Potential Use of Carbocyclic Nucleosides for the Treatment of AIDS: Chemo-enzymatic Syntheses of the Enantiomers of Carbovir

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The lactam (1*R*),(4*S*)-2-azabicyclo[2.2.1]hept-5-en-3-one [(−)-**2**], derived by whole cell enantiospecific hydrolysis of the racemate was converted into (−)-carbovir (−)-**1** in ten steps. Lipase catalysed acetylation of 4-*cis*-hydroxycyclopent-2-enylmethyl triphenylmethyl ether afforded the optically pure ester (+)-**3** and the alcohol (−)-**9**. The former compound was converted into (+)-carbovir (+)-**1** in three steps.

There is current interest in the possible use of carbovir **1** as a chemotherapeutic agent for the treatment of AIDS infections.[1] In this paper we describe, in full,[2,3] two different methods for the preparation of this material in optically active form.

The two routes can be adapted so that each route can be used to prepare either enantiomer of the carbocyclic nucleoside **1** (Fig. 1). One route utilises the γ-lactam 2-azabicyclo[2.2.1]hept-5-en-3-one as the key intermediate and we have converted (1*R*),(5*S*)-2-azabicyclo[2.2.1]hept-5-en-3-one (−)-**2** into (−)-carbovir (−)-**1**. The second route employs the triphenylmethyl (trityl) derivative of *cis*-4-acetoxycyclopent-2-enylmethanol as an important synthon and we show that the (1*R*),(4*S*)-compound (+)-**3** is readily converted into (+)-carbovir (+)-**1**. Note that the availability of both enantiomers of a carbocyclic nucleoside is very important since, remarkably, it has been shown in at least one related case[4] that the two enantiomers of the same chiral compound can display similar biological activity.

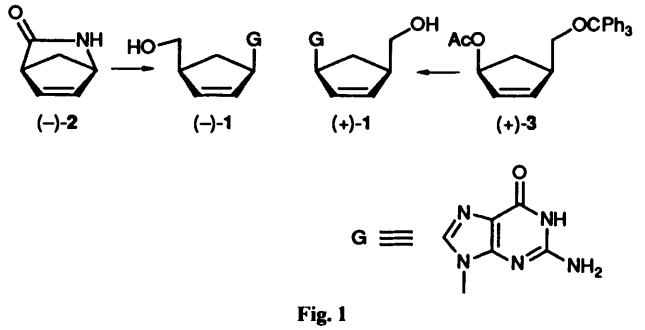
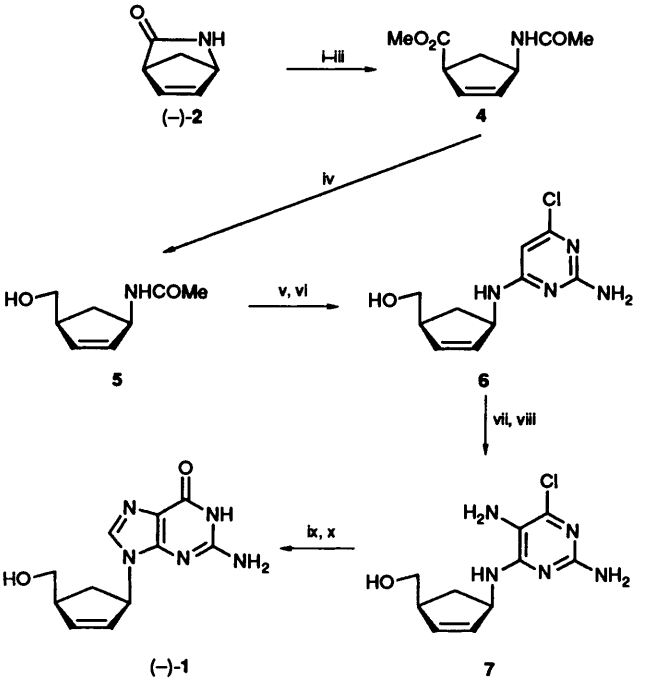


Fig. 1



Scheme 1 *Reagents and conditions:* i, HCl, H_2O reflux; ii, $(MeO)_2CMe_2$, MeOH, HCl; iii, Ac_2O, pyridine, CH_2Cl_2; iv, $Ca(BH_4)_2$, THF, ultrasound; v, HCl, H_2O, EtOH reflux; vi, 2-amino-4,6-dichloropyrimidine, Pr^i_2NEt, BuOH, reflux; vii, 4-$ClC_6H_4N_2^+Cl^-$, HOAc, NaOAc, H_2O; viii, Zn, HOAc, EtOH, H_2O; ix, $(EtO)_3CH$, HCl then HCl, H_2O; x, NaOH, H_2O reflux

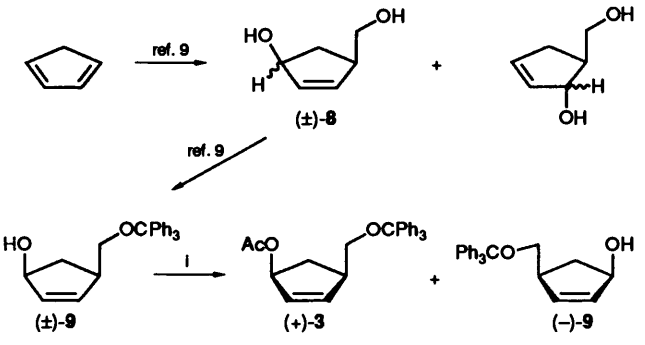
Results and Discussion

The lactam 2-azabicyclo[2.2.1]hept-5-en-3-one is obtained by reaction of toluene-*p*-sulfonyl cyanide and cyclopentadiene followed by acid treatment of the first-formed adduct.[5] The lactam was hydrolysed enantiospecifically by *Pseudomonas solanacearum* NCIMB 40249 to give (−)-lactam (−)-**2** and (1*R*,4*S*)-aminocyclopent-2-enyl-1-carboxylic acid.[2] The lactam was isolated in 45% yield and was shown to be essentially optically pure by NMR spectroscopy employing a chiral shift reagent. The above-mentioned amino acid was also obtained in 45% yield. The absolute configuration of this compound was assigned by comparison of the sign (and magnitude) of the specific rotation of the derived methyl ester acetamide to that described in the literature for an authentic sample.[6]

Hydrolysis of the lactam (−)-**2** gave, after esterification and acetylation, the amido ester **4** (Scheme 1) and this material was reduced by calcium borohydride to provide the alcohol **5**. Deacylation and coupling of the resultant amino alcohol to 2-amino-4,6-dichloropyrimidine furnished the diamine **6**. Diazotization of **6** using 4-chlorophenyldiazonium chloride followed by reduction of the yellow diazo compound with zinc–acetic acid afforded the aromatic amine **7** and this compound was treated with triethyl orthoformate under acidic conditions, and then aqueous sodium hydroxide under reflux, to give the target compound (−)-carbovir (−)-**1** $[\alpha]_D$ −66 (*c* 0.4, methanol). It is noteworthy that an alternative method of preparation of (−)-carbovir, starting from the naturally-occurring carbocyclic nucleoside aristeromycin, has been reported by a group from the Glaxo laboratories.[7] In connection with the present work we found the conditions reported in the following Experimental section more reliable than those published by Vince and Hua[8] for the conversion of racemic 2-azabicyclo[2.2.1]hept-5-en-3-one into (±)-carbovir.

The sequence (−)-**2** ⇒ (−)-**1** represents a workable method for the preparation of the anti-viral compound. However the synthesis has the distinct disadvantage of being linear and hence we explored the possibility of discovering a more convergent approach.

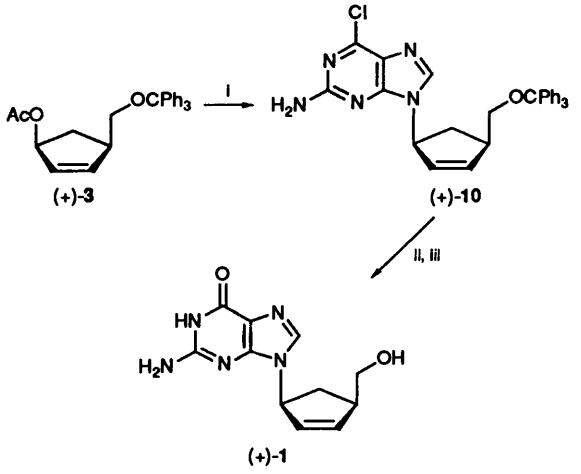
Treatment of cyclopentadiene with formaldehyde and formic acid furnished, after base treatment, a mixture of hydroxycyclopent-2-enylmethanol derivatives **8** from which (±)-4-*cis*-hydroxycyclopent-2-enylmethyl triphenylmethyl ether (±)-**9** was obtained by chromatography and reaction with trityl chloride (Scheme 2).[9] The alcohol (±)-**9** was reacted with vinyl



Scheme 2 *Reagents and conditions:* i, *Pseudomonas fluorescens* lipase, vinyl acetate, 75 h, room temp.

acetate over 75 h using *Pseudomonas fluorescens* lipase as catalyst to afford the ester (+)-**3** and (1*S*,4*R*)-hydroxycyclopent-2-enylmethyl triphenylmethyl ether (−)-**9**. Compounds (+)-**3** and (−)-**9** were easily separated by chromatography and both the ester **3** and the alcohol (−)-**9** showed excellent optical purities (>95% e.e.) as assessed by chiral shift NMR studies. The alcohol (−)-**9** was converted into (−)-**3** in practically quantitative yield by a standard acetylation procedure.

The esters (+)-**3** and (−)-**3** are very valuable precursors to various classes of carbocyclic nucleoside, particularly of the 2′,3′-dideoxydidehydro type. For example, the ester (+)-**3** in the presence of 2-amino-6-chloropurine, sodium hydride and palladium tetrakis(triphenylphosphine) gave the carbocyclic nucleoside (+)-**10** directly in 49% yield.[10] Aromatic nucleophilic substitution by hydroxide ion following deprotection, using acid in the normal way, converted (+)-**10** into (+)-carbovir (+)-**1** {$[\alpha]_D^{20}$ 59.5 (*c* 0.4, methanol)} (Scheme 3).



Scheme 3 *Reagents and conditions:* i, 2-amino-6-chloropurine, NaH, $Pd(PPh_3)_4$, THF; ii, H^+, H_2O; iii, NaOH, H_2O reflux

Conclusions

Two new routes, one linear and one convergent, to optically active carbocyclic nucleosides have been devised. In order to exemplify the methodology the enantiomers of the anti-HIV agent carbovir have been prepared. While this work was in progress it was shown that the laevorotatory enantiomer of carbovir was mainly, if not exclusively, responsible for the observed anti-viral activity.[1c] Like AZT the most likely mechanism of action of carbovir is through inhibition of HIV-reverse transcriptase and, indeed, carbovir 5′-triphosphate is a potent inhibitor of this enzyme.[11]

Experimental

General.—*J* values are given in Hz. $[\alpha]_D$ Values are given in 10^{-1} deg. cm^2 g^{-1}.

(−)-*Methyl* (1S,4R)-4-*Acetamidocyclopent-2-ene*-1-*carboxylate* **4**.—(−)-(1*R*,4*S*)-2-Azabicyclo[2.2.1]hept-5-en-3-one (−)-**2** (1.96 g, 0.018 mol) in 1 mol dm^{-3} aqueous hydrochloric acid (45 cm^3) was refluxed under nitrogen for 1 h. The mixture was concentrated under reduced pressure and the resultant solid stirred in a mixture of methanol (9 cm^3), dimethoxypropane (45 cm^3), and conc. HCl (2.7 cm^3) at room temperature for 20 h. This mixture was concentrated under reduced pressure and dry dichloromethane (45 cm^3), acetic anhydride (2.02 g, 0.0198 mol) then pyridine (3.13 g, 0.0396 mol) added under nitrogen at room temperature. Stirring was continued at this temperature for 24 h when the reaction mixture was diluted with dichloromethane (100 cm^3) and washed with water (50 cm^3). The aqueous layer was extracted with dichloromethane (50 cm^3) and the combined organic layers dried ($MgSO_4$) and concentrated, removing residual pyridine by azeotroping with toluene. Column chromatography (10% acetone–dichloromethane) gave the ester amide **4** (3.16 g, 96%) as a white solid, m.p. 89.5–90.5 °C [lit.,[12] for (±) form, 66–67 °C]; $[\alpha]_D^{24}$ −84.4 (*c* 1, MeOH); $\nu_{max}(CHCl_3)$/cm^{-1} 3437 (NH), 1716 (CO_2Me), 1642 (amide I) and 1499 (amide II); δ_H 6.21 (1 H, br s, NH), 5.82 (2 H, s, 2-H and 3-H), 4.98 (1 H, ddd, *J* 8.5, 8.5, 3.8, 4-H), 3.64 (3 H, s, OMe), 3.44 (1 H, dd, *J* 8.5, 3.8, 1-H), 2.41 (1 H, ddd, *J* 13.9, 8.5, 8.5, 5-H), 1.89 (3 H, s, $NCOCH_3$) and 1.80 (1 H, ddd, *J* 13.9, 3.8, 3.8, 5-H); *m*/*z* 183 (M^+), 140 (M − Ac) and 124 (M − CO_2Me).

(−)-(1S,4R)-4-*Acetamidocyclopent-2-enylmethanol* **5**.—A suspension of calcium chloride (0.999 g, 0.009 mol) and sodium borohydride (0.681 g, 0.018 mol) in dry THF (20 cm^3) was sonicated under nitrogen for 1 h. Ester **4** (1.099 g, 0.006 mol) in dry THF (25 cm^3) was added and sonication continued for a further 3 h at *ca.* 40 °C. The mixture was cooled in an ice bath and saturated ammonium chloride solution (30 cm^3) added very carefully dropwise. The resultant mixture was extracted with dichloromethane (4 × 200 cm^3) and the combined organic layers dried ($MgSO_4$) and concentrated under reduced pressure. Column chromatography (10% EtOH–Et_2O) gave the alcohol **5** (0.677 g, 73%) as a white solid, m.p. 77.5–79.5 °C [Found: m/*z* (CI, NH_3) (M^+ + H), 156.1025. $C_8H_{13}NO_2$ requires (M^+ + H), 156.1024]; $[\alpha]_D^{24}$ −9.4 (*c* 1, MeOH); $\nu_{max}(CHCl_3)$/cm^{-1} 3284 (OH, NH), 1634 (amide I) and 1540 (amide II); δ_H 6.61 (1 H, br d, *J* 8.2, NH), 5.78–5.64 (2 H, m, 2-H and 3-H), 4.92–4.80 (1 H, m, 4-H), 3.63–3.45 (3 H, m, OCH_2CH), 2.77 (1 H, br s, OH), 2.38 (1 H, ddd, *J* 13.5, 8.5, 8.5, 5-H), 1.86 (3 H, s, CH_3) and 1.36 (1 H, ddd, *J* 13.5, 3.8, 3.8, 5-H); δ_C 169.5 (CON), 134.9 and 132.8 (2-C and 3-C), 64.3 (CH_2O), 54.5 (4-C), 46.9 (1-C), 34.2 (5-C) and 23.3 (CH_3).

(−)-(1S,4R)-4-[(2′-*Amino*-6′-*chloropyrimidin*-4′-*yl*)*amino*]-*cyclopent*-2-*enylmethanol* **6**.—Amide **5** (1.17 g, 7.54 mmol) was refluxed under nitrogen in a mixture of ethanol (26 cm^3) and 2

mol dm^{-3} hydrochloric acid (26 cm^3) for 48 h. The solvent was removed under reduced pressure. Butanol (40 cm^3), 2-amino-4,6-dichloropyrimidine (2.47 g, 15.1 mmol) and diisopropylethylamine (12.5 cm^3) were added and the mixture refluxed under nitrogen for 14 h, then poured into water (50 cm^3) and extracted with dichloromethane (4 × 250 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated. Column chromatography (50% EtOAc–light petroleum) gave compound **6** (1.45 g, 80%) as a white foam, m.p. 49–53 °C [lit.[8] for (±) form, 132–134 °C]; $[\alpha]_D^{24}$ −35 (*c* 1, MeOH); ν_{max}-($CHCl_3$)/cm^{-1} 3535, 3424 (NH and OH) and 1580 (pyrimidine); δ_H 5.88–5.71 (3 H, m incorporating s at 5.76, 2-H, 3-H and 5′-H), 5.49 (1 H, br d, *J* 7.4, NH), 5.22 (2 H, br s, NH_2), 4.76 (1 H, br, 4-H), 3.63 (1 H, ddd, *J* 11.0, 11.0, 4.1, CH_aH_bOH), 3.59 (1 H, ddd, *J* 11.0, 11.0, 4.4, CH_aH_bOH), 2.94–2.80 (2 H, m, 1-H and OH), 2.48 (1 H, ddd, *J* 13.7, 8.8, 8.8, 5-H) and 1.47 (1 H, ddd, *J* 13.7, 3.9, 3.9, 5-H); *m/z* 211/209 (M − $HOCH_2$).

(1S,4R)-4-[(2′,5′-*Diamino*-6′-*chloropyrimidin*-4′-*yl*)*amino*]-*cyclopent*-2-*enylmethanol* **7**.—A solution of sodium nitrite (0.524 g, 7.60 mmol) in water (6.2 cm^3) was added dropwise to a solution of 4-chloroaniline (0.869 g, 6.81 mmol) in 2.7 mol dm^{-3} hydrochloric acid (8.3 cm^3) at 9 °C. The resultant solution was added quickly dropwise to a solution of pyrimidine **6** (1.49 g, 6.19 mmol) and sodium acetate (7.45 g) in water (31 cm^3) and glacial acetic acid (31 cm^3) at room temperature. The mixture was stirred vigorously for 27 h then added to ethyl acetate (1100 cm^3). The organic layer was washed with water (200 cm^3) and saturated sodium hydrogen carbonate solution (6 × 200 cm^3). The combined aqueous layers were extracted with ethyl acetate (400 cm^3) which was washed with saturated aqueous sodium hydrogen carbonate (200 cm^3), combined with the other organic layer, dried ($MgSO_4$) and adsorbed onto silica. Column chromatography (50% EtOAc–light petroleum) gave (1*S*,4*R*)-4-({2′-amino-6′-chloro-5′-[(4″-chlorophenyl)azapyrimidin-4′-yl}-amino)cyclopent-2-enylmethanol (1.62 g, 69%) as a bright yellow solid, m.p. 230–232 °C (decomp.) [lit.[8] for (±) form, 229 °C (decomp.)]; $[\alpha]_D^{24}$ +236 (*c* 0.4, DMF).

This azo compound (1.626 g, 4.29 mmol), zinc dust (2.80 g, 42.9 mmol), glacial acetic acid (1.38 cm^3), ethanol (66 cm^3) and water (66 cm^3) was refluxed under nitrogen for 20 min. The mixture was filtered and concentrated, azeotroping several times with ethanol. The residue was dissolved in methanol and absorbed onto silica. Column chromatography (EtOAc) gave the triamine **7** as an off-white solid (0.549 g, 50%), m.p. 158.5–160.5 °C [lit.[8] for (±) compound, 168–170 °C]; $[\alpha]_D^{24}$ *ca.* 0 (*c* 0.4, MeOH); ν_{max}(KBr)/cm^{-1} 3309, 3187 (NH and OH), 1634 and 1558 (C=C, C=N); δ_H([$^2H_6$]-DMSO) 6.36 (1 H, d, *J* 7.6, NH), 5.92–5.85 (1 H, m) and 5.81–5.73 (1 H, m, 2-H and 3-H), 5.58 (2 H, s, NH_2), 5.11–4.98 (1 H, m, 4-H), 4.62 (1 H, t, *J* 5.5, OH), 3.92 (2 H, s, NH_2), 3.40 (2 H, dd, *J* 5.5, CH_2O), 2.76–2.67 (1 H, m, 1-H), 2.41 (1 H, ddd, *J* 13.2, 8.0, 8.0, 5-H) and 1.31 (1 H, ddd, *J* 13.2, 6.5, 6.5); *m/z* 257/255 (M^+), 226/224 (M − $HOCH_2$), 161/159 (M + H − C_6H_9O) and 160/158 (M − C_6H_9O).

(−)-(1S,4R)-4-(2′-*Amino*-1′,9′-*dihydropurin*-6′-*on*-9′-*yl*)-*cyclopent*-2-*enylmethanol*, (−)-*Carbovir*, (−)-**1**.—Pyrimidine **7** 163 mg, 0.637 mmol) was added to conc. hydrochloric acid (*ca.* 0.15 cm^3) in freshly distilled triethyl orthoformate (3.9 cm^3) and the mixture stirred for 18 h at room temperature. The mixture was concentrated and the resultant solid stirred in 0.5 mol cm^{-3} hydrochloric acid (6 cm^3) for 1 h when sufficient 1 mol dm^{-3} sodium hydroxide solution was added to adjust the mixture to pH 8. The mixture was concentrated, dissolved in methanol and absorbed onto silica. Column chromatography (EtOAc) gave (−)-(1*S*,4*R*)-4-(2′-amino-6′-chloro-9′*H*-purin-9′-yl)cyclopent-2-enylmethanol (156 mg, 92%) as a white foam-like solid, m.p. 132–135 °C [lit.[8] for (±) form, 145–147 °C; $[\alpha]_D^{24}$ −75 (*c* 0.9, MeOH). This chloropurine (68.4 mg, 0.257 mmol) was gently refluxed under nitrogen in 0.33 mol dm^{-3} sodium hydroxide (3.5 cm^3) for 3 h. The mixture was neutralized with 0.5 mol dm^{-3} hydrochloric acid, concentrated, dissolved in methanol and absorbed onto silica. Column chromatography (10% MeOH–CH_2Cl_2) yielded compound **1** (46.8 mg, 74%) as a white solid, m.p. 210–220 °C (decomp.) [lit.[8] for (±) form, 254–256 °C (decomp.)]; $[\alpha]_D^{24}$ −66 (*c* 0.4, MeOH) (lit.[7] $[\alpha]_D$ −64); δ_H[$^2H_6$]-DMSO) 10.50 (1 H, br s, NH), 7.57 (1 H, s, 8′-H), 6.46 (2 H, s, NH_2), 6.12 (1 H, ddd, *J* 5.5, 1.8, 1.8) and 5.86 (1 H, ddd, *J* 5.5, 2.0, 2.0, 2-H and 3-H), 5.40–5.29 (1 H, m, 4-H), 4.75–4.65 (1 H, m, OH), 3.47–3.39 (2 H, m, CH_2O), 2.94–2.80 (1 H, m, 1-H), 2.59 (1 H, ddd, *J* 13.5, 8.8, 8.8, 5-H) and 1.58 (1 H, ddd, *J* 13.5, 6.0, 6.0, 5-H).

Enzyme Resolution of (±) 4β-(*Triphenylmethoxymethyl*)-*cyclopent*-2-*en*-1β-*ol* **9**.—*Pseudomonas fluorescens* lipase (22.9 mg) was added to a solution of (±) 4-(triphenylmethoxymethyl)cyclopent-2-en-1-ol **9** (55.4 mg, 0.16 mmol) in vinyl acetate (4 cm^3). After stirring for 46 h at room temperature, the enzyme was removed by filtration and the residue was washed with ethyl acetate. The combined filtrate and washings were concentrated under reduced pressure and the residue (75.0 mg) was purified by chromatography on silica gel (4:1, light petroleum–EtOAc). The first to be eluted was (+) (1*S*,4*R*)-1-acetoxy-4-(triphenylmethoxymethyl)cyclopent-2-ene (+)-**3** (13.6 mg, 22%, R_f 0.42) as an oil; $[\alpha]_D^{27}$ +19.2 (*c* 0.7 in $CHCl_3$) (>95% e.e.); IR, ^{1}H- and ^{13}C-NMR spectra were identical to the racemic compound. This was followed by (−)-(1*R*,4*S*)-(triphenylmethoxymethyl)-cyclopent-2-en-1-ol (−)-**9** (27.7 mg, 50%, R_f 0.16) as a white solid; m.p. 111–112 °C; $[\alpha]_D^{24}$ −52.1 (*c* 1.4 in $CHCl_3$) (82% e.e.); IR, ^{1}H- and ^{13}C-NMR spectra were identical to those for racemic compound.

(+)-cis-2-*Amino*-6-*chloro*-9′-[(4′R)-(*triphenylmethoxymethyl*)*cyclopent*-2′-*en*-1′S)-*yl*]*purine* **10**.—Sodium hydride (60% dispersion in oil, washed with light petroleum, 56.1 mg, 1.4 mmol) was added to a solution of 2-amino-6-chloropurine (241 mg, 1.4 mmol) in dry DMF (1.4 cm^3) under nitrogen. After stirring for 2 h, this solution was added to a suspension of (+)-(1*S*,4*R*)-1-acetoxy-4-triphenylmethoxymethyl)cyclopent-2-ene **3** (283 mg, 0.71 mmol), triphenylphosphine (25.6 mg, 0.098 mmol, 0.14 equiv.) and tetrakis(triphenylphosphine)palladium(0) (408.5 mg, 0.35 mmol) in dry THF (2.2 cm^3). The mixture was immersed in a pre-heated oil bath and stirred for 3 h at 60 °C. After cooling, water (4 cm^3) was added and the mixture was extracted with ethyl acetate (8 × 10 cm^3). The combined extracts were dried, filtered and the filtrate was concentrated under reduced pressure. The residue (969 mg) was chromatographed on silica gel (3:1 to 2:1, hexane–EtOAc) to give (+)-*cis*-2-amino-6-chloro-9-[(4′R)-triphenylmethoxymethyl-cyclopent-2′-en-(1′S)-yl]purine **10** (175.5 mg, 49%, R_f 0.18, hexane–EtOAc, 1:1) as a white foam; m.p. 82–90 °C; $[\alpha]_D^{25}$ +84.6 (*c* 1.2 in MeOH); ν_{max}(KBr)/cm^{-1} 3322 (br s, NH_{str}), 3061 (m, CH_{str}), 2924 (s, CH_{str}), 2866 (s, CH_{str}), 1609, 1560, 1507 (s, $C{=}C_{str}$ and $C{=}N_{str}$), 1455, 1403, 1178 and 1114; λ_{max}-(MeOH)/nm 223.4, 312.0; δ_H(250 MHz; CD_3OD) 7.73 (1 H, s, 8-H), 7.47–7.32 (6 H, m, Ph), 7.29–7.08 (9 H, m, Ph), 6.19 (1 H, ddd, *J* 5.5, 1.5, 1.5, 3′-H), 5.87 (1 H, ddd, *J* 5.5, 1.5, 1.5, 2′-H), 5.41–5.51 (1 H, m, 1′-H), 2.95–3.19 (3 H, m, 5′-CH_2 and 4′-H), 2.63 (1 H, ddd, *J* 14, 8, 8.5, 6′-H) and 1.56 (1 H, ddd, *J* 14, 5, 5.5, 6′-H); δ_C(CD_3OD) 161.39 (C, C-6), 154.85 (C, C-2), 151.55 (C, C-4), 145.35 (C, Ph), 144.89 (C, C-5), 142.09 (CH, C-8), 140.38 (CH, C-3′), 129.88 (CH, C-2′), 129.81 (CH, Ph), 128.80 (CH, Ph), 128.14 (CH, Ph), 87.84 (C, Ph_3C), 67.22 (CH_2, C-5′), 61.17 (CH, C-1′), 47.03 (CH, C-4′) and 35.82 (CH_2, C-6′) (Found:

$[M + H]^+$ 508.1904 calc. for $C_{30}H_{26}ClN_5O$ $[M + H]^+$ 508.1904).

(+)-(1S,4R)-4-(*2'-Amino-1',9'-dihydropurin-6'-on-9'-yl*)-*cyclopent-2-enylmethanol*, (+)-*carbovir* **1**.—A solution of (+)2-amino-6-chloro-9-[(4'R)-(triphenylmethyloxymethyl)cyclopent-2'-en-(1'-*S*)-yl]purine **10** (163 mg, 0.32 mmol) in 80% aqueous acetic acid (3 cm^3) was heated at 50 °C for 4 h. The solvent was removed by evaporation and the residue (207 mg) was purified by chromatography over silica gel (9:1, CH_2Cl_2–MeOH) to give (+)-*cis*-2-amino-6-chloro-9-[(4'*R*)-(hydroxymethyl)cyclopent-2'-en-(1'*S*)-yl]purine (81.2 mg, 96%, R_f 0.27) as a white solid; m.p. 145–148 °C; $[\alpha]_D^{25}$ +75.8 (*c* 0.9 in MeOH); δ_H(250 MHz; [$^2H_6$]-DMSO) 8.04 (1 H, s, 8-H), 6.87 (2 H, br s, NH_2), 6.16 (1 H, ddd, *J* 5.5, 2, 2, 3'-H), 5.92 (1 H, ddd, *J* 5.5, 2, 2.5, 2'-H), 5.52–5.41 (1 H, m, 1'-H), 4.95–4.50 (1 H, br s, OH), 3.47 (2 H, d, *J* 5.5, 5'-CH_2), 2.96–2.83 (1 H, m, 4'-H), 2.64 (1 H, ddd, *J* 13.5, 8.5, 8.5, 6'-H), 1.65 (1 H, ddd, *J* 13.5, 5.5, 5.5, 6'-H) (Found: M^+ 265.0730 calc. for $C_{11}H_{12}ClN_5O$, *M*, 265.0730). A solution of the above (+)-6-chloropurine derivative (73.0 mg, 0.28 mmol) in sodium hydroxide (0.33 mol dm^{-3}; 5 cm^3) was refluxed for 4.5 h. The solvent was removed by evaporation and the residue was chromatographed on silica gel (5:1, $CHCl_3$–MeOH) to give (+)-carbovir (44.7 mg, 66%) as an off-white solid; $[\alpha]_D^{26}$ +59.5 (*c* 0.4 in MeOH); λ_{max}(0.1 mol dm^{-3} HCl)/nm 253.2 and 278.4; δ_H(250 MHz; [$^2H_6$]-DMSO) 10.53 (1 H, br s, 6-OH), 7.58 (1 H, s, 8-H), 6.54 (2 H, br s, NH_2, D_2O-exchangeable), 6.18–6.08 (1 H, m, 3'-H), 5.92–5.82 (1 H, m, 2'-H), 5.40–5.29 (1 H, m, 1'-H), 4.72 (1 H, br s, 5'-OH, D_2O-exchangeable), 3.50–3.40 [2 H, m, 5-CH_2 (d, *J* 5.5 in D_2O)], 2.94–2.80 (1 H, m, 4'-H), 2.59 (1 H, ddd, *J* 13.5, 8.5, 8.5, 6'-H), 1.57 (1 H, ddd, *J* 13.5, 5.5 and 5.5, 6'-H) (Found: $[M + H]^+$ 248.1147 calc. for $C_{11}H_{13}N_5O_2$ $[M + H]^+$ 248.1147).

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References

1 (*a*) E. L. White, N. B. Parker, L. J. Macy, S. C. Shaddix, C. McCaleb, J. A. Secrist III, R. Vince and W. M. Shannon, *Biochem. Biophys. Res. Commun.*, 1989, **161**, 393; (*b*) W. B. Parker, E. L. White, S. C. Shaddin, L. J. Ross, R. W. Buckheit, J. M. Germany, J. A. Secrist, R. Vince and W. M. Shannon, *J. Biol. Chem.*, 1991, **266**, 1754; (*c*) J. A. V. Coates, H. J. Ingall, B. A. Pearson, C. R. Penn, R. Storer, C. Williamson and J. M. Cameron, *Antiviral Res.*, 1991, **15**, 161.
2 Preliminary communication: S. J. C. Taylor, A. G. Sutherland, C. Lee, R. Wisdom, S. Thomas, S. M. Roberts and C. Evans, *J. Chem. Soc., Chem. Commun.*, 1990, 1121; C. T. Evans and S. M. Roberts, Eur. Pat. 0424064/1991.
3 Preliminary communication: S. M. Roberts and K. Shoberu, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2605.
4 J. Balzarini, H. Baumgartner, H. Bodenteich, E. DeClercq and H. Griengl, *J. Med. Chem.*, 1989, **32**, 1861.
5 J. C. Jagt and A. M. van Leusen, *J. Org. Chem.*, 1974, **39**, 564.
6 S. Sicsic, M. Ikbal and F. Le Goffic, *Tetrahedron Lett.*, 1987, **28**, 1887.
7 A. M. Exall, M. F. Jones, C.-L. Mo, P. L. Myers, I. L. Paternoster, H. Singh, R. Storer, G. G. Weingarten, C. Williamson, A. C. Brodie, J. Cook, D. E. Lake, C. A. Meerholz, P. J. Turnbull and R. M. Highcock, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2467; see also M. F. Jones, C. L. Mo, P. L. Myers, I. L. Paternoster, R. Storer, G. G. Weingarten and C. Williamson, *Ann. N.Y., Acad. Sci.*, 1990, 535 and M. F. Jones, P. L. Myers, C. A. Robertson, R. Storer and C. Williamson, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2479.
8 R. Vince and M. Hua, *J. Med. Chem.*, 1990, **33**, 17.
9 H. Paulsen and M. Maass, *Chem. Ber.*, 1981, **114**, 346.
10 *Cf.* B. M. Trost, G.-H. Kuo and T. Benneche, *J. Am. Chem. Soc.*, 1988, **110**, 621.
11 D. Coe, D. C. Orr, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans. 1*, 1991, 3378.
12 S. Daluge and R. Vince, *J. Org. Chem.*, 1978, **43**, 2311.

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