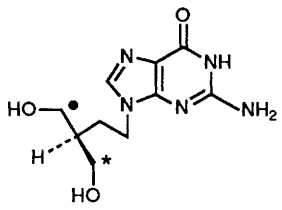
Synthesis of Isotopically Chiral [^{13}C]Penciclovir (BRL 39123) and its Use to determine the Absolute Configuration of Penciclovir Triphosphate formed in Herpes Virus Infected Cells

Richard L. Jarvest,*[a] Roger D. Barnes,[b] David L. Earnshaw,[a] Kevin J. O'Toole,[b] John T. Sime,[b] and R. Anthony Vere Hodge[a]

Beecham Pharmaceuticals Research Division; [a] Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, U.K.; [b] Brockham Park, Betchworth, Surrey RH3 7AJ, U.K.

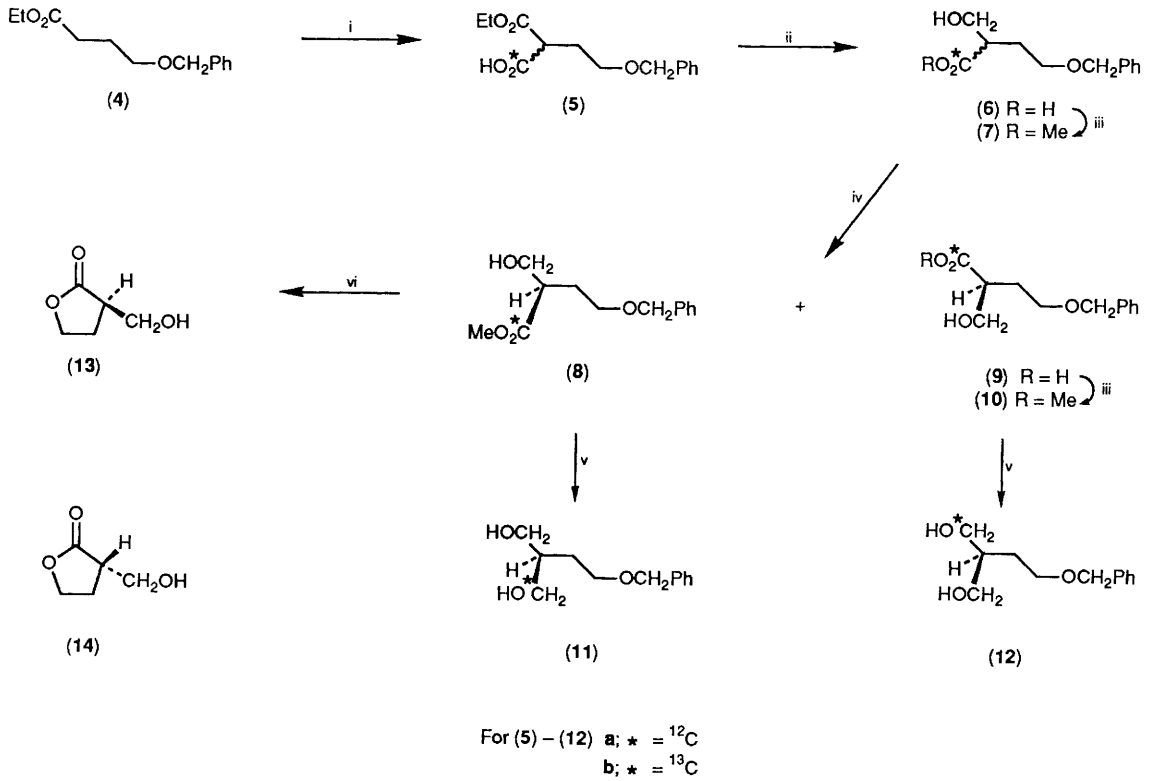
Isotopically chiral [^{13}C]penciclovir (BRL 39123) has been synthesised *via* a stereospecific hydrolysis catalysed by the lipase from *Candida cylindraceae* and has been used to determine, by ^{13}C NMR, that the triphosphate of penciclovir formed in herpes simplex type 1 infected cells has (*S*) stereochemistry with an enantiomeric purity of >95%.

Penciclovir [BRL 39123,9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine] (**1**) is a potent and selective anti-herpes virus agent, particularly against herpes simplex types 1 and 2 (HSV-1 and -2) and varicella zoster virus.[1—3] Currently penciclovir is undergoing clinical trials for efficacy against herpes virus infections in humans. When HSV-1 infected cells are treated with penciclovir, high intracellular concentrations of its triphosphate ester are produced, whereas low or undetectable levels of the triphosphate are formed in uninfected cells.[4] Formation of this triphosphate is believed to be essential for the inhibition of viral DNA synthesis and hence for the observed antiviral effect of penciclovir. The phosphorylation of one of the hydroxymethyl groups of penciclovir creates a chiral centre and in this communication we describe the determination of the stereospecificity and absolute configuration of penciclovir triphosphate formed in HSV-1 infected cells.



(**1**) • = ^{12}C, ⋆ = ^{12}C
(**2**) • = ^{12}C, ⋆ = ^{13}C
(**3**) • = ^{13}C, ⋆ = ^{12}C

We elected to synthesise penciclovir in isotopically chiral form by incorporating ^{13}C into one of the hydroxymethyl groups, namely compounds (**2**) and (**3**). Treatment of the anion of the ester (**4**) with [^{13}C]carbon dioxide afforded the acid-ester (**5b**) (62%) which was reduced with lithium triethylborohydride to the acid-alcohol (**6b**) (Scheme 1). Esterification of (**6b**) afforded the ester-alcohol (**7b**) [31% from (**5b**)] which was subjected to enzymic hydrolysis by the



For (5) – (12) **a**; ⋆ = ^{12}C
b; ⋆ = ^{13}C

Scheme 1. *Reagents*: i, $Li(NPr^i_2)_2$, tetrahydrofuran (THF), then CO_2 or $^{13}CO_2$; ii, $LiEt_3BH$, THF; iii, MeOH, H_2SO_4, reflux; iv, lipase, H_2O, pH 5.0; v, $LiAlH_4$, Et_2O, reflux; vi, H_2, 10% Pd–C, EtOH.

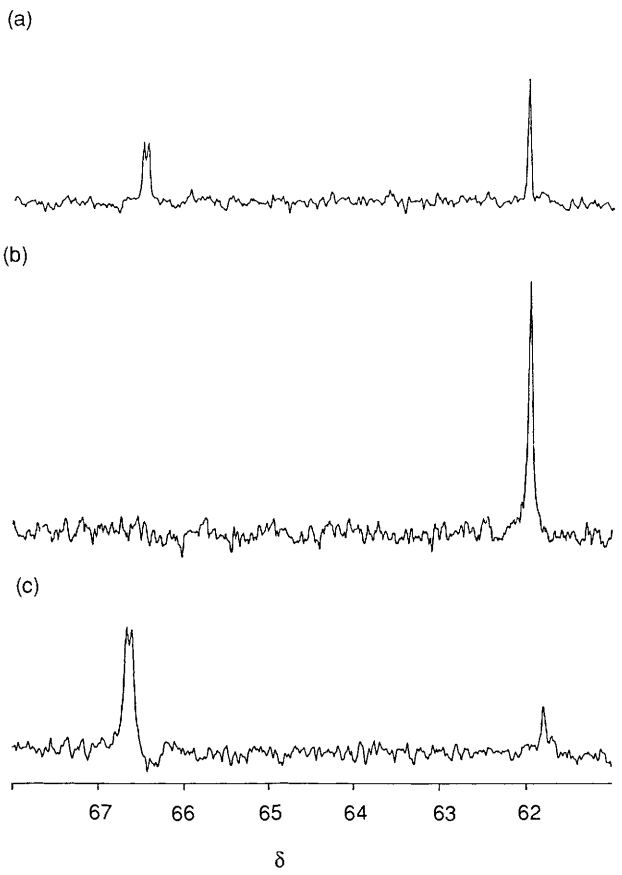
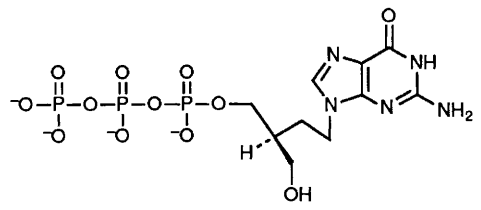


Figure 1. ^{13}C NMR spectra of the triphosphate esters in D_2O recorded at 100.6 MHz on a Bruker AM400 spectrometer. The spectra are referenced to external dioxan. The singlet at δ 62 is the CH_2OH resonance and the doublet at δ 66.7 is the CH_2OP resonance. (a) Synthetic triphosphate of (**1**); (b) biosynthetic triphosphate formed from (**2**); (c) biosynthetic triphosphate formed from an 85 : 15 mixture of (**3**) and (**2**).

lipase from *Candida cylindraceae*. By carrying out the hydrolysis in distilled water at 35 °C whilst maintaining pH 5.0 by titration with 10 mM sodium hydroxide, the recovered ester (**8b**) was obtained with ≥97% enantiomeric purity.† The acid product (**9b**) was re-esterified to (**10b**) which was found to have an enantiomer ratio of 85 : 15.† The unlabelled ester (**8a**) {98 : 2 enantiomer ratio, $[\alpha]_D^{20}$ +16.4° ($CHCl_3$)} was also prepared by a similar route [(**5a**) through to (**8a**)]. The absolute configuration of (**8a**) was assigned as (*R*) by converting it to the lactone (**13**) by catalytic hydrogenolysis: the lactone (**13**) was dextrorotatory {$[\alpha]_D$ +9.7° (EtOH)} in contrast to the known (*S*)-lactone (**14**) {$[\alpha]_D$ −10.6° (EtOH)}.[5] The assignment was confirmed by preparing the (*S*)-lactone (**14**) from (*R*)-2-(benzyloxymethyl)pent-4-en-1-ol[6] and showing that (**13**) and (**14**) were enantiomeric by ^1H NMR spectroscopy in the presence of the chiral solvating agent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol. Reduction of (**8b**) and (**10b**) with lithium aluminium hydride afforded the isotopically enantiomeric diols (**11b**) and (**12b**) which were converted through to (**2**) and (**3**) respectively by known methods.[7]

Cultured MRC-5 cells which had been infected with the HSV-1 strain SC16 were put into a medium containing (**2**) or

† The ratios were determined by HPLC on a Resolvosil-BSA column eluting with 2% propan-2-ol in pH 7.9 sodium phosphate buffer.



(**15**)

(**3**) for 24 h. The intracellular phosphates were extracted with buffered 50% aqueous ethanol and purified by HPLC,[4] collecting the fraction of similar retention time to synthetic (racemic) penciclovir triphosphate.[8] The ^{13}C NMR spectra of the triphosphates are shown in Figure 1. It can be seen that the triphosphate derived from (**2**) has the ^{13}C in the CH_2OH moiety and the triphosphate derived from (**3**) has the ^{13}C in the CH_2OP moiety. The absolute configuration of penciclovir triphosphate produced in HSV-1 infected cells is thus (*S*) as shown in (**15**). From the spectrum of the triphosphate derived from (**2**) (Figure 1b), it is estimated that the enantiomeric purity of the intracellular triphosphate is >95%.[9]

Further work is in progress to determine the stereospecificity of triphosphate formation in HSV-2 infected cells and of the initial phosphorylation step catalysed by the virally encoded thymidine kinase.

We thank Drs K. H. Baggaley, A. G. Brown, M. Cole and M. R. Harnden, and Mr S. W. Elson for encouragement and advice; Miss S. J. Darlison for work on the HPLC isolations; and Dr. S. A. Readshaw for the ^{13}C NMR spectra.

Received, 18th December 1989; Com. 9/05368I

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 R. A. Vere Hodge and R. M. Perkins, *Antimicrob. Agents Chemother.*, 1989, **33**, 223.
5 T. Kaneko, H. Katsura, H. Asano, and K. Wakabayashi, *Chem. Ind.*, 1960, 1187.
6 T. Fukuyama, C.-L. J. Wang, and Y. Kishi, *J. Am. Chem. Soc.*, 1979, **101**, 260. This compound was converted to (**14**) *via* oxidation to the methyl ester (pyridinium dichromate/dimethylformamide then $MeOH/H_2SO_4$) followed by reductive ozonolysis (O_3/EtOH then $NaBH_4$) and debenzylation (H_2, 10% Pd–C,EtOH).
7 M. R. Harnden and R. L. Jarvest, European Patent Appln. 141927A, 1985 (to Beecham Group p.l.c.).
8 Prepared by the reaction of penciclovir toluene-*p*-sulphonate with tripolyphosphate by the general method of V. M. Dixit and C. D. Poulter, *Tetrahedron Lett.*, 1984, **25**, 4055; δ_C (D_2O) 28.8 (CH_2), 39.4 (d, *J* 8.3 Hz, CH), 42.7 (CH_2N), 62.2 (CH_2OH), 66.7 (d, *J* 5.2 Hz, CH_2OP), 117.0 (C-5), 141.2 (C-8), 152.5 (C-4), 154.7 (C-2), and 160.1 (C-6).
9 Phosphorylation of the related acyclonucleoside, ganciclovir, by HSV-1 encoded thymidine kinase has been shown to be stereospecific (J. D. Karkas, J. Germershausen, R. L. Tolman, M. MacCoss, A. F. Wagner, R. Liou, and R. Bostedor, *Biochim. Biophys. Acta*, 1987, **911**, 127). These workers did not determine unambiguously the absolute configuration of the phosphate but inferred from structural comparisons that it was *S*. In our work with penciclovir we have shown that such structural comparisons are not reliable (D. L. Earnshaw and R. A. Vere Hodge, unpublished results).