Synthesis and Biological Activity of the Diphosphorylphosphonate Derivatives of (+)- and (−)-*cis*-9-(4′-Hydroxycyclopent-2′-enyl)guanine

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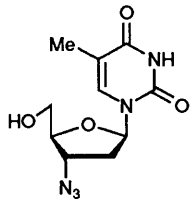
The carbovir triphosphate analogue **10** and its enantiomer **11** have been synthesized and tested as inhibitors of HIV-reverse transcriptase; the enantiomer **11**, more remotely resembling natural nucleotides in stereochemical terms, is surprisingly the more active compound.

Inhibition of the human immunodeficiency virus reverse transcriptase (HIV-rt) enzyme remains a sensible and viable target in the search for chemotherapeutics aimed at combating the HIV viruses.

Very many compounds have been tested as HIV-rt inhibitors and/or as anti-HIV agents and, where comparisons have been made, the optically active compound resembling the natural nucleoside is almost invariably more active than the 'unnatural' enantiomer.[1] For example, azidothymidine (AZT) **1** is a potent



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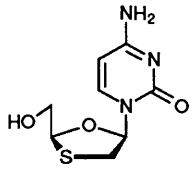

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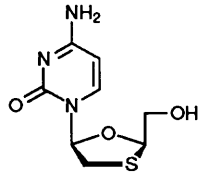

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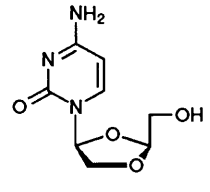
inhibitor of HIV *in vitro* (and is used in the clinical treatment of AIDS) while the enantiomer is very much less effective. The mechanism of action of compounds such as AZT involves formation of the corresponding triphosphate inside the HIV-infected cell followed by inhibition of HIV-rt.

There are some noteworthy exceptions to the above mentioned generalization concerning the stereochemistry of HIV-active compounds. For example the oxathiolanes **2** and **3** are equipotent anti-HIV agents;[2] the latter compound, lamivudine, was taken forward to clinical studies on the basis of its better therapeutic index.[3] The nucleoside analogue **4**, closely related to the oxathiolane **3**, also shows potent activity against HIV-1: the enantiomer of compound **4** is, interestingly, slightly less active.[4]



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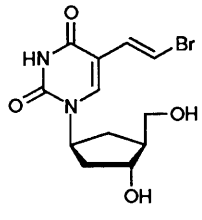

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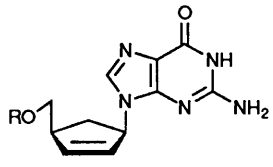
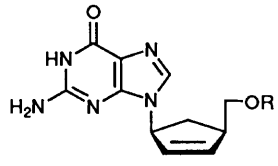
Owing to their stability to metabolism *in vivo*, carbocyclic nucleosides have been identified as a class of compounds of potential interest in antiviral chemotherapy.[5] Comparisons of antiviral activities of enantiomers from this series have been made and, once again, there are isolated reports identifying the 'wrong' enantiomer as an antiviral agent. For example, it has been reported that compound **5** is just one order of magnitude less active against Herpes Simplex virus than is the enantiomer, carbocyclic-BVDU.[6]

Carbovir conforms to the more expected format, the enantiomer **6** being more active as an anti-HIV agent than the compound **8** in *in vitro* tests. However, in recent studies it has been shown that the triphosphates **7** and **9** are approximately equipotent as HIV-rt inhibitors. It seems that the inactivity of compound **8** is due to the lack of phosphorylation *in vivo*.[7]

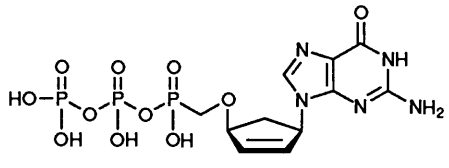


6 R = H
7 R = $H_4P_3O_9$



8 R = H
9 R = $H_4P_3O_9$

We have been interested in the synthesis and biological activity of nucleotide mimics and we have reported that the racemate formed by triphosphate analogues **10** and **11** is a potent inhibitor of HIV-rt.[8] We were intrigued to compare the biological activity of the separate enantiomers in this case.



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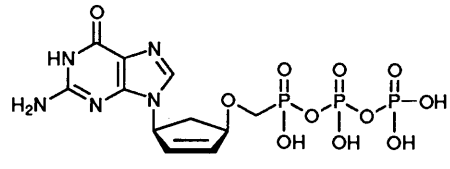

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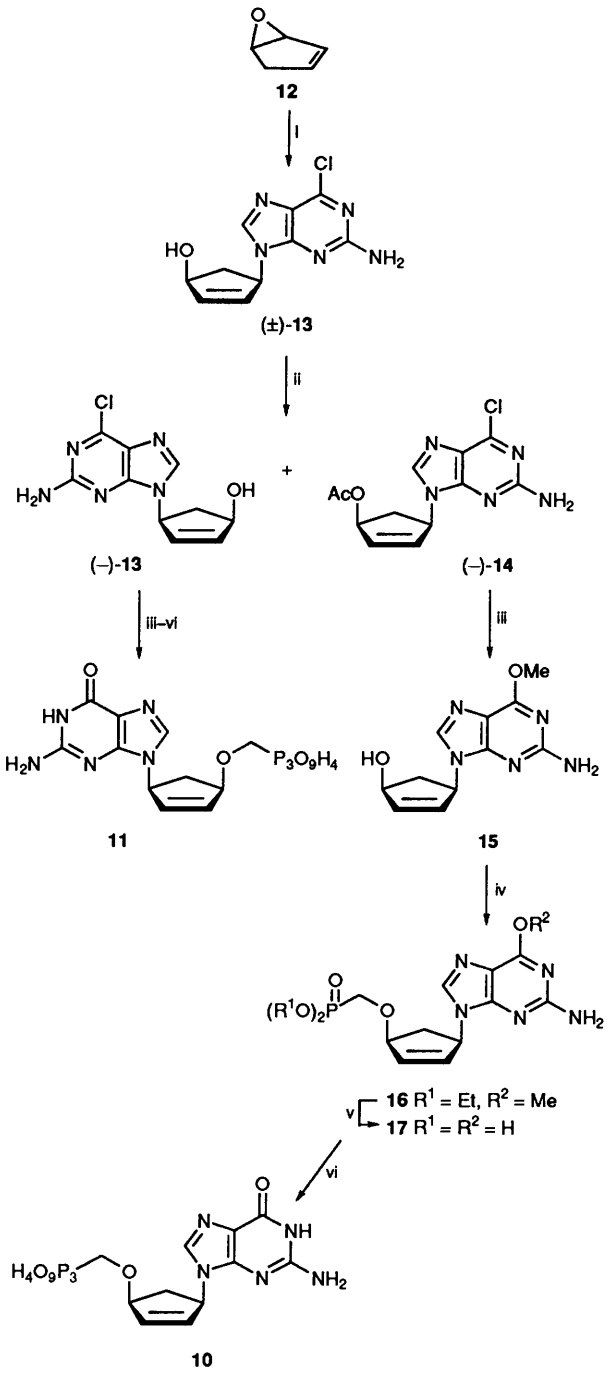
Chemical Synthesis.—The method of preparation of the two enantiomers **10** and **11** utilizes an enzyme-catalysed kinetic resolution.[9] Thus the cyclopentenol (±)-**13** (Scheme 1) (easily



Scheme 1 *Reagents and conditions:* i, $Pd(PPh_3)_4$, 2-amino-6-chloropurine, DMSO, THF, 0 °C–room temp.; ii, *Ps. cepacia* lipase, vinyl acetate, 50 °C, 48 h; iii, K_2CO_3, MeOH, reflux, 2 h; iv, NaH, DMSO; then diethyl (*p*-tolylsulfonyloxymethyl)phosphonate, DMSO, room temp., 18 h; v, Me_3SiI, DMF, room temp., 18 h; vi, morpholine, aq. Bu^{t}OH, reflux, 1 h; then DCC in Bu^{t}OH; evaporation; then tributylammonium pyrophosphate, DMSO, 110 h, room temp.

prepared from 6-oxabicyclo[3.1.0]hex-2-ene **12**) was enantioselectively acetylated using *Pseudomonas cepacia* lipase in vinyl acetate to give the ester (−)-**14** (35%) and the alcohol (−)-**13** (33%), both in states of high optical purity (>90% ee). Methanolysis of the ester (−)-**14** formed the purine derivative **15** (in 94% yield). Deprotonation of the alcohol moiety in compound **15**, followed by reaction with diethyl (*p*-tolylsulfonyloxymethyl)phosphonate provided the phosphonate ester **16** in 57% yield. Cleavage of the phosphate ester groups was accomplished using trimethylsilyl iodide to give the phosphonic acid **17** in 71% yield, whereupon conversion into the diphosphorylphosphonate **10** proceeded smoothly (67% yield) *via* the corresponding morpholidate. The alcohol (−)-**13** was converted into the diphosphorylphosphonate **11** in a similar manner.

Table 1 Effects of (−)-carbovir triphosphate **7** and diphosphorylphosphonates **10** and **11** as HIV-rt inhibitors[a]

Compound	IC_{50} (μg cm^{-3})
7	1.5
10	3.9
11	0.52

[a] The method used for biological testing was the MS2 RNA and oligonucleotide primer assay[10] except that the labelled deoxynucleoside triphosphate was dTTP. In each assay the concentrations of dATP, dGTP and dCTP were 25 micromolar and the concentration of dTTP (8 Ci mmol^{-1}) was 5 micromolar.

Biological Results.—The two enantiomers **10** and **11** were tested as inhibitors of HIV-rt and, as shown in Table 1, the results were most interesting. As expected, compound **10** showed good activity as an inhibitor of HIV-rt, being approximately equipotent as Carbovir. The enantiomer **11** surprisingly showed even greater activity as an inhibitor of HIV-rt. To our knowledge this is the first time that the unnatural enantiomer of a carbocyclic nucleotide mimic has been shown to be significantly more active than the nucleotide mimic itself.

Conclusions.—Previously we have shown that the racemate (±)-**17** is inactive *in vitro* as an anti-HIV agent. The inactivity could be due to the inability of the phosphonate to cross the cell wall and/or the inability of one or both enantiomers to be phosphorylated within the infected cell. We are now concentrating our efforts on the preparation of non-polar pro-drugs of compound **11** in order to overcome these problems. Since compound **11** only remotely resembles a natural nucleotide (almost every part of the molecule has been changed, including, crucially, the absolute configuration) we expect such a compound and its surrogates to show less overt toxicity than agents currently used in the clinic, *e.g.* AZT.

Experimental

Ps. cepacia lipase (PCL) was purchased from the Biocatalysts Co. UV spectra were recorded on a Philips PU 8700 spectrometer and IR spectra were recorded on a Perkin-Elmer 881 spectrometer. NMR spectra (^1H, ^{13}C and ^{31}P) were recorded on a Bruker AM250 or a Bruker AM300 spectrometer at the following frequencies: 300 or 250 MHz for ^1H NMR, 75.5 or 62.9 MHz for ^{13}C NMR, and 121.5 MHz for ^{31}P NMR. All chemical shifts are reported as δ-values in parts per million (ppm) and coupling constants (*J*) are quoted in Hz. Mass spectra (MS) were obtained with a VG 12-253 spectrometer or at the SERC Mass Spectrometry Centre, Swansea, on a VG ZAB-F spectrometer. M.p.s were determined on a Gallenkamp digital apparatus and are uncorrected. Optical rotations $[\alpha]_D$ were measured with an Optical Activity Ltd AA 1000 polarimeter in the indicated solvent. The concentration of the samples was expressed in g/100 cm^3 and the length of the cell used was 0.5 dm. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. TLC was performed on glass plates coated with a 0.25 mm thickness of silica gel 60F-254 (Merck). Paper chromatography (PC) was performed on Whatman no. 1 chromatography paper visualized under UV light (254 nm).

Flash column chromatography was carried out according to the method of Still *et al.*[11] using Merck Kieselgel 60 (230–400

mesh) with the eluent specified. Gel permeation and ion-exchange chromatography were performed on Sephadex LH-20 and Sephadex DEAE A-20, repectively, with the eluent specified.

All solvents were distilled before use. Petroleum spirit refers to the fraction boiling in the range 60–80 °C. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. An inert atmosphere refers to a static atmosphere of argon or nitrogen.

6-Oxabicyclo[3.1.0]*hex-2-ene* **12**.—Peracetic acid (32% w/w in dil. acetic acid; 76.00 g, 0.32 mol) was added, over a period of 45 min to a stirred solution of freshly distilled cyclopentadiene (41.00 g, 0.62 mol) and sodium carbonate (106.00 g, 1 mol) in dichloromethane (500 cm^3). The internal temperature was maintained below 20 °C by intermittent ice cooling. The reaction mixture was stirred at room temperature for an additional hour and then sodium sulfite (81.00 g, 0.64 mol) was added. The reaction mixture was stirred for a further 3 h at room temperature, then kept at room temperature overnight, without being stirred. When no more oxidant was detected by starch–iodide paper analysis, the solid was removed by filtration and washed with dichloromethane (3 × 80 cm^3). The solvent was removed by distillation at atmospheric pressure and the title compound **12** (13.06 g, 50%) was obtained as an oil by distillation under reduced pressure, b.p. 20–26 °C/10–15 mmHg (lit.,[12] 39–41 °C/39–45 mmHg); δ_H(300 MHz; $CDCl_3$) 2.38 (1 H, ddt, *J* 15.8, 2.9 and 2.1, 4β-H), 2.63 (1 H, ddd, *J* 15.8, 1.7 and 1.7, 4α-H), 3.78–3.82 (1 H, m, 1-H), 3.89 (1 H, dt, *J* 2.5 and 2.1, 5-H), 5.95–6.05 (1 H, m, 2-H) and 6.14 (1 H, ddt, *J* 5.0, 1.7 and 1.0, 3-H); δ_C(75.5 MHz; $CDCl_3$) 35.57 (C-4), 56.77 and 59.14 (C-1 and -5) and 131.27 and 137.80 (C-2 and -3).

(±)-2-*Amino*-6-*chloro*-9-(cis-4′-*hydroxycyclopent*-2′-*enyl*)-*purine* **13**.—Tetrakis(triphenylphosphine)palladium(0) (60.0 mg, 0.06 mmol) was added to a suspension of 2-amino-6-chloropurine (1.0 g, 5.84 mmol) in dry dimethyl sulfoxide (DMSO) (12 cm^3). After being stirred for 2 min at room temperature, in the dark, under an inert atmosphere, the reaction mixture was cooled to 0 °C. A solution of 6-oxabicyclo[3.1.0]hex-2-ene **12** (0.53 g, 6.42 mmol) in dry tetrahydrofuran (THF) (14 cm^3) was added dropwise, then the yellow suspension was stirred at 0 °C for 3 h, allowed to warm slowly to room temperature, and stirred overnight. The solvents were removed and the resultant oil was purified by flash chromatography eluting ethyl acetate–methanol (10:1 and then 5:1) as eluent. The *title compound* **13** was obtained as a crystalline solid (1.34 g, 91%), m.p. 156–158 °C (from MeOH); λ_{max}(pH 6 phosphate buffer)/nm 245.6 and 307.8; ν_{max}(KBr)/cm^{-1} 3600–2700s br (NH, OH) and 1610s and 1562s (C=C, C=N); δ_H(300 MHz; [$^2H_6$]DMSO) 1.69 (1 H, dt, *J* 14.0 and 4.4, 5′β-H), 2.86 (1 H, ddd, *J* 14.0, 8.3 and 7.3, 5′α-H), 4.70–4.78 (1 H, m, 4′-H), 5.24 (1 H, d, *J* 6.2, OH), 5.27–5.37 (1 H, m, 1′-H), 6.00 (1 H, ddd, *J* 5.5, 2.2 and 1.1, 2′-H), 6.19 (1 H, dt, *J* 5.5 and 2.0, 3′-H), 6.74 (2 H, s, NH_2) and 8.03 (1 H, s, 8-H); δ_C(75.5 MHz; [$^2H_6$]DMSO) 41.16 (C-5′), 56.86 (C-1′), 73.59 (C-4′), 123.52 (C-5), 130.45 (C-2′), 139.72 (C-3′), 141.23 (C-8), 149.38 (C-4), 153.53 (C-6) and 159.70 (C-2) [Found: (EI) M^+, 251.056 56. $C_{10}H_{10}ClN_5O$ requires M, 251.057 38].

(±)-9-(cis-4′-*Acetoxycyclopent*-2′-*enyl*)-2-*amino*-6-*chloro-purine* **14**.—Acetic anhydride (285.4 mg, 0.264 cm^3, 2.80 mmol) was added to a solution of the purine **13** (469.0 mg, 1.86 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in dry pyridine (30 cm^3). The reaction mixture was stirred for 4.5 h at room temperature and then the solution was concentrated under reduced pressure to give a solid, which was purified by flash chromatography and elution with ethyl acetate–petroleum spirit (8:2). The *title compound* **14** (521.1 mg, 95%) was obtained as a cream solid, m.p. 160–162 °C (from EtOH–EtOAc); λ_{max}(pH 6 phosphate buffer)/nm 194.8, 222.9, 246.4 and 308.0; ν_{max}(KBr)/cm^{-1} 3320 and 3206s (NH_2), 1739s (C=O) and 1625s and 1564s (C=C, C=N); δ_H(300 MHz; [$^2H_6$]DMSO) 1.91 (1 H, dt, *J* 14.4 and 4.2, 5′β-H), 2.02 (3 H, s, Me), 2.99 (1 H, dt, *J* 14.2 and 7.7, 5′α-H), 5.37–5.45 (1 H, m, 1′-H), 5.59–5.66 (1 H, m, 4′-H), 6.23–6.33 (2 H, m, 3′- and 2′-H), 6.92 (2 H, s, NH_2) and 7.95 (1 H, s, 8-H); δ_C(75.5 MHz; [$^2H_6$]DMSO) 20.80 (Me), 37.73 (C-5′), 56.73 (C-1′), 76.84 (C-4′), 123.51 (C-5), 134.31 (C-2′), 134.65 (C-3′), 140.70 (C-8), 149.44 (C-4), 153.65 (C-6), 159.76 (C-2) and 170.02 (C=O) [Found: (EI) M^+, 293.068 93. $C_{12}H_{12}ClN_5O_2$ requires M, 293.067 75].

(1′R,4′S)-9-(4′-*Acetoxycyclopent*-2′-*enyl*)-2-*amino*-6-*chloro-purine* (−)-**14** *and* (1′S,4′R)-2-*Amino*-6-*chloro*-9-(4′-*hydroxy-cyclopent*-2′-*enyl*)*purine* (−)-**13**.—PCL (400.0 mg) was added to a solution of the alcohol (±)-**13** (1.02 g, 4.05 mmol) in vinyl acetate (130 cm^3). The suspension was stirred at 50 °C for 24 h, then further PCL (400 mg) was added and the mixture was stirred for a further 24 h at 50 °C. The enzyme was filtered off and the filtrate was concentrated to give a solid (1.13 g). Conversion was judged to be ~55% by ^1H NMR analysis of the crude material. Purification by flash chromatography and elution with ethyl acetate–petroleum spirit (7:3, then 8:2), then with ethyl acetate, gave the acetate (−)-**14** (420.0 mg, 35%); $[\alpha]_D^{24}$ −60.0 (*c* 0.52, MeOH). The enantiomeric purity of the acetate (ee >90%) was checked by ^1H NMR spectroscopy (300 MHz; $CDCl_3$) in the presence of tris-[3-heptafluoropropyl-hydroxymethylene)-*d*-camphorato]europium(III) [Eu(hfc)$_3$].

A second fraction was obtained by elution with ethyl acetate–methanol (10:1) to give the alcohol (−)-**13** (339.4 mg, 33%), $[\alpha]_D^{26}$ −11.5 (*c* 0.54, MeOH). The alcohol was acetylated (Ac_2O–pyridine, catalytic DMAP) and analysed by the same procedure as above [ee 94%; $[\alpha]_D^{24}$ +64.0 (*c* 0.48, MeOH)].

(1′R,4′S)-2-*Amino*-9-(4′-*hydroxycyclopent*-2′-*enyl*)-6-*meth-oxypurine* **15**.—Potassium carbonate (382.9 mg, 2.77 mmol) was added to a solution of the acetate (−)-**14** (325.5 mg, 1.11 mmol) in dry methanol (10.5 cm^3) and the mixture was refluxed for *ca.* 2 h and then adsorbed onto silica. Flash chromatography with dichloromethane–methanol (15:1) as eluent gave the *title compound* **15** as a solid (258.4 mg, 94%), m.p. 152–153 °C; λ_{max}(pH 6 phosphate buffer)/nm 248.8 and 251.0; ν_{max}(KBr)/cm^{-1} 3600–2800s br (NH, OH) and 1612s and 1580s (C=C, C=N); δ_H(300 MHz; [$^2H_6$]DMSO) 1.64 (1 H, dt, *J* 14.0 and 4.4, 5′β-H), 2.85 (1 H, ddd, *J* 14.0, 8.1 and 7.1, 5′α-H), 3.96 (3 H, s, OMe), 4.68–4.75 (1 H, m, 4′-H), 5.28 (1 H, d, *J* 6.3, OH), 5.25–5.33 (1 H, m, 1′-H), 5.97 (1 H, ddd, *J* 5.5, 2.0 and 1.1, 2′-H), 6.17 (1 H, dt, *J* 5.5 and 1.8, 3′-H), 6.39 (2 H, s, NH_2) and 7.78 (1 H, s, 8-H); δ_C(75.5 MHz; [$^2H_6$]DMSO) 39.67 (C-5′), 51.49 (Me), 54.85 (C-1′), 71.98 (C-4′), 129.10, 136.11 and 137.63 (C-8, -2′, -3′) and 112.34, 151.95, 158.02 and 159.04 (C-2, -4, -5 and -6) [Found: (EI) M^+, 247.1069. $C_{11}H_{13}N_5O_2$ requires M, 247.1069].

(1′*S*,4′*R*)-2-Amino-9-(4′-hydroxycyclopent-2′-enyl)-6-meth-oxypurine *ent*-**15** was obtained from the alcohol (−)-**13** by treatment with potassium carbonate (1.5 mol equiv.) in methanol, in comparable yield.

Diethyl Hydroxymethylphosphonate.—A mixture of diethyl hydrogen phosphite (46.6 cm^3, ~49.96 g, 0.36 mol), paraformaldehyde (11.10 g, 0.36 mol) and triethylamine (5.0 cm^3, ~3.63 g, 0.04 mol) was heated slowly to 50 °C. After the resultant exotherm to ~125 °C, the mixture was heated at 90 °C for 50 min. Purification by distillation gave diethyl hydroxymethyl-phosphonate as a liquid (42.06 g, 68%), b.p. 101–102 °C/0.05 mmHg (lit.,[13] 110 °C/0.5 mmHg); ν_{max}(liquid film)/cm^{-1} 3600–

3100s br (OH), 1231s (P=O) and 1028s (P–O–C); δ_H(250 MHz; $CDCl_3$) 1.28 (6 H, t, *J* 7.0, 2 × $MeCH_2$), 3.84 (2 H, d, *J* 6.0, CH_2P), 4.05–4.20 (4 H, m, 2 × $MeCH_2$) and 4.63 (1 H, br, OH) [Found: (EI) M^+, 168.05510. $C_5H_{13}O_4P$ requires M, 168.055 14].

Diethyl p-*Tolylsulfonyloxymethylphosphonate.*[13]—Triethylamine (1.8 cm^3, ~1.32 g, 1.30 mmol) was added dropwise to stirred solution of diethyl hydroxymethylphosphonate (2.09 g, 12.40 mmol) in dry diethyl ether (15 cm^3). After the mixture had cooled to −10 °C, a solution of toluene-*p*-sulfonyl chloride (2.48 g, 13.00 mmol) in dry diethyl ether (15 cm^3) was added dropwise with the internal temperature maintained at −10 °C. After being stirred at 0 °C for 3 h, the mixture was allowed to warm to room temperature and was then stirred overnight. Diethyl ether (50 cm^3) was added and the solid was filtered off. The solvents were removed under reduced pressure and the oil was purified by flash chromatography with dichloromethane as eluent to afford the *title compound* as an oil (3.91 g, 97%), ν_{max}(liquid film)/cm^{-1} 1380s (SO_2O), 1262s (P=O), 1177s (SO_2O) and 1057s and 1021s (P–O–C, C–O); δ_H(300 MHz; $CDCl_3$) 1.21 (6 H, t, *J* 7.0, 2 × $MeCH_2$), 2.34 (3 H, s, *Me*Ar), 4.05 (6 H, m, 2 × $MeCH_2$, CH_2P), 7.26 (2 H, d, *J* 8.0, ArH) and 7.71 (2 H, d, *J* 8.0, ArH); δ_C(75.5 MHz; $CDCl_3$) 16.18 (Me), 16.25 (Me), 21.53 (MeAr), 60.16 (CH_2P), 63.23 (CH_2), 63.31 (CH_2), 128.08 (CH), 129.96 (CH), 131.68 (C) and 145.49 (C) [Found: M^+, 322.061 13. $C_{12}H_{19}O_6PS$ requires M, 322.064 00].

(1′R,4′S)-2-*Amino*-9-[4′-(*diethoxyphosphorylmethoxy*)*cyclopent*-2′-*enyl*]-6-*methoxypurine* **16**.—Sodium hydride (60% in oil; 130.2 mg, 3.25 mmol) was washed with petroleum spirit (3 × 1 cm^3) and a solution of the alcohol **15** (268.2 mg, 1.08 mmol) in dry DMSO (9.5 cm^3) was added dropwise. The mixture was stirred at room temperature under an inert atmosphere for 2 h. A solution of diethyl *p*-tolylsulfonyloxymethylphosphonate (524.4 mg, 1.63 mmol) in dry DMSO (4.5 cm^3) was added dropwise and the reaction mixture was stirred at room temperature overnight. After the mixture had cooled to 0 °C, glacial acetic acid (0.126 cm^3, 2.10 mmol) was added dropwise, and the resultant suspension was concentrated under reduced pressure to give an oil (1.18 g), which was purified by flash column chromatography and elution with dichloromethane–methanol (15:1). The *title compound* **16** was obtained as a yellow gum (201.0 mg, 47%, 57% based on consumed starting material); λ_{max}(pH 6 phosphate buffer)/nm 249.4 and 281.0; ν_{max}($CHCl_3$)/cm^{-1} 3534w and 3426m (NH), 1608s and 1587s (C=C, C=N) and 1246s br (P=O); δ_H(300 MHz; $CDCl_3$) 1.29–1.40 (6 H, m, 2 × $MeCH_2$), 2.00 (1 H, dt, *J* 14.5 and 3.5, 5′β-H), 2.89 (1 H, ddd, *J* 14.5, 8.0 and 7.0, 5′α-H), 3.81–4.01 (2 H, m, OCH_2P), 4.07 (3 H, s, MeO), 4.09–4.25 (4 H, m, 2 × CH_2Me), 4.67–4.77 (1 H, m, 4′-H), 4.93 (2 H, s, NH_2), 5.39–5.49 (1 H, m, 1′-H), 6.06 (1 H, dd, *J* 5.5 and 2.0, 2′-H), 6.31 (1 H, dt, *J* 5.5 and 2.0, 3′-H) and 7.67 (1 H, s, 8-H); δ_C(75.5 MHz; $CDCl_3$) 16.48 (d, *J* 6.1, $MeCH_2OP$), 37.90 (C-5′), 53.82 (OMe), 56.65 (C-1′), 62.50 (d, *J* 4.5, $MeCH_2OP$), 62.81 (d, *J* 167.6, PCH_2), 84.48 (d, *J* 11.8, C-4′), 133.96, 135.40 and 138.02 (C-2′, -3′ and -8) and 159.30 (C); δ_P(121.5 MHz; $CDCl_3$) 21.85 [Found: (EI) M^+, 397.1515. $C_{16}H_{24}N_5O_5P$ requires M, 397.1515].

(1′*S*,4′*R*)-2-Amino-9-[4′-(diethoxyphosphorylmethoxy)-cyclopent-2′-enyl]-6-methoxypurine was obtained from *ent*-**15** in comparable yield.

(1′R,4′S)-9-[4′-(*Phosphonomethoxy*)*cyclopent*-2′-*enyl*]*guanine* **17**.—Trimethylsilyl iodide (0.445 cm^3, ~0.620 g, 3.13 mmol) was added dropwise to a stirred solution of the phosphonate **16** (62.2 mg, 0.16 mmol) in dry DMF (1.5 cm^3) at 0 °C, in the dark, under an inert atmosphere. The solution was stirred at room temperature overnight. After being cooled to 0 °C, the mixture was treated dropwise with aq. ammonium hydrogen carbonate (0.2 mol dm^{-3}; 2 cm^3) was added dropwise and the mixture was stirred at room temperature for 2.5 h. Ethanol (20 cm^3) was added and the resultant solution was evaporated under reduced pressure. The residue (796.9 mg) was purified by gel permeation chromatography on Sephadex LH-20 and eluted with methanol–aq. formic acid (0.1 mol dm^{-3}) (1:1) to give the yellow *title compound* **17** as a yellow solid (lyophiliate), after lyophilization of appropriate fractions (36.3 mg, 71%). Attempts to separate the product from a phosphorus impurity gave the title compound (24.1 mg, 47%) still not completely pure; λ_{max}(pH 6 phosphate buffer)/nm 253.2 and 280.2; ν_{max}(KBr)/cm^{-1} 3600–2600s (NH, OH) and 1689s and 1607s (C=O, C=C, C=N); δ_H(300 MHz; D_2O) 2.30 (1 H, dt, *J* 15.0 and 4.0, 5′β-H), 3.11 (1 H, dt, *J* 15.0 and 7.5, 5′α-H), 3.83 (2 H, d, *J* 10.0, OCH_2P), 4.60–5.00 (1 H, m, 4′-H), 5.42–5.52 (1 H, m, 1′-H), 6.24–6.31 (1 H, m, 2′-H), 6.49–6.55 (1 H, m, 3′-H) and 8.00 (1 H, s, 8-H); δ_P(121.5 MHz; D_2O) 16.4 (t, *J* 10.0) [Found: (FAB) (M^+ + H), 328.0811. $C_{11}H_{14}N_5O_5P$ requires (M + H), 328.0811].

(1′*S*,4′*R*)-9-[4′-(Phosphonomethoxy)cyclopent-2′-enyl]-guanine *ent*-**17** was obtained from *ent*-**16** in comparable yield.

(1′R,4′S)-9-[4′-(*Diphosphorylphosphonomethoxy*)*cyclopent*-2′-*enyl*]*guanine Triammonium Salt* **10**.—Morpholine (35 mg, 0.40 mmol) was added to a solution of the phosphonic acid **17** (24.1 mg, 0.07 mmol) in *tert*-butyl alcohol–water (1:1) (1.5 cm^3), the mixture was stirred for 1 h at room temperature and was then refluxed. A solution of dicyclohexylcarbodiimide (DCC) (82.5 mg, 0.40 mmol) in *tert*-butyl alcohol (2.5 cm^3) was added dropwise over a period of 2 h. After an additional hour, the solution was cooled and the solvents were removed. The residue was transferred with water (5 cm^3), filtered, and the solid was washed with an additional portion of water (5 cm^3). The combined filtrate was extracted with diethyl ether (3 × 5 cm^3) and then azeotroped with pyridine (3 × 1 cm^3) and finally benzene (1 cm^3). The resultant solid was dissolved in dry DMSO (2.0 cm^3) and added very slowly to a solution of tributylammonium pyrophosphate (0.141 g, 0.39 mmol) in dry DMSO (0.5 cm^3) and the mixture was stirred at room temperature, under an inert atmosphere, for 110 h. The solution was purified by ion-exchange chromatography on Sephadex DEAE A-25 and elution with a linear gradient of aq. ammonium hydrogen carbonate (0–0.4 mol dm^{-3}). Appropriate fractions were evaporated and then lyophilized to give the title compound **10** as a powder (26.4 mg, 67%); λ_{max}(water)/nm 253.9; ν_{max}(KBr)/cm^{-1} 3600–2600s br (NH, OH), 1691s (C=O), 1614w (C=C, C=N), 1237s (P=O) and 905m (P–O–P); δ_H(250 MHz; D_2O) 2.05 (1 H, dt, *J* 14.0 and 5.0, 5′β-H), 3.15 (1 H, dt, *J* 14.0 and 8.0, 5′α-H), 4.02 (2 H, d, *J* 9.0, OCH_2P), 4.93–5.02 (1 H, m, 4′-H), 5.41–5.52 (1 H, m, 1′-H), 6.24 (1 H, d, *J* 5.0, 2′-H), 6.48–6.56 (1 H, m, 3′-H) and 7.97 (1 H, s, 8-H); δ_P(121.5 MHz; D_2O) −21.6 to −22.7 [1 P, m, P(1)], −9.5 to −10.5 [1 P, m, P(2)] and 9.75 [1 P, d, *J* 28.0, P(3)].

(1′*S*,4′*R*)-9-[4′-(Diphosphorylphosphonomethoxy)cyclopent-2′-enyl]guanine triammonium salt **11** was obtained from *ent*-**17** in comparable yield.

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