Chemoenzymatic Approach to the Synthesis of the Antiviral Agents Penciclovir and Famciclovir in Isotopically Chiral [^{13}C] Labelled Form

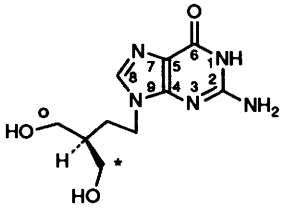
John T. Sime,*[a] Roger D. Barnes,[a] Stephen W. Elson,[a] Richard L. Jarvest[b] and Kevin J. O'Toole[a]

[a] *SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, RH3 7AJ, UK*

[b] *SmithKline Beecham Pharmaceuticals, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, UK*

The antiviral agents penciclovir and famciclovir have been synthesised in isotopically chiral [^{13}C] form. The synthesis of (+)-methyl 4-benzyloxy-2-(hydroxymethyl)butanoate **12a** by use of enzymatic hydrolysis catalysed by the lipase from *Candida cylindracea* is described as is the confirmation of the stereochemistry of this intermediate as *R* by convergent synthetic routes. The butanoate **12a** produced by the enzymic reaction was converted into the (−)-α-hydroxymethyl-γ-butyrolactone which was compared with the (*S*)-(+)-α-hydroxymethyl-γ-butyrolactone synthesised by an alternative, stereodefined route. The products of the enzymic reaction were used as intermediates in the synthesis of the final products.

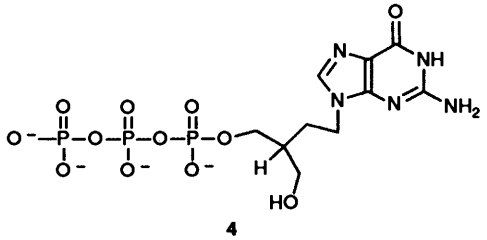
The hydroxyalkylguanine penciclovir 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine **1** has been shown to be a potent and selective antiviral agent with particular activity against



1 o = ^{12}C, * = ^{12}C
2 o = ^{12}C, * = ^{13}C
3 o = ^{13}C, * = ^{12}C

herpes simplex virus (HSV) types 1 and 2, varicella zoster virus and Epstein–Barr virus.[1–4] Studies aimed at determining the mode of action of this compound have indicated that the antiviral potency and selectivity are due to the conversion of penciclovir into its triphosphate ester **4** in virally infected cells.[5] Levels of this triphosphate are very low or undetectable in uninfected cells and it is the triphosphate which is believed to be responsible for the observed antiviral activity of penciclovir.

Phosphorylation of one of the gem-hydroxymethyl groups on the acyclic N(9) substituent creates a carbon centre of asymmetry and since this phosphorylation is enzymically controlled it would not be unusual to expect a degree of stereorecognition to produce an enantiomerically enhanced product **4**.[6] In order to determine whether stereorecognition was detectable, and if so



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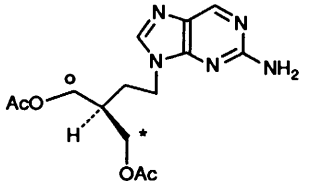
to what extent and which stereopreference was observed it was decided to synthesise penciclovir **1** in isotopically chiral ^{13}C forms **2** and **3**. Treatment of HSV infected cells with these compounds followed by isolation of the resulting triphosphate ester and analysis by ^{13}C NMR would then provide an answer



5 o = ^{12}C, * = ^{12}C
6 o = ^{12}C, * = ^{13}C
7 o = ^{13}C, * = ^{12}C

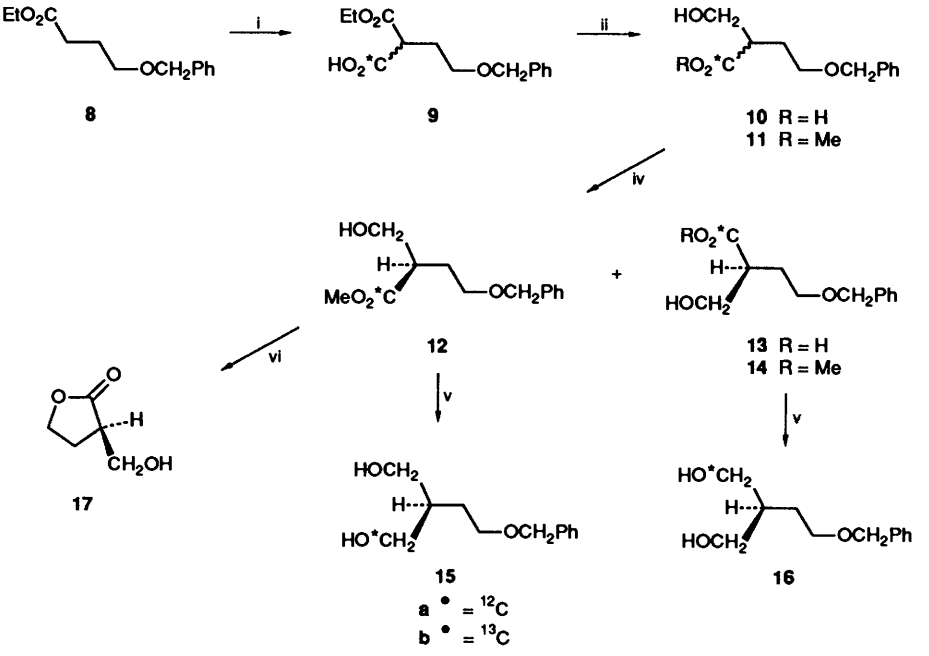
to these questions since the signals due to CH_2OH and CH_2OP are easily distinguishable.[7] Similarly, famciclovir **5**, the oral form of penciclovir, forms chiral metabolites during its conversion into penciclovir, when one of the acetyl ester groups has been hydrolysed. Isotopically ^{13}C labelled forms of famciclovir **6** and **7** can be synthesised by a similar route and used to obtain information on the stereoselectivity of the monoester hydrolysis process.

The following study describes the synthesis of compounds **2** and **3** and also **6** and **7** using an enzymic resolution and determination of the absolute stereochemistry of the synthetic intermediates to confirm the assignment of these enantiomers, and contains a full account of the work on penciclovir previously described in a preliminary communication.[7]

Results and Discussion

In order to incorporate ^{13}C into one of the gem-hydroxymethyl groups in the N(9)-substituent of penciclovir the route outlined in Scheme 1 was employed to obtain compounds **15** and **16** which are reported precursors in the synthesis of penciclovir.[8] The ^{13}C label is incorporated by the treatment of the anion of the ester **8** with [^{13}C]carbon dioxide to give the acid-ester **9** in 62% yield. Selective reduction of the ester function with lithium triethylborohydride followed by esterification of the acid **10** gave the product **11** in 31% yield as a racemate.

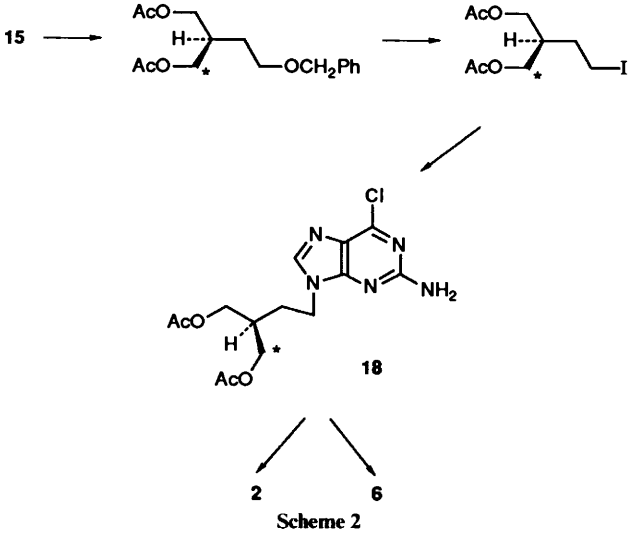
Resolution of enantiomers at this stage made use of the enantioselective esterase activity of the lipase from *Candida cylindracea* to hydrolyse preferentially one isomer. From a limited range of hydrolases tested subtilisin Carlsberg protease, pancreatic lipase and lipases from *Rhizopus javanicus* and *Rhizopus arrhizus* also showed some hydrolysis of the ester substrate, but the rates of reaction were very low and stereoselectivity was not measured. Using the lipase from *Candida*



Scheme 1 *Reagents:* i, $Li(NPr^i_2)_2$, THF, then CO_2 or $^{13}CO_2$; ii, $LiEt_3BH$, THF; iii, MeOH, H_2SO_4, reflux; iv, lipase from *Candida cylindracea*, water, pH 5.0; v, $LiAlH_4$, Et_2O, reflux; vi, H_2, 10% Pd–C, EtOH

cylindracea the enzymation was carried out in deionised water with automatic titration to control the pH and the resulting acid and remaining ester were separated by extraction. The enantioselectivity of the lipase varied little over the pH range 5–7 but at pH 8 was seen to be greatly reduced. An increase in temperature (up to 35 °C) was, surprisingly, found to increase the enantiometic excess produced but a further increase to 40 °C was not beneficial. Even under optimum conditions as shown in Scheme 1 where there was no non-enzymic hydrolysis the hydrolase activity was not stereospecific but showed good stereopreference. If the reaction was allowed to proceed beyond 50% hydrolysis, the dextrorotatory ester **12** with an ee of 94% could be recovered although ee values of >98% were seen when reactions were allowed to proceed to 60% hydrolysis. The recovered, enantiomerically impure acid **13**, when re-esterified to compound **14**, could be enzymated again to provide laevorotatory **14** with an ee around 70%. Enantiomeric excesses were determined by chiral HPLC on a bovine serum albumin column and were confirmed by ^1H NMR in the presence of the chiral solvating reagent 1-(9-anthryl)-2,2,2-trifluoroethanol.

Reduction of compounds **12b** and **14b** gave the products **15b** and **16b**, with enantiomer ratios of 3:97 and 85:15 respectively. Structures of the type **15** (or **16**) represent a common precursor to the synthesis of both penciclovir and famciclovir by published methods.[8] These literature methods allow the activation and coupling of compound **15b** to 2-amino-6-chloropurine to provide compound **18** which can be elaborated to either product **2** or **6** as shown in Scheme 2.[8]

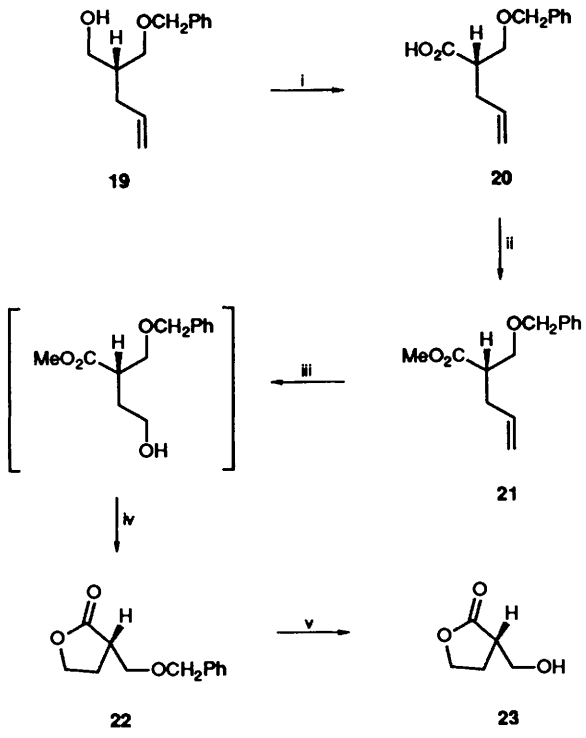


Scheme 2

Treatment of HSV-1 virally infected cells with compound **2** or **3** and isolation of the resulting triphosphates showed, by ^{13}C NMR, that the enzymic phosphorylation was indeed enantioselective.[7] It remained, however, to address the question of which stereochemistry of triphosphate had been produced. Determination of the absolute stereochemistry of the dextrorotatory ester **12b** would answer this but there is not enough precedent for β-hydroxy ester resolutions using *C. cylindracea* lipase to predict the stereocourse of the hydrolytic resolution.

In an attempt to obtain suitable X-ray crystallographic analysis of the resolved material the ester **12a** was converted into an acid and was crystallised with a well defined chiral counterion. The quinine salt was thus prepared and characterised spectroscopically but the resulting needles proved unsuitable for X-ray purposes despite recrystallisations from a range of solvents. An alternative proof of the absolute stereochemistry of compound **12** was required and simple conversion into a compound of reported stereochemistry was chosen.

Enzymically resolved ester **12a** which was shown to be a 2:98 mixture of enantiomers by HPLC was hydrogenolysed, and spontaneous lactonisation of the product gave the lactone **17**. This material was optically active {$[\alpha]_D$ −21.1 ($CHCl_3$); +9.7 (EtOH)} and comparison with the literature value[9] {$[\alpha]_D$ +6.5 (EtOH)} for the *R*-lactone might indicate that the dextrorotatory sample of compound **12a** which was converted into the lactone **17** also had *R* stereochemical configuration. Confirmation of this result was obtained by the synthesis of the lactone **23** with *S* absolute configuration as outlined in Scheme 3. The absolute stereochemistry of the (+)-monobenzyl ether **19** has been established as *R*.[10] This compound was prepared by the literature method to give material which was dextrorotatory in $CHCl_3$. Oxidation of the ether **19** with pyridinium dichromate in dimethylformamide (DMF) produced the acid **20** which has



Scheme 3 *Reagents:* i, Pyridinium dichromate, DMF; ii, MeOH, H_2SO_4; iii, O_3, $NaBH_4$; iv, spontaneous; v, H_2, 10% Pd–C, EtOH.

S configuration. Esterification of the acid **20** gave the ester **21** and ozonolysis of the terminal alkene followed by borohydride reduction of the ozonide with subsequent lactonisation provided the γ-lactone **22**. Hydrogenolysis gave the target *S*-lactone **23** {$[\alpha]_D$ +13.7 ($CHCl_3$); −3.2 (EtOH)} with rotations opposite, if not equal and opposite, to those obtained by the enzymatic synthesis. A greater loss of chiral integrity is to be expected in the chemical route.

The ^1H NMR spectra (in $CHCl_3$) of the dextrorotatory lactone, the laevorotatory lactone and a racemic mixture of compounds **17** and **23** prepared from the unresolved ether **19** were identical. However on addition of the chiral solvating reagent (*R*)-(−)-1-(9-anthryl)-2,2,2-trifluoroethanol the signals due to one of the exocyclic methylene protons moved upfield and the signals arising from each enantiomer were shifted by a sufficiently different amount to allow easy estimation of enantiomer composition. Fig. 1 illustrates the result of such an addition to these samples. Fig. 1(*a*) shows this region of the spectrum for the racemic mixture of compounds **17** and **23** in the absence of chiral reagent and Fig. 1(*b*) shows the clear separation of signals with the chiral reagent. The resolved spectral region shown in Fig. 1(*c*) is that of compound **17** synthesised from a 2:98 enantiomeric mixture of compound **12**. In this the few percent of the higher field enantiomer can be detected as a 'wing' on the signal the other parts of the multiplet being coincidental with signals arising from the lower field enantiomer. Fig. 1(*d*) shows the resolved portion of the spectrum from the lactone **23** produced in Scheme 3 indicating that the major *S*-enantiomer present appears at higher field with some of the lower field enantiomer present. Having adequately proved the stereochemistry of the ester (+)-**12** to be *R* it follows that the labelled penciclovir produced from compound **12b** is also *R*, *i.e.* compound **2** which in turn has shown the phosphorylation of penciclovir to occur on the *pro-S* hydroxymethyl group.[7]

The above reasoning and similar methodology led to the conclusion that during the conversion of famciclovir into penciclovir by an extract of human intestinal wall, the esterase(s)

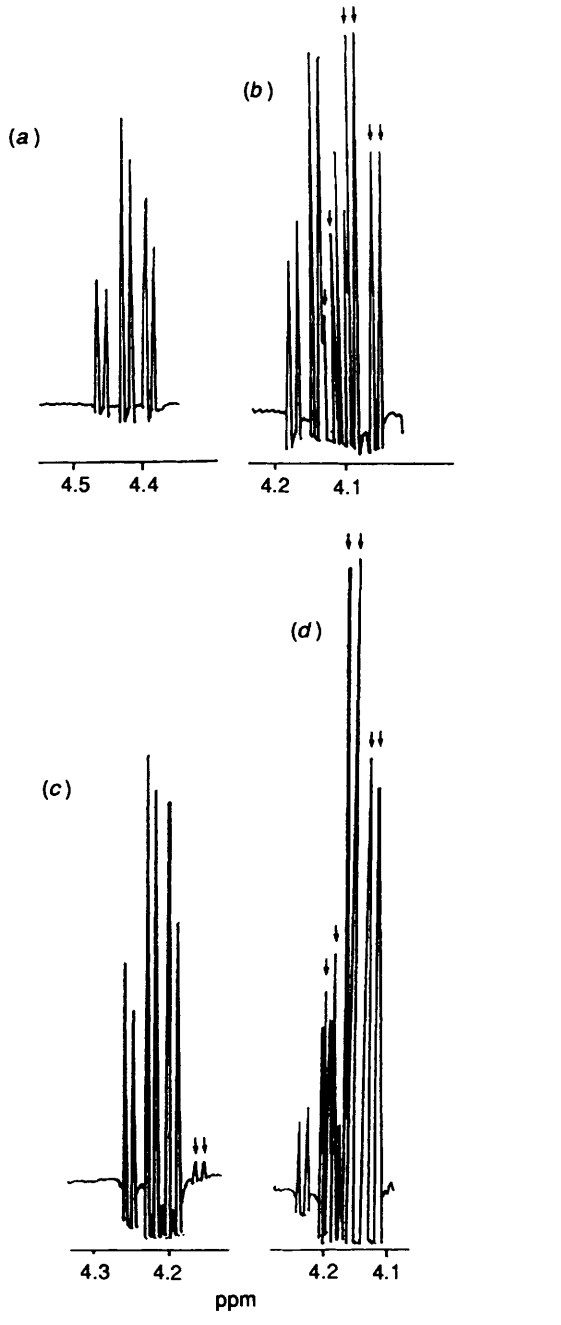


Fig. 1 NMR spectra showing one proton of the lactones **17** and **23**: (*a*) racemate; (*b*) racemate in the presence of chiral solvating reagent; (*c*) Lactone **17** in the presence of chiral solvating reagent; (*d*) lactone **23** in the presence of chiral solvating reagent. In each case the signals have been resolution enhanced and, where appropriate, the signals arising from the higher field enantiomer are marked.

initially hydrolysed the acetyl group preferentially from the *pro-S* acetoxymethyl group of famciclovir.[11]

Experimental

IR Spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on either a Bruker AM 250 or AM 400 spectrophotometer; except where otherwise stated $CDCl_3$ was used as solvent with tetramethylsilane as internal standard. *J* Values are given in Hz. Enantiomeric excess was determined from the ^1H NMR spectra using solutions of (*R*)-1-(9-anthryl)-2,2,2-trifluoroethanol and the compound under study in the ratio 10:1 (w/w) in $CDCl_3$.

Enantiomer ratios for the products of the enzymic resolutions were confirmed by HPLC on a Resolvosil-BSA column eluting with 2% propan-2-ol in sodium phosphate buffer (0.2 mol dm^{-3}; pH 7.9). Mass spectra were recorded on a VG 7070F spectrometer using electron impact (EI). Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Boiling points were determined on a Büchi Kugelruhr GKR-50. All silica gel chromatography was performed using Merck Kieselgel 60 (0.04–0.063 mm) and TLC was carried out with Merck precoated silica gel 60 F_{254} plates (0.2 mm). Plates were visualised with UV light and/or iodine and/or aqueous potassium permanganate (0.1 mol dm^{-3}). All water used was deionised and all organic solvents were of AnalaR quality. Anhydrous sodium sulfate was used for drying organic solutions. Lipases were provided by Biocatalysts Ltd. with the exception of the subtilisin Carlsberg which was obtained from Sigma.

Ethyl 4-(*Benzyloxy*)*butanoate* **8**.—A suspension of diethyl (2-benzyloxyethyl)malonate [12] (10 g, 33.97 mmol) and anhydrous lithium chloride (3 g) in dimethyl sulfoxide (50 cm^3) and water (0.62 cm^3) was heated, with stirring, to 140 °C. The now homogeneous reaction mixture was further heated to 170 °C for 5 h. The mixture was allowed to cool to ambient temperature, diluted with water (50 cm^3) and extracted with diethyl ether (3 × 100 cm^3). The combined organic extracts were dried and evaporated under reduced pressure to yield the title *butanoate* **8** as an orange oil (7.2 g, 95%), b.p. 152 °C (0.5 mmHg) (Found: C, 70.2; H, 8.3. $C_{13}H_{18}O_3$ requires C, 70.2; H, 8.2%); ν_{max}(KBr)/cm^{-1} 1736 (C=O); δ_H(250 MHz) 1.18–1.30 (3 H, t, CH_3), 1.77–2.00 (2 H, m, *J* 7.4, 6.2, 3-H), 2.36–2.48 (2 H, t, *J* 7.4, 2-H), 3.45–3.55 (2 H, t, *J* 6.2, 4-H), 4.05–4.18 (2 H, q, CH_2Me), 4.50 (2 H, s, CH_2Ph) and 7.20–7.40 (5 H, m, Ph); *m/z* 223 (MH^+).

(±)-4-*Benzyloxy*-2-(*ethoxycarbonyl*)*butanoic Acid* **9a**.—Butyllithium in hexane (1.6 mol dm^{-3}; 6 cm^3) was added slowly, with stirring, to a solution of diisopropylamine (1.4 cm^3) in tetrahydrofuran (THF) (40 cm^3) at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred for a further 30 min at 4 °C and then cooled to −60 °C. A solution of the butanoate **8** (1.0 g, 4.50 mmol) in THF (10 cm^3) was added slowly the reaction temperature being maintained below −60 °C. The reaction mixture was then stirred for 1 h at −60 °C before CO_2 was bubbled through it for 45 min. After warming to ambient temperature the mixture was evaporated under reduced pressure to give a foam-like residue which was dissolved in water (10 cm^3); the solution, at pH 11, was then extracted into ethyl acetate (3 × 10 cm^3). The aqueous phase was adjusted to pH 2 by the addition of HCl (0.1 mol dm^{-3}) and further extracted into ethyl acetate (3 × 10 cm^3). The dried evaporated product was a foam from which the unchanged starting material was separated by silica gel column chromatography eluting with ethyl acetate–hexane to yield the title acid **9a** as an oil (923 mg, 77%); ν_{max}(KBr)/cm^{-1} 2600br (CO_2H), 1736 (CO_2Et) and 1632 (CO_2H); δ_H(250 MHz) 1.20–1.32 (3 H, t, CH_2CH_3), 2.20–2.35 (2 H, m, 3-H), 3.50–3.61 (2 H, m, 4-H), 3.61–3.68 (1 H, t, *J* 6.9, 2-H), 4.10–4.25 (2 H, m, CH_2Me), 4.50 (2 H, s, CH_2Ph) and 7.23–7.40 (5 H, m, Ph) (Found: M^+, 266.1157. *M*, 266.1154).

(±)-[1-^{13}C]-4-*Benzyloxy*-2-(*ethoxycarbonyl*)*butanoic Acid* **9b**.—This compound was prepared according to the methodology above using $^{13}CO_2$ to quench the anion to yield the title acid **9b** (62%); δ_H(400 MHz) as for compound **9a** except 3.61–3.68 (1 H, q, *J* 7.0, 2-H); δ_C(100 MHz) 14.00 (CH_3), 29.55 (3-C), 61.82 (4-C), 67.69 (MeCH_2), 72.96 (PhCH_2), 127.63 (*p*-Ph), 127.71 (*m*-Ph), 128.39 (*o*-Ph), 138.23 (*ipso*-Ph) and 174.49 ($^{13}CO_2H$).

(±)-*Methyl* 4-*Benzyloxy*-2-(*hydroxymethyl*)*butanoate* **11a**.—The acid **9a** (233 mg, 0.875 mmol) was dissolved in THF (3.5 cm^3) and cooled to 4 °C under an atmosphere of nitrogen. To this stirred solution was slowly added lithium triethylborohydride in THF (1 mol dm^{-3}; 3.5 cm^3) the reaction temperature being maintained below 10 °C. After warming to ambient temperature the reaction mixture was stirred for 18 h before being cooled to 4 °C and quenched with water. Evaporation and dissolution of the residue in water (10 cm^3) was followed by extraction of the aqueous phase at pH 12 into ethyl acetate (2 × 20 cm^3). Acidification of the aqueous phase to pH 2 by the addition of HCl (0.1 mol dm^{-3}) and extraction into ethyl acetate (2 × 20 cm^3) yielded (±)-4-benzyloxy-2-(hydroxymethyl)butanoic acid **10a** as an oil which was used without further purification.

Compound **10** was dissolved in methanol (10 cm^3) and the solution heated to reflux with conc. sulfuric acid (50 mm^3) for 3 h. Evaporation gave a yellow oil which was dissolved in water (10 cm^3) and extracted into diethyl ether (2 × 10 cm^3) to yield, after drying and evaporation of the extract, a yellow oil which was purified by silica gel chromatography eluting with ethyl acetate–hexane to give the title ester **11a** (68.8 mg, 33% from **9a**) as an oil; ν_{max}(KBr)/cm^{-1} 1733 (CO_2Me); δ_H(250 MHz) 1.82–2.10 (2 H, m, 3-H), 2.72–2.84 (1 H, m, 2-H), 3.49–3.61 (2 H, m, 4-H), 3.69 (3 H, s, CO_2CH_3), 3.80 (2 H, d, CH_2OH), 4.50 (PhCH_2) and 7.20–7.40 (5 H, m, Ph) (Found: M^+, 238.1219. *M*, 238.1205).

(±)-*Methyl* [1-^{13}C]-4-*Benzyloxy*-2-(*hydroxymethyl*)*butanoate* **11b**.—This compound was prepared from compound **9b** according to the above methodology (31%); δ_H(400 MHz) as for compound **11a** except for 3.69 (3 H, d, CO_2CH_3) and 3.80 (2 H, dd, CH_2OH); δ_C(100 MHz) 28.70 (3-C), 44.89 (d, *J* 42.5, 2-C), 51.82 (d, *J* 5.4 CH_3), 62.98 (4-C), 67.84 (d, *J* 2.5, CH_2OH), 73.15 (PhCH_2), 127.75, 128.44 and 138.03 (Ph) and 175.32 ($^{13}CO_2H$).

Enzymic Resolution of (+) *and* (−)-*Methyl* 4-*Benzyloxy*-2-(*hydroxymethyl*)*butanoate* **12a** *and* **14a**.—The butanoate **11a** (103 mg, 0.43 mmol) was suspended in water (15 cm^3) and the pH adjusted to 5.0. This was added to a solution of *Candida cylindracea* lipase (70 mg, 3750 units of esterase activity) in water (5 cm^3) at pH 5.0. The reaction mixture was stirred at 35 °C and the pH maintained at 5.0 by the addition of aqueous NaOH (0.01 mol dm^{-3}). After 0.5 molar equivalents of base had been added (*ca.* 29.5 h) the reaction mixture was extracted into ethyl acetate (3 × 60 cm^3) and the organic phase re-extracted into saturated aqueous sodium hydrogen carbonate (2 × 10 cm^3, 1 × 15 cm^3). The organic phase was dried and evaporated to afford the title dextrorotatory methyl ester **12a** (26.6 mg, 25%) as a colourless oil, $[\alpha]_D$ +16.40 (*c* 1, $CHCl_3$); enantiomeric excess (ee) 94% by HPLC. The basic aqueous phase from the extraction was acidified to pH 1.9 by the addition of HCl (5 mol dm^{-3}) and then extracted into ethyl acetate (2 × 30 cm^3) to give, after drying and evaporation, the acid **13a** (58.7 mg, 60%) as a colourless oil. This was re-esterified ($MeOH–H_2SO_4$) and the product re-enzymated as above to give further quantities of compound **12a** and laevorotatory butanoate **14a**, ee 70% by HPLC.

Quinine–4-*Benzyloxy*-2-(*hydroxymethyl*)*butanoate*.—The resolved ester **12a** (80 mg, 0.33 mmol) which was shown by HPLC to be >99% enantiomerically pure dextrorotatory isomer was boiled for 3 h in THF (2 cm^3) with HCl (5 mol dm^{-3}; 2 cm^3). The reaction mixture was extracted into ethyl acetate (2 × 10 cm^3) and the organic phase washed with water. The acid product was extracted into saturated aqueous sodium hydrogen carbonate (2 × 5 cm^3) and this was acidified to pH 2 by the addition of HCl (5 mol dm^{-3}). Extraction into chloroform gave, after drying and evaporation, a clear oil (42.5 mg) which was one spot by TLC. This was dissolved in ether (1 cm^3) and to it was added a solution of quinine (67.7 mg, 0.37 mmol) which

had been dissolved in ether (1 cm^3) and ethanol (0.2 cm^3). The resulting mixture was filtered, diluted with hexane (0.7 cm^3) and left at ambient temperature for 4 days over which time a white crystalline solid formed (Found: C, 68.8; H, 7.2; N, 4.9. $C_{32}H_{40}N_2O_6 \cdot 0.5H_2O$ requires C, 68.9; H, 7.4; N, 5.0%); δ_H(400 MHz) 1.83 (1 H, ABq, 3-H), 2.11 (1 H, ABq, 3-H), 2.57 (1 H, m, 2-H), 3.59 (2 H, AA', 4-H), 3.70 (1 H, ABq, CH_2OH), 3.78 (1 H, ABq, CH_2OH), 4.48 (2 H, s, $PhCH_2$), 7.30 (5 H, m, Ph) and the signals arising from the quinine moiety.

(+)-[1-^{13}C]-4-*Benzyloxy*-2-(*hydroxymethyl*)*butanol* **15b**.—A solution of compound **12b** (0.21 g, 0.879 mmol) in diethyl ether (10 cm^3) was added slowly to a stirred suspension of lithium aluminium hydride (0.4 g, 10.54 mmol) in diethyl ether (10 cm^3). The reaction mixture was heated to reflux for 4 h, cooled (ice) and the excess of lithium aluminium hydride destroyed by the addition of water (0.4 cm^3), aqueous sodium hydroxide (5 mol dm^{-3}; 0.4 cm^3) and water (1.2 cm^3). The white granular precipitate was filtered off and washed thoroughly with diethyl ether. The combined ether extracts were dried and evaporated to give the title butanol **15b** (123 mg, 93%) as a colourless oil; ee 94%. Spectral data were consistent with those of the unlabelled diol.[12]

(−)-[1-^{13}C]-4-*Benzyloxy*-2-(*hydroxymethyl*)*butanol* **16b**.—This compound was prepared from the butanoate **11b** *via* the butanoate **14b** as described above to give the title product **16b** with ee 70%.

1-*Hydroxymethylbutan*-4-*olide* **17**.—The butanoate **12a** (30.5 mg, 0.128 mmol) was dissolved in methanol (10 cm^3) and shaken for 2.5 h under an atmosphere of hydrogen with 10% palladium–carbon catalyst (10 mg). The reaction mixture was filtered through Celite and the filtrate evaporated to give the lactone **17** (10.1 mg, 68%) as a gum, $[\alpha]_D$ +9.74 (*c* 1, EtOH) (lit.,[9] +6.5 for *R* enantiomer), $[\alpha]_D$ −21.1 (*c* 4.2, $CHCl_3$).

9-{[4-^{13}C]-4-*Acetoxy*-3-(*acetoxymethyl*)*butyl*}-2-*amino*-6-*chloro*-9H-*purine* **18**.—This compound was prepared from compound **15b** *via* the iodo derivative (0.175 g, 0.556 mmol) according to published methodology[8] to give the product **18** (125 mg, 63%). All compounds gave spectroscopic data consistent with those published and showed enrichment at one C atom in the ^{13}C NMR.

9-{[4-^{13}C]-4-*Hydroxy*-3-(*hydroxymethyl*)*butyl*}-9H-*guanine* **2**.—This compound was prepared from the purine **18** (0.12 g, 0.337 mmol) according to published methodology[8] to give the product **2** (53 mg, 62%), with spectral data as published but showing enrichment of one C atom in the NMR at δ_C 61.38 (CH_2O); *m/z* M^+ 254.

9-{[4-^{13}C]-4-*Acetoxy*-3-(*acetoxymethyl*)*butyl*}-2-*amino*-9H-*purine* **6**.—This compound was prepared from compound **18** (0.122 g, 0.342 mmol) according to published methodology[8] to give the product (88.1 mg, 80%), with spectral data as published, but showing enrichment of one C atom in the NMR at δ_C 63.68 (CH_2O); *m/z* M^+ 322.

(S)-2-*Benzyloxymethylpent*-4-*enoic Acid* **20**.—A solution of (*R*)-2-benzyloxymethylpent-4-enol **19**[10] (100 mg, 0.485 mmol) in DMF (1 cm^3) was added to a solution of pyridinium dichromate (640 mg, 1.70 mmol) in DMF (1 cm^3). The reaction mixture was stirred at ambient temperature for 16 h and then poured into cold water (10 cm^3) and extracted into diethyl ether. The organic extract was dried and evaporated to afford the acid **20** (98 mg, 91%) as a brown oil, b.p. 212–215 °C (0.2 mmHg) (Found: C, 70.65; H, 7.5. $C_{13}H_{16}O_3$ requires C, 70.9; H, 7.3%); $[\alpha]_D$ −24.4 (*c* 2.67, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2600 (CO_2H) and 1705 (C=O); δ_H (250 MHz) 2.40 (2 H, dd, 3-H), 2.80 (1 H, $CHCO_2H$), 3.65 (2 H, d, CH_2O), 4.55 (2 H, s, OCH_2Ph), 5.08 (2 H, m, 5-H), 5.77 (1 H, m, 4-H) and 7.32 (5 H, m, Ph).

(S)-*Methyl* 2-*Benzyloxymethylpent*-4-*enoate* **21**.—The acid **20** (50 mg, 0.227 mmol) was dissolved in methanol (5 cm^3) containing 3 drops of concentrated sulfuric acid and the solution heated to reflux for 2 h. The reaction mixture was neutralised with sodium hydrogen carbonate, evaporated, resuspended in ethyl acetate and filtered through a plug of silica gel. Evaporation gave the title pent-4-enoate **21** (48.5 mg, 91%) as a yellow oil, b.p. 180–183 °C (0.5 mmHg) (Found: C, 72.3; H, 8.0. $C_{14}H_{18}O_3$ requires C, 71.8; H, 7.7%); $[\alpha]_D$ +3.2 (*c* 4.00, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1740 (C=O) and 1640 (C=CH_2); δ_H (250 MHz) 2.29 (2 H, m, 3-H), 2.74 (1 H, m, 2-H), 3.52 (2 H, m, CH_2O), 3.62 (3 H, s, CO_2CH_3), 4.45 (2 H, s, OCH_2Ph), 4.98 (2 H, m, CH=CH_2), 5.67 (1 H, m, CH=CH_2) and 7.26 (5 H, m, Ph).

(S)-2-*Benzyloxymethylbutan*-4-*olide* **22**.—The methyl ester **21** (126 mg, 0.538 mmol) was dissolved in ethanol (15 cm^3) and the solution cooled to −70 °C. Ozone was bubbled through the reaction mixture until a permanent blue colour remained. After purging the reaction mixture with oxygen and argon, sodium borohydride (25 mg, 0.67 mmol) in ethanol (2 cm^3) was added whilst the temperature was maintained at −70 °C. The reaction temperature was then allowed to increase to ambient over 1 h and the solution acidified with HCl (5 mol dm^{-3}; 10 cm^3) and extracted into diethyl ether. The dried, evaporated organic phase gave the title lactone **22** (79.1 mg, 71%) as a gum; $[\alpha]_D$ −3.9 (*c* 2.1, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1774 (lactone); δ_H(250 MHz) 2.36 (2 H, m, 3-CH_2), 2.83 (1 H, m, 2-H), 3.65 (2 H, m, 4-H), 4.27 (2 H, m, CH_2OH), 4.55 (2 H, d, OCH_2Ph) and 7.35 (5 H, m, Ph); (M^+, 206.0945. *M*, 206.0943).

(S)-2-*Hydroxymethylbutan*-4-*olide* **23**.—The benzyl ether **22** (48.2 mg, 0.289 mmol) was dissolved in ethanol (10 cm^3) and hydrogenated for 2.5 h over 10% palladium–carbon catalyst (10 mg). The reaction mixture was filtered through Celite and the filtrate evaporated to yield the title lactone **23** (18.2 mg, 54%) as a gum; $[\alpha]_D$ +13.7 (*c* 9.1, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1764 (lactone); δ_H(250 MHz) 2.32 (2 H, m, 3-H), 2.80 (1 H, m, 2-H), 3.83 (1 H, m, 4-CH), 3.98 (1 H, m, 4-CH), 4.26 (1 H, m) and 4.44 (1 H, m) (together CH_2OH); (M^+, 117.0552. *M*, 117.0552).

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References

1 M. R. Harnden, R. L. Jarvest, T. H. Bacon and M. R. Boyd, *J. Med. Chem.*, 1987, **30**, 1636.
2 M. R. Boyd, T. H. Bacon, D. Sutton and M. Cole, *Antimicrob. Agents Chemother.*, 1987, **31**, 1238.
3 M. R. Boyd, T. H. Bacon and D. Sutton, *Antimicrob. Agents Chemother.*, 1988, **32**, 358.
4 M. R. Harnden, *Drugs of the Future*, 1989, **14**, 347.
5 R. A. Vere Hodge and R. M. Perkins, *Antimicrob. Agents Chemother.*, 1989, **33**, 223.
6 J. D. Karkas, W. T. Ashton, L. F. Canning, R. Liou, J. Germershausen, R. Bostedor, A. K. Field and R. L. Tolman, *J. Med. Chem.*, 1986, **29**, 842.
7 R. L. Jarvest, R. D. Barnes, D. L. Earnshaw, K. J. O'Toole, J. T. Sime and R. A. Vere Hodge, *J. Chem. Soc., Chem. Commun.*, 1990, 555.

8 M. R. Harnden and R. L. Jarvest, European Patent Appl., 141927A, 1985 (to Beecham Group plc); G. R. Geen, T. J. Grinter, P. M. Kincey and R. L. Jarvest, *Tetrahedron*, 1990, **46**, 6903.
9 T. Kaneko, H. Katsura, H. Asano and K. Wakabayashi, *Chem. Ind. (London)*, 1960, 1187.
10 T. F. Fukuyama, C.-L. J. Wang and Y. Kishi, *J. Am. Chem. Soc.*, 1979, **101**, 260.
11 R. A. Vere Hodge, D. L. Earnshaw, R. L. Jarvest and S. A. Readshaw, *Antiviral Res.*, 1990, Suppl. 1, 87.
12 W. H. Rastetter and D. P. Phillion, *J. Org. Chem.*, 1981, **46**, 3204.

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