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Non-thiazolidinedione Antihyperglycaemic Agents. Part 3: The Effects of Stereochemistry on the Potency of α-Methoxy-β-phenylpropanoic Acids†

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Abstract—*Rhizopus delemar* lipase catalysed ester hydrolysis of the α-methoxy-β-phenylpropanoate **1** affords the (*R*)-(+) and (*S*)-(−) isomers in >84% enantiomeric excess. Absolute stereochemistry was determined by a single crystal X-ray analysis of a related synthetic analogue. The activity of these two enantiomers on glucose transport in vitro and as anti-diabetic agents in vivo is reported and their unexpected equivalence attributed to an enzyme-mediated stereospecific isomerisation of the (*R*)-(+) isomer. Binding studies using recombinant human PPARγ (peroxisomal proliferator activated receptor γ), now established as a molecular target for this compound class, indicate a 20-fold higher binding affinity for the (*S*) antipode relative to the (*R*) antipode.

Introduction

Type 2 (non-insulin-dependent) diabetes mellitus is a chronic metabolic disorder characterised by a progressive resistance of peripheral tissues to the metabolic effects of insulin.[1] Insulin resistance interferes with glucose homeostasis through impaired uptake and utilisation of glucose in skeletal muscle and adipose tissues and through reduced control of hepatic glucose output.[2] Impaired release of insulin in response to elevated plasma glucose is a feature of Type 2 diabetes which leads to persistent hyperglycaemia and long term diabetic complications.[3] Moreover, several prospective studies indicate that the development of insulin resistance is an early event in the progression to Type 2 diabetes,[4,5] suggesting that drugs which are capable of improving insulin sensitivity should be useful in the treatment of the disease. Indeed, a number of thiazolidinedione derivatives typified by ciglitazone,[6] pioglitazone,[7] troglitazone[8] and rosiglitazone (BRL 49653),[9,10] have been shown to increase insulin sensitivity and normalize glycaemic control in rodent models of diabetes. Similar results have also been reported with troglitazone from clinical studies in human Type 2 diabetic patients.[11]

Until recently, attempts to identify potent insulin sensitisers not containing the thiazolidinedione pharmacophore have been largely unsuccessful.[10,12] Work from our own laboratories, however, has recently shown that this ring system may be effectively replaced by a variety of α-functionalized carboxylic acid derivatives.[13,14] Mechanistic studies suggest that, in common with the thiazolidinediones, these compounds act by the specific activation of a peroxisome proliferator-activated receptor (PPARγ), a member of the steroid nuclear hormone receptor superfamily.[15] Of particular interest were α-alkoxy derivatives such as the α-methoxypropanoic acid **1**, since many compounds of this series were up to ten-fold more potent in the *ob*/*ob* mouse than the corresponding thiazolidinedione, BRL 48482.[10,13] In contrast to the thiazolidinediones, which undergo a facile pH-dependent racemization,[16] it was anticipated that the enantiomers of **1** would be less labile and thus permit an

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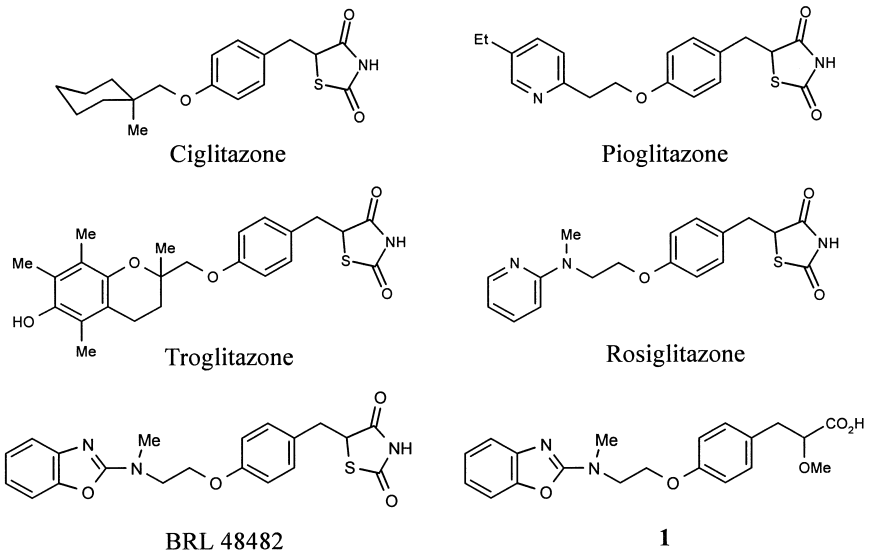


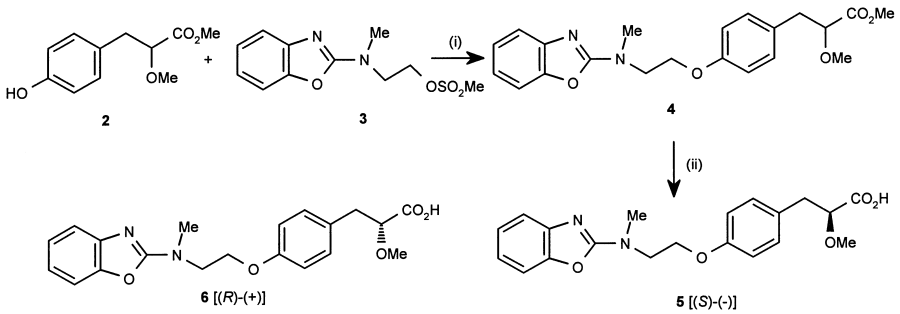
investigation into the enantiomeric dependence of biological activity. In this paper we discuss the synthesis of the enantiomers of the α-methoxypropanoic acid **1** and some related compounds and discuss their activity in vitro and in in vivo models of diabetes.

Chemistry

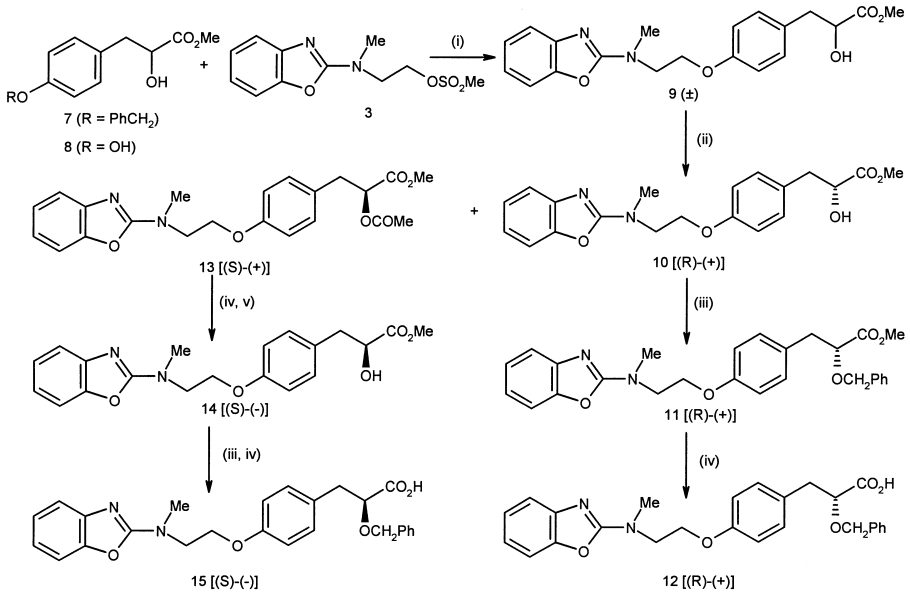
Rhodium acetate-mediated insertion of methanol into diazotyrosine methyl ester following the procedure of Moody et al.[17] gave the α-methoxy precursor **2**, which on further reaction with the mesylate **3**[10] afforded the racemic α-methoxypropanoate **4** in good yield (Scheme 1). Since enzyme catalysed enantioselective ester formation and hydrolysis is a well-established procedure for the resolution of carboxylic acids,[18] we decided to investigate the hydrolysis of **4** using a variety of commercially available lipases, having first established an efficient HPLC assay for the isomers of **4** based on their differential elution on a chiral α_1-acid glycoprotein column. From this range of enzymes, *Rhizopus delemar* lipase was found to effect preferential hydrolysis of the (*S*) ester under conditions of partial hydrolysis (66%) when the pH was maintained at 7.0 by the addition of sodium hydroxide. By this procedure unchanged (*R*) ester was recovered in 84% ee and was converted into the (*R*) acid **6** (84% ee) by exhaustive hydrolysis using *R. delemar* lipase. The (*S*) acid **5** (40% ee) isolated from the initial hydrolysis was re-esterified with methanolic hydrogen chloride and rehydrolysed to 32% completion with *R. delemar* lipase to furnish the (*S*)-acid **5** with 86% ee. The absolute stereochemistry of **5** and **6** was initially deduced by comparison with analogous reactions,[19] but was subsequently confirmed by comparison with an authentic sample of **5** (vide infra).

The corresponding α-benzyloxypropanoic acids **12** and **15** were prepared as illustrated in Scheme 2. In this instance, the *O*-protected phenol **7**[20] was hydrogenolytically debenzylated to the phenol **8**, which on coupling with the mesylate **3** gave the racemic α-hydroxypropanoate **9**. Treatment of **9** with vinyl acetate in the presence of *Pseudomonas fluorescens* lipase[21] selectively acetylated the (*S*) isomer, leaving the unchanged (*R*) enantiomer **10** in high purity (99% ee). Alkylation of **10** with benzyl bromide then gave the ester **11**, which was hydrolysed to the requisite (*R*) acid **12** without loss of chiral integrity. The absolute stereochemistry of this acid was established by conversion of the ester **10** into the crystalline oxazolidinedione **16** by reaction with (*S*)-α-methylbenzyl isocyanate. A single crystal X-ray diffraction study of this adduct (Fig. 1) clearly established the stereochemistry of the unknown center as (*R*).

Exhaustive hydrolysis of the (*S*)-acetate **13** (produced on treatment of hydroxyester **9** with vinyl acetate and *P. fluorescens* lipase) followed by re-esterification afforded



Scheme 1. *Reagents*: (i) NaH, DMF, 80 °C, 16 h; (ii) *R delemar* lipase, 0.1 M NaOH, aq acetone.



Scheme 2. *Reagents*: (i) NaH, DMF, 80 °C, 16 h; (ii) *P. fluorescens* lipase, vinyl acetate; (iii) $PhCH_2Br$, NaH, Ag_2O, DMF, RT, 8 h; (iv) HCl, dioxan, H_2O, 80 °C, 4.5 h; (v) MeOH, 4-$MePhSO_3H$, 70 °C, 4 h.

the (*S*) isomer **14** which was converted into the (*S*) enantiomer **15** (96% ee) by benzylation and hydrolysis. Enantiomeric purity was again assessed by HPLC using a chiral α_1-acid glycoprotein column. Furthermore, methylation and subsequent hydrolysis of **14** provided an authentic sample of the (*S*) α-methoxy acid, which was identical with compound **5**, thus confirming the stereochemistry of this series.

Finally, the α,α-dimethoxypropanoic acid **21** was prepared following the route shown in Scheme 3. Reaction of the benzyl chloride **17** with the silyl enolate of methyl dimethoxyacetate gave a modest yield of the acetal **18**, which was hydrogenolytically debenzylated to the phenol **19**. Subsequent reaction of the anion of **19** with mesylate **3** followed by mild hydrolysis then gave a good yield of the requisite acid **21**.

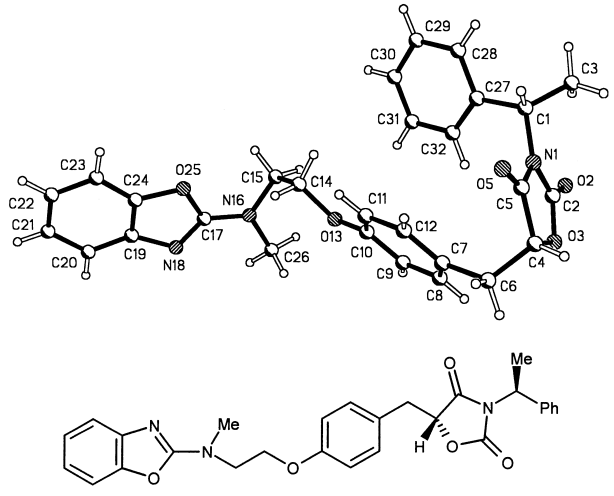


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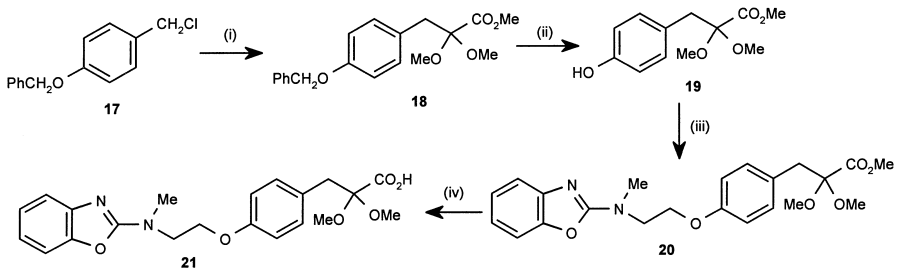
Figure 1. X-ray structure of oxazolidinedione **16**.

Results and Discussion

It is well-documented that anti-diabetic thiazolidinediones readily racemize in solution[16] and it is this lability that has largely confounded attempts to establish whether they show enantiomeric dependence at their site of action. The identification of a series of α-alkoxypropanoic acids related to one of the more potent compounds, BRL 48482, but lacking the thiazolidinedione moiety,[13] suggested that this intriguing question could now be answered. Rather surprisingly, however, resolution of **1**, the α-methoxy member of this series, failed to show any distinction between the two enantiomers, **5** and **6** in vivo in the *ob*/*ob* mouse (Table 1), despite their apparent aqueous stability.[22] Furthermore, essentially identical activity was observed for both compounds in their ability to stimulate 2-deoxyglucose uptake in differentiated 3T3-L1 adipocytes (Table 1), an in vitro assay of glucose transport[23] which correlates with in vivo potency.[24] In the belief that steric integrity was indeed maintained under the assay conditions we prepared the α,α-dimethoxy compound **21** in an attempt to determine whether there was steric tolerance for both (*R*) and (*S*) methoxy groups at this position, but this compound was inactive in vivo (Table 1).

In order to establish whether the similarity between compounds **5** and **6** was a general phenomenon we prepared the (*R*) and (*S*) enantiomers, **12** and **15**, respectively, of the related α-benzyloxy compound, which was previously shown to be a potent anti-hyperglycaemic agent.[13] By contrast with the α-methoxy antipodes, **5** and **6**, compounds **12** and **15** were readily distinguishable in vivo in that the (*S*) isomer **15** was 100-fold more potent as an antihyperglycaemic agent (Table 1).

In view of these results we examined the possibility that the enantiomers of **1** may racemize under the conditions



Scheme 3. Reagents: (i) LDA, TMSCl, $(MeO)_2CHCO_2Me$, then $ZnCl_2$, CH_2Cl_2; (ii) H_2, Pd/C, MeOH; (iii) NaH, DMF, then **3**; (iv) LiOH, MeOH, THF, H_2O.

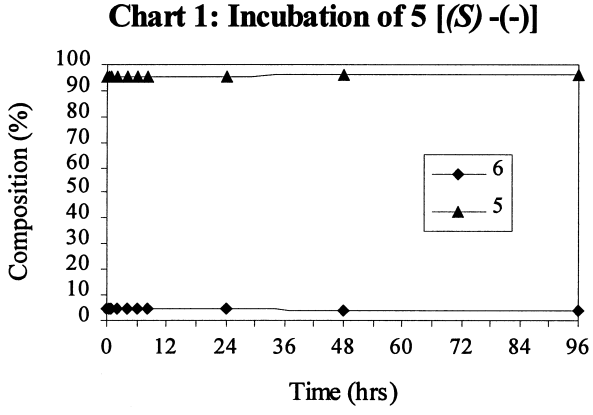
Table 1. Evaluation of α-alkoxy-β-phenylpropanoic acids[a]

Compound	ee (%)	In vivo potency *ob*/*ob* mouse ED_{25} (μmol kg^{-1} diet)	Glucose transport 3T3-L1 cells EC_{50} (nM)	PPARγ Binding *E. coli* expressed GST-hPPARγ LBD IC_{50} (nM)
1 (*R,S*)	—	3	3.1 ± 0.1 (*n* = 3)	15 (*n* = 1)
5 (*S*)	86.0	3	6.3 ± 0.4 (*n* = 3)	3.9 (*n* = 1)
6 (*R*)	84.0	3	4.9 ± 0.2 (*n* = 3)	82 ± 26 (*n* = 3)
12 (*R*)	98.0	30	nd[b]	nd[b]
15 (*S*)	96.0	0.3	nd[b]	nd[b]
21	—	Inactive @ 100	nd[b]	nd[b]
BRL48482	—	3	1.8 ± 0.2 (*n* = 5)	22 (*n* = 1)

[a]See Experimental for definitions.
[b]Not determined.

of the 3T3-L1 and *ob*/*ob* mouse experiments, while retaining at least partial steric integrity during the shorter binding studies. Each enantiomer was therefore independently incubated with differentiated 3T3-L1 adipocytes at nominal initial concentrations of 1.08 μM (400 ng mL^{-1} (Fig. 2) and 5.40 μM (2000 ng mL^{-1}) at 37 °C over a 96-h period. At both concentrations, the (*S*)-enantiomer **5** was unaffected, whereas the (*R*)-enantiomer **6** showed almost complete (>90%), unidirectional stereoinversion to **5**. Neither compound showed any change when incubated in the cell culture medium in the absence of 3T3-L1 cells. The time for 50% conversion of the (*R*)- into the (*S*)-enantiomer was estimated at ca. 36 h at the lower concentration and ca. 42 h at the higher concentration. The sigmoidal shape of the enantiomer interconversion curve is consistent with the probable involvement of a two-stage enzyme mediated reaction. In a similar experiment, **5** and **6** were incubated with cultured rat hepatocytes over a 3-h period at initial concentrations of 20 μM and 50 μM. Again, unidirectional stereoinversion of the (*R*)-enantiomer into the (*S*)-antipode was observed only in the presence of hepatocytes.

Similar unidirectional stereoinversion is well documented for non-steroidal anti-inflammatory drugs such as (*R*)-(−)-ibuprofen, which have been shown to invert via the formation and epimerization of a coenzyme-A thioester.[25] Given the demonstrated inversion of the (*R*)-enantiomer **6** in vitro, it is likely that a similar enzyme mediated conversion occurs in vivo during the evaluation of the enantiomers in the *ob*/*ob* mouse, thus accounting for their identical anti-hyperglycaemic activity. However, this epimerisation mechanism



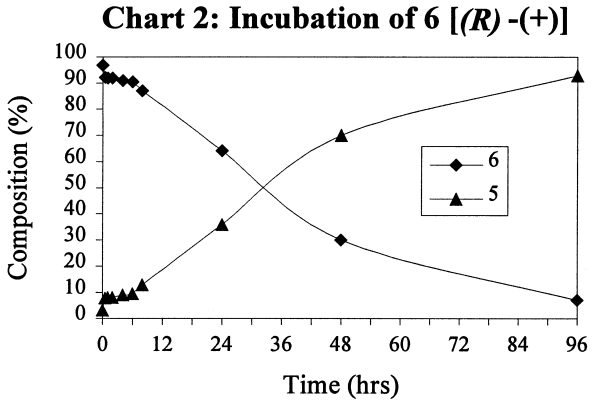


Figure 2. Incubation of **5** and **6** with 3T3-L1 Adipocytes. Compounds **5** and **6** were incubated individually with 3T3-L1 adipocytes at a concentration of 1.08 μM. Samples were withdrawn at intervals and derivatised for GC/MS assay by the method described in the Experimental.

appears to be highly substrate dependent, since the (*R*) and (*S*) enantiomers of the α-benzyloxy acid, **12** and **15** respectively, did not undergo stereoinversion when incubated with rat hepatocytes for 24 h under conditions similar to those described above. Very recently a related α-ethoxy compound was also reported to be configurationally stable under conditions of the 3T3-L1 assay,[26] adding support to the structural dependence observed in this work.

Towards the completion of our work a strong correlation was demonstrated between the anti-hyperglycaemic activity of the thiazolidinediones in rodents and their binding affinity and agonist potency on PPARγ, and there is now compelling evidence that these agents exert their action predominantly through the activation of this class of nuclear hormone receptors.[15] What had still to be established, however, was direct evidence that PPARγ receptors elicit enantiomeric discrimination, although such discrimination has previously been demonstrated at related PPARα receptors.[27] We therefore evaluated the binding affinity of **5** and **6** at recombinant human PPARγ whereby, in clear contrast to the 3T3-L1 and *ob*/*ob* mouse experiments, a marked differentiation between the two enantiomers was observed. Thus at this receptor the (*S*) enantiomer **5** had an IC_{50} of 3.9 nM ($n=1$), whereas the (*R*) antipode **6** had an IC_{50} of 82 ± 26 nM ($n=3$). It is possible that the binding affinity of the latter enantiomer **6** (84% ee) is due to the presence of the small amount of **5** in the sample, however this 20-fold greater binding affinity of **5** over **6**, taken together with our recently published work on a related α-(trifluoroethoxy) analogue,[28] implicitly confirms enantiomer discrimination at this receptor.

Conclusion

From detailed experiments in rodent adipocytes we have unequivocally demonstrated that the unexpected equivalence of the two enantiomers **5** and **6** of the potent insulin sensitiser **1** on glucose transport in vitro results from an enzyme mediated stereospecific inversion of the (*R*)-(+) antipode **6**. In view of this observation it is likely that the equivalence of these enantiomers as anti-diabetic agents in vivo is attributable to a similar enzyme mediated conversion. By contrast, the related α-benzyloxy acids **12** and **15** did not show bioequivalence in vivo, a result which is consistent with the finding that they did not undergo stereoinversion in vitro. We have further shown that the (*S*)-(−) isomer **5** ($IC_{50}=3.9$ nM) shows a 20-fold greater binding than the (*R*)-(+) isomer at human PPARγ, therefore providing confirmatory evidence for stereospecific binding at this receptor.

Experimental

General experimental details

Mass spectroscopy was conducted using electron impact (EI), chemical ionisation (CI), with ammonia as the reagent gas, or fast atom bombardment (FAB) in a 3-nitrobenzyl alcohol–sodium acetate (NOBA–Na) matrix. Compounds characterised by high resolution mass measurement were homogeneous by TLC. ^{1}H NMR spectra were recorded at 270 MHz in $CDCl_3$ solution. Chemical shifts are given in δ (ppm) relative to TMS and coupling constants *J* are given in Hz. $[\alpha]_D^{25}$ values are given in deg cm^2 g^{-1}. Dry solvents refers to the use of Aldrich Sure-Seal® dried solvents. All organic solutions obtained from aqueous extractions were dried over $MgSO_4$. Chromatography refers to flash chromatography on silica gel.

Determination of enantiomeric excess

Enantiomeric excess of chiral compounds was determined by HPLC using a chiral α_1-acid glycoprotein (chiral AGP) column. HPLC methods and conditions, including eluents used, solvent flow rate and detection wavelength were:- (A): MeCN-0.01M aq Na_3PO_4, pH 7.0 (12:88 v/v), 0.8 mL/min, $\lambda=245$ nm; (B): MeCN-0.01M aq Na_3PO_4, pH 7.0 (5:95 v/v), 0.5 mL/min, $\lambda=225$ nm; (C): MeCN-0.01 M aq Na_3PO_4, pH 7.0 (10:90 v/v), 1 mL/min, $\lambda=245$ nm and (D): MeCN-0.01 M aq Na_3PO_4, pH 5.7 (12:88 v/v), 1 mL/min, $\lambda=245$ nm. The method appropriate to the analysis of each chiral compound is indicated within the text.

Biological procedures

Determination of anti-hyperglycaemic activity. C57 Bl/6 *ob*/*ob* mice were obtained at 9–10 weeks of age from Harlan Olac Ltd., Oxon, U.K. and were maintained at 26 ± 2 °C on a 12 h light/12 h dark cycle. The animals were provided with powdered RM3 diet (Special Diet Services, Witham, Essex, U.K.) and water ad libitum. Compounds were administered by dietary admixture for 8 days. Diets supplemented with compound were stored at 4 °C, and mice were provided with fresh food each day. Six to eight mice were used in each treatment group and none of the compounds tested caused significant changes in body weight gain during the treatment period. After 8 days of compound administration, mice were fasted for 5 h from 08.00 hrs after which time a blood sample (10 μL) was taken for glucose analysis from the cut tip of the tail. Glucose (3 g/kg of body weight) was then administered by oral gavage. Additional blood samples (10 μL) for glucose analysis were then taken at 45, 90 and 135 min after glucose challenge. Blood samples were added to 1 mL of haemolysis reagent (50 mg/L digitonin, 100 mg/L maleimide), and the glucose concentration in the haemolysed sample was determined spectrophotometrically with hexokinase/glucose-6-phosphate dehydrogenase[29] using a Ciba-Corning 550 express clinical chemistry analyser.

Anti-hyperglycaemic activity is defined as the percentage of reduction in the area under the blood glucose versus time curve (AUC) relative to control animals. The AUC was calculated trigonometrically using the trapezoid rule.[30] The ED_{25} dose is that dose of compound that produces an approximate 25–30% reduction in the AUC and results in a glucose tolerance profile equivalent to that of normoglycaemic (+/?) lean littermates.

3T3-L1 cell experiments

Glucose uptake. The 3T3-L1 fibroblast cell line was obtained from the American Type Culture Collection. Cells were grown to confluence, differentiated according to established procedures[31] and used 8–10 days post differentiation. Compounds were dissolved in DMSO (final concentration of DMSO$\leq$0.2%) and incubated with 3T3-L1 cells for 48 h prior to assay of 2-deoxy-1-[^{14}C]-glucose transport.[31,32] The EC_{50} is defined as the concentration of test compound giving a 50% stimulation of glucose transport.

Bioconversion experiments. On the day of assay, cells were washed in serum free medium and then exposed to either compound **5** or compound **6** at nominal concentrations of 400 and 2000 ng mL^{-1}. Samples were then incubated at approximately 37 °C over a period of 96 h with aliquots being removed at specific times for isomer analysis. The separate isomers were also incubated at the same nominal concentrations in cell culture medium in the absence of 3T3-L1 cells over a period of 120 h at approximately 37 °C to assess their potential to interconvert under cell-free conditions. Control samples (non-incubated) were also prepared in the cell culture medium and analysed immediately prior to the incubation samples to confirm that no interconversion occurred during the period of analysis. These were prepared in triplicate and randomly placed throughout the run sequence.

Assay procedure. Samples of the incubation media (500 μL) containing the test compound were acidified with hydrochloric acid (1.0 M, 100 μL) and extracted with toluene (2 mL). The mixture was centifuged (~3000 g, 10 min) and the toluene layer was separated and evaporated in a gentle stream of nitrogen at 60 °C. The resulting material was derivatised by treatment with oxalyl chloride (50 μL, 15 min) followed, after re-evaporation, by (*R*)-1-cyclohexylethylamine (1% v/v in toluene, 100 μL). After brief whirlimixing the resulting amides were analysed by capillary GC/MS using a conventional non-chiral silica column (25 m×0.32 mm, HP-1 {methyl silicone}, 0.52 μm phase thickness).

PPARγ binding assays. Binding assays were performed in 96-well plates on crude extracts of XL-1 blue *E.Coli.* cells expressing a fusion protein comprising GST and the ligand binding domain of human PPARγ (GST-hPPARγLBD). 1.43 μg of total protein was present in a final volume of 50 μL. Radioligand [^{125}I]-SB 236636 {3-[4-[2-[N-(2-benzoxazolyl)-N-methylamino]ethoxy]-3-iodophenyl]-2-ethoxypropanoic acid,[28] K_d 8 nM at GST-hPPARγLBD, ED_{25} 3 μmol.kg^{-1} diet in *ob*/*ob* mice} was present at 145 pM. Competing compounds were dissolved in DMSO (concentration of DMSO$\leq$0.1%) and binding was allowed to reach equilibrium by incubation for 18 h at 4 °C. Bound ligand was separated from free on mixed cellulose ester filters. Each assay was performed in triplicate.

Screen of enzymes for ester hydrolysis activity. A mixture of the test enzyme (2–5 mg; below), potassium phosphate buffer (0.1 M, pH 7.0; 0.475 mL) and a solution of racemic ester **4** in acetone (7 mg/mL; 0.025 mL) was shaken at ambient temperature and monitored by TLC for evidence of ester hydrolysis.

Enzymes screened. *Candida cylindracea* lipase, pancreatic lipase, *Mucar javanicus* lipase, *Rhizopus javanicus* lipase, *Rhizopus arrhizus* lipase, *Pen. roqueforte* lipase, *Pen. cyclopium* lipase, *Pseudomonas fluorescens* lipase. *Rhizopus niveus* lipase, *Rhizopus delemar* lipase, *Candida lipolytica* lipase, *Rhizopus sp.* LIP F3, *Rhizopus sp.* LIP F4, acetylesterase (orange peel) and *Aspergillus sp.* lipase.

Methyl 3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]ethoxy]-phenyl]-2-methoxypropanoate 4. Sodium hydride (60% dispersion in mineral oil; 0.92 g, 23 mmol) was added to a solution of phenol **2**[17] (4.34 g, 21 mmol) in dry DMF (50 mL) at room temperature under argon. The mixture was stirred for 30 min at room temperature prior to the addition of a solution of mesylate **3**[10] (5.67 g, 21 mmol) in DMF (50 mL) and then heated at 80 °C for 16 h before being cooled and diluted with water (1.4 L). The mixture was acidified to pH 2 with hydrochloric acid then extracted with ethyl acetate (3×400 mL). The combined ethyl acetate solutions were washed with water (4×1 L) and brine (1 L), dried and evaporated. The resulting gum was chromatographed using methanol-dichloromethane (1.5/98.5 v/v) as eluent to afford the racemic methoxy-ester **4**, (5.77 g, 72%), a colourless gum. [Found $(M+H)^+$ (CI) 385.1759. $C_{21}H_{24}N_2O_5$ requires $[M+H]^+$ 385.1763]; ν_{max} (Film)/cm^{-1} 1740 (CO); δ_H (270 MHz, $CDCl_3$) 2.95 (2H, m, $ArCH_2$), 3.33 (3H, s, NMe), 3.34 (3H, s, OMe), 3.70 (3H, s, OMe), 3.93 (3H, m, NCH_2 and $ArCH_2CH$), 4.23 (2H, t, *J* 5.2, OCH_2), and 6.70–7.40 (8H, m, aryl-H); *m*/*z* (CI) 385 [$(M+H)^+$, 100%].

***Rhizopus Delemar* lipase resolution of 4: (*S*)-(−)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2-methoxypropanoic acid 5 and (*R*)-(+)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2-methoxypropanoic acid 6.** *Rhizopus delemar* lipase (0.680 g, ex Biocatalysts Ltd.) was stirred in deionised water (380 mL) at 23 °C and the mixture adjusted to pH 7.0. A solution of racemic methoxy-ester **4** (1.0 g, 2.6 mmol) in acetone (20 mL) was added and the mixture stirred and maintained at pH 7.0 by autotitration with 0.1 M sodium hydroxide solution until a 66% molar equivalent of base had been added. The solution was acidified to pH 2.0 with dilute hydrochloric acid (2 M) and extracted with dichloromethane (400 mL). The dichloromethane solution was washed with 50% saturated sodium bicarbonate solution (200 mL) and water, dried and evaporated to afford the enriched (*R*)-(+)-*methoxy-ester* [0.350 g, 34%; a colourless oil; 90% ee by HPLC (Method A, retention time 15.7 min)]. Acidification of the sodium bicarbonate solution and re-extraction with dichloromethane afforded the enriched (*S*)-(−)-*methoxy-acid* (0.530 g, 55%; a colourless solid) which was dissolved in methanol presaturated with hydrogen

chloride gas, stirred at room temperature for 3 h and then concentrated. The residue was partitioned between water and ethyl acetate and the organic solution was dried and evaporated to afford the enriched (*S*)-(−)-*methoxy-ester* (0.362 g, 36% based on initial racemic ester used; 40% ee by HPLC (Method A, retention time 12.5 min).

The reaction was repeated and the respective enriched (*R*)-(+)-*ester* (0.334 g, 33%; 80% ee) and enriched (*S*)-(−)-*ester* (0.590 g, 59%; ee not determined) combined with those isolated above.

(*S*)-(−)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2-methoxypropanoic acid 5. A solution of the combined enriched (*S*)-(−)-*esters* (0.950 g, 2.4 mmol) in acetone (20 mL) was treated with *R. delemar* lipase (0.500 g) in deionised water (380 mL) at pH 7.0 as before and autotitrated with 0.1 M sodium hydroxide solution until a 32% molar equivalent of base had been added. The mixture was acidified to pH 2.0 with hydrochloric acid and extracted into dichloromethane. The dichloromethane solution was extracted with 50% saturated sodium bicarbonate solution and the dichloromethane solution discarded. The sodium bicarbonate solution was re-acidified and re-extracted with dichloromethane to afford the crude product which was triturated with hexane to afford the (*S*)-(−)-methoxy-acid **5** (0.256 g, 13% based on total racemic ester used), a colourless solid, mp 116–119 °C. [Found C, 64.5; H, 5.9; N, 7.5%; M^+ (EI) 370.1530. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%; *M* 370.1529]; $[\alpha]_D^{25}$ −10° (*c* 0.55 in MeOH); 86% ee by HPLC (Method B, retention time 9.4 min); ν_{max} (KBr)/cm^{-1} 2900–2500 (COOH) and 1710 (CO); δ_H (270 MHz, $CDCl_3$) 2.95 (1H, dd, *J* = 14.0 and 6.9 Hz, ArC*H*H), 3.07 (1H, dd, *J* = 14.0 and 5.0 Hz, ArCH*H*), 3.31 (3H, s, NMe), 3.40 (3H, s, OMe), 3.90 (2H, m, NCH_2), 3.96 (1H, dd, *J* = 6.9 and 5.0 Hz, $ArCH_2CH$), 4.15 (2H, m, *J* = 5.2 Hz, OCH_2), 6.30 (1H, br, exchanges with D_2O, COOH) and 6.77–7.40 (8H, m, aryl-H); *m*/*z* (EI) 370 (M^+, 100%).

(*R*)-(+)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2-methoxypropanoic acid, 6. In a similar manner, the combined enriched (*R*)-(+)-*esters* (0.684 g, 1.78 mmol) were exhaustively hydrolysed by *R. delemar* lipase (0.300 g) by addition of 0.1 M sodium hydroxide solution to maintain pH 7.0 to afford, after extractive workup, the (*R*)-(+)-methoxy-acid **6** (0.490 g, 25% based on total racemic ester used), a colourless solid, mp 121–123 °C. [Found C, 64.6; H, 5.9; N, 7.5%. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%]; $[\alpha]_D^{25}$ +13° (*c* 0.5 in MeOH); 84% ee by HPLC (Method B, retention time 8.2 min); spectroscopically identical with **5**.

Methyl 2-hydroxy-3-(4-hydroxyphenyl)propanoate 8. A solution of benzyl ether **7**[20] (10.06 g, 35 mmol) in methanol (100 mL) was hydrogenated over 10% Palladium-charcoal (0.5 g) at room temperature and pressure. The mixture was filtered and concentrated to afford the phenol **8** (6.60 g, 97%) as a colourless oil. ν_{max} (Film)/cm^{-1} 3400 (OH) and 1725 (CO); δ_H (270 MHz, $CDCl_3$) 2.90 (1H, dd, *J* = 14.0 and 6.3 Hz, ArCH*H*), 3.05 (1H, dd, *J* = 14.0 and 4.4 Hz, ArC*H*H), 3.77 (3H, s, OMe), 4.42 (1H, dd, *J* = 6.3 and 4.4 Hz, $ArCH_2CH$) 5.50 (2H, br, exchanges with D_2O, 2×OH), 6.70 (2H, d, *J* = 8.8 Hz, aryl 3-H & 5-H) and 7.05 (2H, d, *J* = 8.8 Hz, aryl 2-H & 6-H); *m*/*z* (CI) 214 [$(M + NH_4)^+$, 100%], 197 [$(M + H)^+$, 6], 179 (5) and 107 (34).

Methyl 3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2-hydroxypropanoate 9. Phenol **8** (2.38 g, 12 mmol) was reacted with mesylate **3** (3.24 g, 12 mmol) in DMF in a manner analogous to that described for the preparation of α-methoxy-ester **4** (above). The resulting gum was chromatographed using ethyl acetate/hexane (50/50 v/v) as eluent to afford the racemic hydroxy-ester **9**, (1.52 g, 37%), a colourless solid, mp 130–132 °C. [Found C, 65.0; H, 6.1; N, 7.5%; M^+ (EI) 370.1526. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%; *M* 370.1529]; ν_{max} (KBr)/cm^{-1} 3400 (OH) and 1730 (CO); δ_H (270 MHz, $CDCl_3$) 2.88 (1H, dd, *J* = 14.0 and 6.6 Hz, ArCH*H*), 3.02 (2H, m, ArC*H*H and OH), 3.31 (3H, s, NMe), 3.73 (3H, s, OMe), 3.90 (2H, t, *J* = 5.2 Hz, NCH_2), 4.20 (2H, t, *J* = 5.2 Hz, OCH_2), 4.42 (1H, m, $ArCH_2CH$) and 6.70–7.40 (8H, m, aryl-H); *m*/*z* (FAB) 393 [$(M + Na)^+$, 75%] and 371 [$(M + H)^+$, 100].

***P. Fluorescens* lipase resolution of methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2-hydroxy-propanoate 9.** *Pseudomonas Fluorescens* lipase (7.1 g) was added to a solution of the racemic hydroxy-ester **9** (5.08 g, 13.7 mmol) in vinyl acetate (350 mL) and the mixture stirred at room temperature for 6 days, then filtered and concentrated. The residue was chromatographed using ethyl acetate/hexane (gradient: from 50/50 v/v to 60/40 v/v) as eluent to afford firstly the (*S*)-acetate **13** (2.43 g, 43%), followed by the (*R*)-(+)-hydroxy-ester **10** (1.97 g, 39%).

(*S*)-(+)-Methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methyl-amino]ethoxy]phenyl]-2-acetoxy-propanoate 13. An oil. [Found M^+ (EI) 412.1635. $C_{22}H_{24}N_2O_6$ requires *M* 412.1635]; $[\alpha]_D^{25}$ +1° (*c* 1.0 in $CHCl_3$); 98% ee by HPLC (Method C, retention time 24.7 min; (*R*)-enantiomer retention time 33.7 min); δ_H (270 MHz, $CDCl_3$) 2.06 (3H, s, COMe), 3.05 (1H, dd, *J* = 14.3 and 8.2 Hz, ArCH*H*), 3.10 (1H, dd, *J* = 14.3 and 4.7 Hz, ArC*H*H), 3.33 (3H, s, NMe), 3.69 (3H, s, OMe), 3.92 (2H, t, *J* = 5.2 Hz, NCH_2), 4.23 (2H, t, *J* = 5.2 Hz, OCH_2), 5.15 (1H, dd, *J* = 8.2 and 4.7 Hz, $ArCH_2CH$) and 6.70–7.40 (8H, m, aryl-H); *m*/*z* (FAB) 435 [$(M + Na)^+$, 36%], 413 [$(M + H)^+$, 100], 369 (4), 329 (10), 307 (7) and 175 (47).

(*R*)-(+)-Methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methyl-amino]ethoxy]phenyl]-2-hydroxypropanoate 10. A colourless solid, mp 130–131 °C. [Found C, 64.9; H, 6.05; N, 7.6%. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%]; $[\alpha]_D^{25}$ +10° (*c* 0.85 in $CHCl_3$); 99% ee by HPLC (Method C, retention time 12.4 min); spectroscopically identical with the racemic hydroxy-ester **9**.

(*R*)-(+)-Methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methyl-amino]ethoxy]phenyl]-2-benzyloxypropanoate 11. To a solution of the (*R*)-(+)-hydroxy-ester **10** (99% ee,

0.72 g, 1.96 mmol) and benzyl bromide (3.35 g, 19.6 mmol) in DMF (14 mL) was added sodium hydride (60% dispersion in mineral oil, 0.072 g, 1.8 mmol) followed by silver(I) oxide (0.458 g, 1.96 mmol). The mixture was stirred at room temperature for 8 h before being poured into a mixture of brine (60 mL), ethyl acetate (80 mL) and dilute hydrochloric acid (2 M, 5 mL). The organic solution was separated and washed with brine (4×60 mL), dried and concentrated. Chromatography of the residue using ether/hexane (67/33 v/v) as eluent afforded the (*R*)-(+)-benzyloxy-ester **11** (0.63 g, 76% based on sodium hydride used) as a colourless gum. [Found M^+ (EI) 460.2000. $C_{27}H_{28}N_2O_5$ requires *M* 460.1999]; $[\alpha]_D^{25}$ +35° (*c* 0.85 in $CHCl_3$); ee not determined; ν_{max} (Film)/cm^{-1} 1740 (CO); δ_H (270 MHz, $CDCl_3$) 2.98 (2H, m, ArCH_2CH), 3.35 (3H, s, NMe), 3.69 (3H, s, OMe), 3.94 (2H, t, *J* = 5.2 Hz, NCH_2), 4.10 (1H, dd, *J* = 7.7 and 5.5 Hz, $ArCH_2CH$), 4.24 (2H, t, *J* = 5.2 Hz, OCH_2), 4.35 (1H, d, *J* = 11.9 Hz, OC*H*HPh), 4.64 (1H, d, *J* = 11.9 Hz, OCH*H*Ph), 6.79 (2H, d, *J* = 8.8 Hz, aryl 3-H and 5-H) and 6.95–7.40 (11H, m, aryl-H)]; *m*/*z* (FAB) 483 [$(M+Na)^+$, 18%] and 461 [$(M+H)^+$, 100].

(*R*)-(+)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2-benzyloxypropanoic acid 12. A solution of the (*R*)-(+)-benzyloxy-ester **11** (0.61 g, 1.33 mmol) in dioxan (15 mL) and dilute hydrochloric acid (2 M, 15 mL) was heated at 80 °C for 4.5 h, then cooled and concentrated. The residue was partitioned between ethyl acetate and brine and the organic solution dried and evaporated. The crude acid thus obtained was dissolved in ethyl acetate (15 mL) and (*S*)-(−)-α-methylbenzylamine (0.169 g, 1.39 mmol) was added. The mixture was stirred for 2 h and the resulting white solid was filtered off, washed with ether, then partitioned between ethyl acetate (50 mL), brine (50 mL) and dilute hydrochloric acid (2 M, 3 mL). The ethyl acetate solution was dried and concentrated to yield the (*R*)-(+)-benzyloxy-acid **12**, a foam (0.54 g, 91%). Crystallisation from ether-hexane gave a white solid, mp 76–78 °C. [Found C, 69.1; H, 5.8; N, 6.2%; M^+ (EI) 446.1844. $C_{26}H_{26}N_2O_5{\cdot}0.25\ H_2O$ requires C, 69.2; H, 5.9; N, 6.2%; *M* 446.1842]; $[\alpha]_D^{25}$ +23° (*c* 0.7 in $CHCl_3$); 98% ee by HPLC (Method D, retention time 13.3 min); ν_{max} (Nujol)/cm^{-1} 2800–2400 (COOH) and 1722 (CO); δ_H (270 MHz, $CDCl_3$) 3.05 (2H, m, ArCH_2CH), 3.31 (3H, s, NMe), 3.90 (2H, t, *J* = 5.4 Hz, NCH_2), 4.15 (3H, m, $ArCH_2CH$ and OCH_2CH_2N), 4.45 (1H, d, *J* = 11.5 Hz, PhC*H*HO), 4.70 (1H, d, *J* = 11.5 Hz, PhCH*H*O), 6.70–7.40 (13H, m, aryl-H) and 9.80 (1H, br, exchanges with D_2O, COOH); *m*/*z* (EI) 446 (8%), 311 (36), 175 (83), 161 (38), 148 (100).

(*S*)-(−)-Methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2-hydroxypropanoate 14. A solution of the (*S*)-acetate **13** (98% ee, 2.16 g, 5.24 mmol) in dilute hydrochloric acid (2 M, 30 mL) and dioxan (30 mL) was heated at 80 °C for 4.5 h, cooled and concentrated and the residue suspended in benzene (50 mL) and re-evaporated. The resulting solid was dissolved in methanol (150 mL) containing *p*-toluenesulfonic acid (ca. 0.1 g) and heated at reflux for 4 h, then cooled and concentrated. The residue was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution, the organic solution dried and concentrated and the crude product purified by chromatography using ethyl acetate/hexane (60/40 v/v) as eluent to give (*S*)-(−)-hydroxy-ester **14** (1.54 g, 79%), a colourless solid, mp 129–130 °C. [Found C, 64.7; H, 6.1; N, 7.6%; M^+ (EI) 370.1528. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%; *M* 370.1529]; $[\alpha]_D^{25}$ −9° (*c* 0.8 in $CHCl_3$); 97% ee by HPLC (Method C, retention time 25.8 min); spectroscopically identical with racemic alcohol **9**.

(*S*)-(−)-3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2-benzyloxypropanoic acid 15. The (*S*)-(−)-benzyloxy-acid **15** was prepared from the (*S*)-(−)-hydroxy-ester **14** (97% ee) in an analogous manner to that described above for the (*R*)-(+)-enantiomer **12**, and was obtained as a colourless foam. [Found M^+ (EI) 446.1844. $C_{26}H_{26}N_2O_5$ requires *M* 446.1842]; $[\alpha]_D^{25}$ −23 (*c* 0.8 in $CHCl_3$); 96% ee by HPLC (Method D, retention time 10.5 min); spectroscopically identical with the (*R*)-(+)-enantiomer **12**.

Preparation of (*S*)-(−)-3-[4-[2-[N-(2-benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2-methoxypropanoic acid 5 by alkylation and hydrolysis of (*S*)-(−)-hydroxy-ester 14. Methylation of the (*S*)-(−)-hydroxy-ester **14** (98% ee) with methyl iodide in an analogous manner to that described above for the (*R*)-(+)-benzyloxy-ester **11** afforded the (*S*)-(−)-*methoxy-ester* [A colourless gum; $[\alpha]_D^{25}$ −10° (*c* 1.23 in $CHCl_3$); 93% ee by HPLC (see above); spectroscopically identical with the racemic methoxy-ester **4**]. Subsequent hydrolysis in the manner described for the (*R*)-(+)-benzyloxy-acid **12** afforded the (*S*)-(−)-methoxy-acid **5** as a colourless solid, mp 118–120 °C (dichloromethane/hexane). [Found C, 64.5; H, 5.9; N, 7.6%; M^+ (EI) 370.1528. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.6%; M 370.1529]; $[\alpha]_D^{25}$ −12° (*c* 1.08 in MeOH); 92% ee by HPLC (see above); spectroscopically identical with that prepared via the *R. delemar* lipase procedure.

[3(1*S*),5*R*]-5-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]-ethoxy]benzyl]-3-(1-phenylethyl)oxazolidin-2,4-dione 16. Sodium hydride (60% dispersion in mineral oil, 0.011 g, 0.28 mmol) was added to a solution of (*S*)-(−)-α-methylbenzylisocyanate (0.046 mL, 0.31 mmol) and the (*R*)-(+)-hydroxy-ester **10** (99% ee, 0.105 g, 0.28 mmol) in THF (3 mL). The mixture was stirred for 5 min then acidified with dilute hydrochloric acid (2 M), extracted with ethyl acetate and the organic solution dried and concentrated. The residue was purified by preparative silica gel thin layer chromatography using diethyl ether as eluent to afford the product, a colourless gum (0.115 g, 83%), as a 4/1 mixture of diastereoisomers. Crystallisation from hexane-ethyl acetate afforded the major [3(1*S*),5*R*]-diastereoisomer **16** as a colourless solid, mp 107–108 °C. [Found C, 69.2; H, 5.65; N, 8.7%; M^+ (EI) 485.1949. $C_{28}H_{27}N_3O_5$ requires C, 69.3; H, 5.6; N, 8.7%; M 485.1951]; δ_H (270 MHz, $CDCl_3$) 1.58 (3H, d, *J* = 7.1 Hz, Me), 3.11 (1H, dd, *J* = 14.6 and 3.85 Hz, ArC*H*H), 3.23 (1H, dd, *J* = 14.6 and 4.4 Hz, ArCH*H*), 3.37 (3H, s, NMe), 3.96 (2H, t, *J* = 5.0 Hz, NCH_2), 4.20 (2H, t, *J* = 5.0 Hz, OCH_2), 4.92

(1H, dd, $J=4.4$ and 3.85 Hz, oxazolidine 5-H), 5.07 (1H, q, $J=7.1$ Hz, PhCH) and 6.70–7.40 (13H, m, aryl-H); m/z (CI) 486 [$(M+H)^+$, 100%].

Methyl 3-(4-benzyloxyphenyl)-2,2-dimethoxypropanoate 18. A solution of methyl dimethoxyacetate (3.0 g, 22.4 mmol) in dry THF (5 mL) was added slowly to a −70 °C solution of lithium diisopropylamide [prepared from *n*-butyllithium (2.5 M in hexane, 8.95 mL, 22.4 mmol) and diisopropylamine (3.13 mL, 22.4 mmol) in THF (10 mL)] under argon. The mixture was stirred at −70 °C for 1 h prior to the addition of chlorotrimethylsilane (2.84 mL, 22.4 mmol) and stirring continued for an additional 50 min at −70 °C, warmed to room temperature and stirring continued for a further 30 min. The mixture was concentrated and the residue suspended in dry diethyl ether (50 mL), filtered and the filtrate concentrated to afford the crude silyl enol ether as a yellow oil (3.541 g). The oil was dissolved in dry dichloromethane (15 mL) and 4-benzyloxybenzyl chloride (3.77 g, 22.4 mmol) and zinc(II) chloride (0.22 g, 2.24 mmol) added. The mixture was stirred at room temperature overnight then washed with water (2×20 mL), dried and evaporated. The resulting gum was chromatographed twice, first using ethyl acetate/hexane (gradient: from 5/95 v/v to 30/70 v/v) followed by acetone-hexane (gradient: from 5/95 v/v to 10/90 v/v) as eluent to afford the propanoate **18** (1.145 g, 16%), a colourless solid, mp 49–50 °C. [Found C, 67.9; H, 7.2%; M^+ (EI) 330.1467. $C_{19}H_{22}O_5$ requires C, 67.95; H, 7.2%; M 330.1467]; ν_{max} (Nujol)/cm^{-1} 1750 (CO); δ_H (270 MHz, $CDCl_3$) 3.12 (2H, s, ArCH_2C), 3.37 (6H, s, 2×OMe), 3.61 (3H, s, COOMe), 5.02 (2H, s, ArCH_2O) and 6.87–7.44 (9H, m, aryl-H); m/z (FAB) 353 [$(M+Na)^+$, 100%].

Methyl 3-(4-hydroxyphenyl)-2,2-dimethoxypropanoate 19. A solution of benzyl ether **18** (1.016 g, 3.1 mmol) in methanol (20 mL) was hydrogenated over 10% palladium-charcoal (0.1 g) at room temperature and ambient pressure. The mixture was filtered and concentrated to afford the phenol **19** (0.583 g, 79%), a pale-yellow solid, mp 115–116 °C. [Found M^+ (EI) 240.0998. $C_{12}H_{16}O_5$ requires M 240.0998]; ν_{max} (Nujol)/cm^{-1} 3900 (OH) and 1750 (CO); δ_H (270 MHz, $CDCl_3$) 3.09 (2H, s, ArCH_2C), 3.35 (6H, s, 2×OMe), 3.63 (3H, s, COOMe), 5.50 (1H, br, exchanges with D_2O, OH), 6.64 (2H, d, $J=7.98$ Hz, aryl 3-H and 5-H) and 6.95 (2H, d, $J=7.98$ Hz, aryl 2-H and 6-H); m/z (CI) 258 [$(M+NH_4)^+$, 52%], 226 (29) and 194 (100).

Methyl 3-[4-[2-[N-(2-benzoxazolyl)-N-methylamino]-ethoxy]phenyl]-2,2-dimethoxypropanoate 20. Phenol **19** (0.522 g, 2.2 mmol) was reacted with mesylate **3** (0.590 g, 2.2 mmol) in a manner analogous to that described previously for methoxy-ester **4**. Dimethoxy-ester **20** (0.662 g, 73%) was obtained as a colourless oil which crystallised on standing, mp 77–78 °C. [Found M^+ (EI) 414.1786. $C_{22}H_{26}N_2O_6$ requires M 414.1791]; ν_{max} (Nujol)/cm^{-1} 1760 (CO); δ_H (270 MHz, $CDCl_3$) 3.10 (2H, s, ArCH_2C), 3.34 (3H, s, NMe), 3.36 (6H, s, 2×OMe), 3.61 (3H, s, COOMe), 3.93 (2H, t, $J=5.2$ Hz, NCH_2), 4.22 (2H, t, $J=5.2$ Hz, OCH_2) and 6.78–7.37 (8H, m, aryl-H); m/z (FAB) 437 [$(M+Na)^+$, 100%] and 415 [$(M+H)^+$, 62].

3-[4-[2-[N-(2-Benzoxazolyl)-N-methylamino]ethoxy]phenyl]-2,2-dimethoxypropanoic acid 21. A mixture of the dimethoxy-ester **20** (0.30 g, 0.73 mmol), lithium hydroxide monohydrate (0.033 g, 8.0 mmol), methanol (2 mL), THF (2 mL) and water (2 mL) was stirred at room temperature for 18 h. A further portion of lithium hydroxide (8.0 mmol) was added and stirring continued for an additional 18 h. The mixture was concentrated and the residue diluted with water (15 mL), acidified to pH 4.5 with dilute hydrochloric acid (0.001 M) and extracted with ethyl acetate (2×30 mL). The ethyl acetate solution was dried and concentrated and the resulting gum triturated with isohexane-ether to afford the dimethoxy-acid **21** (0.262 g, 90%), a white solid, mp 134–135 °C. [Found C, 62.9; H, 6.2; N, 6.95%; M^+ (EI) 401.1711. $C_{21}H_{24}N_2O_6$ requires C, 63.0; H, 6.0; N, 7.0%; M 401.1713]; ν_{max} (Nujol)/cm^{-1} 3400–2500 (COOH) and 1740 (CO); δ_H (270 MHz, $CDCl_3$) 3.16 (2H, s, ArCH_2C), 3.27 (3H, s, NMe), 3.42 (6H, s, 2×OMe), 3.80 (2H, t, $J=5.2$ Hz, NCH_2), 3.93 (2H, t, $J=5.2$ Hz, OCH_2) and 6.72–7.75 (9H, m, reduces to 8H on exchange with D_2O, aryl-H and COOH); m/z (CI) 401 [$(M+H)^+$, 100%].

X-ray crystallography of oxazolidinedione 16. Oxazolidinedione **16** was crystallised from ethanol containing a trace of acetonitrile. The molecule crystallised with two independent molecules in the crystallographically independent unit. The two molecules had identical stereochemistry and differed conformationally only slightly. The largest difference between molecules in any torsion angle was 17°. One of the molecules is shown in Figure 1. Lattice parameters were determined from the setting angles of 25 reflections well distributed in reciprocal space measured on an Enraf–Nonius CAD-4 diffractometer. Intensity data were collected on the diffractometer using graphite monochromated copper radiation and an ω-2θ variable speed scan technique. The structure was solved by direct methods using the SHELXS program series and refined using the SHELXL-93 refinement program. Hydrogen atoms were included in idealized positions which rode on the atom to which they are attached.

Crystal data

$C_{28}H_{27}N_3O_5$; $M=485.53$; $T=223(2)$K; $\lambda=1.54178$ Å; crystal size 0.54×0.50×0.12 mm; monoclinic; space group $P2_1$; unit cell dimensions $a=7.652(4)$Å, $b=6.355(2)$Å, $c=49.45(5)$Å, $\beta=91.22(2)°$; $V=2404(3)$Å^3; $Z=4$; $D_{calc}=1.341$ Mg/m^3; $\mu=0.761$ mm^{-1}; F(000) = 1024; θ range for data collection 1.79 to 62.56°; index ranges $-8\leq h\leq 8$, $0\leq k\leq 7$, $-56\leq l\leq 56$; reflections collected 8451; independent reflections 4231 ($R_{int}=0.0421$); refinement method full-matrix least-squares on F^2; data/restraints/parameters 4230/1/653; goodness-of-fit on F^2 1.079; final R indices: 4062 data; $I>2\sigma(I)$ R1 = 0.070, wR2 = 0.194, all data R1 = 0.072, wR2 = 0.197; absolute structure parameter −0.1(4); largest diff. peak and hole 0.69 and −0.37 eÅ^{-3}.

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References and Notes

1. Reaven, G. M. *Diabetes* **1988**, *37*, 1595.
2. Moller, D. E.; Flier, J. S. *N. Engl. J. Med.* **1991**, *325*, 938.
3. DeFronzo, R. A. *Diabetes* **1988**, *37*, 667.
4. Warram, J. H.; Martin, B. C.; Krolewski, A. S.; Soeldner, J. S.; Kahn, C. R. *Ann. Intern. Med.* **1990**, *113*, 909.
5. Lillioja, S.; Mott, D. M.; Spraul, M., Ferraro, R.; Foley, J. E.; Ravussin, E.; Knowler, W. C.; Bennett, P. H.; Bogardus, C. *N. Engl. J. Med.* **1993**, *329*, 1988.
6. (a) Sohda, T.; Mizuno, K.; Imamiya, E.; Sugiyama, Y.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1982**, *30*, 3580. (b) Fujita, T.; Sugiyama, Y.; Taketomi, S.; Sohda, T.; Kawamatsu, Y.; Iwatsuka, H.; Suzuoki, Z. *Diabetes* **1983**, *32*, 804.
7. Momose, Y.; Meguro, K.; Ikeda, H.; Ot, S.; Sohda, T. *Chem. Pharm. Bull.* **1991**, *39*, 1440.
8. Yoshioka, T.; Fujita, T.; Kanai, T.; Aizawa, Y.; Kurumada, T.; Hasegawa, K.; Horikoshi, H. *J. Med. Chem.* **1989**, *32*, 421.
9. Cantello, B. C. C.; Cawthorne, M. A.; Haigh, D.; Hindley, R. M.; Smith, S. A.; Thurlby, P. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1181.
10. Cantello, B. C. C.; Cawthorne, M. A.; Cottam, G. P.; Duff, P. T.; Haigh, D.; Hindley, R. M.; Lister, C. A.; Smith, S. A.; Thurlby, P. L. *J. Med. Chem.* **1994**, *37*, 3977.
11. (a) Nolan, J. J.; Ludvik, B.; Beerdsen, P.; Joyce, M.; Olefsky, J. *N. Engl. J. Med.* **1994**, *331*, 1188. (b) Kumar, S.; Boulton, A. J. M.; Beck-Nielsen, H.; Berthezene, F.; Muggeo, M.; Persson, B.; Spinas, G. A.; Donoghue, S.; Lettis, S.; Stewart-Long, P. *Diabetologia* **1996**, *39*, 701.
12. The thiazolidinedione moiety has been replaced by a variety of other heterocyclic systems, although few of these modifications have given compounds with high anti-hyperglycaemic potency. See for example: Hulin, B.; McCarthy, P. A.; Gibbs, E. M. *Curr. Pharmaceut. Design* **1996**, *2*, 85.
13. Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2121.
14. Buckle, D. R.; Cantello, B. C. C.; Cawthorne, M. A.; Coyle, P. J.; Dean, D. K.; Faller, A.; Haigh, D.; Hindley, R. M.; Jefcott, L. J.; Lister, C. A.; Pinto, I. L.; Rami, H. K.; Smith, D. G.; Smith, S. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2127.
15. (a) Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A., *J. Biol. Chem.* **1995**, *270*, 12953. (b) Forman, B. M.; Tontonoz, P.; Chen, J.; Brun, R. P.; Spiegelman, B. M.; Evans, R. M. *Cell* **1995**, *83*, 803. (c) Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D.; Moore, L. B.; Kliewer, S. A.; Lehmann, J. M. *J. Med. Chem.* **1996**, *39*, 665.
16. Sohda, T.; Mizuno, K.; Kawamatsu, Y. *Chem. Pharm. Bull.* **1984**, *32*, 4460.
17. Cox, G. G.; Haigh, D.; Hindley, R. M.; Miller, D. J.; Moody, C. J. *Tetrahedron Lett.* **1994**, *35*, 3139.
18. (a) Boland, W.; Frossl, C.; Lorenz, M. *Synthesis*, **1991**, 1049. (b) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071. (c) Sih, C. J.; Wu, S-H. *Topics in Stereochemistry*, **1989**, *19*, 63.
19. (a) Delinck, D. L.; Margolin, A. L. *Tetrahedron Lett.* **1990**, *31*, 6797. (b) Sih, J. C.; Gu, R. L. *Tetrahedron: Asymmetry*, **1995**, *6*, 357. (c) Lefker, B. A.; Hada, W. A.; McGarry, P. J. *Tetrahedron Lett.* **1994**, *35*, 5205. (d) Kato, Y.; Ohta, H.; Tsuchihashi, G. *Tetrahedron Lett.* **1987**, *28*, 1303.
20. Haigh, D.; Jefcott, L. J.; Magee, K.; McNab, H. *J. Chem. Soc. Perkin Trans 1* **1996**, 2895.
21. (a) *Preparative Biotransformations—Whole Cell and Isolated Enzymes in Organic Synthesis*; Roberts, S. M., Ed.; John Wiley: New York. 1992. Section 1:6.41–1:6.48. (b) Wong, C. H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Oxford: Pergamon, 1994; p 82.
22. The failure to demonstrate significant base-induced deuterium exchange of the α-hydrogen atom under NMR conditions has been used to confirm the stability of this stereo center in the racemate **1**; Haigh, D. Unpublished results.
23. (a) Frost, S. C.; Lane, M. D. *J. Biol. Chem.* **1985**, *260*, 2646. (b) Gibbs, E. M.; Leinhard, G. E.; Gould, G. W. *Biochemistry* **1988**, *27*, 6681.
24. Holder, J. C.; Lister, C. A.; Smith, S. A.; Young, P. W. Unpublished results.
25. (a) Knihinicki, R. D.; Williams, K. M.; Day, R. O. *Biochem. Pharmacol.* **1989**, *38*, 4389. (b) Wechter, W. J. *J. Clin. Pharmacol.* **1994**, *34*, 1036.
26. Hulin, B.; Newton, L. S.; Lewis, D. M.; Genereux, P. E.; Gibbs, E. M.; Clark, D. A. *J. Med. Chem.* **1996**, *39*, 3897.
27. (a) Boie, Y.; Adam, M.; Rushmore, T. H.; Kennedy, B. P. *J. Biol. Chem.* **1993**, *268*, 5530, (b) Yu, K.; Bayona, W.; Kallen, C. B.; Harding, H. P.; Ravera, C. P.; McMahon, G.; Brown, M.; Lazar, M. A. *J. Biol. Chem.* **1995**, *270*, 23975.
28. Young, P. W.; Buckle, D. R.; Cantello, B. C. C.; Chapman, H.; Clapham, J. C.; Coyle, P. J.; Haigh, D.; Hindley, R. M.; Holder, J. C.; Kallender, H.; Latter, A. L.; Laurie, K. W. W.; Mossakowska, D.; Murphy, G. J.; Cox, L. R.; Smith, S. A. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 751.
29. Bergmeyer, H.-U; Bernt, E.; Schmidt, F. H.; Stork, H. In *Methods of Enzymatic Analysis*; Academic: New York, 1974; p 1196.
30. Phillips, G. M.; Taylor, P. J. *Theory and Applications of Numerical Analysis*; Academic: New York 1973; p 131.
31. Frost, S. C.; Lane, M. D. *J. Biol. Chem.* **1985**, *260*, 2646.
32. Gibbs, E. M.; Leinhard, G. E.; Gould, G. W. *Biochemistry* **1988**, *27*, 6681.