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An Optically Pure 1,4-Dihydropyridine from a Resolution Catalysed by Rabbit Liver Esterase

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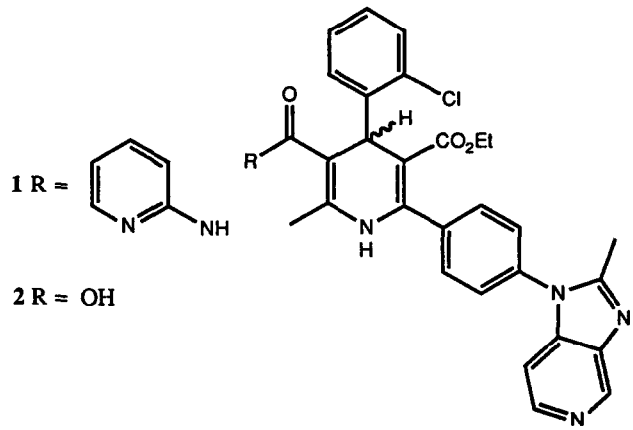
Abstract: The kinetic resolution of a precursor of the dihydropyridine UK-74,505, **1**, an antagonist of platelet activating factor, is described, in which rabbit liver esterase was used to catalyse the hydrolysis of an ester function linked to the chiral centre *via* a spacer arm of appropriate length.

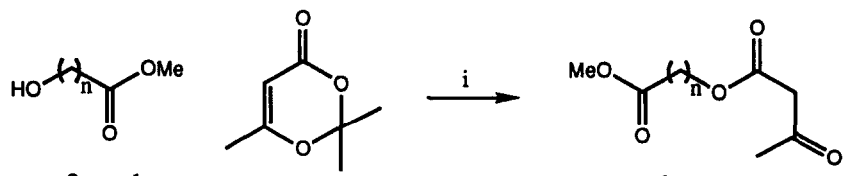
The 1,4-dihydropyridines are an important class of calcium antagonists which have found widespread use in the treatment of various cardiovascular disorders.[1] Examples of such compounds include both symmetrical, and hence achiral, 1,4-dihydropyridines and those possessing a chiral centre at C-4. Compound UK-74,505 **1** is a chiral 1,4-dihydropyridine which lacks calcium antagonistic activity, but is a potent antagonist of platelet activating factor (PAF)[2]. PAF has been implicated in a variety of pathophysiological conditions such as asthma, inflammation and shock, and a number of PAF antagonists, including dihydropyridine **1**, are currently undergoing clinical evaluation. The importance of chirality to pharmacological activity may be a purely quantitative phenomenom. However, examples of the two enantiomers of a dihydropyridine exhibiting antagonistic and agonistic behaviour have been reported.[3] Methods for the preparation of single enantiomers of dihydropyridines are therefore of importance.

Chiral 1,4-dihydropyridines have been obtained in high optical purity both by resolution of the corresponding dihydropyridinecarboxylic acids through the formation of diasteromeric salts with chiral amines[4] and *via* enantioselective syntheses.[4] More recently the hydrolysis, with stereodifferentiation between enantiotopic ester groups, of prochiral 1,4-dihydropyridines by lipases has been reported[5,6]. However we were intrigued to discover whether the enzyme-catalysed kinetic resolution of large, unsymmetrically substituted dihydropyridines such as

compounds **13-15** could be accomplished. It was hoped that selective hydrolysis would afford either the carboxylic acids **16-18** or residual starting material in an enantiomerically enriched form, which could be subsequently converted into **1** *via* carboxylic acid **2**[7]. The C-3 and C-5 esters, being vinylogous carbamates, are largely resistant to hydrolysis. However by introducing an extended methyl ester chain at C-5 this problem could be circumvented.



Synthesis of the dihydropyridines **13-15** proceeded by acetoacetylation of the hydroxy methyl esters **3-5** with 2,2,6-trimethyl-1,3-dioxen-4-one in refluxing toluene. This afforded the corresponding methyl 3-oxobutyl alkanedioates **6-8** in almost quantitative yield (Scheme 1). Treatment of the alkanedioates **6-8** with ammonium acetate in refluxing toluene with azeotropic removal of water[8] gave the enamines **9-11** in good yield (Scheme 2). Finally, Hantzsch condensation of the enamines **9-11** with the Knoevenagel intermediate **18** in refluxing methanol afforded the desired 1,4-dihydropyridines **13-15** in moderate yield (Scheme 3).

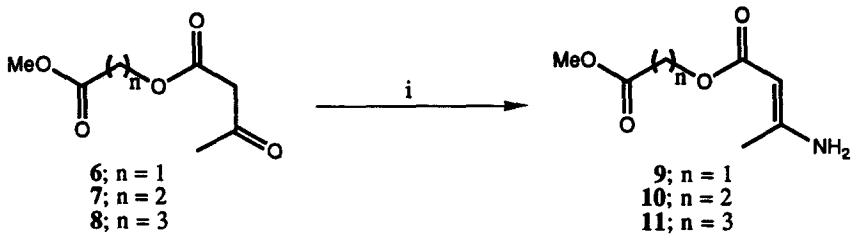


3; n = 1
4; n = 2
5; n = 3

6; n = 1
7; n = 2
8; n = 3

Scheme 1 *Reagents and conditions:* i, Toluene, reflux, 3 h

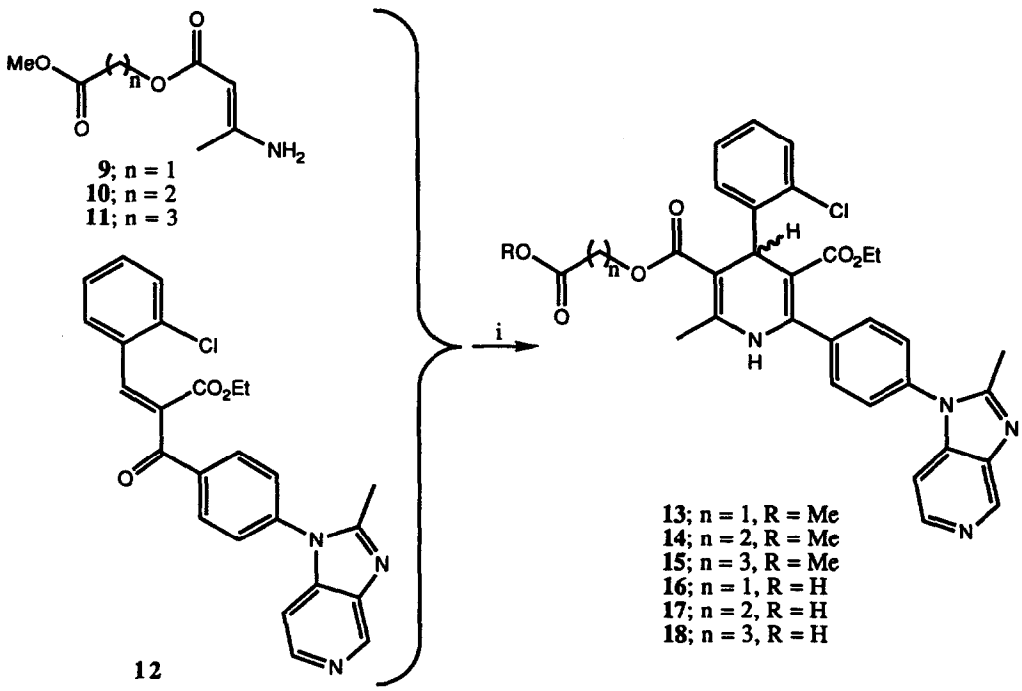
Our initial experiments focused on the use of pig liver esterase which has extensively been employed for the selective hydrolysis of prochiral esters[9] *via* the 'meso trick'[10] and for the resolution of racemates[11].



6; n = 1
7; n = 2
8; n = 3

9; n = 1
10; n = 2
11; n = 3

Scheme 2 *Reagents and conditions:* i, Toluene, CH_3COOH, $CH_3COO^-NH_4^+$, azeotropic removal of H_2O.



9; n = 1
10; n = 2
11; n = 3

12

13; n = 1, R = Me
14; n = 2, R = Me
15; n = 3, R = Me
16; n = 1, R = H
17; n = 2, R = H
18; n = 3, R = H

Scheme 3 *Reagents and conditions:* i, MeOH, CH_3COOH, reflux, 48 h.

When applied to the resolution of dihydropyridine **13**, pig liver esterase showed no enantioselectivity, catalysing its complete hydrolysis to the acid **16**. Dihydropyridine **13** and its hydrolysis product **16**, isolated from reactions terminated prior to completion, were both found to be racemic. Similar results were obtained when the hydrolysis was performed with crude horse liver esterase.

In contrast to the widely reported use of pig liver esterase[10] and to a lesser extent horse liver esterase[12] as stereoselective catalysts, there are very few examples of the application of rabbit liver esterase in this context[13]. When applied to the resolution

of dihydropyridine **13**, rabbit liver esterase was found to exert a moderate degree of selectivity. Initial experiments allowed the isolation of unreacted **13** in 80% enantiomeric excess. The hydrolysis product **16** was isolated in 22% enantiomeric excess. A conversion (c) and enantiomeric ratio (E)[14] of 0.78 and 3 respectively were determined from the optical purity of unreacted dihydropyridine **13** and product **16**. The determined E value was used to predict that optically pure unreacted dihydropyridine **13** could be obtained if the hydrolysis was permitted to proceed to a conversion of approximately 0.9[14] Indeed, when this prediction was tested experimentally, optically pure unreacted dihydropyridine **13** was isolated (Figure 1).

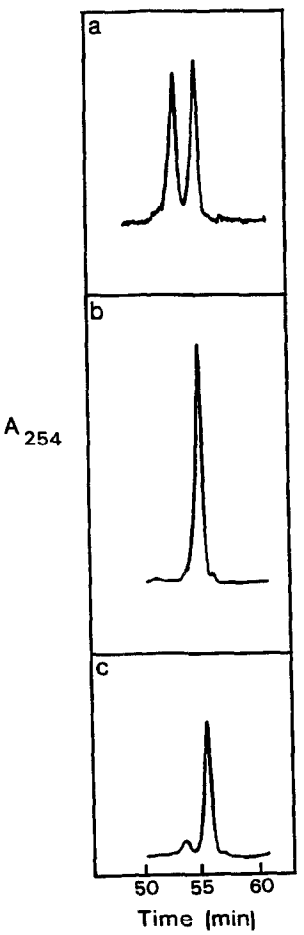


Figure 1. Resolution of dihydropyridine **13** by h.p.l.c on a Cyclobond PT column: a. racemic dihydropyridine **13**; b. unreacted dihydropyridine **13** from the rabbit liver esterase-catalysed resolution; c. unreacted dihydropyridine **13** with added racemate.

The hydrolysis of the higher homologues **14** and **15** to the corresponding carboxylic acids **17** and **18** by rabbit liver esterase proceeded with almost no enantioselectivity. The loss of enantioselectivity is perhaps not surprising, since the site of hydrolysis is progressively one carbon further from the centre of chirality in substrates **14** and **15** Presumably as the length of the alkyl spacer arm increases, there is less interaction between the enzyme and the chiral centre, and hence the factors governing selectivity are diminished.

The need for a high degree of conversion in order to obtain unreacted dihydropyridine **13** in an optically pure form somewhat limits the preparative value of this resolution unless the product is remethylated and further enzymatic hydrolyses performed. However the large and sterically demanding nature of this molecule does illustrate the diversity of structures which may serve as substrates in esterase-catalysed reactions. Clearly, rabbit liver esterase has potential as an alternative to the more widely used pig liver esterase for enantioselective biotransformations.

Experimental

UV-visible spectra were recorded on a Philips PU 8720 spectrometer in spectroscopic grade ethanol. ^{1}H NMR spectra were recorded on a Bruker ACF 250 (250.134 MHz) or WH-400 (400.13 MHz) spectrometer as solutions in $CDCl_3$. *J* Values are given in Hz. ^{13}C NMR were recorded on the same instruments at 62.9 MHz and 100.62 MHz respectively as solutions in $CDCl_3$. Mass spectra were obtained on a Kratos MS-80 or Finnigan 4000 spectrometer. H.p.l.c analyses were performed on a Gilson 305/306 system. Compound **12** (UK-78,274) was a kind gift from Pfizer Central Research the synthesis of which has been described[7].

Methyl 3-hydroxypropanoate 4.

β-Propiolactone (5.73 g, 79.5 mmol) was added dropwise to a stirred solution of dry methanol (12.1 cm^3) and NaOH (0.16 g, 3.98 mmol) at 0°C. After 1.5 h the base was neutralised by the addition of one equivalent of conc. HCl. Filtration followed by distillation under reduced pressure afforded a colourless oil, (2.99 g, 36%); b.p. 74-76°C/15 mm Hg; δ_H(400 MHz; $CDCl_3$) 3.73 (2H, t, *J* 5.8 Hz; CH_2OH), 3.58 (3H, s, OCH_3), 2.70 (1H, br s, *OH*), 2.44 (2H, t, *J* 5.9Hz, $COCH_2$); δ_C(100 MHz; $CDCl_3$) 172.8, 57.6, 51.4, 36.5; *m/z* (EI) 105 (MH^+, 100%), 87 (13%), 73 (71%), 68 (1.3%), 61 (3%), 59 (10%), 55 (17%), 45 (16%), 43 (53%) [Found (MH^+) 105.0546 $C_4H_9O_3$ requires 105.0552].

Methyl 4-hydroxybutanoate 5.
γ-Butyrolactone (43 g, 0.5 mol) was added dropwise to a stirred solution of sodium methoxide (1 g, 0.02 mol) in methanol (100 cm^3) at -78°C. After 2 h the base was neutralised by the addition of one equivalent of conc HCl. The solution was diluted with water and extracted with dichloromethane. The organic layer was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. Distillation under reduced pressure afforded a colourless oil (7.26 g, 12%); b.p. 87-89°C/7 mm Hg; δ_H(250 MHz; $CDCl_3$) 3.56 (3H, s, OCH_3), 3.52 (2H, t, *J* 6.2 Hz, CH_2OH), 3.35 (1H, br s, O*H*), 2.32 (2H, t, *J* 7.3 Hz, $COCH_2$), 1.75 (2H, m, $CH_2CH_2CH_2$); δ_C(62.9 MHz; $CDCl_3$) 174.4, 61.2, 51.5, 30.4, 27.4; *m/z* (EI) 119 (MH^+, 21%), 101 (46%), 88 (61%), 87 (100%), 74 (70%), 69 (19%), 57 (33%) [Found (MH^+) 119.0702 $C_5H_{11}O_3$ requires 119.0708].

Methyl 3-oxobutyl ethanedioate 6.
Methyl glycolate (4.41 g, 49 mmol) and toluene (15 cm^3) were heated to reflux and 2,2,6-trimethyl-1,3-dioxen-4-one (6.96 g, 49 mmol) added dropwise over a 30 minute period The acetone produced was continuously removed by distillation. After 3 h the mixture was cooled and toluene evaporated under reduced pressure to yield an orange oil (7.44 g, 94%); δ_H(250 MHz; $CDCl_3$) 4.72 (2H, s, $COCH_2O$), 3.80 (3H, s, OCH_3), 3.60 (2H, s, $COCH_2CO$), 2.35 (3H, s, CH_3CO); δ_C(100 MHz; $CDCl_3$) 199.7, 167.6, 166.3, 60.9, 52.2, 49.4, 29.9; *m/z* (EI) 174 (M^+, 5%), 143 (3%), 132 (14%), 115(6%), 100 (10%), 91 (6%), 73 (5%), 59 (2%), 43 (100%) [Found (M^+) 174.0540 $C_7H_{10}O_5$, requires 174.0528]

Methyl 3-oxobutyl propanedioate 7.
This compound was prepared as described for methyl 3-oxobutyl ethanedioate from methyl 3-hydroxypropanoate (2.8 g, 26.9 mmol) and 2,2,6-trimethyl-1,3-dioxen-4-one (3.82 g, 26.9 mmol). The product was obtained as an orange oil (4.8g, 95%); δ_H(250 MHz; $CDCl_3$) 4.22 (2H, t, *J* 6.3 Hz, CH_2,O), 3.53 (3H, s, OCH_3), 3.31 (2H, s, $COCH_2CO$), 2.52 (2H, t, *J* 6.3 Hz, $COCH_2$), 2.08, (3H, s, CH_3CO); δ_C(62.9 MHz; $CDCl_3$) 200.2, 170.6, 166.7, 60.3, 51.5, 49.5, 33.1, 29.7; *m/z* (EI) 188 (M^+, 9%), 156 (6%), 146 (100%), 114 (52%), 105 (23%), 85 (90%), 73 (34%), 59 (41%), 42 (41%) [Found (M^+) 188.0684 $C_8H_{12}O_5$ requires 188.0685].

Methyl 3-oxobutyl butanedioate 8.
This compound was prepared as described for methyl 3-oxobutyl ethanedioate from methyl 4-hydroxybutanoate (3.63 g, 0.03 mol) and 2,2,6-trimethyl-1,3-dioxen-4-one (4.27 g, 0.03 mol). The product was obtained as an orange oil (5.83 g, 96%); δ_H(250 MHz; $CDCl_3$) 4.23 (2H, t, *J* 6.3 Hz, CH_2,O), 3.71 (3H, s, OCH_3), 3.5 (2H, s, $COCH_2CO$), 2.45 (2H, t, *J* 7.3 Hz, $COCH_2$), 2.3 (3H, s, CH_3CO), 2.01 (2H, m, $CH_2CH_2CH_2$); δ_C(100 MHz; $CDCl_3$) 200.4, 172.9, 166.9, 64.0, 51.4, 49.7, 30.1, 29.9, 23.6; *m/z* (EI) 203

(MH$^+$, 12%), 160 (9%), 129 (3%), 119 (7%), 101 (100%), 85 (29%), 69 (4%), 43 (85 %) [Found (MH$^+$) 203.0929 $C_9H_{15}O_5$ requires 203.0919].

Methyl 3-amino-2-butenyl ethanedioate 9.

To dry toluene (50 cm^3) was added glacial acetic acid (1 cm^3, 17.5 mmol), ammonium acetate (1.95 g, 25 mmol) and methyl 3-oxobutyl ethanedioate (2.3 g, 13.2 mmol). The mixture was heated to reflux with azeotropic removal of water. After 3.5 h the reaction mixture was cooled, washed with saturated $NaHCO_3$ and dried over anhydrous $MgSO_4$. The toluene was evaporated under reduced pressure to yield a yellow solid (1.53 g, 70%); m.p. 100-101°C (from water); δ_H(400 MHz; $CDCl_3$) 7.80 (1H, br s, N*H*), 5.04 (1H, br s, N*H*), 4.56 (1H, s, vinylic proton), 4.54 (2H, s, COC*H*$_2$O), 3.71 (3H, s, OC*H*$_3$), 1.88 (3H, s, C*H*$_3$CNH$_2$); δ_C(62.9 MHz; $CDCl_3$) 169.6, 168.8, 161.7, 82.2, 59.2, 51.9, 22.1; *m/z* (EI) 173 (M$^+$, 79%), 142 (29%), 84 (100%), 68 (35%), 57 (40%), 54 (23%), 42 (61%), 28 (10%) [Found (M$^+$) 173.0688 $C_7H_{11}NO_4$ requires 173.0688].

Methyl 3-amino-2-butenyl propanedioate 10.

This compound was prepared as described for methyl 3-amino-2-butenyl ethanedioate from methyl 3-oxobutyl propanedioate (4 g, 21 mmol). The product was obtained as a yellow oil (3.53 g, 90%); δ_H(250 MHz; $CDCl_3$) 7.74 (1H, br s, N*H*), 5.08 (1H, br s, N*H*), 4.34 (1H, s, vinylic proton), 4.18 (2H, t, *J* 6.4 Hz, OC*H*$_2$), 3.57 (3H, S, OC*H*$_3$), 2.53 (2H, t, *J* 6.4 Hz, COC*H*$_2$), 1.78 (3H, s, C*H*$_3$CNH$_2$); δ_C(100 MHz; $CDCl_3$) 171.3, 169.3, 160.3, 82.7, 57.6, 51.3, 33.7, 21.7; *m/z* (EI) 187 (M$^+$, 21%), 114 (2%), 100 (1%), 84 (100%), 68 (5%), 57.1 (46%), 42 (26%) [Found (M$^+$) 187.0846 $C_8H_{13}NO_4$ requires 187.0844].

Methyl 3-amino-2-butenyl butanedioate 11.

This compound was prepared as described for methyl 3-amino-2-butenyl ethanedioate from methyl 3-oxobutyl butanedioate (4.24 g, 21 mmol). The product was obtained as a yellow oil (3.27 g, 77%); δ_H(250 MHz; $CDCl_3$) 7.75 (1H br s, N*H*), 5.06 (1H, br s, N*H*), 4.33 (1H, s, vinylic proton), 3.92 (2H, t, *J* 6.3 Hz, OC*H*$_2$), 3.53 (3H, s, OC*H*$_3$), 2.27 (2H, t, *J* 7.4 Hz, COC*H*$_2$), 1.81 (2H, m, CH$_2$C*H*$_2$CH$_2$), 1.76 (3H, s, C*H*$_3$CNH$_2$); δ_C(100 MHz; $CDCl_3$) 173.2, 169.6, 160.0, 82.8, 61.0, 51.1, 30.2, 24.0, 21.7; *m/z* (EI) 201 (M$^+$, 11%), 128 (1%), 101 (29%), 84 (100%), 68 (2%), 57 (31%), 42 (16%) [Found (M$^+$) 201.1010 $C_9H_{15}NO_4$ requires 201.1001].

4-(2-Chlorophenyl)-1,4-dihydro-3-ethoxycarbonyl-6-methyl-2-[4-(2-methylimidazo[4,5-c]pyrid-1-yl)phenyl]pyridine-5-methoxycarbonyl-methylcarboxylate 13.

Methyl-3-amino-2-butenylethanedioate (1.16 g, 6.7 mmol) and **18** (1.5 g, 3.35 mmol) were dissolved in methanol (100 cm^3) containing a catalytic amount of glacial acetic acid. After heating at reflux for 48 h the solvent was evaporated under

reduced pressure. The product was purified by flash chromatography with dichloromethane/methanol (95:5) as eluant to give the product as a yellow solid (1.03 g, 51 %); λ_{max} (EtOH)/nm 202.5, 239.6, 369.8; δ_H(250 MHz; $CDCl_3$) 8.95 (1H, s, C_5H_3N), 8.49 (1H, s, N*H*), 7.89 (1H, d, *J* 5.6 Hz, C_5H_3N), 7.59 (1H, m, C_6H_4Cl), 7.54, 7.51, 7.22 and 7.19 (q, 4H, *para*-substituted aromatic), 7.26 (1H, m, C_6H_4Cl), 7.17 (1H, m, C_6H_4Cl), 7.06 (1H, m, C_6H_4Cl), 6.91 (1H, d, *J* 5.6 Hz, C_5H_3N), 5.60 (1H, s, 4-*H*), 4.62, 4.56, 4.54 and 4.47 (2H, AB quartet, $COCH_2O$), 3.83 (2H, q, *J* 7.1 Hz, $CO_2CH_2CH_3$), 3.62 (3H, s, OCH_3), 2.46 (3H, s, imidazo-Me), 2.43 (3H, s, 6-Me), 0.91 (3H, t, *J* 7.1 Hz, $CO_2CH_2CH_3$,); δ_C(100 MHz; $CDCl_3$) 168.5, 166.4, 166.2, 153.3, 146.5, 144.7, 144.4, 140.9, 140.7, 140.5, 139.5, 137.9, 134.5, 132.7, 131.6, 130.1, 129.4, 127.4, 126.5, 125.7, 105.3, 104.8, 101.5, 59.8, 59.5, 51.8, 38.1, 18.8, 14.2, 13.6; *m/z* (FAB) 601 (MH^+, 74%), 543 (23%), 489 (17%), 154 (100%), 89 (58%), 77 (68%), 51 (46%) [Found (MH^+) 601.1854 $C_{32}H_{30}N_4O_6Cl$ requires 601.1853].

4-(2-Chlorophenyl)-1,4-dihydro-3-ethoxycarbonyl-6-methyl-2-[4-(2-methylimidazo[4,5-c]pyrid-1-yl)phenyl]pyridine-5-methoxycarbonyl-ethylcarboxylate 14.

This compound was prepared as described for dihydropyridine **13** from methyl 3 amino-2-butenylpropanedioate (1.26 g, 6.74 mmol) and **18** (1.5 g, 3.35 mmol). The product was purified by flash chromatography with dichloromethane/methanol (95:5) as eluant to give the product as a yellow solid (0.58 g, 28 %); λ_{max} (EtOH)/nm 206.9, 238.4, 365.7; δ_H(250 MHz; $CDCl_3$) 8.75 (1H, s, C_5H_3N), 8.31 (1H, s, N*H*), 7.84 (1H, d, *J* 5.6 Hz, C_5H_3N), 7.51 (1H, m, C_6H_4Cl), 7.48, 7.45, 7.16 and 7.13 (4H, q, *para*-substituted aromatic), 7.21 (1H, m, C_6H_4Cl), 7.12 (1H, m, C_6H_4Cl), 7.02 (1H, m, C_6H_4Cl), 6.85 (1H, d, *J* 5.5 Hz, C_5H_3N), 5.44 (1H, s, 4-*H*), 4.22 (2H, t, *J* 6.4 Hz, CH_2CH_2O), 3.78 (2H, q, *J* 7.1 Hz, $COCH_2CH_3$), 3.58 (3H, s, OCH_3), 2.56 (2H, t, *J* 6.5 Hz, $COCH_2CH_2$), 2.41 (3H, s, imidazo-Me), 2.36 (3H, s, 6-Me), 0.86 (3H, t, *J* 7.1 Hz, $COCH_2CH_3$); δ_C(100 MHz; $CDCl_3$) 171.2, 167.1, 166.5, 153.3, 145.9, 144.8, 144.7, 141.2, 140.8, 140.7, 139.6, 138.1, 134.7, 132.6, 131.8, 130.2, 129.6, 127.5, 126.7, 125.8, 105.4, 104.5, 102.1, 59.6, 59.1, 51.6, 38.3, 33.5, 18.9, 14.3, 13.7; *m/z* (FAB) 615 (MH^+, 100%), 503 (38%), 154 (73%), 89 (46%), 77 (73%) [Found (MH^+) 615.2010 $C_{33}H_{32}N_4O_6Cl$ requires 615.20098].

4-(2-Chlorophenyl)-1,4-dihydro-3-ethoxycarbonyl-6-methyl-2-[4-(2-methylimidazo[4,5-c]pyrid-1-yl)phenyl]pyridine-5-methoxycarbonyl-propylcarboxylate 15.

This compound was prepared as described for dihydropyridine **13** from methyl 3-amino-2-butenyl butanedioate (1.35 g, 6.71 mmol) and **18** (1.5 g, 3.35 mmol). The product was purified by flash chromatography with dichloromethane/methanol (95:5) as eluant to give the product as a yellow solid (0.81 g, 38%); λ_{max} (EtOH)/nm 205.9, 238.7, 364.8; δ_H(250 MHz; $CDCl_3$) 8.81 (1H, s, C_5H_3N), 7.93 (1H, d, *J* 5.1Hz,

C_5H_3N), 7.80 (1H, s, N*H*), 7.47 (1H, m, C_6H_4Cl), 7.46, 7.44, 7.19 and 7.16 (4H, q, *para*-substituted aromatic), 7.22 (1H, m, C_6H_4Cl), 7.12 (1H, m, C_6H_4Cl), 7.00 (1H, m, C_6H_4Cl), 6.88 (1H, d, *J* 5.4 Hz, C_5H_3N), 5.44 (1H, s, 4-*H*), 3.99 (2H t, *J* 6.3 Hz, $CH_2CH_2CH_2O$), 3.79 (2H, q, *J* 7.1 Hz, $CO_2CH_2CH_3$), 3.57 (3H, s, OCH_3), 2.43 (3H, s, imidazo-Me), 2.35 (3H, s, 6-Me), 2.16 (2H, t, *J* 7.7 Hz, $COCH_2CH_2CH_2$), 1.85 (2H, m, $CH_2CH_2CH_2$), 0.86 (3H, t, *J* 7.1 Hz, $CO_2CH_2CH_3$); δ_C(100 MHz; CDCl3) 173.7, 167.1, 166.4, 153.0, 144.8, 144.5, 144.0, 141.9, 141.5, 140.7, 139.7, 138.1, 135.2, 132.8, 131.7, 130.0, 129.7, 127.6, 126.7, 126.2, 105.2, 104.9, 102.8, 62.8, 59.8, 51.5, 38.5, 30.4, 23.9, 19.3, 14.4, 13.8; m/z (FAB) 629 (MH^+, 24%), 307 (14%), 154 (100%), 89 (54%), 77 (65%) [Found (MH^+) 629.2170 $C_{34}H_{34}N_4O_6Cl$ requires 629.2166].

Rabbit liver esterase-catalysed hydrolysis of dihydropyridine esters. To 3 cm^3 of phosphate buffer (0.1 M, pH 7.0) was added 2 cm^3 of DMSO in which the dihydropyridine ester (10 mg, 16.6 μmoles) was dissolved. The mixture was stirred vigorously and rabbit liver esterase (Sigma, crystalline suspension in 3.6 M $(NH_4)_2SO_4$, 50 U) added. Reactions were performed at 30°C. The hydrolysis was monitored by tlc on slica gel plates developed in dichloromethane/methanol (95:5). Unreacted dihydropyridine and product acid were extracted with dichloromethane and separated by preparative tlc using the above solvent system. The product acid was dissolved in dichloromethane/methanol (1:1) and remethylated by treating with an etheral solution of diazomethane. The enantiomeric composition of the unreacted dihydropyridine and that obtained by re-methylation of the hydrolysis product was determined by h.p.l.c (Cyclobond PT column, 25 cm x 4 mm, isocratic elution with hexane/ethanol (75:25), 0.3 ml min^{-1}). Dihydropyridines were detected at 254 nm.

We thank Pfizer Central Research for financial support, and for the supply of compound **18** (UK-78,274).

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