The Potential of Carbocyclic Nucleosides for the Treatment of AIDS: Synthesis of Carbocyclic 6'-Fluoro-2',3'-dideoxythymidine

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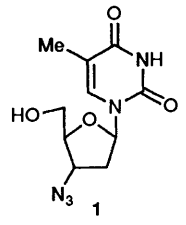
The carbocyclic nucleoside **4** and the corresponding triphosphate **5** were prepared from cyclopent-2-enylmethanol **6** in 8 and 10 steps respectively. The key step in the synthesis is the reaction of the azido alcohol **9** with diethylaminosulphur trifluoride: participation of the azido group led to the formation of the isomeric fluorides **10** and **11** (ratio *ca.* 2:1). The triphosphate **5** was found to be a poor inhibitor of HIV-coded reverse transcriptase.

Human immunodeficiency virus (HIV) has been identified as the cause of the acquired immunodeficiency syndrome (AIDS). HIV is a retrovirus in which genetic information is carried as RNA rather than DNA. A virtually coded enzyme, DNA polymerase, is responsible for converting the viral genetic information into DNA, making a single-strand DNA copy of the viral RNA. An associated enzyme, ribonuclease, destroys the original RNA and the polymerase makes an additional copy of the DNA using the first polynucleotide as a template. The polymerase and the ribonuclease are often referred to collectively as the HIV reverse transcriptase.

The reverse transcriptase has been considered a good target in the quest for a therapy for AIDS because of the unique association of this protein with the retrovirus. In particular attention has been focussed on the inhibitory properties of dideoxynucleosides such as 3'-azido-2',3'-dideoxythymidine (AZT) **1**, 2',3'-dideoxythymidine **2** and 2',3'-dideoxydidehydrothymidine **3**. All three compounds are potent inhibitors of HIV replication.[1]



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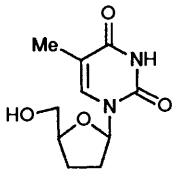

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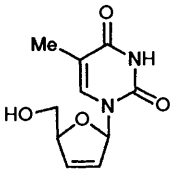

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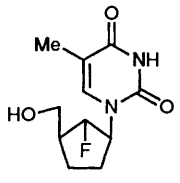
The active form of the nucleoside mimic is the corresponding triphosphate; this is formed in the cell *via* the mono- and diphosphates by the action of various cellular kinases. The nucleoside triphosphates act as competitive inhibitors of the natural substrate, thymidine triphosphate, at the active site of virally-coded DNA polymerase. In addition, incorporation of the nucleotide mimic into a growing strand of viral DNA results in chain termination because of the absence of the hydroxy group at the 3'-position required for chain elongation. There is some evidence that reverse transcriptase accepts nucleoside analogues more readily than do mammalian DNA polymerases. This preference explains why the replication of HIV in infected cells is affected to a greater degree than the replication of uninfected cells.[2]

AZT (also known as Zidovudine and Retrovir) has been approved for use in the clinic as an anti-AIDS agent; however this drug is not ideal. AZT has a very short life in the body and has to be ingested every 4 h. It can also be toxic to bone marrow causing anaemia and leukopenia often resulting in the need for the patient to undergo frequent blood transfusions. Therefore there is a need to develop a potent anti-HIV chemotherapeutic agent with a lower toxicity, greater stability and hence a longer half-life than AZT.

Greater stability of such bio-active nucleosides can be achieved by replacing the furanose unit with a cyclopentane ring. Such carbocyclic analogues of nucleosides are not subject to cleavage by nucleoside phosphorylases because they possess a cycloalkyl-heterocycle bond in place of the glycosidic bond of the true nucleoside.[3] However replacement of the heterocyclic oxygen atom by a methylene group causes a profound stereoelectronic change in the rest of the molecule. A smaller change would be expected on replacement of the oxygen atom by a fluoromethylene group; this is because of the closer electronic relationship between a fluoromethylene group and an oxygen atom.[4] Application of this idea to the preparation of nucleosides has been carried out previously and has resulted in the preparation of novel compounds active against Herpes simplex virus type-1 (HSV-1).[5]

The preparation of carbocyclic nucleosides and nucleotides that may be active against HIV is being investigated in a number of laboratories.[6] The ability of a nucleoside analogue to prevent DNA-chain elongation can be measured by enzyme inhibition tests involving reverse transcriptase. In these tests the ability of the triphosphate of the nucleoside to halt the elongation of a growing DNA chain is measured relative to the inhibition observed for the triphosphate of AZT.[7] This gives an indication of the potential of the carbocyclic nucleoside as an anti-HIV agent.

Carbocyclic 6'-α-fluoro-2',3'-dideoxythymidine **4** (an analogue of the active compound **2**) and the corresponding triphosphate **5** have been prepared from cyclpentadiene, as described below and the anti-viral activity of this carbocyclic 2',3'-dideoxyribonucleoside has been compared with that of AZT **1**.



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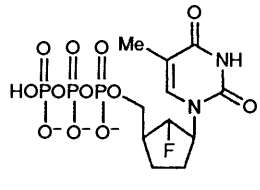

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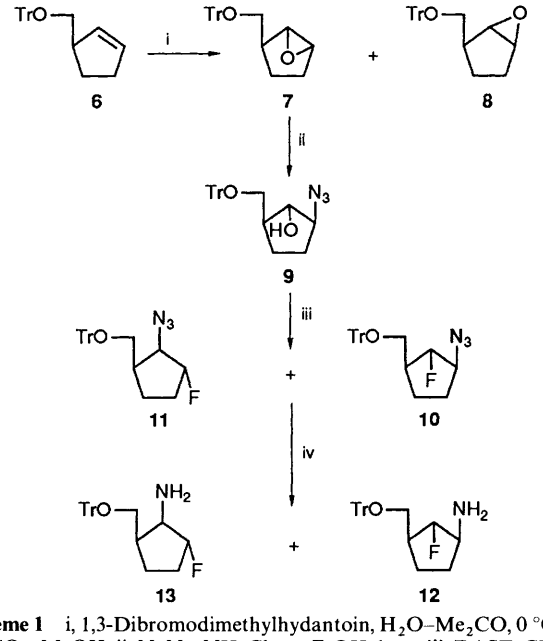
Discussion

3-Chlorocyclopentene was prepared by the addition of hydrogen chloride gas to cyclopentadiene,[8] and was then treated with magnesium at carefully controlled low temperature to form cyclopent-2-enylmagnesium chloride. This Grignard reagent was added to solid carbon dioxide to obtain cyclopent-

2-enecarboxylic acid.[9] The alternative method of preparing cyclopent-2-enecarboxylic acid from 3-chlorocyclopent-2-ene using Rieke-magnesium[10] was not used due to the large amount of potassium metal that would have been required. Reduction of the acid was accomplished using lithium aluminium hydride and resulted in the formation of cyclopent-2-enylmethanol which was converted into the corresponding triphenylmethyl (trityl) ether **6** using triphenylmethyl chloride and triethylamine in the presence of a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP).[11]

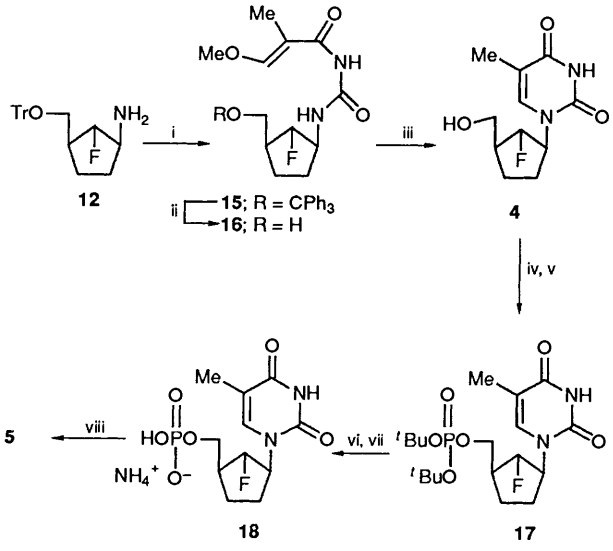
Functionalisation of the remaining double bond was achieved by the reaction of the alkene **6** with *N*,*N*-dibromodimethylhydantoin in aqueous acetone and the resulting bromohydrin(s) was (were) converted into the epoxide **7** on treatment with base (81% yield from the alkene **6**). Note that electrophilic attack by the bromonium ion occurs from, what seems at first sight, the more hindered *exo*-face of the molecule: in a similar fashion reaction of the alkene **6** with *meta*-chloroperoxybenzoic acid (MCPBA) afforded mainly the unwanted isomeric epoxide **8**. We believe that the trityloxymethyl substituent occupies a pseudo-equatorial position on the β-face of the five-membered ring and offers protection of the α-face of the molecule by a suitable orientation of one of the aromatic rings.

Ring opening of the epoxide **7** with sodium azide and ammonium chloride in ethanol–water gave the desired azido alcohol **9**. In this reaction the regioisomer, (1α,2β,3β)-2-azido-3-triphenylmethyloxymethylcyclopentanol, is also produced in a small amount. The alcohol was converted into the alkyl fluorides **10**, **11** using diethylaminosulphur trifluoride (DAST)[12] (Scheme 1). The replacement of the hydroxy group by the fluorine atom occurs with retention of configuration at the reacting carbon centre or with concomitant migration of the neighbouring azido group. Such a neighbouring group effect has been noted previously in reactions involving DAST.[5,13] The mixture of fluoro azides **10** and **11** proved difficult to separate by flash chromatography, although repeated chromatography did allow isolation of the minor product. This could be further facilitated by partial removal of some of the undesired isomer **11** by crystallisation from ethanol and chromatographing the evaporated residue. Azide **10** could be readily reduced in quantitative yield with hydrogen using Lindlar's catalyst[14] giving the amine **12**. An alternative procedure was to reduce the mixture of fluoro azides **10** and **11** by catalytic hydrogenation and then separate the amines **12** and **13**.



Scheme 1 i, 1,3-Dibromodimethylhydantoin, H_2O–Me_2CO, 0 °C then K_2CO_3, MeOH; ii, NaN_3, NH_4Cl, aq. EtOH, heat; iii, DAST, CH_2Cl_2, 0 °C; iv, H_2, Lindlar catalyst, ethyl acetate, room temp.

The thymine moiety was constructed according to the procedure of Shaw and Warrener[15] (Scheme 2). β-Methoxy-α-methylacryloyl isocyanate **14**, prepared from silver cyanate and β-methoxy-α-methylacryloyl chloride, was condensed with the amine **12** to give the compound **15**. Deprotection of **15** with toluene-*p*-sulphonic acid (*p*-TSA) resulted in the formation of the required alcohol **16**. Cyclisation of **16** was achieved using aqueous hydrochloric acid.[16]



Scheme 2 *Reagents:* i, CH(OMe)C(Me)CONCO **14**, DMF, −10 °C; ii, *p*-TSA, CH_2Cl_2, MeOH, room temp.; iii, 2 mol dm^{-3} HCl, 110 °C; iv, Di-*tert*-butyl *N*,*N*-diethyl phosphoramidite, 1*H*-tetrazole, THF, room temp.; v, MCPBA, CH_2Cl_2, −40 °C; vi, CF_3CO_2H, CH_2Cl_2, room temp.; vii, NH_3, EtOH, 4 °C; viii, 1,1′-carbonyldiimidazole, DMF, room temp., then $P_2O_7H_4$ Bu_3N, DMF, room temp.

The final three steps of the synthesis involved converting the fluorocarbocyclic nucleoside **4** into the corresponding triphosphate. Thus di-*tert*-butyl phosphate **17** was prepared by reaction of the nucleoside with di-*tert*-butyl *N*,*N*-diethylphosphoramidite and 1*H*-tetrazole followed by oxidation of the resultant phosphite ester with MCPBA.[17] Hydrolysis of the phosphate ester **17** using trifluoroacetic acid and subsequent reaction with ammonia resulted in the formation of the monoammonium salt **18**.

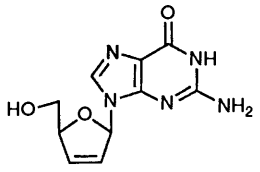
The triphosphate **5** was formed by treating the salt **18** with 1,1′-carbonyldiimidazole, followed by monotributylammonium pyrophosphate,[18] a method that works under mild conditions and is easily carried out on a small scale. In some attempts the triphosphate obtained contained an unknown phosphorus impurity, as indicated by ^{31}P NMR spectroscopy. The formation of this impurity was circumvented by using freshly prepared monotributylammonium pyrophosphate and by using a shallow gradient in the ion exchange chromatography.

Biological Activity

The triphosphate **5** was a poor inhibitor of HIV-coded reverse transcriptase (IC_{50} = 200 μg cm^{-3}) compared to AZT-triphosphate (IC_{50} = 0.05 μg cm^{-3}). In line with this result, the nucleoside **4** shows no activity against HIV-infected cells *in vitro*. When these data are considered with other results reported earlier[19] it is clear that carbocyclic analogues that are very closely related to bio-active sugars show disappointing anti-retroviral activity. It is possible that, as in the area of herpes

chemotherapy, the carbocyclic nucleosides have their own structure–activity pattern which does not match the structure–activity relationships that are seen in the sugar series.

As a broad generalisation, nucleosides showing anti-herpes activity have a pyrimidine base attached to the sugar unit; carbocyclic nucleosides often display enhanced activity for the purine based derivatives. The same may be true of reverse transcriptase inhibitors and certainly carbovir **19** acts as a good inhibitor *in vitro* (IC_{50} of the triphosphate = 0.05 μg cm^{-3} against HIV-RT) while the corresponding dideoxydidehydrosugar is much less active.



19

Our work aimed at exploring this postulate will be disclosed in due course.

Experimental

The IR spectra were recorded on a Perkin-Elmer 881 spectrometer; the absorption peaks are quoted in reciprocal centimetres. The ^1H NMR spectra were recorded on either a Hitachi Perkin-Elmer or Bruker AM 250 spectrometer at 60 or 250 MHz respectively using deuteriochloroform as the solvent unless otherwise stated. The ^{31}P NMR spectrum was taken at 100 MHz. All chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are quoted in Hz. The m.p.s were determined on a capillary apparatus, and the b.p.s are uncorrected and are reported in °C. Light petroleum refers to the fraction of b.p. 60–80 °C. Benzene, ether and tetrahydrofuran (THF) were distilled from $LiAlH_4$ or sodium wire and benzophenone immediately before use. Dichloromethane and dimethylformamide (DMF) were distilled from CaH_2 and stored over 4 Å molecular sieves. Pyridine was distilled from KOH.

3-*Chlorocyclopent*-2-*ene*.—Hydrogen chloride gas was bubbled rapidly through freshly distilled cyclopentadiene (155 g, 2.15 mol) at *ca.* −35 °C with vigorous stirring. Addition was continued until weight measurements indicated an uptake of about 90% of the theoretical quantity of HCl. Distillation afforded 3-chlorocyclopent-2-ene (152.48 g, 1.48 mol, 69%) as a colourless liquid, b.p. 22–24 °C/18 mmHg (lit.,[8] 18–25 °C/5 mmHg). The unstable compound was stored at temperatures below −10 °C until required; distillation residues and unused material were destroyed with aqueous $NaHCO_3$; δ_H(60 MHz) 6.2–5.8 (2 H, m, CH=CH), 5.05 (1 H, m, $CHCl_3$) and 2.7–2.0 (4 H, m, 2 × CH_2).

Cyclopent-2-*enecarboxylic Acid*.—A solution of 3-chlorocyclopent-2-ene (152.48 g, 1.48 mol) in dry THF (*ca.* 350 cm^3) was added dropwise to magnesium turnings (36.52 g, 1.5 mol) in dry THF (250 cm^3) with stirring under an atmosphere of nitrogen. During the addition the temperature was maintained in the range −12 to −7 °C by external cooling (acetone–CO_2). After being stirred for 1 h the solution was added to freshly crushed dry ice (*ca.* 500 g) and allowed to warm to room temp. overnight. The resultant mixture was poured onto crushed ice (100 cm^3), and concentrated hydrochloric acid was added to dissolve the excess of magnesium. The solution was extracted with ether (3 × 400 cm^3) and the combined ether layers were extracted with aqueous sodium hydroxide (2 mol dm^{-3}; 3 × 300 cm^3). The aqueous solution was extracted with ether (400 cm^3) and acidified to pH 1 with conc. hydrochloric acid with external cooling. The product was extracted into ether (3 × 500 cm^3) and the combined ether layers were washed with water (600 cm^3) and brine (600 cm^3), and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure at <4 °C resulted in a dark brown liquid. Distillation yielded cyclopent-2-enecarboxylic acid (42.5 g, 0.38 mol, 26%) as a pale yellow liquid, b.p. 76–78 °C/1 mmHg (lit.,[9] 103–104 °C/11 mmHg); δ_H(60 MHz) 12.0 (1 H, s, CO_2H), 6.1–5.6 (2 H, m, CH=CH), 3.6 (1 H, m, $CHCO_2$) and 2.8–1.8 (4 H, m, 2 × CH_2).

Cyclopent-2-*enylmethanol*.—A solution of cyclopent-2-enecarboxylic acid (10.25 g, 0.09 mol) in dry ether (25 cm^3) was added dropwise to a solution of lithium aluminium hydride (3.47 g, 0.09 mol) in dry ether (75 cm^3) at 0 °C with stirring. After 30 min, water (3.5 cm^3) was added slowly, followed by aqueous sodium hydroxide (4 mol dm^{-3}; 3.5 cm^3) and additional water (10.5 cm^3). The reaction mixture was filtered and the filtrate dried ($MgSO_4$). Evaporation of the solvent under reduced pressure (<4 °C) afforded a pale yellow liquid, which was dissolved in dichloromethane (40 cm^3) and dried ($MgSO_4$). Cyclopent-2-enylmethanol (6.04 g, 0.06 mol; 68%) was obtained as a colourless liquid by evaporation of the solvent and Kugelrohr distillation (98–100 °C/4 mmHg) (lit.,[9] 58–59 °C/9 mmHg); $\nu_{max}(CHCl_3)/cm^{-1}$ 3626 and 3450; δ_H(60 MHz) 6.0–5.5 (2 H, m, HC=CH), 3.5 (2 H, d, J 6, CH_2O) and 3.3–1.2 [6 H, m, $(CH_2)_2CH$, OH].

Triphenylmethoxymethylcyclopent-2-*ene* **6**.—To a stirred solution of cyclopent-2-enylmethanol (2.70 g, 0.028 mol) in dry dichloromethane (30 cm^3) containing a catalytic amount of DMAP at 0 °C under an inert atmosphere was added triethylamine (11.4 cm^3, *ca.* 6.46 g, 0.081 mol) and triphenylmethyl chloride (11.46 g, 0.041 mol). The reaction mixture was allowed to warm to room temperature overnight and then partitioned between ethyl acetate (120 cm^3) and water (120 cm^3). The organic phase was washed with brine (100 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed over silica using light petroleum–ethyl acetate (19:1) as eluent. *Triphenylmethoxymethylcyclopent*-2-*ene* **6** was obtained as white crystals (9.3 g, 0.027 mol, 98%), m.p. 70–72 °C, when the solution was concentrated and set aside (Found: C, 88.45; H, 7.25. $C_{25}H_{24}O$ requires C, 88.19; H, 7.11%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3064 and 1598; δ_H(250 MHz) 7.63–7.15, 7.47–7.28 (6 H and 9 H, respectively, 2 × m, ArH), 5.82 (2 H, s, 2-H, 3-H), 3.05 (3 H, m, CH_2O, 1-H), 2.34 (2 H, m, 4-H and 5-H), 2.05 (1 H, m, 4-H or 5-H) and 1.55 (1 H, m, 4-H or 5-H).

2-exo-*Triphenylmethoxymethyl*-6-*oxabicyclo*[3.1.0]*hexane* **7**.—To a stirred solution of **6** (3.00 g, 8.82 mmol) in acetone–water (55 cm^3, 10:1) at 0 °C was added dibromodimethylhydantoin (1.26 g, 4.42 mmol) portionwise over *ca.* 6 h. The solvent was evaporated under reduced pressure and the resultant slurry was partitioned between ethyl acetate (50 cm^3) and water (50 cm^3). The organic layer was washed with brine (2 × 50 cm^3), dried ($MgSO_4$) and evaporated to give a pale yellow gum. To a solution of this gum in methanol (120 cm^3) was added potassium carbonate (1.5 g) and the reaction mixture was stirred overnight at room temp. The residue obtained on evaporation of the solvent was dissolved in ethyl acetate (50 cm^3) and the solution washed with water (3 × 25 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. Recrystallisation of the residue from hexane yielded the title compound **7** (2.74 g, 7.68 mmol, 87%) as a white solid, m.p. 116–118 °C (Found: C, 84.1; H, 6.95. $C_{25}H_{24}O_2$ requires C, 84.24; H, 6.79%); $\nu_{max}(CHCl_3)/cm^{-1}$ 835; δ_H(250 MHz) 7.46 and 7.26

(6 H and 9 H respectively, 2 × m, Ar-H), 3.49 (2 H, m, 1-H, 5-H), 3.11 (1 H, dd, *J* 9, 6, CH_2O), 3.0 (1 H, dd, *J* 9, 7, CH_2O), 2.54 (1 H, m, 2-H), 1.94 (1 H, m, 3-H or 4-H) and 1.68–1.30 (3 H, m, 3-H or 4-H).

(1α,2β,5β)-2-*Azido*-5-*triphenylmethyloxymethylcyclopentanol* **9**.—To a stirred solution of **7** (3.0 g, 8.4 mmol) in ethanol–water (100 cm^3, 4:1) was added sodium azide (2.19 g, 0.03 mol) and ammonium chloride (2.19 g). The mixture was refluxed at 100 °C for 72 h and evaporated. The residue was partitioned between ethyl acetate (100 cm^3) and water (75 cm^3). The organic phase was washed with brine (2 × 75 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The yellow oil obtained was chromatographed over silica using light petroleum–ethyl acetate (7:1) as eluent. The *title compound* **9** (2.82 g, 7.03 mmol, 84%) was obtained as a colourless oil; $\nu_{max}(CHCl_3)/cm^{-1}$ 3600–3200 and 2112; δ_H(250 MHz) 7.43 and 7.29 (6 H and 9 H respectively, 2 × m, ArH), 3.75 (2 H, m, 1-H and 2-H), 3.38 and 3.09 (2 × 1 H, m, CH_2O), 2.74 (1 H, m, OH), 2.08 (2 H, m, 3-H, 5-H), 1.78 (1 H, m, 4-H), 1.56 (1 H, m, 3-H) and 1.33 (1 H, m, 4-H).

Fluorination of the Azido Alcohol **9** *with Diethylaminosulphur Trifluoride* (*DAST*).—A solution of **9** (0.260 g, 0.65 mol) in dry dichloromethane (2.5 cm^3) was added dropwise to a solution of DAST (0.172 cm^3, *ca.* 0.2098 g, 1.3 mmol) in dry dichloromethane (3 cm^3) at 0 °C with stirring. The mixture was stirred for 10 min after which excess of DAST was quenched with ice; the mixture was then partitioned between ethyl acetate (10 cm^3) and sodium hydrogencarbonate (10 cm^3). The organic phase was washed with brine (10 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. Repeated chromatography of the residue over silica using light petroleum–ethyl acetate (10:1) as eluent afforded (1β,2α,3β)-1-azido-2-fluoro-3-triphenylmethyloxymethylcyclopentane **10** (0.053 g, 0.13 mmol, 21%); $\nu_{max}(CHCl_3)/cm^{-1}$ 2106; δ_H(250 MHz) 7.47 and 7.27 (6 H and 9 H respectively, 2 × m, ArH), 4.75 (1 H, ddd, *J* 53, 5, 5, 2-H), 4.01 (1 H, m, 1-H), 3.16 (2 H, m, CH_2O), 2.39 (1 H, m, 3-H), 2.04 (1 H, 5-H), 1.93 (1 H, m, 5-H), 1.69 (1 H, m, 5-H) and 1.57 (1 H, m, 4-H); and (1α,2β,3β)-2-*azido*-1-*fluoro*-3-*triphenylmethyloxymethylcyclopentane* (0.144 g, 0.36 mmol, 55%) as a white solid, m.p. 125–126 °C (Found: C, 74.7; H, 6.2; N, 10.45. $C_{25}H_{24}FN_3O$ requires C, 74.79; H, 6.03; N, 10.47%); $\nu_{max}(CHCl_3/cm^{-1}$ 2110; δ_H(250 MHz) 7.47 and 7.30 (6 H and 9 H respectively, 2 × m, Ar-H), 5.02 (1 H, m, 1-H), 4.26 (1 H, dd, *J* 11, 5, 2-H), 3.30 (1 H, dd, *J* 9, 6, CH_2O), 3.16 (1 H, dd, *J* 9, 9, CH_2O), 2.62 (1 H, m, 3-H), 2.14 (1 H, m, 5-H), 1.96 (1 H, m, 5-H), 1.85 (1 H, m, 4-H) and 1.39 (1 H, m, 4-H).

(1β,2α,3β)-2-*Fluoro*-3-*triphenylmethoxymethylcyclopentylamine* **12**.—A solution of **10** (0.210 g, 0.57 mmol) in ethanol (5 cm^3) was hydrogenated over Lindlar catalyst (*ca.* 0.015 g) at atmospheric pressure for 3 h. The catalyst was removed by filtration using Celite and the filtrate evaporated under reduced pressure. The title compound **12** (0.21 g, 0.56 mmol, 99%) was obtained as a colourless gum which was used without further purification; $\nu_{max}(CHCl_3)/cm^{-1}$ 3624; δ_H(250 MHz) 7.47 and 7.27 (6 H and 9 H respectively, 2 × m, Ar-H), 4.86 (2 H, br s, NH_2), 4.71 (1 H, ddd, *J* 54, 7, 7, 2-H), 3.53 (1 H, m, 1-H), 3.17 (2 H, d, *J* 6, CH_2O), 2.32 (1 H, m, 3-H), 2.06 (1 H, m, 5-H or 4-H), 1.87 (1 H, m, 5-H or 4-H) and 1.60 (2 H, m, 5-H or 4-H).

N-[(1′β,4′β,6′α)-6′-*Fluoro*-4′-*triphenylmethoxymethylcyclopentylaminocarbonyl*]-3-*methoxy*-2-*methylprop*-2-*enamide* **15**.—To a suspension of silver cyanate (1 g, dried P_2O_5, 4 h, 120 °C, 0.2 mmHg) in dry benzene (8 cm^3) was added β-methoxy-α-methylacryloyl chloride[15] (0.34 cm^3) with stirring. The mixture was refluxed for 30 min with careful exclusion of moisture. The resultant cooled supernatant contained β-methoxy-α-methylacryloyl isocyanate **14** at a concentration of 0.44 mol cm^{-3}.

To a solution of **12** (0.1731 g, 0.46 mol) in dry DMF (1 cm^3) was added the above solution of isocyanate **14** (2.1 cm^3, 0.92 mmol) with stirring at −10 °C. The resultant mixture was warmed slowly to room temp. and stirred overnight. The solvent was evaporated (Kugelrohr 60 °C, 0.2 mmHg) and the residue co-distilled with ethanol (2 × 5 cm^3). Chromatography over silica using ethyl acetate–light petroleum (5:1) as eluent yielded the *title compound* **15** (0.1951 g, 0.37 mmol, 82%) as a white solid, m.p. 68–70 °C; $\nu_{max}(CHCl_3)/cm^{-1}$ 3274, 1688, 1617 and 1543; δ_H(250 MHz) 8.87 [1 H, d, *J* 8, N(1′)-H], 8.63 [1 H, s, N(3′)-H], 7.37 (15 H, m, Ar-H), 4.77 (1 H, ddd, *J* 53, 5, 5, 6′-H), 4.29 (1 H, m, 1′-H), 3.87 (3 H, m, OCH_3), 3.15 (2 H, m, CH_2O), 2.39 (1 H, m, 4′-H) and 2.24–1.44 (7 H, m, C=CCH_3, 2 × 2′-H and 2 × 3′-H); δ_F(235 MHz) −19.11 (ddd, *J* 53, 25, 16).

Note: the numbering in this and subsequent compounds is consistent with the nomenclature advocated in ref. 19.

N-[(1β,4′β,6′α)-6′-*Fluoro*-4′-(*hydroxymethyl*)*cyclopentylaminocarbonyl*]-3-*methoxy*-2-*methylprop*-2-*enamide* **16**.—To a solution of **15** (0.1951 g, 0.37 mmol) in dichloromethane–methanol (10 cm^3, 1:1) was added toluene-*p*-sulphonic acid (0.025 g). After the mixture had stirred at room temp. for 3 h triethylamine (0.2 cm^3) was added. The solution was evaporated under reduced pressure and the residue purified by chromatography over silica using light petroleum–ethyl acetate (1:1 followed by 1:2) as eluent. The title compound **16** (0.0911 g, 0.33 mol, 89%) was obtained as a white solid, m.p. 117–119 °C; $\nu_{max}(CHCl_3)/cm^{-1}$ 3274, 1688, 1616 and 1543; δ_H(250 MHz) 8.86 [1 H, d, *J* 9, N(1)-H], 7.90 [1 H, s, N(3)-H], 7.33 (1 H, m, HC=C), 4.81 (1 H, ddd, *J* 53, 5, 5, 6′-H), 4.34 (1 H, m, 1′-H), 3.86 (3 H, m, OCH_3), 3.71 (2 H, m, CH_2O) and 2.46–1.53 (9 H, m, 5-CH_3, 2 × 2′-H, 2 × 3′-H, 4′-H, OH).

(1′β,4′β,6′α)-1-[6′-*Fluoro*-4′-(*hydroxymethyl*)*cyclopentyl*]-5-*methylpyrimidine*-2,4(1H,3H)-*dione* **4**.—Aqueous hydrochloric acid (2 mol dm^{-3}; 5 cm^3) was added to **16** (0.1149 g, 0.42 mmol) and the resultant mixture was refluxed with stirring at 110 °C for 30 min. The solution was evaporated under reduced pressure and the resultant gum was chromatographed over silica using dichloromethane–methanol (9:1) as eluent. The *title compound* **4** (0.0896 g, 0.37 mmol, 88%) was obtained as a white solid, m.p. 188–190 °C; λ_{max}(MeOH)/270; ν_{max}(KBr)/cm^{-1} 3394 and 1702; δ_H(250 MHz, [$^2H_6$]-DMSO) 11.25 (1 H, br s, NH), 7.89 (1 H, s, 6-H), 5.24–4.03 (3 H, m, 1′-H, 6′-H and OH), 3.51 (2 H, m, CH_2O), 2.33–2.06 (1 H, m, 4′-H) and 2.04–1.46 (7 H, m, 5-CH_3, 2 × 2′-H and 2 × 3′-H) [Found: M^+ + H, 243.11042. $C_{11}H_{16}FN_2O_3$ requires (*M* + H), 243.10947].

Formation of the Phosphate Ester **17**.—1*H*-Tetrazole (0.0273 g, 0.39 mmol) was added in one portion to a stirred solution of **4** (0.0307 g, 0.13 mmol) and di-*tert*-butyl-*N*,*N*-diethylphosphoramidite (0.018 g, 0.195 mmol),[15] in dry THF (0.3 cm^3). Stirring was continued at room temp. for 15 min. The colourless solution was cooled to −40 °C and rapidly treated with a solution of 85% MCPBA (0.0448 g, 0.26 mmol) in dry dichloromethane (0.2 cm^3). The solution was warmed to room temp., treated with 10% aqueous sodium sulphite (2 cm^3) and stirred for 10 min. The aqueous phase was extracted with dichloromethane (3 × 5 cm^3) and the combined organic layers were washed with 50% aqueous sodium hydrogencarbonate (10 cm^3). The aqueous layer was back extracted with dichloromethane (4 cm^3) and the combined organic layers were dried ($MgSO_4$) and evaporated

under reduced pressure. Chromatography of the residue over silica using dichloromethane–ethyl acetate (2:3) followed by neat ethyl acetate as eluent afforded the *tert*-butyl phosphate **17** (0.0344 g, 0.08 mmol, 62%) as a white solid, m.p. 118–120 °C (decomp.); λ_{max}(MeOH)/nm 270; ν_{max}($CHCl_3$)/cm^{-1} 3398, 1692, 1256 and 997; δ_H(250 MHz) 8.08 (1 H, s, NH), 7.06 (1 H, d, *J* 1, 6-H), 5.11 (1 H, ddd, *J* 55, 7, 7, 6′-H), 4.81 (1 H, m, 1′-H), 4.08 (2 H, m, CH_2O), 2.45 (1 H, m, 4′-H), 2.28–1.54 (7 H, m, 5-CH_3, 2 × 2′-H and 2 × 3′-H) and 1.51 [18 H, s, 2 × $(CH_3)_3C$] [Found: $M^+ + NH_4$, 452.2326. $C_{19}H_{32}FN_2O_6P$ requires ($M + NH_4$) 452.2326].

Hydrolysis of the Phosphate Ester **17**.—A solution of the ester **17** (0.0344 g, 0.08 mmol) in dichloromethane (0.2 cm^3) and trifluoroacetic acid (0.05 cm^3) was stored at room temp. for 1 h. The yellow solution was evaporated under reduced pressure and the residue was co-distilled with ethanol (2 × 1 cm^3) and then dissolved in concentrated ammonia (3 drops) and diluted with ethanol (0.8 cm^3). After 2 h at 5 °C the supernatant was removed and the white solid washed with ethanol and dried. The ammonium salt of the monophosphate **18** (0.0258 g, 0.076 mmol, 96%) was obtained as a white solid, m.p. 115–118 °C; λ_{max}(MeOH)/nm 270; ν_{max}(KBr)/cm^{-1} 3600–2500, 1686 and 1013; δ_H(250 MHz; CD_3OD) 7.50 (1 H, s, 6-H), 5.30–4.87 (2 H, m, 1′-H, 6′-H), 4.07 (2 H, m, CH_2O), 2.45 (1 H, m, 4′-H) and 2.24–1.68 (7 H, m, 5-CH_3, 2 × 2′-H and 2 × 3′-H) [Found: $M^+ - NH_3 + H$, 323.0808. $C_{11}H_{19}FN_3O_6P$ requires ($M - NH_3 - H$) 323.0808].

Preparation of the Triphosphate **5**.—A solution of tetrasodium pyrophosphate decahydrate (0.892 g, 2.0 mmol) in warm water (7 cm^3) was passed through a column of Dowex 50W-X4 (pyridinium form) resin (30 cm^3) using water as the eluent. To the eluate (60 cm^3) was added tributylamine (0.48 cm^3, 2.0 mmol) and the solution was evaporated under reduced pressure. The syrupy residue was dried by repeated addition and evaporation of dry pyridine (5 cm^3) and DMF (3 × 5 cm^3) and finally dissolved in DMF (20 cm^3).

To a solution of the anhydrous monoammonium salt **18** (0.0153 g, 0.045 mmol) [prepared by addition and evaporation of dry pyridine (1 cm^3) and DMF (1 cm^3)] in DMF (0.5 cm^3) was added at room temperature a solution of 1,1′-carbonyldiimidazole (0.0366 g, 0.23 mmol) in DMF (0.5 cm^3) under an inert atmosphere. The solution was stirred at room temp. for 6.5 h and then treated with methanol (15 mm^3, 0.36 mmol). After 30 min at room temp. the above solution of tributylammonium pyrophosphate in DMF (2.3 cm^3, 0.23 mmol) was added and the white suspension was stirred for 24 h. The precipitate, imidazolium pyrophosphate, was filtered off and washed with DMF (3 × 1 cm^3). The filtrate was diluted with methanol (7 cm^3) and evaporated under reduced pressure (Kugelrohr, 45 °C/0.1 mmHg). The residue was dissolved in water (*ca.* 1 cm^3) and applied to a DEAE-A25 Sephadex column (15 × 400 mm, *ca.* 9 g gel) and eluted with a linear gradient of aqueous $Et_3NH_2CO_3$ (0–04 mol dm^{-3} in 400 cm^3, then 0.4 mol dm^{-3}, pH 7.5, flow rate 90 cm^3 h^{-1}). The column was connected to a UV detector (λ 254 nm). Appropriate fractions (9 cm^3) were evaporated under reduced pressure and the residue co-distilled with methanol (3 × 5 cm^3). The tris(triethylammonium) salt of the triphosphate **5** was obtained as a colourless gum (0.0338 g 0.043 mmol, 96%); λ_{max}(MeOH)/nm 271; ν_{max}(CH_3CN)/cm^{-1} 3632, 1691 and 1235; δ_H(250 MHz; CD_3OD), 7.64 (1 H, s, 6-H), 5.17 (1 H, m, 6′-H), 4.16 (2 H, m, CH_2O), 3.12 [18 H, m, 3 × $N(C_2H_5)_3$], 2.45 (1 H, m, 4′-H), 2.21–1.76 (7 H, m, 5-CH_3, 2 × 2′-H and 2 × 3′-H) and 1.30 [27 H, m, 3 × $N(C_2H_5)_3$]; δ_P(101 MHz; CD_3OD) −8.7, −9.3 [2 P, each d, *J* 24, P(1) and P(3)] and −21.8 [1 P, t, *J* 24, P(2)] (Found: M^+, 786. $C_{29}H_{63}FN_5O_{12}P_3$ requires *M*, 786).

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