5-Cl | 3.5 ± 0.37 | B | C, H, N |
| **65** | 6 | H | 5-Cl | 3.2 ± 0.32 | C | C, H, N |
| **66** | 5 | H | 5,7-diCl | 1.8 ± 0.2 | B | C, H, N |
| **67** | 5 | H | 6-Cl | 41 ± 3.6 | B | C, H, N, Cl |
| **68** | 5 | 3-$CH_2$Ph | 5-Cl | 2.9 ± 0.2 | B | C, H, N[c] |
| **69** | 5 | 3-Me | 5-Cl | 9.5 ± 0.8 | B | C, H, N[d] |
| **70** | 5 | 3-Ph | 5-Cl | 11 ± 1.0 | B | C, H, N[e] |
| **71** | 5 | 3-$NO_2$ | 5-Cl | 64 ± 10 | A | C, H, N |
| **72** | 5 | 2-Ph | 5-Cl | 0.37 ± 0.03 | A | C, H, N |
| **73** | 5 | 2-Me | 5-Cl | 13 ± 1.2 | A | C, H, N, Cl |

[a] See Scheme 4. [b] Microanalyses reported for the disodium salts. Most compounds contained varying levels of $H_2O$ of crystallization. [c] Analysis for $1H_2O{\cdot}2.4SiO_2$. [d] Analysis for $1H_2O{\cdot}0.6NaOAc$. [e] Analysis for $1H_2O{\cdot}0.8SiO_2$.

**Table 5.** Tricyclic Compounds

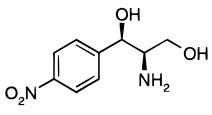


| # | X | $K_i$ (μM) | Analyses[a] |
|---|---|---|---|
| 74 |  | 2.9 ± 0.25 | C, H, N |
| 75 |  | 0.71 ± 0.05 | C, H, N |
| 76[b] |  | 0.22 ± 0.015 | C, H, Cl |

[a] Microanalyses reported for the disodium salts. Most compounds contained varying levels of $H_2O$ of crystallization. [b] 1:1 mixture of 3*R**,5*S**,9′*R** and 3*R**,5*S**,9′*S** diastereomers.

was then converted to the corresponding dihydroisoxazole intermediate by procedures similar to those shown for the indole in Scheme 4c and elaborated to compound **76** in the normal way.

**Resolution of Compounds 45 and 58.** Initial batches (ca. 10 mg) of the resolved enantiomers of compounds **45** and **58** were obtained by chiral HPLC separation of the methyl esters of the corresponding γ-lactones **77** and **80** (Table 6), followed by hydrolysis to the hydroxy acid in the normal way. We later discovered a highly efficient resolution through formation of the diastereomeric salt of the lactone, **77** or **80**, with chloramphenicol base (D-(−)-*threo*-2-amino-3-(4-nitrophenyl)propane-1,3-diol, **40**). This produced the



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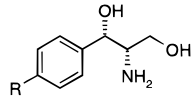
enantiomerically pure (+)-enantiomer from a single crystallization. The (−)-enantiomer was readily obtained in a similar manner by use of the L-(+)-enantiomeric base **41a**. This was readily synthesized by



**41a**: R = $NO_2$, **41b**: R = H

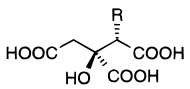
nitration of commercially available (1*S*,2*S*)−(+)-1-phenyl-2-aminopropane-1,3-diol **41b** after first protecting as its triacetyl derivative. A typical resolution gave excellent yields, with ca. 80% isolation of each pure enantiomer from the racemate. Chiral purity of the resultant hydroxy acids after hydrolysis was established by means of a micellar electrokinetic capillary chromatography (MECC) assay developed within our laboratories.[6] X-ray crystallographic analysis on the chloramphenicol base salt of (+)-**77** permitted the assignment of its absolute stereochemistry as 3*S*,5*R*.

## Results and Discussion

The compounds synthesized in this investigation are listed in Tables 1−5. Enzyme inhibitory activities are presented as reversible $K_i$'s for inhibition of human recombinant ACL. The latter superceeded our original screening protocol which employed purified rat enzyme. Where comparisons were made, potent compounds had almost identical $K_i$'s against both forms of the enzyme.

In the preceding paper[2a] we described the design of inhibitors of ACL that was based upon the concept of linking a lipophilic group to a citrate-like "head". The best compounds **1a**−**1c** were 2-hydroxybutanedioic acids substituted in the 2-position with an eight-atom spacer chain linked to a 2,4-dichlorophenyl group. Enzyme kinetic analysis was consistent with the hypothesis that the compounds were interacting with the enzyme by binding of the butanedioic acid moiety to the citrate site, while the aryl ring located an adjacent hydrophobic domain.

(−)-Hydroxycitrate[7,2a] **42a** is a known, naturally oc-



**42a**: R = OH

**42b**: R = $NH_2$

**42c**: R = SH

**42**

curring, competitive inhibitor of ACL.[8] Its $K_i$ (0.15 $\mu$M, rat ACL), compared to the $K_m$ for citrate (100 $\mu$M),[2e] suggests that the hydroxyl group is imparting a specific, favorable interaction to the molecule when binding to the enzyme. Both the corresponding amino (**42b**),[9] ($K_i$ = 11 $\mu$M[10]) and thio (**42c**) ($K_i$ = 58 $\mu$M)[2b] citrate analogues are much less potent rat ACL inhibitors, corroborating this view. As our compounds were citrate competitive inhibitors,[2a] we anticipated that their potency might be improved further by introducing appropriately positioned hydroxyl group(s) in the vicinity of the "citrate-head". Table 1 shows enzyme inhibitory activities for a number of hydroxylated analogues that were prepared for this purpose.

Introduction of a hydroxyl group in the 5-position, as in compound **45**, a single racemic diastereomer whose

**Scheme 5.** Synthesis of the Fluorene **76**

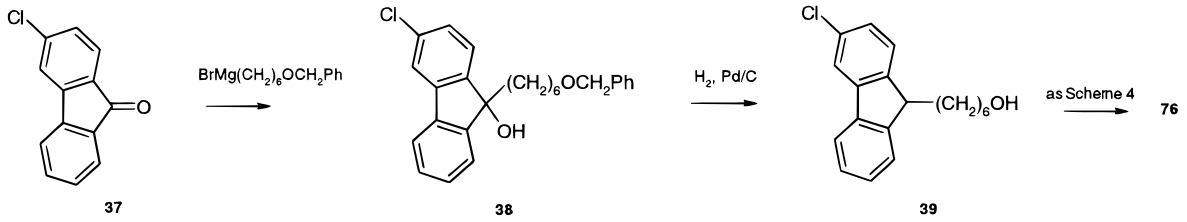
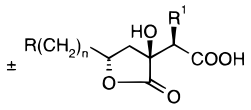



**Table 6.** Compounds Assayed in HepG2 Cells and/or in Vivo in the Rat




| | | | | | HepG2 cells | | | | % reduction of plasma triglycerides in the rat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | R | *n* | $R^1$ | γ-lactone of compd | concn ($\mu$M) | cholesterol synthesis % control | fatty acid synthesis % control | ATP levels % control | 37.5 mg/kg/d | 75 mg/kg/d | analyses |
| **42a** ((−)-hydroxycitrate) | | | | 100 | 110 ± 19 | 87 ± 8 | 100 ± 3 | nd | nd | C, H | |
| | | | | | 300 | 40 ± 6 | 79 ± 11 | 103 ± 3 | | | |
| | | | | | 1000 | 15 ± 9 | 57 ± 4 | 104 ± 5 | | | |
| **45** | | | | | 100 | 84 ± 13 | 99 ± 4 | 104 ± 4 | nd | nd | C, H |
| **77** | 2,4-$Cl_2C_6H_3$ | 6 | H | **45** | 3 | 98 ± 5 | 114 ± 14 | 97 ± 4 | 31 | 56[a] | C, H, Cl |
| | | | | | 10 | 53 ± 7[c] | 74 ± 19[a] | 97 ± 3 | | | |
| | | | | | 30 | 9 ± 2[c] | 18 ± 3[c] | 94 ± 2[a] | | | |
| **(+)-77** | | | | (+)-**45** | 5 | 95 ± 6 | 113 ± 7 | 101 ± 3 | nd | nd | C, H |
| | | | | | 15 | 52 ± 7[c] | 81 ± 9[b] | 100 ± 4 | | | |
| | | | | | 15 | 41 ± 8[c] | 69 ± 7[c] | 98 ± 2 | | | |
| | | | | | 30 | 11 ± 6[c] | 25 ± 7[c] | 99 ± 2 | | | |
| **(−)-77** | | | | (−)-**45** | 5 | 105 ± 13 | 123 ± 8 | 101 ± 6 | nd | nd | C, H |
| | | | | | 15 | 37 ± 7[c] | 90 ± 9 | 99 ± 5 | | | |
| | | | | | 15 | 33 ± 7[c] | 82 ± 8[b] | 98 ± 3 | | | |
| | | | | | 30 | 9 ± 3[c] | 39 ± 3[c] | 98 ± 2 | | | |
| **78** | 2,4-$Cl_2C_6H_3$ | 6 | OH | **50** | 1 | | | 103 ± 10 | nd | nd | C, H |
| | | | | | 3 | 69 ± 13[a] | 74 ± 11 | 100 ± 6 | | | |
| | | | | | 10 | 86 ± 69 | 76 ± 13 | 106 ± 7 | | | |
| **79** | 2-Ph,4-$ClC_6H_3$ | 6 | H | **51** | 3 | 101 ± 28 | 108 ± 4 | 98 ± 3 | nd | nd | C, H |
| | | | | | 10 | 65 ± 9[b] | 75 ± 9[b] | 96 ± 2 | | | |
| | | | | | 10 | 60 ± 6[c] | 88 ± 6[a] | 100 ± 4 | | | |
| | | | | | 30 | nd | nd | 95 ± 6 | | | |
| **80** | 2-(3,4-dimethylpyrrol-1-yl)-4-$ClC_6H_3$ | 6 | H | **58** | 3 | 104 ± 13 | 102 ± 8 | 98 ± 4 | 10 | 20 | C, H, N |
| | | | | | 10 | 82 ± 9[a] | 75 ± 8[b] | 96 ± 5 | | | |
| | | | | | 10 | 82 ± 5[a] | 89 ± 2 | 101 ± 4 | | | |
| | | | | | 28.2 | nd | nd | 96 ± 4 | | | |
| **81** | 2-($C_6H_5OCH_2$)-4-$ClC_6H_3$ | 6 | H | **62** | nd | nd | nd | nd | 22 | 34[a] | C, H |
| **82** | 3-Cl-carbazol-9-yl | 5 | H | **75** | nd | nd | nd | nd | 16 | 33[a] | C, H, N |
| **83** | 3-Cl-fluoren-9-yl | 5 | H | **76** | nd | nd | nd | nd | 5 | 16 | C, H |

[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$. nd, not determined.

relative stereochemistry has been determined as 3*R**,5*S** by X-ray crystallographic analysis, gives a marginal increase in potency relative to **1c**. Although the 3*R**,5*R** diastereomer **46** could not be independently synthesized, the $K_i$ of 2 $\mu$M for the 1:1 mixture of diastereomers, relative to a $K_i$ of 1 $\mu$M for **45**, indicates that the 3*R**,5*R** diastereomer is at least a 5−10-fold less active inhibitor. Introduction of the hydroxyl group α to the tertiary center gives compounds which can be considered to have a hydroxycitrate-like, rather than a citrate-like, head linked to the lipophilic group. When the OH group was located on the lipophilic group side (4-position) in compound **47**, activity was reduced 5−6-fold compared to compound **45**. Relocation of the hydroxyl group to the 2-position was easiest examined in derivatives of **45**. However, both of the possible diastereomers, **49** and **50**, showed no distinct advantages in potency over **45**, and certainly not the desired 1−2 orders of magnitude difference in affinity demonstrated by compound **42a** over citrate.

Even so, compound **45** was one of our most potent inhibitors of the enzyme. Like **1a**, the compound demonstrates an essentially competitive type of inhibition with respect to citrate.[2a] Not surprisingly, like our earlier compounds,[2a] it too was ineffective in the HepG2 cell at inhibiting cholesterol or fatty acid synthesis at concentrations up to 100 $\mu$M (Table 6). However, this compound has the potential for lactonization to either a five- or six-membered ring lactone which could serve as a prodrug for the active hydroxy acid form of the inhibitor. Lactonization of **45** in 25% aqueous HCl or aqueous HCl/THF, at ca. 60 °C, gave exclusively the thermodynamically favored five-membered ring lactone **77**.[11] The latter, as expected, was inactive as an inhibitor of the isolated enzyme. Encouragingly, compound **77** was found to be efficacious as an inhibitor of de novo lipid synthesis in HepG2 cells. The $IC_{50}$'s for inhibition of de novo cholesterol and fatty acid synthesis were in the 10−30 $\mu$M range (Table 6).[12] At higher concentrations (>30 $\mu$M), in the absence of albumin, the

compound was found to be toxic to the cells, as judged by the effect on cellular ATP levels. The concentration of (−)-hydroxycitrate **42a** required to produce an equivalent reduction in lipid synthesis was at least 10-fold greater (Table 6). The results are thus consistent with the lactone facilitating entry into the cell where hydrolysis to the active hydroxy acid form of the inhibitor occurs.

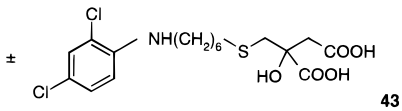
Encouraged by the results presented above, we continued our SAR investigation in the hydroxy acid/lactone class of inhibitor, represented by **45**/**77**, to identify the best candidate to evaluate in vivo.

**Enantiomers of 45.** Both the (+)- [3*S*,5*R*] and (−)-[3*R*,5*S*] enantiomers of compound **45** demonstrated equivalent potency against both the rat and human recombinant enzyme (Table 2). Their corresponding lactones, (+)-**77** and (−)-**77**, repectively, demonstrated no clear differences in efficacy on de novo lipid synthesis in HepG2 cells (Table 6). In a similar fashion, the separate enantiomers of compound **58** (see later) were both potent inhibitors of hrACL (Table 2). Considering the stereospecific requirement for exclusive modification of the citrate *pro-S* carboxylate in the ACL-catalyzed cleavage reaction,[2b,13] and the deductions that this class of inhibitor is binding to the citrate site of the enzyme,[2a] it is surprising that both enantiomers are equally effective inhibitors. However, this outcome allowed us to evaluate the single diastereomeric, but racemic, analogues in our subsequent SAR studies.

**Further SAR.** In a preceding paper, in analogues of compound **1a**, we established a requirement for a 2,4-disubstituted aryl ring, with a preference for a lipophilic halogen substituent in the para position.[2a] Less clear was the scope for modifying the ortho substituent, although the limited number investigated suggested that more diverse substituents could be incorporated in this position. For example, both *o*-phenyl and *o*-nitro gave improvements in activity over the unsubstituted parent. Continuation of this study in the hydroxy acid class of inhibitor established that the 2-Ph, 4-Cl substitution pattern in **51** gave a compound equipotent with **45**. However, further substitution in the second phenyl ring led to decreases in activity (Table 3), which may be related to the increased bulk of the substituent. The scope for greater diversity in this substituent was also demonstrated with the benzyloxy substituent in compound **62**. A role for the benzyl group in increasing the affinity of the inhibitor is indicated by the order of magnitude improvement in activity relative to a methoxy group in this position in compound **61**.

We considered that a pyrrol-1-yl group might approximate as a hybrid of a phenyl ring and nitro group. This group is reasonably lipophilic ($\pi$ = 0.95) and electron withdrawing ($\sigma_p$ = 0.37, cf. $NO_2$ = 0.78) on a phenyl ring.[14] Encouragingly, the prototype **57** was a reasonably potent inhibitor ($K_i$ = 3.9 $\mu$M). An order of magnitude improvement in potency was achieved on increasing lipophilicity, by the introduction of methyl groups in the 3- and 4-positions of the pyrrole ring in compound **58**. This activity was abolished by relocation of the methyl groups to the α-position in compound **59**, suggesting that the two aryl rings need to lie coplanar in ortho biaryl systems of this type.

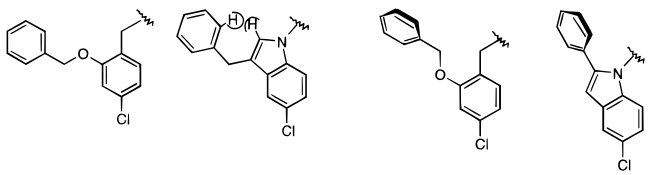
In a complementary approach, we investigated some N-linked indole analogues as potential inhibitors of the enzyme. These were regarded as conformationally restricted derivatives of a moderately potent aniline **43**




**43**

(rat ACL, $K_i$ = 12 $\mu$M) which was prepared in the earlier S-linked series of compounds.[2a] The compounds synthesized are listed in Table 4. The conformational restriction imposed by the indole nucleus in **64** did indeed confer an improvement in activity relative to the corresponding aniline **43**. The critical nature of the chain length linking the head to the aryl group was further emphasized with the dramatic loss of activity of the lower homologue **63**. Introduction of a second chlorine into the indole **66** made only a marginal difference to activity, unlike the case for compound **45**, suggesting that the indole 3-position is likely to correspond to the normal ortho position in the latter compound. On the basis of this analysis, substitution in the 3-position was investigated as a means of improving activity. However, the compounds in Table 4 indicate that this strategy is not favored, as can be seen from the lower activity of the 3-methyl (**69**), phenyl (**70**), and nitro (**71**) analogues. Nevertheless, the 3-benzyl substituent in compound **68** overcomes this and restores activity to the level of the parent compound. This suggests that there may indeed be an additional binding area for the aryl ring, as discussed earlier for the benzyloxy compound **62**. Thus, identifying ways to correctly position the phenyl ring was pursued as a strategy which had high expectations for significantly improving activity.

On comparing compounds **68** and **62**, most low energy conformations of the flexible benzyloxy group in **62** can be mimicked very well by **68**. An exception is an extended all planar conformation (**44a**) of the benzyloxy




**44**

group of **62** which would be a relatively high energy one for **68**, due to steric interaction between the phenyl and indole rings (**44b**). An alternative, but probably higher energy conformation of the benzyloxy group is that represented by **44c**. This is best mimicked in indole compounds by substitution in the 2-position (e.g., **44d**). This proved to be the case when the 2-phenyl group in compound **72** increased activity to give the most potent compound in the series ($K_i$ = 0.37 $\mu$M). This is ca. 30 times more potent than the corresponding 3-phenyl analogue and 1 order of magnitude better than the unsubstituted parent. By comparison with the 2-methylindole, **73** ($K_i$ = 13 $\mu$M), it is most likely that the phenyl group is improving activity by making an ad-

ditional binding interaction, rather than by its ability to control the linking side chain conformation.

By regarding 2,3-ring fused indoles as conformationally restricted analogues of the *o*-biaryl inhibitors, we decided to prepare the tricyclic carbazole and fluorene compounds **74**−**76** (Table 5). From this study, the fluorene **76** ($K_i$ = 0.22 $\mu$M) was identified as the most potent of all the inhibitors of hrACl that we had synthesized.

## Biology

Compounds with $K_i$'s ≤ 1 $\mu$M (**50, 51, 58, 62, 75, 76**) were converted to their respective five-membered ring lactones **78**−**83** and compared with **77** in their efficacy in HepG2 cells at inhibiting de novo lipid synthesis and/or in their ability to reduce plasma triglyceride concentrations in the rat after oral administration.[15] The latter in vivo screen was established to determine the ability of compounds to reduce plasma triglyceride concentrations 4 h after the second of two doses of compound given 24 h apart. No compounds were more active than compound **77** in the HepG2 cell assay (Table 6). Although a number of compounds did demonstrate hypolipidemic activity in the rat in vivo assay, none were more potent than **77** (Table 6).

**Pharmacology of 77.** Compound **77** was chosen for more detailed pharmacological evaluation in the rat and the dog. The results of these studies will be reported in detail elsewhere, so are only briefly outlined in this paper.[16] After oral administration to rats, we established that the compound is efficiently absorbed by the liver and converted to the corresponding hydroxy acid **45**. Feeding of **77** to rats for a period of 7 days, in amounts of 0.05 to 0.25% w/w in the diet, resulted in a dose-dependent decrease in plasma cholesterol (up to 46%) and triglycerides (up to 80%). The rate of VLDL triglyceride synthesis also decreased, by up to 48%, suggesting that a decrease in the rate of VLDL production contributed to the observed hypolipidemic response. Administration of **77** to dogs, once a day at doses of 25 mg/kg/day for 15 days, further confirmed the hypolipidemic properties of the compound. At this dose, plasma cholesterol and triglyceride concentrations were decreased by up to 23% and 38% respectively.

## Summary and Conclusions

A series of (3*R**,5*S**)-$\omega$-substituted-3-carboxy-3,5-dihydroxyalkanoic acids have been synthesized as inhibitors of ATP-citrate lyase and their SAR investigated. The best compounds have $K_i$'s for inhibition of human recombinant enzyme in the 200−1000 nM range. Due to limited cellular penetration, compounds of this structural class are ineffective in reducing cholesterol or triglyceride synthesis in cell-based systems. However, as their internal, thermodynamically favored, $\gamma$-lactone prodrugs, a number of compounds demonstrate activity in HepG2 cells and/or are able to reduce plasma triglyceride levels in rats after oral administration. The best of these, compound **77**, is able to induce clear hypocholesterolemic and hypotriglyceridemic responses when administered orally to rat and dog. The biological data on compound **77** is consistent with our hypothesis that inhibition of ACL in vivo will result in combined hypocholesterolemic and hypotriglyceridemic effects. Inhibitors of ATP-citrate lyase, like compound **77,** thus have the potential to be a novel class of hypolipidemic agent.

## Experimental Section

**General Procedures.** Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. Elemental analyses were measures in the Analytical Science Department of SmithKline Beecham and are within 0.4% of theoretical values unless otherwise indicated. $^1$H NMR spectra were determined on Brucker AM250 or AM360 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. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. All structural assignments were consistent with NMR, IR, and mass spectra.

**Original Procedure for the Preparation of (±)-(3*R**,5*S**)-3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (45).** Sodium borohydride (1.47 g, 38.8 mmol) was added in portions to a stirred solution of (±)-methyl 11-(2,4-dichlorophenyl)-3-hydroxy-3-(methoxycarbonyl)-5-oxoundecanoate[2a] (16.0 g, 36.9 mmol) and cerium(III) chloride heptahydrate (14.4 g, 38.8 mmol) in methanol (200 mL) at 0 °C. The solution was stirred for 0.5 h and then quenched with aqueous HCl. The mixture was diluted with water, and extracted with ether. The extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$), and the solvent was removed under vacuum. Aqueous NaOH (1.5 M, 100 mL, 150 mmol) was added to a stirred solution of the crude hydroxy ester in ethanol (100 mL) at 0 °C. The mixture was stirred for 4 h, diluted with ethanol (200 mL), and filtered. The solid was recrystallized (aqueous ethanol) to give the title compound (11.9 g, a monohydrate, 69%) as a white solid, mp indeterminate: $^1$H NMR ($D_2O$, 250 MHz) $\delta$ 7.51 (s, 1H) and 7.30 (s, 2H) (Ar H's), 3.90 (m, 1H, C*H*OH), 2.69 and 2.49 (doublets, 1H each, $J$ = 15.6 Hz, C$H_2$-$CO_2$Na), 2.72 (t, 2H, $J$ = 7.5 Hz, Ar C$H_2$), 1.81 (d, 2H, $J$ = 5.7 Hz, C$H_2$C(OH)($CO_2$Na)), 1.6 (m, 2H, C$H_2$CH) and 1.7−1.5 (m, 8H, (C$H_2$)$_4$). Anal. ($C_{18}H_{22}Cl_2O_6Na_2$·$H_2O$) C, H.

**Typical Procedure for the Preparation of (3*R**,5*S**)-$\omega$-Substituted-3-carboxy-3,5-dihydroxyalkanoic Acids: (±)-(3*R**,5*S**)-3-Carboxy-3,5-dihydroxy-11-[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]undecanoic Acid, Disodium Salt (58). (i) [[4-Chloro-2-(3,4-dimethylpyrrol-1-yl)-phenyl]methyl]triphenylphosphonium Bromide (5, R = 3,4-dimethylpyrrol-1-yl).** 4-Chloro-2-(3,4-dimethylpyrrol-1-yl)benzoic acid[17a] was converted to its methyl ester ($CH_2N_2$ in $Et_2O$) and reduced to the benzyl alcohol using DIBAL, and this was converted to 4-chloro-2-(3,4-dimethylpyrrol-1-yl)benzyl bromide (NBS/$PPh_3$ in $CH_2Cl_2$) by standard procedures (e.g., ref 2a). A solution of this bromide (1.76 g, 5.9 mmol) and triphenylphosphine (1.54 g, 5.9 mmol) in acetonitrile (30 mL) was heated at reflux for 7 h. The mixture was concentrated and the residual oil triturated with ether and then filtered to give the title compound as a white solid (3.00 g, 95%).

**(ii) (±)-5-(Carbomethoxymethyl)-3-(5-hydroxypentyl)-5-(methoxycarbonyl)-4,5-dihydroisoxazole (3).** Diisobutylaluminum hydride (1.0 M in $CH_2Cl_2$, 125 mL) was added by cannula to a vigorously stirred solution of $\epsilon$-caprolactone (13.0 g, 0.114 mol) in $CH_2Cl_2$ (100 mL) under argon at −78 °C. After 20 min water (41 mL) was added and the mixture allowed to warm to room temperature. Ethyl acetate was added to make the viscous mixture stirrable followed by solid sodium bicarbonate and this mixture was stirred for 10 min, filtered through Celite, and concentrated to give an oil. This crude lactol was dissolved in ether (200 mL), and solid hydroxylamine hydrochloride (25.30 g, 0.364 mol) was added followed by slow addition of aqueous sodium carbonate (21.2 g, 0.200 mol in 100 mL) and the mixture stirred vigorously for 3 h. Sodium chloride was added to saturate the aqueous layer and the ethereal layer separated. The aqueous layer was further extracted with 1-butanol (3×), and all nonaqueous fractions were then combined, washed with brine (1×), dried ($MgSO_4$),

and concentrated. The residue was azeotroped with toluene to remove traces of 1-butanol to give the crude oxime intermediate. Sodium hypochlorite (15% aqueous solution, 175 mL) was added dropwise to a stirred mixture of crude oxime, dimethyl itaconate (22.1 g, 0.140 mL), triethylamine (1 mL, 0.0136 mol), $CH_2Cl_2$ (100 mL), and water (20 mL), controlling the temperature at ambient during addition with a water bath. After 1 h, the reaction mixture was filtered through Celite and the organic layer separated. The aqueous layer was extracted with $CH_2Cl_2$ (2×), and the combined organic extracts were then washed with water (1×) and brine (1×), dried ($MgSO_4$), and concentrated. The residue was dissolved in ether and then poured into 3 volumes of petroleum ether (40−60 °C). After standing, the supernatant was decanted and discarded and the precipitation procedure applied for a second time to the residual oil. The residue was dissolved in toluene, filtered through silica, washing well with 50% ether/petroleum ether (40−60 °C), followed by ether and then ethyl acetate, to yield the title compound on concentration as a colorless oil (22−58 g, 69%).

**(iii) (±)-5-(Carbomethoxymethyl)-5-(methoxycarbonyl)-3-(5-oxopentyl)-4,5-dihydroisoxazole (4).** Dry DMSO (1.1 mL, 0.0156 mol) was added dropwise to a stirring solution of oxalyl chloride (0.99 g, 0.0078 mol) in $CH_2Cl_2$ (15 mL) at −78 °C under argon. After 10 min, **3** (1.6 g, 0.056 mol) in $CH_2Cl_2$ (15 mL) was added, and stirring was continued for 1 h at −78 °C. Triethylamine (3.4 mL, 0.024 mol) was added, and after 10 min the reaction was allowed to warm to room temperature, poured into 1 M aqueous HCl, and extracted with $CH_2Cl_2$ (2 × 25 mL). The combined organic extracts were washed with water (2×) and brine (1×), dried ($MgSO_4$), and concentrated to give the title compound as a yellow oil, which was used directly for next stage.

**(iv) (±)-5-(Carbomethoxymethyl)-3-[6-[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]hex-5-enyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole (6, R = 3,4-dimethylpyrrol-1-yl).** Sodium hydride (60% dispersion in oil, 0.23 g, 0.058 mol) was stirred with dry DMSO (15 mL) at 80 °C under argon until hydrogen evolution had ceased. The solution was placed in an ice bath, a solution of [[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]methyl]triphenylphosphonium bromide (3.04 g, 0.056 mol) in dry DMSO (25 mL) was added, and the mixture was then allowed to warm to room temperature and stir for 30 min. After the mixture was recooled to 0 °C, a solution of **4** (0.056 mol) in dry DMSO (10 mL) was added and the mixture stirred overnight at room temperature. The reaction was poured into 1 M HCl and extracted with ether (3 × 25 mL). The combined ether extracts were washed with $H_2O$ (3×) and brine (1×), dried ($MgSO_4$), and concentrated to give a yellow oil. This oil was purified by silica gel chromatography (40% ether/petroleum ether (40−60 °C)) to give title compound as a yellow oil (mixture of *E* and *Z* isomers) (1.58 g, 58%).

**(v) (±)-Methyl 11-[4-Chloro-2-(3,4-dimethylpyrrol-1-yl)-phenyl]-3-hydroxy-3-methoxycarbonyl-5-oxoundecanoate (7, R = 3,4-Dimethylpyrrol-1-yl).** A solution of (±)-5-(carbomethoxymethyl)-3-[6-[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]hex-5-enyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole (1.64 g, 0.0034 mol) and boric acid (0.83 g, 0.014 mol) in methanol/water (25 mL:3 mL) over Raney nickel (0.02 g) was shaken under hydrogen for 3 h. The reaction mixture was filtered through Celite, poured into water, and extracted with ethyl acetate (3 × 25 mL). The organic solution was washed with brine (1×), dried ($MgSO_4$), and concentrated to give an oil. This crude product was dissolved in methanol (30 mL), $Pt_2O$ (ca. 0.05 g) was added, and this mixture was shaken under hydrogen at 50 psi for 1.5 h. The mixture was filtered through Celite and concentrated to give a colorless oil. The title compound was obtained by silica gel chromatography (40% ether/petroleum ether (40−60 °C)) as a colorless oil (1.08 g, 65%).

**(vi) (±)-(3*R**,5*S**)-3-carboxy-3,5-dihydroxy-11-[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]undecanoic Acid, Disodium Salt (58).** Solid sodium borohydride (0.32 g, 8.5 mmol) was added in portions to acetic acid (6 mL) at 0 °C. After addition the mixture was left stirring at room temperature for 20 min. To this mixture (recooled to 0 °C) was added a solution of (±)-methyl 11-[4-chloro-2-(3,4-dimethylpyrrol-1-yl)phenyl]-3-hydroxy-3-(methoxycarbonyl-5-oxoundecanoate (1.05 g, 2.13 mmol) in acetic acid (6 mL), and this mixture was allowed to stir for 1 h at room temperature. The mixture was poured into ice water and extracted with ether (3 × 10 mL). The combined ether extracts were washed with water (1×) and brine (1×), dried ($MgSO_4$), and concentrated to give a colorless oil. Aqueous sodium hydroxide (0.26 g, 6.4 mmol in 3 mL) was added to the crude hydroxy ester in ethanol (25 mL) at 0 °C. The resulting mixture was left to stir overnight at room temperature, diluted with ethanol (25 mL), and filtered. The solid was recrystallized from aqueous ethanol to give the title compound as a white solid (0.74 g, 68%): NMR ($D_2O$, 250 MHz) $\delta$ 7.30 (m, 2H) and 7.16 (s, 1H) (Ar H's), 6.58 (s, 2H, pyrrole-H's), 3.87 (m, 1H, C*H*OH), 2.66 (doublet, 1H, $J$ = 15.6 Hz) and 2.49 (m 3H) ($CH_2CO_2Na$ + $ArCH_2$), 2.03 (s, 6H, 2 × $CH_3$), 1.81 (br s, 2H, $CH_2C(OH)(CO_2Na)$), 1.38−1.17 (m, 10H, $(CH_2)_5$). Anal. ($C_{24}H_{30}ClNO_6Na_2 \cdot 1.6H_2O$) C, H, N.

**Typical Procedure for Preparation of γ-lactones: (±)-(3*R**,5*S**)-3-(Carboxymethyl)-5-[6-(2,4-dichlorophenyl)-hexyl]-3-hydroxytetrahydrofuran-2-one (77).** A mixture of **45** (disodium salt) (11.8 g, 25.1 mmol), aqueous HCl (3 M, 200 mL), and tetrahydrofuran (200 mL) was heated at 60 °C for 6 h and then cooled. Most of the tetrahydrofuran was removed under vacuum. The residual 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 vacuum, and the residue was recrystallized (ether/petroleum ether (40−60 °C)) to give the title compound (8.80 g, 90%) as a white solid: mp 87−89 °C; $^1$H NMR (DMSO-$d_6$, 360 MHz) $\delta$ 7.55 (s, 1H) and 7.36−7.39 (m, 2H) (Ar H's), 4.53 (m, 1H, C*H*), 2.65−2.75 (m, 4H, $ArCH_2$ + $CH_2COOH$), 2.26 (dd, 1H, $J$ = 13.4, 5.6 Hz) and 2.06 (dd, 1H, $J$ = 13.4, 9.8 Hz) (ring $CH_2$), 1.62−1.54 (m, 4H) and 1.34 (br m, 6H) $(CH_2)_5$); MS (FAB positive ion) *m/z* 389 ($(M + H)^+$); IR (KBr) 3297, 1759, 1707 $cm^{-1}$. Anal. ($C_{18}H_{22}Cl_2O_5$) C, H.

**(Phenylmethyl)triphenylphosphonium Bromides.** The intermediate phosphonium salts (**5**), whose syntheses are not described herein, which were required for the syntheses of the compounds of Table 3, were prepared in three steps from the appropriately substituted alkyl benzoates, by analogous procedures to those described above. Those containing an ortho (substituted) phenyl group were readily prepared by Suzuki coupling of the corresponding triflate with the appropriate boronic acid using standard procedures. The 2-(pyrrol-1-yl)-4-chlorobenzoates were prepared from the corresponding 2-amino-4-chlorobenzoates by standard literature procedures.[17]

**Synthesis of (±)-(3*R**,5*R**)-3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (46) (as a 1:1 Mixture with Its 3*R**,5*S** Diastereomer, 45). (i) 7-(2,4-Dichlorophenyl)-1-heptanal (11).** Swern oxidation of 7-(2,4-dichlorophenyl)-1-heptanol[2a] as described for **4** gave the title compound which was used without further purification.

**(ii) (±)-Methyl 11-(2,4-Dichlorophenyl)-5-hydroxy-3-oxoundecanoate (12).** Methyl acetoacetate (0.247 mL, 2.29 mmol) was added dropwise to a stirred suspension of sodium hydride (61 mg, mass after removal of mineral oil, 2.52 mmol) in THF (2 mL) at 0 °C under argon. The solution was stirred for 0.5 h at room temperature and cooled to 0 °C, and *n*-butyllithium (2.5 M in hexanes, 1.01 mL, 2.52 mmol) was added. This solution was stirred for 0.25 h at room temperature and cooled to −78 °C, and a solution of the crude aldehyde in tetrahydrofuran (3 mL) was added. The mixture was stirred for 0.3 h at −78 °C, allowed to warm to room temperature, poured into 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 vacuum, and the residue was purified by chromatography on silica gel (60−100% ether/petroleum ether (40−60 °C)) to give the title compound (563 mg, 79%) as an oil.

**(iii) 1:1 Mixture of (±)-(3*R**,5*R**)- and (±)-(3*R**,5*S**)-3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (46).** **12** was converted to a 1:1 mixture of the title compound diastereomers via cyanohydrin formation ($KCN/NaH_2PO_4$) and acid hydrolysis, as described previously.[2a] Anal. ($C_{18}H_{22}Cl_2O_6 \cdot NNa_2 \cdot 1.3H_2O$) C, H.

**Procedure for the Syntheses of 47 and 48: Synthesis of 48. (i) (*E*)-Methyl 12-(2,4-Dichlorophenyl)-3-(methoxycarbonyl)-3-dodecenoate (15, *n* = 8).** Swern oxidation, analogous to **11**, of 9-(2,4-dichlorophenyl)-1-nonanol (2.00 g, 6.91 mmol) (see ref 2a for a typical synthesis) gave the corresponding crude aldehyde, which was heated in toluene (25 mL) with 1,2-bis(methoxycarbonyl)ethylenetriphenylphosphorane (**14**) (4.21 g, 10.4 mmol) at 100 °C for 24 h and then cooled. The solvent was removed under vacuum, and the residue was purified by chromatography on silica gel (10−40% ether/petroleum ether (40−60 °C)) to give the title compound (2.46 g, 86%) as an oil.

**(ii) (±)-(3*R**,4*S**)-Methyl 12-(2,4-Dichlorophenyl)-3,4-dihydroxy-3-(methoxycarbonyl)dodecanoate (16, *n* = 8).** A mixture of (*E*)-methyl 12-(2,4-dichlorophenyl)-3-(methoxycarbonyl)-3-dodecenoate (1.50 g, 3.61 mmol), osmium tetroxide (0.229 mL of a 2% solution in *tert*-butyl alcohol, 0.018 mmol), *N*-methylmorpholine *N*-oxide (634 mg, 5.42 mmol), water (2 mL), and acetone (2 mL) was stirred at room temperature for 64 h. Aqueous $NaHSO_3$ (1.1 M, 6 mL) was added, and the mixture filtered, after 20 min, through a plug of silica gel. The silica gel plug was washed with ether, and then the filtrate was washed with aqueous HCl, water, and saturated aqueous NaCl. After drying ($MgSO_4$), the solvent was removed under vacuum, and the residue was purified by chromatography on silica gel (50−100% ether/petroleum ether (40−60 °C)) to give the title compound (1.33 g).

**(iii) (±)-(4*R**,5*S**)-4-Carboxy-5-[8-(2,4-dichlorophenyl)-octyl]-4-hydroxytetrahydrofuran-2-one (γ-Lactone of 48).** (±)-(3*R**,4*S**)-Methyl 12-(2,4-dichlorophenyl)-3,4-dihydroxy-3-(methoxycarbonyl)-3-dodecanoate (0.95 g, 2.11 mmol) was heated under reflux in aqueous HCl (7.7 M) for 3.5 h. The mixture was cooled, diluted with water, and extracted with ether. The extracts were washed with aqueous NaOH, and then the aqueous extracts were washed with ether, acidified (aqueous HCl), and extracted again with ether. The combined extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under vacuum. The crude diacid was stirred in ether with silica gel (5 g) impregnated with 0.5 mL of 2 M aqueous $H_2SO_4$ at room temperature for 18 h, and then the mixture was filtered and the solvent removed under vacuum from the filtrate. The residue was recrystallized (dichloromethane/petroleum ether (40−60 °C)) to give the title compound (485 mg, 57%): $^1$H NMR (DMSO-$d_6$, 250 MHz) $\delta$ 7.55 (s, 1H) and 7.36 (s, 2H) (Ar H's), 6.20 br s, 1H, O*H*), 4.26 (m, 1H, C*H*), 2.98 and 2.65 (doublets, 1H each, $J$ = 17 Hz, ring C$H_2$), 2.63 (t, 2H, $J$ = 8 Hz, ArC$H_2$), 1.55−1.20 (m, 14H, (C$H_2$)$_7$); MS (FAB positive ion) *m*/*z* 403 ((M + H)$^+$); IR (KBr) 3489, 1731 cm$^{-1}$. Anal. ($C_{19}H_{24}Cl_2O_5$) C, H.

This lactone was hydrolyzed with excess aqueous NaOH to generate the corresponding hydroxy acid (**48**) prior to adding to the buffer mix for enzyme screening.

**Syntheses of 49 and 50: (i) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (17).** Diazomethane, generated from diazald (1.37 g, 6.38 mmol) and 60% aqueous KOH (6 mL) in carbitol (6 mL) and ether (6 mL), was bubbled in a stream of ether saturated nitrogen through a solution of **77** (1.24 g, 3.19 mmol) in 10% methanol/ether (12 mL). When a yellow color appeared in the solution, the excess diazomethane was quenched with acetic acid, and then the solvent was removed under vacuum. The residue was purified by chromatography on silica gel (50−100% ether/petroleum ether (40−60 °C)) to give the title compound (1.15 g, 90%) as an oil.

**(ii) (±)-(1′R*,3*R**,5*S**) and (±)-(1′*R**,3*S**,5*R**) Diastereomers of 3-[(Carbomethoxy)hydroxymethyl]-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (18).** *n*-Butyllithium (3.27 mL, 8.18 mmol, 2.5 M in hexanes) was added into a stirred solution of hexamethyldisilazane (1.73 mL, 8.18 mmol) in tetrahydrofuran (15 mL) at 0 °C under argon. After 5 min, the solution was cooled to −78 °C and a solution of **17** (1.15 g, 2.85 mmol) in tetrahydrofuran (10 mL) was added via cannula slowly. After 1 h at −78 °C, a solution of 2-(benzenesulfonyl)-3-phenyloxaziridine[5a] (1.07 g, 4.09 mmol) in tetrahydrofuran (7 mL) was added by cannula. The solution was stirred at −78 °C for 2 h and at −50 °C for 2.5 h, and then allowed to warm to 0 °C. Aqueous HCl was added, 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 vacuum, and the residue was purified by chromatography on silica gel (60−100% ether/petroleum ether (40−60 °C)) to afford the separated (1′*R**,3*R**,5*S**) (236 mg, 20%) and (1′*R**,3*S**,5*R**) (145 mg, 12%) diastereoisomers, both as oils.

**(iii) (±)-(2*R**,3*R**,5*S**)-3-Carboxy-11-(2,4-dichlorophenyl)-2,3,5-trihydroxyundecanoic Acid, Disodium Salt (49).** Aqueous NaOH (1 M, 2.25 mL, 2.25 mmol) was added dropwise to a stirred solution of (±)-(1′*R**,3*R**,5*S**)-3-[(carbomethoxy)hydroxymethyl]-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (235 mg, 0.56 mmol) in methanol (6 mL) at 0 °C. The solution was stirred for 10 min at 0 °C and then for 3 h at room temperature. Methanol was removed under vacuum, and the residue was diluted with water and acidified with aqueous HCl. The mixture was extracted with ether, and the extracts were washed with water. The solvent was removed under vacuum, and the wet residue was dissolved in ethanol (20 mL). Aqueous NaOH (1 M, 1.4 mL, 1.4 mmol) was added. The mixture was allowed to stand for 45 min, then boiled, and cooled to 0 °C. The solid was filtered off, washed with 5% water/ethanol, and dried to give the title compound (181 mg, 69%), mp indeterminate: $^1$H NMR ($D_2O$, 250 MHz) $\delta$ 7.42 (s, 1H) and 7.23(s, 2H) (Ar H's), 4.09 (s, 1H, C*H*(OH)$CO_2$Na), 3.81 (m, 1H, $CH_2$C*H*(OH)$CH_2$), 2.65 (t, 2H, $J$ = 7.5 Hz, ArC$H_2$), 2.00 (m, 2H, C$H_2$C(OH)($CO_2$Na)), 1.6−1.3 (m, 10H, (C$H_2$)$_5$); MS (FAB positive ion) *m*/*z* 467 ((M + H)$^+$); IR (KBr) 3399, 1599 cm$^{-1}$. Anal. ($C_{18}H_{22}Cl_2Na_2O_7 \cdot 0.84H_2O$) C, H.

**(iv) (±)-(2*R**,3*S**,5*R**)-3-Carboxy-11-(2,4-dichlorophenyl)-2,3,5-trihydroxyundecanoic Acid, Disodium Salt (50).** (±)-(1′*R**,3*S**,5*R**)-3-[(Carbomethoxy)hydroxymethyl]-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (145 mg, 0.346 mmol) was hydrolyzed by a procedure analogous to that described above for **49** to give **50** (111 mg, 69%), contaminated with 20% of the (2*R**,3*R**,5*S**) diastereoisomer, mp indeterminate: $^1$H NMR ($D_2O$, 250 MHz) $\delta$ 7.43 (s, 1H) and 7.25 (s, 2H) (Ar H's), 4.14 (s, 1H, C*H*(OH)$CO_2$Na), 3.81 (m, 1H, $CH_2$C*H*(OH)$CH_2$), 2.69 (t, 2H, $J$ = 7.5 Hz, ArC$H_2$), 1.96 (m, 2H, C$H_2$C(OH)($CO_2$Na)), 1.6−1.3 (m, 10H, (C$H_2$)$_5$); MS (FAB positive ion) *m*/*z* 467 ((M + H)$^+$); IR (KBr) 3403, 1597 cm$^{-1}$. Anal. ($C_{18}H_{22}Cl_2O_7Na_2 \cdot 0.9H_2O$) C, H.

**(±)-(3*R**,5*S**)-11-[2-(Benzyloxy)-4-chlorophenyl]-3-carboxy-3,5-dihydroxyundecanoic Acid, Disodium Salt (62). (i) [2-(Benzyloxy)-4-chlorobenzyl]triphenylphosphonium Bromide (21).** This was prepared from ethyl 2-(benzyloxy)-4-chlorobenzoate by the general procedure exemplified for 4-chloro-2-(3,4-dimethylpyrrol-1-yl)benzyl bromide above.

**(ii) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-3-hydroxy-5-(5-hydroxypentyl)tetrahydrofuran-2-one (20).** The dihydroisoxazole **3** was converted to the lactone **19** by the general procedures described above. This was converted to the title methyl ester using MeOH/$H_2SO_4$, followed by chromatography on silica gel (0−80% EtOAc/$Et_2O$).

**(iii) (±)-(3*R**,5*S**)-5-[6-[2-(Benzyloxy)-4-chlorophenyl]-hexyl]-3-(carbomethoxymethyl)-3-hydroxytetrahydrofuran-2-one (22).** Swern oxidation of **20** (0.5 g, 1.92 mmol), as described above for **4**, gave the corresponding aldehyde. Sodium hydride (60% oil suspension, 63 mg, 1.58 mmol) was washed with petroleum ether (40−60 °C) under argon, and then heated with DMSO (2 mL) at 70−90 °C until all solid had dissolved. After the mixture was cooled in a water bath, a solution of **21** (0.86 g, 1.5 mmol) in DMSO (10 mL) was added

by cannula, and the mixture was stirred at room temperature for 15 min. A solution of the aldehyde in DMSO (4 mL) was added, and the mixture was stirred for 1 h, 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 columned on silica gel (50−90% ether/petroleum ether (40−60 °C)) to give (±)-(3*R**,5*S**)-5-[6-[2-(benzyloxy)-4-chlorophenyl]-5-hexenyl]-3-(carbomethoxymethyl)-3-hydroxytetrahydrofuran-2-one (0.42 g, 65%) as a mixture of *E* and *Z* isomers. This compound (0.4 g, 0.83 mmol) in methanol (5 mL) was shaken with $PtO_2$ under hydrogen at 50 psi for 3 h, and then the hydrogen replaced with nitrogen. The catalyst was filtered off through Hyflo, and the solvent was removed under reduced pressure. The residue was columned on silica gel (ether) to give the title compound (0.39 g, 99%) as an oil.

**(iv) (±)-(3*R**,5*S**)-11-[2-(Benzyloxy)-4-chlorophenyl]-3-carboxy-3,5-dihydroxyundecanoic Acid, Disodium Salt (62).** A solution of NaOH (95 mg, 2.37 mmol) in aqueous ethanol (1:1, 2 mL) was added dropwise to a stirred solution of **22** (282 mg, 0.594 mmol) in ethanol (6 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C and then for 5 h at room temperature. Ethanol (6 mL) was added and the solid filtered off and then recrystallized (water) to give the title compound (217 mg, 70%) as a solid: mp >250 °C; $^1$H NMR ($D_2O$, 360 MHz) $\delta$ 7.47 (m, 5H, OBz), 7.20 (d, 1H, $J$ = 8 Hz), 7.13 (s, 1H) and 7.01 (d, 1H, $J$ = 8 Hz) (Ar H's), 5.25 (s, 2H, ArC$H_2$O), 3.88 (m, 1H, C*H*OH), 2.66 and 2.48 (doublets, 1H each, $J$ = 15.3 Hz, C$H_2CO_2$Na), 2.58 (t, 2H, $J$ = 7.5 Hz, ArC$H_2$), 1.82 (d, 2H, $J$ = 5.7 Hz, C$H_2$C(OH)($CO_2$Na)), 1.53 (m, 2H, ArCH$_2$C$H_2$), 1.37−1.27 (m, 8H, $(CH_2)_4$); MS (FAB negative ion) *m/z* 477 (free acid, $(M - H)^-$); IR (Nujol mull) 1572, 1457 cm$^{-1}$. Anal. ($C_{25}H_{29}ClO_7Na_2 \cdot 0.85H_2O$) C, H.

**(±)-(3*R**,5*S**)-11-(2-Hydroxy-4-chlorophenyl)-3-carboxy-3,5-dihydroxyundecanoic Acid (60). (i) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-5-[6-(4-chloro-2-hydroxyphenyl)-hexyl]-3-hydroxytetrahydrofuran-2-one (23).** A solution of **22** (300 mg, 0.634 mmol) in methanol (20 mL) containing concentrated HCl (0.02 mL) was shaken with palladium on charcoal (5%, 125 mg, 0.059 mmol) under hydrogen at 50 psi for 7 h. The atmosphere was then replaced with nitrogen and the catalyst filtered off through Hyflo. The solvent was removed from the filtrate under reduced pressure and the residue columned on silica gel (70−90% ether/petroleum ether (40−60 °C)) to give the title compound (190 mg, 78%) as an oil.

**(ii) (±)-(3*R**,5*S**)-3-(Carboxymethyl)-5-[6-(4-chloro-2-hydroxyphenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (24).** A solution of NaOH (73 mg, 1.83 mmol) in aqueous ethanol (1:1, 2 mL) was added dropwise to a stirred solution of **23** (176 mg, 0.457 mmol) in ethanol (6 mL) at 0 °C, and the mixture was stirred at room temperature for 20 h. Ethanol (6 mL) was added, and the solid was filtered off. The crude disodium salt was heated in aqueous HCl (10 mL) at 60 °C for 18 h, then cooled, 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 columned on silica gel (0−40% ethyl acetate/ether + 0.5% acetic acid). The material was further purified by recrystallization (chloroform/hexane) to give the title compound (120 mg, 71%) as a solid: mp 156−158 °C; $^1$H NMR (DMSO-$d_6$, 360 MHz) $\delta$ 7.05 (d, 1H, $J$ = 8 Hz), 6.79 (s, 1H) and 6.74 (d, 1H, $J$ = 8 Hz) (Ar H's), 4.54 (m, 1H, C*H*), 2.69 (s, 2H, C$H_2CO_2$H), 2.48 (t, 2H, ArC$H_2$), 2.25 (dd, 1H, $J$ = 13.4, 5.6 Hz) and 2.05 (dd, 1H, $J$ = 13.4, 9.8 Hz) (ring C$H_2$), 1.23−1.62 (m, 10H, $(CH_2)_5$); MS (EI) *m/z* 370 ($M^+$), 352, 334, 306, 141; IR (Nujol mull) 1746, 1693 cm$^{-1}$. Anal. ($C_{18}H_{23}ClO_6$) C, H.

**(iii) (±)-(3*R**,5*S**)-11-(2-Hydroxy-4-chlorophenyl)-3-carboxy-3,5-dihydroxyundecanoic Acid (60).** The lactone **24** was hydrolyzed to the hydroxy acid form for enzyme screening by analogous procedures to those described above.

**(±)-(3*R**,5*S**)-3-Carboxy-11-(4-chloro-2-methoxyphenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (61). (i) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-5-[6-(4-chloro-2-methoxyphenyl)hexyl]-3-hydroxytetrahydrofuran-2-one (25).** Iodomethane (0.045 mL, 0.728 mmol) was injected into a stirred mixture of **23** (200 mg, 0.52 mmol), potassium carbonate (108 mg, 0.780 mmol), and dimethylformamide (3 mL) under argon, and the mixture was stirred for 5 h. Aqueous HCl was added and the mixture 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 columned on silica gel (50−70% ether/petroleum ether (40−60 °C)) to give the title compound (181 mg, 87%) as an oil.

**(ii) (±)-(3*R**,5*S**)-3-Carboxy-11-(4-chloro-2-methoxyphenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (61).** Hydrolysis of **25** as described for other lactones above gave the title compound as a solid, mp >250 °C. Anal. ($C_{19}H_{25}ClO_7Na_2$) C, H.

**Typical Procedures for the Syntheses of the Indoles of Table 4. Indole Intermediates (29).** 5,7-Dichloroindole,[18] 3-methyl-5-chloroindole,[19] and 3-phenyl-5-chloroindole[20] were prepared by published procedures.

**3-Nitro-5-chloroindole.** A solution of benzoyl chloride (0.64 g, 4.55 mmol) in $CH_3CN$ (1 mL) was added to a stirred mixture of silver nitrate (0.85 g, 5 mmol) and $CH_3CN$ (5 mL) at 0 °C, and the mixture was stirred for 20 min. Stirring was stopped and the precipitate allowed to settle. The supernatant liquor was transferred to a stirred solution of 5-chloroindole (647 mg, 4.27 mmol) in acetonitrile (8 mL) cooled in an ice/salt bath at −5 °C. The resulting solution was stirred under argon for 30 min at −5 °C and at room temperature for 18 h and then poured into water. The mixture was extracted with ether, and the extracts were washed with aqueous $Na_2CO_3$, water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was columned on silica gel (60−100% ether/petroleum ether (40−60 °C)) to give the title compound (328 mg, 39%).

**2-Phenyl-5-chloroindole.** A mixture of 4-chloroaniline (10.0 g, 78.4 mmol), 1-phenylethanediol (4.33 g, 31.4 mmol), bis(triphenylphosphine)ruthenium dichloride (300 mg, 0.314 mmol), and 2-methoxyethyl ether (20 mL) was heated at reflux with stirring under argon for 18 h, cooled, and poured into aqueous HCl. The mixture was extracted with ether, and the extracts were washed with water and saturated aqueous NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue columned on silica gel twice (10−100% ether/petroleum ether (40−60 °C), then 10−50% dichloromethane/petroleum ether) to give the title compound (0.47 g, 7%) as a solid, mp 186−191 °C.

**(a) Method A (Scheme 4a): (±)-(3*R**,5*S**)-3-Carboxy-10-(5-chloro-3-nitroindol-1-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (71). (i) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-3-hydroxy-5-(5-iodopentyl)tetrahydrofuran-2-one (28, *n* = 5).** *N*-Bromosuccinimide (752 mg, 4.22 mmol) was added to a stirred solution of **20** (1.00 g, 3.84 mmol) and triphenylphosphine (1.11 g, 4.22 mmol) in $CH_2Cl_2$ (25 mL) at 0 °C under argon. After 1 h, toluene (5 mL) was added to the solution, and the $CH_2Cl_2$ was removed under reduced pressure. The residual solution was loaded onto a column of silica gel and the bromide eluted with 60−100% ether/petroleum ether (40−60 °C). Sodium iodide (4.55 g, 30.3 mmol) was added to a stirred solution of the bromide in acetone (20 mL) under argon. The mixture was stirred for 18 h, and then the solvent was removed under reduced pressure. The residue was partitioned between water and ether, and the ethereal extracts were washed with water and saturated aqueous NaCl and then dried ($MgSO_4$). The solvent was removed under reduced pressure to give the title compound (1.11 g, 78%) as an oil.

**(ii) (±)-(3*R**,5*S**)-3-(Carbomethoxymethyl)-5-[5-(5-chloro-3-nitroindol-1-yl)pentyl]-3-hydroxytetrahydrofuran-2-one (30, *n* = 5, R = 3-$NO_2$, $R^1$ = 5-Cl).** A solution of 3-nitro-5-chloroindole (100 mg, 0.509 mmol) in dimethylformamide (2 mL) was added to a stirred suspension of sodium hydride (13

mg of a 60% oil dispersion, 0.56 mmol) in dimethylformamide (1 mL) at room temperature. The mixture was stirred for 5 min, and then a solution of (±)-(3*R**,5*S**)-3-(carbomethoxymethyl)-3-hydroxy-5-(5-iodopentyl)tetrahydrofuran-2-one (207 mg, 0.56 mmol) in dimethylformamide (2 mL) was added. The resulting solution was stirred under argon for 55 h and then poured into dilute aqueous HCl. The aqueous mixture was extracted with ether, and 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 columned on silica gel (40−80% ethyl acetate/hexane) to give the title compound (146 mg, 65%) as an oil.

**(iii) (±)-(3*R**,5*S**)-3-Carboxy-10-(5-chloro-3-nitroindol-1-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (71).** Aqueous NaOH (7.10 mL, 1 M, 7.10 mmol) was added dropwise to a stirred solution of (±)-(3*R**,5*S**)-3-(carbomethoxymethyl)-5-[5-(5-chloro-3-nitroindol-1-yl)pentyl]-3-hydroxytetrahydrofuran-2-one (779 mg, 1.78 mmol) in ethanol (50 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at room temperature for 24 h. Ethanol (50 mL) was added, and then the solid was filtered off and recrystallized ($H_2O$/EtOH) to give the title compound (742 mg, 86%) as a solid: mp > 250 °C; $^1$H NMR ($D_2O$, 400 MHz) $\delta$ 8.45 (s, 1H, indole-2H), 8.03 (s, 1H, indole-4H), 7.55 (d, 1H, $J$ = 9 Hz, indole-7H), 7.37 (d, 1H, $J$ = 9 Hz, indole-6H), 4.25 (t, 2H, $J$ = 7 Hz, NC$H_2$), 3.86 (m, 1H, C*H*OH), 2.68 and 2.47 (doublets, 1H each, $J$ = 15.6 Hz, C$H_2$-$CO_2$Na), 1.90−1.83 (m, 4H, NCH$_2$C$H_2$ and C$H_2$C(OH)($CO_2$Na)), 1.46−1.30 (m, 6H, (C$H_2$)$_3$); MS (FAB positive ion) *m*/*z* 465 ((M + H)$^+$ of monosodium salt); IR (Nujol mull) 1573, 1526, 1456 cm$^{-1}$. Anal. ($C_{19}H_{21}ClN_2O_8Na_2$·0.5$H_2O$) C, H, N.

**(b) Method B (Scheme 4b): (±)-(3*R**,5*S**)-3-Carboxy-10-(5,7-dichloroindol-1-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (66). (i) (±)-3-(5-Bromopentyl)-5-(carbomethoxymethyl)-5-(methoxycarbonyl)-4,5-dihydroisoxazole (32).** *N*-Bromosuccinimide (4.50 g, 25.3 mmol) was added in portions to a stirred solution of **3** (6.61 g, 23.0 mmol) and triphenylphosphine (6.64 g, 25.3 mmol) in $CH_2Cl_2$ (70 mL) at 0 °C. The solution was stirred at room temperature under argon for 1.5 h, then toluene (20 mL) was added, and the $CH_2$-$Cl_2$ was removed under reduced pressure. The residual solution was loaded onto a column of silica gel, and the products were eluted with 50−80% ether/petroleum ether (40−60 °C). This gave the title compound (6.93 g, 86%) as an oil.

**(ii) (±)-5-(Carbomethoxymethyl)-3-[5-(5,7-dichloroindol-1-yl)pentyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole (33, R = H, R$^1$ = 5,7-diCl).** Freshly ground potassium hydroxide (206 mg, 3.67 mmol) was stirred in DMSO (20 mL) under argon for 5 min, 5,7-dichloroindole[18] (580 mg, 3.12 mmol) was added, and the mixture was stirred for 45 min. A solution of **32** (1.31 g, 3.74 mmol) in DMSO (5 mL) was added by cannula, and the mixture was stirred for 1 h. Water was added, 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 columned on silica gel (50% ether/petroleum ether (40−60 °C)) to give the title compound (1.07 g, 75%) as an oil.

**(iii) (±)-(3*R**,5*S**)-3-Carboxy-10-(5,7-dichloroindol-1-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (66).** The dihydroisoxazole intermediate **33** (R = H, R$^1$ = 5,7-diCl) was converted to the title compound, mp >250 °C, by the general procedures described above: $^1$H NMR ($D_2O$, 360 MHz) $\delta$ 7.58 (s, 1H, indole 6-H), 7.34 (d, 1H, $J$ = 3 Hz, indole 2-H), 7.25 (s, 1H, indole 4-H), 6.52 (d, 1H, $J$ = 3 Hz, indole 3-H), 4.48 (t, 2H, $J$ = 7 Hz, NC$H_2$), 3.85 (m, 1H, C*H*OH), 2.68 and 2.47 (doublets, 1H each, $J$ = 15.6 Hz, C$H_2$$CO_2$Na), 1.80 (m, 4H, NCH$_2$C$H_2$ and C$H_2$C(OH)($CO_2$Na)), 1.41−1.28 (m, 6H, (C$H_2$)$_3$); MS (FAB positive ion) *m*/*z* 432 ((M + H)$^+$ of free acid); (FAB negative ion) *m*/*z* 430 (M − H)$^-$ of free acid); IR (Nujol mull) 1574, 1463 cm$^{-1}$. Anal. ($C_{19}H_{21}Cl_2NO_6Na_2$·1.1$H_2O$) C, H, N.

**(c) Method C (Scheme 4c): (±)-(3*R**,5*S**)-3-Carboxy-11-(5-chloroindol-1-yl)-3,5-dihydroxyundecanoic Acid, Disodium Salt (65).** 5-Chloroindole was alkylated with 7-bromoheptanol using KOH in DMSO by the procedure used above for (±)-5-(carbomethoxymethyl)-3-[5-(5,7-dichloroindol-1-yl)pentyl]-5-(methoxycarbonyl)-4,5-dihydroisoxazole. This was converted to the title compound, via the dihydroisoxazole intermediate, by the general procedures described above. Anal. ($C_{20}H_{24}ClNO_6Na_2$·$H_2O$) C, H, N.

**Syntheses of 74 and 75.** The procedure described for **66** was employed, using either 3-chlorocarbazole[21] or 6-chlorotetrahydrocarbazole[22] in place of the indole.

**(±)-(3*R**,5*S**,9′*R**)- and (±)-(3*R**,5*S**,9′*S**)-3-Carboxy-10-(3′-chlorofluoren-9′-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (as a 1:1 Mixture of Diastereomers) (76). (i) 3-Chloro-9-[6′-(benzyloxy)hex-1′-yl]-9-hydroxyfluorene (38).** 6-(Benzyloxy)hexyl bromide (3.00 g, 0.011mol) was added to magnesium turnings (2.98 g, 0.122 mol) in anhydrous ether (10 mL), under an argon atmosphere. Reaction was initiated by adding a crystal of iodine and heating. Further 6-(benzyloxy)hexyl bromide (28.68 g, 0.106 mol) in anhydrous ether (50 mL) was added at such a rate to maintain reflux. After addition, the reaction was chilled (−78 °C), 3-chlorofluorenone (12.07 g, 0.056 mol) in THF (50 mL) was added by cannula, and the mixture was left to warm to room temperature and stir overnight. 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 to give an oil. This was purified by silica gel chromatography (20% ether/petroleum ether (40−60 °C)) to give the title compound as a colorless gum (15.86 g, 33%).

**(ii) (±)-6-(3′-Chlorofluoren-9′-yl)hexan-1-ol (39).** A solution of (±)-3-chloro-9-[6′-(benzyloxy)hex-1′-yl]-9-hydroxyfluorene (15.76 g, 0.0387 mol) in methanol (100 mL) and concentrated aqueous HCl (3 mL), mixed with 10% palladium on carbon (2.0 g), was shaken at room temperature under hydrogen at 50 psi for a total of 24 h. The reaction mixture was filtered through Celite, poured into water, and extracted with ether (2×). The combined ether extracts were washed with water (2×) and brine (1×), dried ($MgSO_4$), and concentrated to give an oil. This was purified by silica gel chromatography (20% ether and then 50% ether/petroleum ether (40−60 °C)) to give the title compound as an oil (5.16 g, 44%).

**(iii) (±)-(3*R**,5*S**,9′*R**)- and (±)-(3*R**,5*S**,9′*S**)-3-Carboxy-10-(3′-chlorofluoren-9′-yl)-3,5-dihydroxydecanoic Acid, Disodium Salt (as 1:1 Mixture of Diastereomers) (76). 39** was converted to the title compound, via the corresponding dihydroisoxazole intermediate, using the general procedures described above.

$^1$H NMR ($D_2O$, 400 MHz at 340 K) $\delta$ 8.02 (m, 2H), 7.82 (m, 1H), 7.74 (d, 1H, $J$ = 8 Hz), 7.63−7.56 (m, 2H) and 7.55 (d, 1H, $J$ = 8 Hz) (all fluorene ring H's), 4.22 (m, 1H, C*H*), 3.97 (m, C*H*OH), 2.80 and 2.62 (doublets, 1H each, $J$ = 15.3 Hz, C$H_2$$CO_2$Na), 2.20 (m, 2H, CHC$H_2$), 1.93 (m, C$H_2$C(OH)($CO_2$-Na), 1.49−1.17 (m, 8H, (C$H_2$)$_4$); MS (FAB negative ion) *m*/*z* 427 ((M − H)$^-$ − $H_2O$), 393 (Cl/H ex); IR (Nujol mull) 1572, 1457, 1445 cm$^{-1}$. Anal. ($C_{24}H_{25}ClO_6Na_2$·0.84$H_2O$) C, H, Cl.

**Resolution of 77 and 45. (*S*)−(3*R**,5*S**)-3-(Carboxymethyl)-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one ((+)-77).** The racemic lactone **77** (15 g, 38.5 mmol) was dissolved in ethanol−water (96:4) (50 mL). To this was added a solution of d-(−)-*threo*-2-amino-1-(4-nitrophenyl)propane-1,3-diol (8.17 g, 38.5 mmol) in hot ethanol−water (96:4) (210 mL). This was allowed to cool to room temperature, depositing a precipitate, and left to stand overnight in the body of a refridgerator. The crystallized solid (10.31 g) was collected, washed with ethanol−water (96:4), and dried in vacuo. This salt was suspended in water and 2 M HCl was added to pH 1−2. This was extracted with ether (3×), and the combined extracts were washed with water (1×) and dried over $MgSO_4$. Concentration gave a white solid (6.40 g) which was recrystallized from $CHCl_3$/hexane to give the pure (+)-**77** (6.23 g, 16 mmol, 83% of theoretical); mp 87−88.5 °C; $[\alpha]^{25}_D$ = +19.4 ($c$ = 0.5% w/v; EtOH). Anal. ($C_{18}H_{22}Cl_2O_5$) C, H.

**(*R*)-(3*R**,5*S**)-3-(Carboxymethyl)-5-[6-(2,4-dichlorophenyl)hexyl]-3-hydroxytetrahydrofuran-2-one ((−)-77).**

The ethanol−water liquors containing the enrched (−)-**77** salt from the above were concentrated in vacuo and to the residue added water and then 2 M aqueous HCl to acidify. This was extracted with ether (3×), and the extracts were washed with 2 M HCl and then water and dried over $MgSO_4$. Concentration gave an oil which was triturated with hexane to give enriched (−)-**77** (8.12 g, 20.8 mmol) as a solid. This was dissolved in ethanol−water (96:4) (25 mL), and a hot solution of L-(+)-*threo*-2-amino-1-(4-nitrophenyl)propane-1,3-diol, **41a** (4.41 g, 20.8 mmol), in the same solvent (120 mL) was added. The resulting precipitate was collected, after standing overnight in the body of a refridgerator, and the pure (−)-**77** (5.83 g, 15 mmol, 78% of theoretical after recrystallization) was regenerated from the salt as described above: mp 87−88.5 °C; $[\alpha]^{25}_D = -19.5$ ($c$ = 0.5% w/v; EtOH). Anal. ($C_{18}H_{22}Cl_2O_5$) C, H.

**(*S*)-(3*R**,5*S**)-3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt ((+)-45).** (+)-**77** (160 mg) was dissolved in ethanol (2.5 mL), and 5% aqueous NaOH solution (0.64 mL) in water (1.86 mL) was added with stirring. After ca. 10 min, the precipitated solid was collected, washed with 1:1 EtOH−$H_2O$, and recrystallized from water−ethanol to give the (+)-**45** (100 mg): $[\alpha]^{25}_D = +23.3$ ($c$ = 0.16% w/v; $H_2O$). Anal. ($C_{18}H_{22}Cl_2O_6Na_2 \cdot H_2O$) C, H.

**(*R*)-(3*R**,5*S**)-3-Carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic Acid, Disodium Salt ((−)-45).** Hydrolysis of (−)-**77** (100 mg) as described for (+)-**77** above gave (−)-**45** (65 mg), $[\alpha]^{25}_D = -20.6$ ($c$ = 0.1% w/v; $H_2O$). Anal. ($C_{18}H_{22}Cl_2O_6Na_2 \cdot H_2O$) C, H.

**Estimation of Enantiomeric Purity of Resolved Enantiomers by Micellar Electrokinetic Capillary Chromatography (MECC).** Resolved lactone chloramphenicol base salts of **77** and **80** were analyzed for enantiomeric purity by MECC prior to regeneration of the free lactone or subsequent hydrolysis to the hydroxy acid. Free hydroxy acids were dissolved in water, and the lactones, or their salts, were hydrolyzed to the hydroxy acids by addition of excess NaOH to the compound in water. MECC separation was carried out on this solution according to the method developed by Okafo et al.[6] on a Beckman P/ACE instument 570 mm × 50 $\mu$m id capillary, using the following conditions: capillary, 570 mm × 50 $\mu$m id (effective length 500 mm); buffer, 30 mM sodium phosphate, 10 mM boric acid, 50 mM taurodeoxycholic acid, 20 mM $\beta$-cyclodextrin, adjusted to pH 7 with 0.1 M NaOH; applied voltage, 25 kV; detection, UV absorbance at 214 nM; temperature, 25 °C. The resolved (+) and (−) enantiomers of both **45** and **58**, obtained from hydrolysis of **77** and **80**, repectively, showed one single peak by MECC, with no trace of the other enantiomer (level of detection >99.5%).

**Enzyme Assay and HepG2 Cell Assay.** Both assays were carried out as described previously.[2]

**Measurement of Effect of Compounds on In Vivo Triglyceride Concentrations in Rats.** The procedures involving animals in this study were subject to both internal review and UK Home Office regulations. Male Sprague−Dawley rats were maintained on a reverse light cycle (12 h light, 12 h dark) and fed a standard RM1 powdered diet (Special Diet Service, Witham, Essex, UK) with free access to food and water. Compound was administered as two doses, given 24 h apart by oral gavage at the dose levels indicated in the table. Blood samples were obtained mid dark cycle (4 h post second dose), placed into EDTA plasma tubes and plasma isolated by centrifugation at 1500$g$ for 15 min. Plasma triglyceride concentrations were measured enzymatically, using the Merck GPO-PAP test kit for trigylceride. Results are expressed as the percentage reduction of plasma triglyceride concentration from control levels. Significant changes from control were determined by analysis of variance.

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**Supporting Information Available:** Experimental details of the X-ray structure determination of (+)-**77**, including tables of fractional atomic coordinates, interatomic distances and angles, and thermal parameters (11 pages). Ordering information is given on any current masthead page.

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