Synthesis of Two 6'α-Fluorocarbocyclic Nucleosides and Incorporation into Short Segments of DNA

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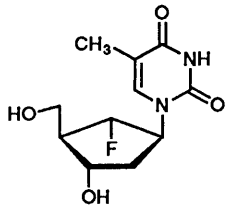
The 6'-fluorocarbocyclic nucleosides **1** and **2** have been synthesized from the bicyclic ketone **3** in 16 and 2% yield respectively. The nucleoside **1** has been incorporated into short chains of DNA using an automated synthesizer and these oligonucleotides have been shown to hybridize with (fluorine containing) complementary strands.

We have been interested for some time in the synthesis of mimics of (deoxy)ribose derivatives[1] particularly with regard to the anti-Herpes[2] and anti-HIV activity[3] of the surrogate compounds. The work has been concentrated on the synthesis of carbocyclic nucleosides with the oxygen atom of the (deoxy)ribose sugar unit replaced by a fluoromethylene unit.[4] Thus the fluorocarbocyclic nucleosides **1**, **2** are typical of the



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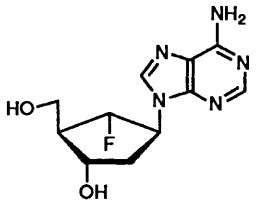

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compounds that have attracted our interest. In this paper we describe a very efficient synthesis of optically active **1**, a less efficient though quite satisfactory preparation of the nucleoside analogue **2**, and incorporation of the thymidine analogue **1** into short strands of DNA.

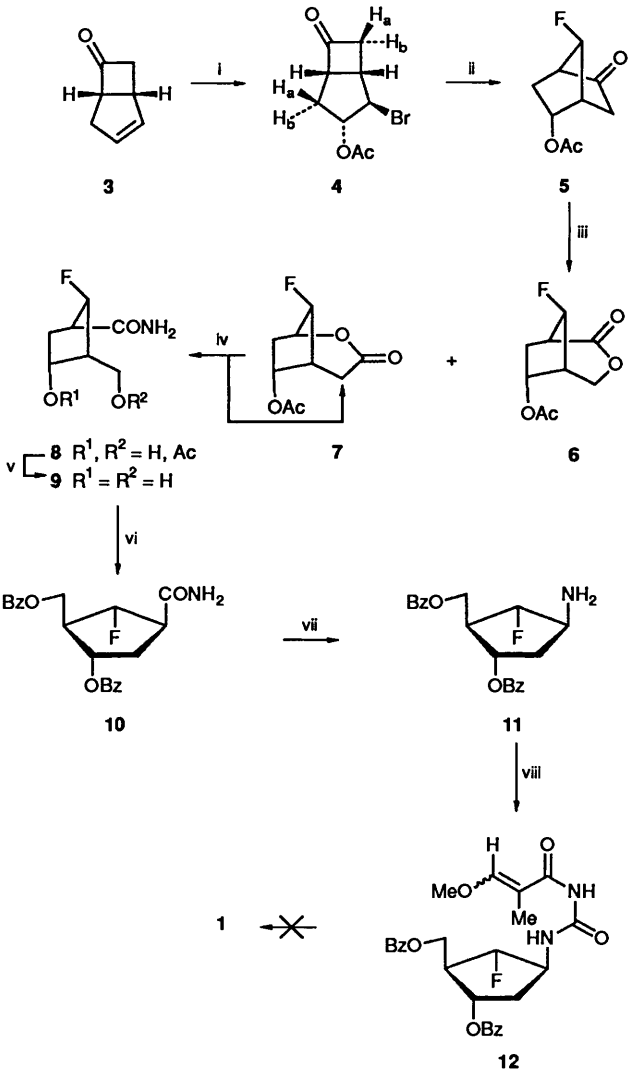
Results and Discussion

The compound **1** was prepared by adaptation of previously reported methodology.[5] Thus the optically active ketone **3** was converted into the bromoacetate **4** (90%).[6] Treatment of the ester **4** with potassium *tert*-butoxide followed by triethylamine tris-hydrofluoride gave the fluoroester **5** (83%).[7] Baeyer–Villiger oxidation of the ketone **5** using *meta*-chloroperoxybenzoic acid (*m*CPBA) and sodium hydrogen carbonate in dichloromethane gave the isomeric lactones **6** and **7** in the ratio 2.85:1 and in 94% yield.[8]

The lactones **6** and **7** were not separated: the mixture was treated with liquid ammonia at −70 °C for 1 h. As expected the predominant lactone was converted into the amides **8** while the lactone **7** was unaffected. The yield of the esters **8** from the ketone **5** was a highly satisfactory 69%. Hydrolysis of the mixture of acetates **8** afforded the diol **9** which, in turn, furnished the dibenzoate **10** (50%) under Mitsunobu conditions.

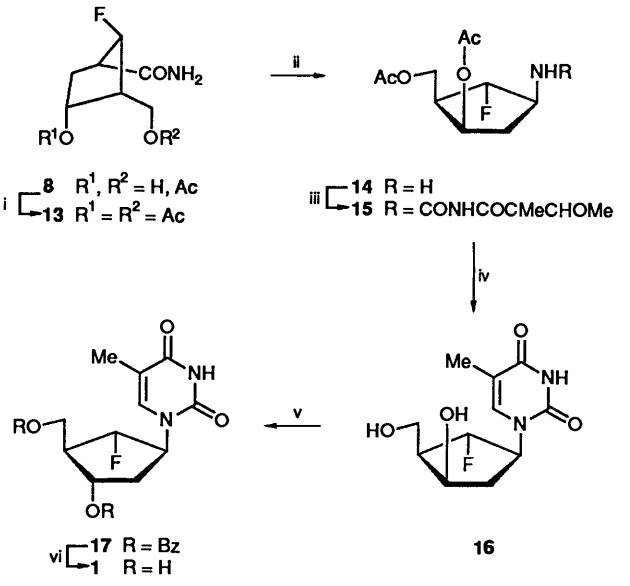
The amide **10** was subjected to a modified Hofmann reaction using Loudon's[9] and Koser's[10] reagents. The former reagent gave the amine **11** in 86% yield after chromatography, while the Koser reagent gave the same compound in a slightly reduced yield (81%). We favoured the use of Koser's reagent since it is commercially available and the reaction entailed fewer components, leading to more facile purification of the product.

Conversion of the amine **11** into the acryloyl urea **12** was accomplished in moderate yield (61%) but attempts to convert



Scheme 1 *Reagents and conditions:* i, NBA, AcOH, room temp., 16 h, 90%; ii, Bu^{t}OK, Et_2O, −78 °C–room temp., 4 h then $Et_3N{\cdot}3HF$, CH_2Cl_2, room temp., 72 h, 83%; iii, *m*CPBA, $NaHCO_3$, CH_2Cl_2, room temp., 9 h, 94%; iv, $NH_3(l)$, −70 °C, 1 h, **8** 74%, **7** 26%; v, NH_3, MeOH, room temp., 18 h, 79%; vi, PPh_3, $PhCO_2H$, $EtO_2CN{=}NCO_2Et$, THF, room temp., 12 h, 49%; vii, PhI(OH)OTos, MeCN, reflux, 2 h, 81%; viii, MeOCHCMeCONCO, PhH, DMF, −20 °C–room temp., 16 h, 61%

the diester **12** into the targest molecule [using hot aqueous ammonia, aqueous sodium hydroxide under reflux and aqueous hydrochloric acid (4 mol dm^{-3}) under reflux] proved fruitless. Thus attempted cyclization to form the heterocyclic unit under the normal conditions[11] led to recovery of starting material.



Scheme 2 *Reagents and conditions:* i, $(MeCO)_2O$, DMAP, pyridine, room temp., 5 min, 99%; ii, $PhI(OCOCF_3)_2$, MeCN, H_2O, pyridine, 4 h, room temp.; iii, MeOCHCMeCONCO, PhH, DMF, −20 °C–room temp., 16 h, 66% from **13**; iv, HCl, H_2O, dioxane, 100 °C, 2 h, 82%; v, PPh_3, $PhCO_2H$, $EtO_2CN{=}NCO_2Et$, THF, room temp., 18 h, 70%; vi, K_2CO_3, MeOH, room temp., 2 h, 83%

We reasoned that the required ring closure was prohibited by the bulky benzoyloxy group at C-5′. Attempts to remove the ester protecting groups using methanolic potassium carbonate led to complete decomposition of the molecule.

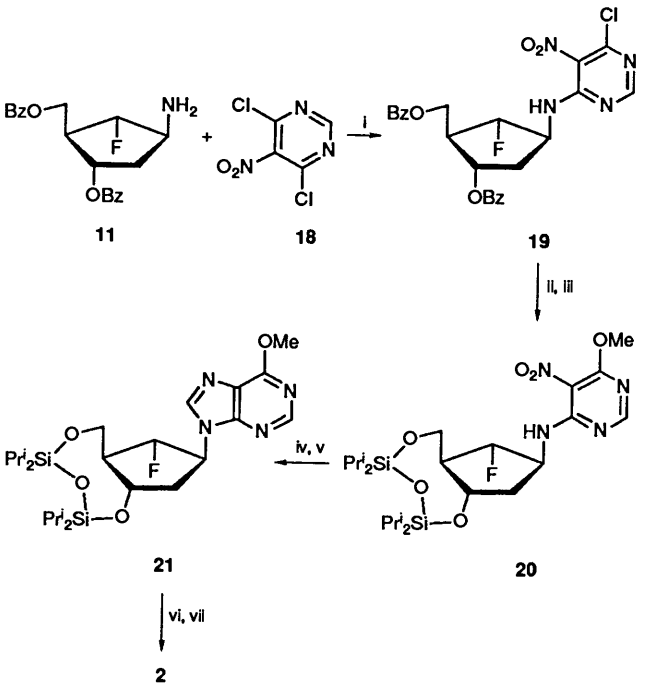
In order to circumvent this problem the mixture of amido esters **8** was converted into the diacetate **13** (Scheme 2). The latter compound was transformed into the amine **14** using the Hofmann–Loudon strategy; in this case, the Koser protocol led to extensive decomposition, probably due to the more acidic conditions that are employed.

Formation of the urea **15** was uneventful (66% from **8**) and, thankfully, formation of the heterocyclic ring (with concomitant loss of the two acetate groups) proceeded smoothly to afford the diol **16** in 82% yield. Mitsunobu inversion and hydrolysis furnished the required carbocyclic nucleoside **1** (58% for two steps).

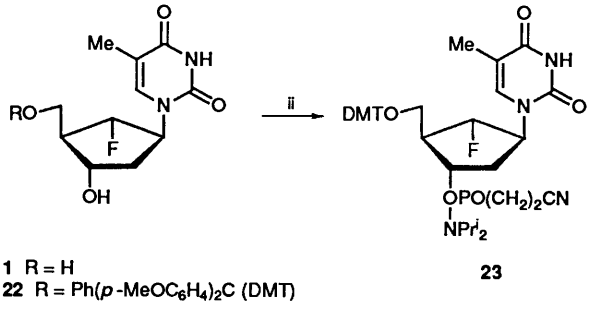
The second target molecule **2** was prepared from the dibenzoate **11**. Thus the diester was treated with 4,6-dichloro-5-nitropyrimidine **18** to give the desired compound **19** (Scheme 3). Since reduction of the nitro group in compound **19** proved problematic the benzoyl groups were removed using methoxide ion and replaced by the tetraisopropyldisilyloxa (TIPS) protecting group to afford compound **20** (53% overall yield). Reduction of the nitro group was then accomplished by hydrogenation over Raney nickel (66%) and cyclization was effected by heating the reduction product with diethoxymethyl acetate to afford the purine **21** (91%). Substitution of the methoxy unit by an amino group followed by fluoride-mediated desilylation furnished the deoxyadenosine analogue **2** (54% for the last two steps).

In summary the nucleoside analogue **1** was obtained from the ketone **3** in 11 steps (16% overall yield) while the adenine derivative was obtained from **3** in a longer (15 step) less efficient (2% yield) process.

The diol **1** was transformed into the dimethoxytrityl derivative **22** and then the phosphoramidite **23** using standard conditions (Scheme 4).[12] The latter compound was used to construct several strands of modified DNA **24**–**32** containing one or more units of the fluorocarbocyclic nucleoside. The sequences **24**–**32** were prepared on a 0.2 μmol scale using a



Scheme 3 *Reagents and conditions:* i, $EtPr^i_2N$, CH_2Cl_2, room temp., 36 h, 75%; ii, K_2CO_3, MeOH, room temp., 3 d, 79%; iii, $TIPSCl_2$, imidazole, DMF, room temp., 1 h, 66%; iv, Raney Ni, H_2, EtOH, room temp., 0.5 h, 66%; v, $(EtO)_2CHOAc$, 140 °C, 6 h, 91%; vi, $NH_3(l)$, 60 °C, 30 atm, 5 d, 58%; vii, Bu_4NF, THF, room temp., 12 h, 93%



Scheme 4 *Reagents and conditions:* i, 4,4′-Dimethoxytrityl chloride, pyridine, molecular sieves, room temp., 12 h, 76%; ii, $Pr^i_2NP(Cl)O(CH_2)_2CN$, Pr^i_2EtN, CH_2Cl_2, room temp., 45 min, 79%

Biosearch Cyclone DNA synthesiser using the standard manual protocols. The coupling efficiencies were determined by UV analysis of the released dimethoxytrityl moiety that was collected during the deprotection steps. The average efficiency of the F^T coupling step was 85% as opposed to 98–99% for the other coupling steps. The lower coupling efficiency for the F^T moiety was expected in view of similar observations made by Imbach *et al.* during the preparation of other carbocyclic oligothymidylates.[13] The fluorine-containing oligonucleotides were removed from the synthesis column anchoring beads using

24 5′TTTTF^{T}TTTT3′	
25 GCTTF^{T}AAAG	
26 GCTF^{T}TAAAG	Nucleoside
27 GCF^{T}F^{T}F^{T}AAAG	T = thymidine
28 GCAAGCF^{T}TCG	G = guanidine
29 GCAAGCTF^{T}CG	A = adenosine
30 CGAAGCTF^{T}GC	C = cytosine
31 CGAAGCF^{T}TGC	F^T = compound **1**
32 TGCAF^{T}CTGA	
33 CGAAGCTTGC	

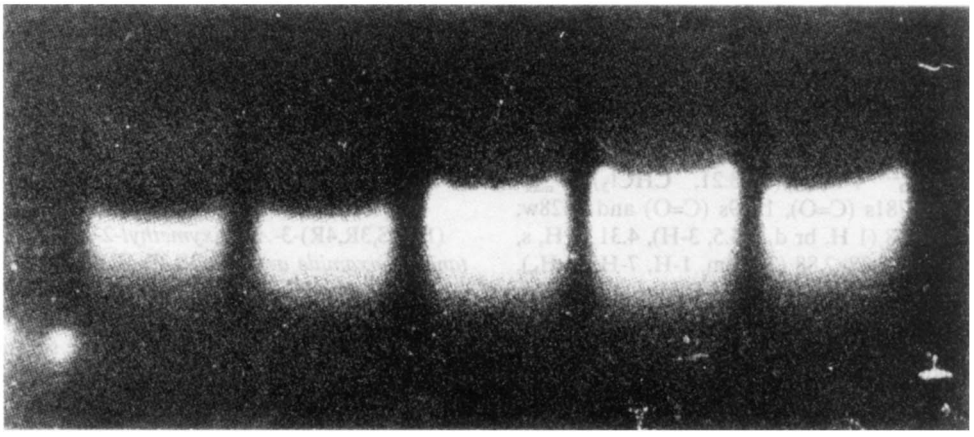
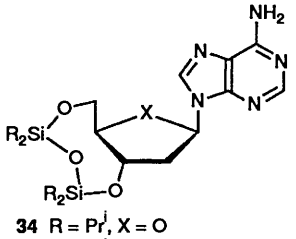


Fig. 1 Results of hybridization of fluorocarbocyclic nucleosides with complementary strands, polyacrylamide gel electrophoresis, treatment with ethidium bromide and visualization with UV light. The lanes contain (left to right): 1, compounds **33** and GCAAGCTTCG; 2, compounds **33** and **28**; 3, compounds **28** and **30**; 4, compounds **31** and **28**; 5, compounds **29** and **30**.

concentrated aqueous ammonium hydroxide (30% v/v). This reagent also removed the β-cyanoethyl protecting groups and, on heating at 55 °C for 5 h, led to removal of acyl protecting groups from the purine residues. The ammonium hydroxide was evaporated under reduced pressure and the samples lyophilized.

Purification of the oligonucleotides was achieved by polyacrylamide gel electrophoresis under urea-denaturing conditions.[14] Isolation of the appropriate band and desalting by Sephadex ion-exchange chromatography gave the pure oligonucleotides.

The sequence **33** was also prepared and the hybridization of some of the complementary strands was investigated in a preliminary fashion. Thus the complementary strands 28:33, 28:30, 28:31, 29:30 were mixed and the products analysed using a 20% polyacrylamide gel with 5% cross linking. Evidence for hybridization of the complentary strands was accrued by visualization of the plate with ethidium bromide, an intercalating agent that only highlights double stranded oligonucleotides when irradiated with UV light (Fig. 1). Clearly



34 R = Pr^i, X = O
35 R = Pr^i, X = CHF (F-alpha)

hybridization of a natural DNA oligomer and a fluorinated nucleotide mimic had occurred, as had hybridization of two complementary fluorine-containing strands.

Incorporation of a fluorine atom into nucleosides, nucleotides and oligonucleotides allows an opportunity to detect changes in the magnetic environment at the very heart of the pseudo-sugar unit. The signal due to the ^{19}F atom in the nucleoside analogue **17** dissolved in C_6D_6 appears as a doublet split to a triplet at −17.6 ppm. On addition of 1 mol equiv. of the compound **34** the signal shifts to −17.7 ppm, possibly due to a change in the orientation of the thymine unit on base-pairing. A similar shift in the position of the ^{19}F resonance of compound **35** is seen on adding an equimolar quantity of compound **17**.

Finally the synthetic oligonucleotide **32** in deuterium oxide displayed a signal due to ^{19}F at −17 ppm. The signal was distinct but broad, presumably due to slow tumbling of the molecule.

We are now starting to investigate, *inter alia*, the susceptibility of the oligonucleotide strands such as **24**–**32** to cleavage by restriction enzymes.

Experimental

Ether refers to diethyl ether and light petroleum was the fraction with b.p. 40–60 °C. Light petroleum, ethyl acetate and dichloromethane were distilled prior to use. Anhydrous solvents were stored under a dry nitrogen atmosphere and prepared as follows: THF and ether were freshly distilled from sodium benzophenone ketyl; dichloromethane was freshly distilled from calcium hydride; benzene was distilled from lithium aluminium hydride and stored over activated 4 Å molecular sieves; triethylamine, pyridine and diisopropylethylamine were distilled from calcium hydride and stored over potassium hydroxide pellets; dimethylformamide was distilled from barium oxide and stored over activated 4 Å molecular sieves; methanol was distilled from magnesium methoxide and stored over activated 4 Å molecular sieves.

Thin layer chromatography (TLC) was performed on precoated glass backed plates (Merck 60F-254, 0.25 mm Art 5715). Flash chromatography was performed on Camlab silica gel 60 (230–400 mesh). Solvent compositions are volume percentages. ^1H (250 MHz), ^{13}C (62.9 MHz), ^{19}F (235 MHz) and ^{31}P (101 MHz) NMR spectra were recorded on a Bruker AM 250 spectrometer using Me_4Si (^1H and ^{13}C), C_6F_6 (^{19}F), and H_3PO_4 (^{31}P) as external references. Coupling constants (J) are recorded in Hz.

IR spectra were recorded on a Perkin-Elmer 881 grating spectrophotometer. UV spectra were recorded on a Phillips PU:8720 scanning spectrophotometer and optical rotations were measured using an AA-1000 automatic polarimeter. High resolution mass spectra were run by members of the SERC mass spectrometry service at Swansea using a VG ZAB-F spectrometer or a VG12-253 spectrometer.

Elemental analyses were performed by Butterworths Laboratories, Middlesex. M.p.s are uncorrected.

(+)-(1S,2R,3R,5S)-3-*Acetoxy*-2-*bromobicyclo*[3.2.0]*heptan*-6-*one* **4**.—Freshly distilled (+)-bicyclo[3.2.0]hept-2-en-6-one **3** (1.32 g, 12.22 mmol) was dissolved in glacial acetic acid (20 cm^3) at room temp. *N*-Bromoacetamide (2.10 g, 15.28 mmol) was added and the mixture stirred for 16 h. The mixture was diluted with ether (50 cm^3) and extracted with aqueous saturated

sodium hydrogen carbonate (500 cm^3). The aqueous phase was re-extracted with ether (3 × 30 cm^3). The organic phases were combined, dried ($MgSO_4$) and solvent evaporated under reduced pressure. Flash chromatography (20% EtOAc–light petroleum) gave the bromoacetate **4** as a white solid (2.71 g, 10.99 mmol; 90%), R_f 0.52 (33% EtOAc–light petroleum); m.p. 37–38 °C (ether); $[\alpha]_D^{22.5}$ +14.6 (*c* 3.21, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3011w, 1781s (C=O), 1739s (C=O) and 1428w; δ_H($CDCl_3$; 250 MHz) 5.38 (1 H, br d, *J* 4.5, 3-H), 4.31 (1 H, s, 2-H), 3.80 (1 H, m, 5-H), 3.38–2.88 (3 H, m, 1-H, 7-H_a, 7-H_b), 2.53 (1 H, ddd, *J* 15, 10 and 5, 4-H_a), 2.26 (1 H, dm, *J* 15, 4-H_b) and 1.94 (3 H, s, CH_3); δ_C($CDCl_3$; 62.9 MHz) 209.56 (C), 169.02 (C), 82.94 (CH), 63.65 (CH), 54.26 (CH), 39.28 (CH), 34.18 (CH_2) and 20.89 (CH_3) [Found: M + NH_4^+ (CI, NH_3), 264.0235. $C_9H_{11}BrO_3$ requires *M* + NH_4^+, 264.0235].

(−)-(1R,4R,5R,7S)-5-endo-*Acetoxy*-5-anti-*fluorobicyclo*[2.-2.1]*heptan*-2-*one* **5**.—Potassium *tert*-butoxide (7.57 g, 67.46 mmol) was stirred in dry freshly distilled (from $LiAlH_4$) ether (1.8 dm^3) at 20 °C for 20 min and then cooled to −70 °C. A solution of bromoacetate **4** (13.77 g, 55.73 mmol) in dry ether (20 cm^3) was added over 1 h. The suspension was maintained at −70 °C for 0.5 h and then warmed to room temp. over 4 h.

The suspension was filtered through a Celite pad and the excess of ether evaporated under reduced pressure to afford 3-acetoxytricyclo[3.2.0.$0^{2,7}$]heptan-6-one. The crystals were dissolved in dry dichloromethane (40 cm^3) and triethylamine trihydrofluoride (35 cm^3) was added. The solution was stirred at room temp. for 72 h and then diluted with water (10 cm^3) and dichloromethane (100 cm^3). The mixture was extracted with water (2 × 50 cm^3) and the aqueous phase re-extracted with dichloromethane (3 × 25 cm^3). The combined dichloromethane phases were dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was subjected to flash chromatography (20% EtOAc–light petroleum) to give starting material **4** (2.76 g, 11.17 mmol, 20%) and the title compound **5** as a white solid (6.88 g, 36.96 mmol, 66%); R_f 0.23 (20% EtOAc–light petroleum); m.p. 49.5–50 °C (dichloromethane) (Found: C, 57.9; H, 6.0. $C_9H_{11}FO_3$ requires C, 58.06; H, 5.95%); $[\alpha]_D^{22.5}$ −8.6 (*c* 4.36, $CHCl_3$); ν_{max}(film)/cm^{-1} 3503w, 2988w, 1760s (ketone C=O) and 1735s (ester C=O); δ_H($CDCl_3$; 250 MHz) 5.38–5.28 (1 H, dddm, *J* 9, 4, 3.5, 5-H), 5.00 (1 H, ddd, *J* 55.5, 2 and 2, 7-H), 3.00 (1 H, dm, *J* 2, 4-H), 2.71 (1 H, m, 1-H), 2.61 (1 H, ddd, *J* 14, 6 and 5, 6-H_{exo}), 2.50 (1 H, d, *J* 6.2, 3-H_{endo}), 2.00 (1 H, m, 3-H_{exo}), 1.97 (3 H, s, CH_3) and 1.47 (1 H, dm, *J* 14, 6-H_{endo}); δ_F($CDCl_3$; 235 MHz) −38.8 (ddm, *J* 55.5, 4.8 and 1.5) [Found: M^+ (EI), 186.0692. $C_9H_{11}FO_3$ requires *M*, 186.0692].

(1R,5R,6R,8S)-6-endo-*Acetoxy*-8-anti-*fluoro*-3-*oxabicyclo*-[3.2.1]*octan*-2-*one* **6** *and* (1S,5R,6R,8S)-6-endo-*Acetoxy*-8-anti-*fluoro*-2-*oxabicyclo*[3.2.1]*octan*-3-*one* **7**.—*m*CPBA (17.45 g, 86 mmol; approx. 85% pure) was added portionwise to a cooled (0 °C), stirred suspension of the ketone **5** (10.0 g, 53.7 mmol), sodium hydrogen carbonate (16.11 g, 191.8 mmol) and dry dichloromethane (215 cm^3). After the mixture had been stirred at room temp. for 9 h dilute (10% w/v) aqueous sodium sulfite (140 cm^3) was added dropwise. The aqueous phase was washed with dichloromethane (2 × 30 cm^3) and the combined organic phases were extracted with saturated aqueous sodium hydrogen carbonate (3 × 100 cm^3). Re-extraction of the combined aqueous phases with dichloromethane (2 × 30 cm^3) and drying ($MgSO_4$) and evaporation under reduced pressure of the extract gave a pale yellow solid. Flash chromatography (33% EtOAc–light petroleum) of this gave the lactones **6** and **7** as an inseparable mixture [ratio **6**:**7** 2.85:1 (^1H NMR)] (10.18 g, 50.38 mmol, 94%); R_f 0.15 (25% EtOAc–light petroleum); ν_{max}($CHCl_3$)/ cm^{-1} 2974w, 1741s (C=O), 1369m and 1208s (C–F); δ_H($CDCl_3$; 250 MHz) 5.34 [1 H, m, 6-H (**6** and **7**)], 5.18 [1 H, dm, *J* 52, 8-H (**6** and **7**)], 4.67 [0.266 H, m, 1-H (**7**)], 4.37 [0.75 H, ddd, *J* 11.5, 7.5 and 1, 4-H (**6**)], 4.15 [0.77 H, dd, *J* 11.5 and 3, 4-H (**6**)], 3.17–2.62 [3.4 H, m, 2 × 4-H (**7**), 2 × 7-H (**7**), 2 × 7-H (**6**) and 1-H (**6**)], 2.08–1.80 [4.05 H, m, 2 × CH_3 (**6** and **7**) and 5-H (**7**)] and 1.89 [0.78 H, dd, *J* 15 and 3, 5-H (**6**)] [Found: M + NH_4^+ (CI, NH_3), 220.0985. $C_9H_{11}FO_4$ requires *M* + NH_4^+, 220.0985].

(1R,2S,3R,4R)-3-*Acetoxymethyl*-2-*fluoro*-4-*hydroxycyclopentanecarboxamide and* (1R,2S,3R,4R)-4-*Acetoxy*-2-*fluoro*-3-*hydroxymethylcyclopentanecarboxamide* **8**.—The lactones **6** and **7** (10.14 g, 50.16 mmol) were dissolved in an excess of liquid ammonia (100 cm^3) at −70 °C and the solution stirred at this temperature for 1 h. Removal of the ammonia under reduced pressure at −60 °C, addition of dry dichloromethane (100 cm^3) and evaporation of the solvent under reduced pressure at −60 °C gave an oil. Purification by flash chromatography (EtOAc) gave the title carboxamides **8** (8.2 g, 37.4 mmol, 74%) and unchanged lactone **7** (2.63 g, 13.04 mmol; 26%).

The amides **8** gave physical data as follows: R_f 0.26 (EtOAc); ν_{max}($CHCl_3$)/cm^{-1} 3522m (OH), 3409m (NH), 2952 (NH) 1731s (ester C=O) and 1682s (amide C=O); δ_H(CD_3OD; 250 MHz) 5.31–4.89 (1.9 H, m, 4-H and 2-H), 4.42–4.21 (0.36 H, m, OCH_2), 3.84–3.66 (1.75 H, m, OCH_2), 3.08–2.84 (0.97 H, m, 3-H), 2.60–2.32 (1.97 H, m, 5-H and 1-H) and 2.10–1.79 (4.24 H, m, CH_3 and 5-H) (Found: M + H^+, 220.0985. $C_9H_{13}FNO_4$ requires *M* + H^+, 220.0985).

The bicyclooctanone **7** had the following properties: R_f 0.15 (25% EtOAc–light petroleum); m.p. 112–114 °C (dichloromethane) (Found: C, 53.35; H, 5.6. $C_9H_{11}FO_4$ requires C, 53.47; H, 5.48%); $[\alpha]_D^{26.5}$ +28.51 (*c* 1.05, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1742 (C=O); δ_H($CDCl_3$, 250 MHz) 5.34 (1 H, ddd, *J* 10, 5.5 and 4, 6-H), 5.17 (1 H, dd, *J* 50 and 1, 8-H), 4.63 (1 H, m, 1-H), 2.97–2.59 (4 H, m, 5-H, 2 × 7-H and 4-H) and 2.08–1.95 [4 H, m (including δ 2.05, 3 H, s), 4-H and CH_3]; δ_C($CDCl_3$; 62.9 MHz) 170.20 (C), 167.36 (d, *J* 2, C), 93.68 (d, *J* 188, CH), 73.05 (CH), 39.65 (d, *J* 18, CH), 36.70 (CH_2), 30.90 (d, *J* 10, CH_2) and 20.60 (CH_3) [Found: M + NH_4^+ (CI, NH_3) 220.0985. $C_9H_{11}FO_4$ requires *M* + NH_4^+, 220.0985].

(−)-(1R,2S,3R,4R)-2-*Fluoro*-4-*hydroxy*-3-*hydroxymethylcyclopentanecarboxamide* **9**.—The acetates **8** (237 mg, 1.081 mmol) were dissolved in methanol saturated with ammonia (20 cm^3) and stirred at room temp. for 18 h. Evaporation of the solvent under reduced pressure and purification by flash chromatography (5% MeOH–EtOAc) gave the diol **9** (152 mg, 0.858 mmol, 79%) as a colourless gum; R_f 0.18 (5% MeOH–EtOAc); $[\alpha]_D^{25}$ −20.1 (*c* 2.42, CH_3CN); ν_{max}(CH_3CN)/cm^{-1} 3486m (OH/NH), 3370m (OH/NH) and 1674s (C=O); δ_H(CD_3OD; 250 MHz) 5.08 (1 H, ddd, *J* 54.5, 7.5 and 6, 2-H), 4.35 (1 H, m, 4-H), 3.93–3.70 (2 H, m, OCH_2), 2.95 (1 H, dddd, *J* 23, 10, 7.5 and 6, 3-H), 2.41 (1 H, ddd, *J* 16, 10 and 6, 5α-H), 2.33 (1 H, dtt, *J* 22, 7.5 and 6, 1-H) and 1.83 (1 H, ddd, *J* 14, 7 and 4, 5β-H); δ_C(CD_3OD; 62.9 MHz) 174.64 (C), 99.27 (d, *J* 183, C-2), 72.14 (d, *J* 8, CH), 60.11 (CH_2), 54.04 (d, *J* 17, CH), 50.63 (d, *J* 2, CH) and 37.32 (d, *J* 4, 5-CH_2) [Found: M + H^+ (CI, NH_3), 178.0879. $C_7H_{12}FNO_3$ requires *M* + H^+, 178.0879].

(+)-(1R,2S,3R,4S)-4-*Benzoyloxy*-3-*benzoyloxymethyl*-2-*fluorocyclopentanecarboxamide* **10**.—The diol **9** (65 mg, 0.367 mmol), triphenylphosphine (198 mg, 1.101 mmol) and benzoic acid (92 mg, 1.101 mmol) were dissolved in dry THF (1.5 cm^3). To this was added dropwise a solution of diethyl azodicarboxylate (119 mm^3, 1.101 mmol) in dry THF (160 mm^3) over 5 min. The solution was stirred in the dark at room temp. for 12 h. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography (20% EtOAc–light

petroleum) to give the title compound **10** as a white solid (70 mg, 0.181 mmol, 49%); R_f 0.75 (EtOAc); m.p. 106 °C (dichloromethane–light petroleum) (Found: C, 65.45; H, 5.2. $C_{21}H_{20}FNO_5$ requires C, 65.26; H, 5.05%); $[\alpha]_D^{24.4}$ +41.2 (*c* 1.32, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3526 and 3410 (NH) and 1710s (C=O); $\delta_H(CDCl_3$; 250 MHz) 8.10–7.26 (10 H, m, 2 × C_6H_5), 6.26 (1 H, br s, NH), 6.05 (1 H, br s, NH), 5.32 (1 H, ddd, *J* 6.5, 3.5 and 3.5, 4-H), 5.16 (1 H, ddd, *J* 54, 6.5 and 6.5, 2-H), 4.55 (2 H, m, OCH_2), 3.29 (1 H, m, 3-H), 2.85 (1 H, dddd, *J* 22.5, 10.5, 6 and 6, 1-H), 2.46 (1 H, ddd, *J* 14.5, 10.5 and 6.5, 5β-H) and 2.35–2.22 (1 H, m, 5α-H); $\delta_C(CDCl_3$; 62.9 MHz) 173.79 (C), 166.37 (C), 165.89 (C), 133.28 (CH), 133.18 (CH), 129.76 (CH), 129.67 (CH), 129.63 (CH), 128.44 (CH), 96.88 (d, *J* 187, C-2), 75.07 (d, *J* 5, C-4), 63.10 (CH_2), 51.35 (d, *J* 21, CH), 49.28 (d, *J* 20, CH) and 33.06 (d, *J* 10, C-5); *m/z* (EI) 385.5 (M^+) and 280.5 ($M - C_6H_5CO^+$).

(+)-(1S,2S,3R,4S)-4-*Benzoyloxy*-3-*benzoyloxymethyl*-2-*fluorocyclopentylamine* **11**.—The amide **10** (598 mg, 1.552 mmol) was suspended in acetonitrile (6.75 cm^3) and to this solution was added hydroxy(tosyloxy)iodobenzene (1.063 g, 2.710 mmol). The mixture was brought rapidly to reflux and maintained at that temperature for 2 h. The solvent was evaporated under reduced pressure and the residue dissolved in dichloromethane (100 cm^3). The solution was extracted with dilute aqueous sodium hydroxide (2 mol dm^{-3}; 30 cm^3) and this aqueous phase then re-extracted with dichloromethane (4 × 20 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. Flash chromatography (EtOAc) of the residue gave the title compound **11** as a pale yellow gum (450 mg, 1.259 mmol, 81%); R_f 0.46 (5% MeOH–EtOAc); $[\alpha]_D^{28}$ +41.0 (*c* 1.40, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2959w (NH) and 1719s (C=O); $\delta_H(CDCl_3$; 250 MHz) 8.07–7.26 (10 H, m, 2 × Ph), 5.35 (1 H, ddd, *J* 7, 5.6 and 5.6, 4-H), 4.61 (1 H, ddd, *J* 53.8, 6.4 and 6.4, 2-H), 4.59 (2 H, d, *J* 6, OCH_2), 3.80 (1 H, m, 1-H), 2.50–2.28 (3 H, m, 3-H, NH_2), 2.27 (1 H, ddd, *J* 14.5, 7.8 and 3, 5α-H) and 2.15–1.98 (1 H, m, 5β-H); $\delta_C(CDCl_3$; 62.9 MHz), 166.32 (C), 166.02 (C), 133.18 (CH), 133.09 (CH), 129.85 (CH), 129.80 (CH), 129.62 (CH), 128.39 (CH), 128.11 (CH), 100.18 (d, *J* 186, C-2), 74.05 (d, *J* 6, C-4), 63.67 (d, *J* 2, CH_2), 55.58 (d, *J* 22, CH), 50.01 (d, *J* 21, CH) and 37.50 (d, *J* 6, C-5) [Found: M + H^+ (CI, NH_3), 358.1456. $C_{20}H_{21}FNO_4$ requires *M* + H^+, 358.1455].

(+)-(1S,2S,3R,4S)-4-*Benzoyloxy*-3-*benzoyloxymethyl*-2-*fluoro*-N-(3-*methoxy*-2-*methylpropenoylcarbamoyl*)*cyclopentylamine* **12**.—The amine **11** [0.516 mmol (based on amide **10** prior to Hofmann rearrangement)] was dissolved in dry DMF (1.43 cm^3) and cooled to −20 °C. A solution of β-methoxy-α-methylacryloyl isocyanate (2.68 cm^3, 1.12 mmol) in benzene was added dropwise over 10 min. The solution was allowed to warm to room temp. and was stirred for 16 h. After this it was evaporated under reduced pressure and the residue purified by flash chromatography (EtOAc) to yield the acryloyl urea **12** (166 mg, 0.333 mmol, 64% based on amide) as white needles after recrystallisation from ethanol; R_f 0.81 (EtOAc); m.p. 142–143 °C (EtOH); $[\alpha]_D^{26.5}$ +6.95 (*c* 0.99, $CHCl_3$); λ_{max}(EtOH)/nm 231 and 254; $\nu_{max}(CHCl_3)/cm^{-1}$ 3443w (NH), 1721s (C=O) and 1711s (C=O); $\delta_H(CDCl_3$; 250 MHz) 9.26 (1 H, d, *J* 6.5, NH), 8.95 (1 H, s, NH), 8.08–7.34 (11 H, m, 2 × C_6H_5, 3′-H), 5.36 (1 H, ddd, *J* 7, 3.5 and 3.5, 4-H), 4.92 (1 H, ddd, *J* 46, 6.5 and 6.5, 2-H), 4.70 (1 H, dddd, *J* 15, 15, 10 and 8, 1-H), 4.58 (2 H, d, *J* 5, OCH_2), 3.84 (3 H, s, OCH_3), 2.85 (1 H, dddd, *J* 22, 10.5, 5 and 5, 3-H), 2.52–2.39 (1 H, m, 5α-H), 2.24 (1 H, ddd, *J* 15, 9.5 and 7, 5β-H) and 1.79 (3 H, d, *J* 1, 2′-CH_3); $\delta_C(CDCl_3$; 62.9 MHz), 169.84 (C), 166.24 (C), 165.86 (C), 158.63 (CH), 154.64 (C), 133.26 (CH), 133.11 (CH), 129.71 (C), 129.66 (CH), 128.43 (CH), 128.42 (CH), 107.54 (C), 97.54 (d, *J* 190, C-2), 73.38 (d, *J* 6, CH), 63.18 (CH_2), 61.45 (CH_3), 54.20 (d, *J* 23, CH), 49.78 (d, *J* 21, CH), 35.51 (d, *J* 5, C-5) and 8.72 (CH_3) [Found: M + H^+ (FAB, NOBA), 499.1881. $C_{26}H_{27}FN_2O_7$ requires *M* + H^+, 499.1881].

(−)-(1R,2S,3R,4R)-4-*Acetoxy*-3-*acetoxymethyl*-2-*fluorocyclopentanecarboxamide* **13**.—The amide mixture **8** (410 mg, 1.870 mmol) and DMAP (23 mg, 0.19 mmol) were dissolved in dry pyridine (1.9 cm^3) and stirred at room temp. Distilled acetic anhydride (353 mm^3) was added and after 5 min the solvent was evaporated under reduced pressure. Flash chromatography (EtOAc) gave the diacetate **13** as a colourless gum (483 mg, 1.849 mmol, 99%); R_f 0.45 (EtOAc); $[\alpha]_D^{22}$ −35.9 (*c* 1.87, $CHCl_3$); $\nu_{max}(CDCl_3)/cm^{-1}$ 1736 (C=O) and 1693 (C=O); $\delta_H(CHCl_3$; 250 MHz) 5.95 (1 H, br s, NH), 5.79 (1 H, br s, NH), 5.24–5.00 [2 H, m (including ddd, *J* 54.5, 8 and 7, 2-H), 4-H], 4.24 (2 H, d, *J* 7, OCH_2), 2.88 (1 H, dddd, *J* 22.5, 10.5, 8 and 7, 3-H), 2.70–2.40 (2 H, m, 1-H and 5α-H), 2.10 (1 H, ddd, *J* 15, 8 and 3.5, 5β-H), 2.03 (3 H, s, CH_3) and 2.01 (3 H, s, CH_3); $\delta_C(CDCl_3$; 62.9 MHz) 174.43 (C), 170.80 (C), 170.12 (C), 97.02 (d, *J* 186, C-2), 71.75 (d, *J* 8, C-4), 60.51 (CH_2), 48.25 (d, *J* 20, CH), 47.65 (d, *J* 19, CH), 32.48 (d, *J* 5, C-5), 20.78 (CH_3) and 20.68 (CH_3) [Found: M + H^+ (CI, NH_3), 262.1091. $C_{11}H_{16}FNO_5$ requires *M* + H^+, 262.1091]; *m/z* (CI, NH_3) 279 (89%, M + NH_4^+), 262 (100, M + H^+), 242 (7, M − F^+) and 202 (72, M − $CH_3CO_2^+$).

(−)-(1S,2S,3R,4R)-4-*Acetoxy*-3-*acetoxymethyl*-2-*fluorocyclopentylamine* **14**.—The amide **13** (3.084 g, 11.808 mmol) was dissolved in distilled acetonitrile (23.7 cm^3) and stirred at room temp. in the dark. Freshly prepared bis(trifluoroacetoxy)iodobenzene (10.234 g, 23.616 mmol) was added followed immediately by distilled pyridine (1.80 cm^3) and glass distilled water (9.57 cm^3). After 4 h in the dark the solvent was evaporated under reduced pressure and the residue subjected to flash chromatography (5% MeOH–EtOAc). The resultant gum was dissolved in dichloromethane (200 cm^3) and the solution shaken vigorously with dilute aqueous ammonium hydroxide (2 mol dm^{-3}; 100 cm^3) until the organic phase was clear. The organic layer was separated and dried ($MgSO_4$) and evaporated under reduced pressure to give the amine **14** as a pale yellow gum; R_f 0.14 (EtOAc); $[\alpha]_D^{22}$ −34.8 (*c* 2.98, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1735 (C=O); $\delta_H(CDCl_3$; 250 MHz) 5.11 (1 H, m, 4-H), 4.42 (1 H, m, 2-H), 4.08 (2 H, m, OCH_2), 3.25 (1 H, m, 3-H), 2.40 (2 H, m, 5-H and 1-H), 1.90 (6 H, 2 × s, 2 × CH_3), 1.60 (2 H, br s, NH_2) and 1.34 (1 H, m, 5-H) [Found: M + H^+ (EI), 234.1142. $C_{10}H_{16}FNO_4$ requires *M* + H^+, 234.1142].

(−)-(1S,2S,3R,4R)-4-*Acetoxy*-3-*acetoxymethyl*-2-*fluoro*-N-(3-*methoxy*-2-*methylpropenoylcarbamoyl*)*cyclopentylamine* **15**.—A solution of the amine **14** (assumed 11.808 mmol) in dry DMF (26 cm^3) was cooled to −20 °C. A solution of β-methoxy-α-methylacryloyl isocyanate (0.418 mol dm^{-3}; 48.7 cm^3, 20.36 mmol) in dry benzene was added dropwise at a rate sufficient to maintain a reaction temperature of −20 °C. The resulting solution was warmed to room temperature overnight with constant stirring. The solvent was evaporated under reduced pressure (1 mmHg) and the residue purified by flash chromatography (33% EtOAc–light petroleum) to yield the acryloyl urea **15** (2.806 g, 7.495 mmol; 63% based on the amide **13** as starting material) as a white solid; R_f 0.50 (66% EtOAc–light petroleum); m.p. 100–101 °C (EtOH) (Found: C, 51.3; H, 6.2; N, 7.45. $C_{16}H_{23}FN_2O_7$ requires C, 51.3; H, 6.19; N, 7.48%); $[\alpha]_D^{23}$ −22.5 (*c* 2.52, $CHCl_3$); λ_{max}(MeOH)/nm 254; $\nu_{max}(CHCl_3)/cm^{-1}$ 1737 (C=O) and 1698 (C=O); $\delta_H(CDCl_3$; 250 MHz) 9.26 (1 H, d, *J* 6.5, NH), 9.17 (1 H, s, NH), 7.37 (1 H, d, *J* 1, 3′-H), 5.39 (1 H, ddd, *J* 5, 5 and 1.5, 4-H), 4.84 (1 H, ddd, *J* 52.2, 5.5 and 3, 2-H), 4.44 (1 H, m, 1-H), 4.26–4.10 (2 H, m, OCH_2), 3.79 (3 H, s,

OCH_3), 2.55–2.39 (2 H, m, 5α-H and 3-H), 2.08 (3 H, s, CH_3CO), 2.01 (3 H, s, CH_3CO), 1.81 (1 H, dm, *J* 14, 5β-H) and 1.73 (3 H, s, 2′-CH_3); δ_C($CDCl_3$; 62.9 MHz) 170.63 (C), 170.05 (C), 169.72 (C), 158.29 (CH), 154.34 (C), 107.82 (C), 100.34 (d, *J* 187, C-2), 73.50 (d, *J* 7, CH), 61.40 (CH_3), 60.92 (d, *J* 2, OCH_2), 54.45 (d, *J* 27, CH), 48.53 (d, *J* 22, CH), 36.90 (d, *J* 3, C-5), 20.79 (CH_3), 20.67 (CH_3) and 8.69 (CH_3).

(−)-(1′S,3′R,4′R,6′S)-1-(6′-*Fluoro*-3′-*hydroxy*-4′-*hydroxymethylcyclopentyl*)*thymine* **16**.*— Acryloyl urea **15** (382 mg, 1.020 mmol) was dissolved in 1,4-dioxane (8 cm^3) and aqueous dilute hydrochloric acid (4 mol dm^{-3}; 8 cm^3) was added. The mixture was refluxed for 2 h and then evaporated under reduced pressure and the residue purified by flash chromatography (5% MeOH–EtOAc) to give the title compound **16** (216 mg, 0.836 mmol, 82%) as a white solid; R_f 0.38 (5% MeOH–EtOAc); m.p. 166–167 °C (decomp., MeOH) (Found: C, 51.1; H, 6.0; N, 10.9. $C_{11}H_{15}FN_2O_4$ requires C, 51.16; H, 5.85; N, 10.85%); $[\alpha]_D^{29.5}$ −1.81 (*c* 4.62, MeOH); λ_{max}(EtOH)/nm 271; ν_{max}(CH_3CN)/cm^{-1} 1689 (C=O); δ_H([$^2H_6$]-DMSO; 250 MHz) 11.25 (1 H, s, NH), 7.62 (1 H, d, *J* 1, 6-H), 5.22 (1 H, br d, *J* 3.5, OH), 5.18–4.83 [2 H, m (including 1 H, ddd, *J* 47, 9 and 6.5, 6′-H), 1′-H], 4.49 (1 H, br m, OH), 4.22 (1 H, br m, 3′-H), 3.82–3.53 (2 H, m, OCH_2), 2.54 (1 H, m, 2′α-H), 2.18 (1 H, m, 4′-H), 1.79 (3 H, d, *J* < 1, 5-CH_3) and 1.60 (1 H, ddd, *J* 14, 8.5 and 2.5, 2′β-H); δ_H([$^2H_6$]-DMSO + D_2O; 250 MHz) *inter alia* 3.78–3.53 (2 H, ABXq, *J* 11, 8 and 5.5, OCH_2); δ_C(CD_3OD; 62.9 MHz) 166.25 (C), 153.11 (C), 140.22 (CH), 112.43 (C), 99.71 (d, *J* 186, C-6′), 70.57 (d, *J* 8, CH), 61.24 (d, *J* 24, CH), 59.81 (CH_2), 52.25 (d, *J* 17, CH), 38.68 (d, *J* 6, C-2′) and 12.42 (CH_3) [Found: M^+ (EI), 258.1016. $C_{11}H_{15}FN_2O_4$ requires M^+, 258.1016].

(+)-(1′S,3′S,4′R,6′S)-1-(3′-*Benzoyloxy*-4′-*benzoyloxymethyl*-6′-*fluorocyclopentyl*)*thymine* **17**.—The diol **16** (164 mg, 0.637 mmol), triphenylphosphine (501 mg, 1.911 mmol) and benzoic acid (233 mg, 1.911 mmol) were dissolved in dry THF (3.7 cm^3). A solution of diethyl azodicarboxylate (301 mm^3, 1.911 mmol) in dry THF (0.4 cm^3) was added at room temp. over 0.25 h. After 17.5 h stirring in the dark the solvent was evaporated under reduced pressure and the residue dissolved in dichloromethane (50 cm^3). This solution was extracted vigorously with saturated sodium hydrogen carbonate (30 cm^3) for 2 min. The aqueous phase was back-extracted with dichloromethane (2 × 20 cm^3). The organic phases were combined and the solvent was evaporated under reduced pressure. The residue was subjected to flash chromatography (20% EtOAc–light petroleum) to give the dibenzoate **17** (207 mg, 0.444 mmol, 70%) as a white solid; R_f 0.75 (EtOAc); m.p. 194–195 °C (dichloromethane–light petroleum); $[\alpha]_D^{30.5}$ +10.56 (*c* 2.76, $CHCl_3$); λ_{max}(MeOH)/nm 229 and 270; ν_{max}($CDCl_3$)/cm^{-1} 1713 (C=O) and 1689 (C=O); δ_H($CDCl_3$; 250 MHz) 9.55 (1 H, s, NH), 8.10–7.30 (10 H, m, 2 × C_6H_5), 7.03 (1 H, d, *J* 1, 6-H), 5.47 (1 H, ddd, *J* 54.6, 6.8 and 6.8, 6′-H), 5.49 (1 H, m, 3′-H), 4.75 (1 H, dddd, *J* 21.2, 9.7, 9.7 and 6.8, 1′-H), 4.64 (2 H, d, *J* 5.9, OCH_2), 2.93 (1 H, m, 4′-H), 2.80 (1 H, ddd, *J* 14.4, 9.7 and 7.3, 2′α-H), 2.38 (1 H, ddd, *J* 14.4, 9.7 and 3.5, 2′β-H) and 1.90 (3 H, d, *J* 1, 5-CH_3); δ_C($CDCl_3$; 62.9 MHz) 166.28 (C), 165.77 (C), 164.00 (C), 150.61 (C), 139.50 (CH), 133.37 (CH), 133.19 (CH), 129.70 (C), 129.54 (CH), 128.47 (CH), 128.43 (CH), 111.37 (C), 95.09 (d, *J* 188, C-6′), 73.14 (d, *J* 5, C-3′), 65.00 (d, *J* 23, C-1′), 62.88 (CH_2), 49.53 (d, *J* 20, C-4′), 33.59 (d, *J* 6, C-2′) and 12.28 (CH_3) [Found: M + NH_4^+ (CI, NH_3), 484.1884. $C_{25}H_{23}FN_2O_6$ requires *M* + NH_4^+, 484.1884].

(+)-(1′S,3′S,4′R,6′S)-1-(6′-*Fluoro*-3′-*hydroxy*-4′-*hydroxymethyl*)*cyclopentylthymine* **1**.—The dibenzoate **17** (276 mg, 0.592 mmol) was dissolved in methanol (25 cm^3) containing potassium carbonate (2% w/v) and stirred for 2 h at room temp. Citric acid (150 mg) was added, the suspension filtered through a Celite bed, the bed rinsed with methanol (300 cm^3) and the solvent evaporated from the filtrate under reduced pressure. Flash chromatography (5% MeOH–EtOAc) gave the title diol **1** (127 mg, 0.492 mmol, 83%) as a white solid; R_f 0.23 (5% MeOH–EtOAc); m.p. 210–211 °C (EtOH–pentane) (Found: C, 50.9; H, 5.8; N, 10.9. $C_{11}H_{15}FN_2O_4$ requires C, 51.16; H, 5.85; N, 10.85%); $[\alpha]_D^{23}$ +48.15 (*c* 0.162, H_2O); λ_{max}(EtOH)/nm 271; ν_{max}(CH_3CN)/cm^{-1} 1694s (C=O); δ_H(CD_3OD; 250 MHz) 7.46 (1 H, d, *J* 1, 6-H), 5.15 (1 H, ddd, *J* 55, 7 and 7, 6′-H), 5.00 (1 H, m, 1′-H), 4.20 (1 H, ddd, *J* 7, 5.5 and 4, 3′-H), 3.76 (2 H, d, *J* 5, OCH_2), 2.90 (1 H, m, 4′-H), 2.26 (1 H, ddd, *J* 14, 10 and 7, 2′α-H), 2.22–1.99 (2 H, m, 3-H and 2′β-H) and 1.88 (3 H, d, *J* 1, 5-H); δ_H([$^2H_6$]-DMSO; 250 MHz) *inter alia* 11.25 (1 H, br s, NH) and 4.80 (2 H, br s, 2 × OH) [Found: M + H^+ (CI, NH_3), 259.1094. $C_{11}H_{15}FN_2O_4$ requires *M* + H^+, 259.1094].

(−)-(1′S,3′S,4′R,6′S)-4-(3′-*Benzoyloxy*-4′-*benzoyloxymethyl*-6′-*fluorocyclopentylamino*)-6-*chloro*-5-*nitropyrimidine* **19**.—A solution of the amine **11** (61 mg, 0.171 mmol) and diisopropylethylamine (119 mm^3, 0.683 mmol) in dry dichloromethane (2 cm^3) was added by syringe pump over 3 h to a stirred solution of 4,6-dichloro-5-nitropyrimidine (165 mg, 0.854 mmol) in dry dichloromethane (7 cm^3) at room temp. The resultant solution was stirred for a further 36 h and then evaporated under reduced pressure. The residue was purified by flash chromatography (25% EtOAc–light petroleum) to give the title compound **19** (66 mg, 0.128 mmol, 75%); R_f 0.39 (20% EtOAc–light petroleum); m.p. 122–123 °C (solid foam); $[\alpha]_D^{22}$ −5.8 (*c* 0.38, $CHCl_3$); λ_{max}(EtOH)/nm 231 and 348; ν_{max}($CHCl_3$)/cm^{-1} 1720s (C=O) and 1586s (C=C); δ_H($CDCl_3$; 250 MHz) 8.43 (1 H, s, 2-H), 8.10–7.97 (4 H, m, part of 2 × C_6H_5), 7.71 (1 H, d, *J* 6.5, NH), 7.64–7.37 (6 H, m, part of 2 × C_6H_5), 5.42 (1 H, m, 3′-H), 5.08 (1 H, dddd, *J* 15, 15, 7 and 7, 1′-H), 5.06 (1 H, ddd, *J* 55, 7 and 7, 6′-H), 4.60 (2 H, d, *J* 5.5, OCH_2), 2.93 (1 H, dddd, *J* 21, 11, 5 and 5, 4′-H), 2.61 (1 H, m, 2′α-H) and 2.28 (1 H, ddd, *J* 16, 9 and 7, 2′β-H); δ_C($CDCl_3$; 62.9 MHz) 166.27 (C), 165.94 (C), 158.03 (C), 155.96 (C), 133.47 (CH), 133.37 (CH), 129.72 (CH), 129.60 (CH), 129.49 (C), 128.52 (CH), 96.72 (d, *J* 193, C-6′), 72.74 (d, *J* 6, CH), 62.65 (CH_2), 56.13 (d, *J* 23, CH), 49.46 (d, *J* 21, CH) and 35.38 (d, *J* 6, C-2′) [Found: M + H^+ (CI, NH_3), 515.1134. $C_{24}H_{20}ClFN_4O_6$ requires *M* + H^+, 515.1134].

(−)-(1′S,3′S,4′R,6′S)-4-[6′-*Fluoro*-3′,5′-*O*-(1,1,3,3-*tetraisopropyl*-1,3-*disiloxanediyl*)*cyclopentylamino*]-6-*methoxy*-5-*nitropyrimidine* **20**.—The dibenzoate **19** (355 mg, 0.689 mmol) was dissolved in methanol (20 cm^3) containing potassium carbonate (2% w/v) and stirred at room temp. for 72 h. The reaction mixture was quenched with Amberlite IR-120 (plus) ion exchange resin, filtered and the solvent evaporated from the filtrate under reduced pressure. The residue was purified by flash chromatography (EtOAc) to give (+)-(1′*S*,3′*S*,4′*R*,6′*S*)-4-(6′-fluoro-3′-hydroxy-4′-hydroxymethylcyclopentylamino)-6-methoxy-5-nitropyrimidine as a pale yellow gum (164 mg, 0.543 mmol, 79%); R_f 0.44 (EtOAc); $[\alpha]_D^{29}$ +32.4 (*c* 1.45, $CHCl_3$); λ_{max}(EtOH)/nm 230, 277 and 345; δ_H($CDCl_3$; 250 MHz) 8.53 (1 H, d, *J* 7.5, NH), 8.27 (1 H, s, 2-H), 5.10–4.69 [2 H, m, (including ddd, *J* 53, 5 and 5, 6′-H), 1′-H], 4.36 (1 H, aq, *J* 6.5, 3′-H), 4.08 (3 H, s, OCH_3), 3.90 (2 H, m, OCH_2), 2.76 (2 H, br s, 2 × OH) and 2.40–1.99 (3 H, m, 4′-H and 2 × 2′-H); δ_C($CDCl_3$; 62.9 MHz) 164.55 (C), 158.79 (CH), 156.67 (C), 98.34 (d, *J* 186, C-6′), 71.68 (d, *J* 5, CH), 61.30 (d, *J* 4, CH_2), 55.87 (CH), 55.44 (CH_3), 54.60 (d, *J* 20, CH) and 38.97 (d, *J* 4, C-2′)

* Although straightforward application of the IUPAC rules would suggest that cyclopentane derivatives have no 6′ position in the ring, the numbering system used in the present paper for nucleosides has been retained in order to allow for ease of reference with related compounds in the literature.

[Found: M + H$^+$ (CI, NH_3), 303.1105. $C_{11}H_{15}FN_4O_5$ requires M + H$^+$, 303.1105).

This diol (143 mg, 0.474 mmol) in dry DMF (5 cm^3) with imidazole (161 mg, 2.369 mmol) was stirred at room temp. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (197 mm^3, 0.616 mmol) in dry THF (300 mm^3) was added dropwise and the resultant solution stirred for 1 h and then evaporated under reduced pressure (0.2 mmHg) and the residue purified by flash chromatography (11% EtOAC–light petroleum) to give the title compound **20** as a pale yellow gum (172 mg, 0.315 mmol, 66%); R_f 0.21 (66% dichloromethane–light petroleum); $[\alpha]_D^{23}$ −0.41 (*c* 1.47, $CHCl_3$); λ_{max}(EtOH)/nm 230, 277 and 343; δ_H($CDCl_3$; 250 MHz) 8.47 (1 H, br d, *J* 6, NH), 8.27 (1 H, s, 2-H), 4.92–4.62 (2 H, m, 1′-H and 6′-H), 4.37 (1 H, q, *J* 8.5, 3′-H), 4.10 (3 H, s, OCH_3), 3.96 (2 H, m, OCH_2), 2.41 (1 H, ddd, *J* 13, 9 and 9, 2′α-H), 2.14 (1 H, m, 4′-H), 1.99 (1 H, ddd, *J* 12.5, 7.5 and 4, 2′β-H) and 1.27–1.00 (28 H, m, 4 × Pr^i) [Found: M + H$^+$ (CI, NH_3), 545.2627. $C_{23}H_{41}FN_4O_6Si_2$ requires M + H$^+$, 545.2627].

(+)-(1′S,3′S,4′R,6′S)-9-[6′-*Fluoro*-3′,5′-O-(1,1,3,3-*tetraisopropyl*-1,3-*disiloxanediyl*)*cyclopentylamino*]-6-*methoxy*-9H-*purine* **21**.—The nitropyrimidine **20** (160 mg, 0.294 mmol) was dissolved in ethanol (15 cm^3) and Raney nickel (2 cm^3) added. The mixture was degassed under reduced pressure for 2 min and then stirred under a hydrogen atmosphere (balloon) for 0.5 h, after this the catalyst was filtered off through a Celite pad. The Celite pad was rinsed with ethanol (5 × 10 cm^3) and the combined organic phases evaporated under reduced pressure. Flash chromatography (25% EtOAc–light petroleum) gave the corresponding amino pyrimidine as a colourless gum (101 mg, 0.196 mmol, 66%); R_f 0.34 (25% EtOAc–light petroleum); $[\alpha]_D^{27}$ +15.96 (*c* 2.02, $CHCl_3$); λ_{max}(EtOH)/nm 279; δ_H($CDCl_3$; 250 MHz) 8.01 (1 H, s, 2-H), 4.79 (1 H, ddd, *J* 54.5, 9 and 6, 6′-H), 4.79 (1 H, br d, *J* 6, NH), 4.54–4.34 (2 H, m, 1′-H and 3′-H), 4.02–3.85 (5 H, m, OCH_2 and OCH_3), 2.89 (2 H, br s, NH_2), 2.33 (1 H, ddd, *J* 13.5, 11 and 9, 2′α-H), 2.18–1.91 (2 H, m, 2′β-H and 4′-H) and 1.12–0.97 (28 H, m, 4 × Pr^i); δ_C($CDCl_3$; 62.9 MHz) *inter alia* 154.40 (C), 149.20 (CH), 109.28 (C), 95.84 (d, *J* 188, C-6′), 67.22 (d, *J* 9, CH), 58.23 (C-5′), 54.57, (d, *J* 22, CH), 53.84 (d, *J* 17, CH), 38.25 (d, *J* 5, 2′-CH_2), 17.47–17.01 (8 × CH_3), 13.42 (CH), 13.27 (CH), 12.91 (CH) and 12.59 (CH) [Found: M$^+$ (EI), 514.2807. $C_{23}H_{43}FN_4O_4Si_2$ requires M, 514.2807].

The aminopyrimidine (98 mg, 0.190 mmol) was dissolved in diethoxymethyl acetate (5 cm^3) and heated at 140 °C for 6 h. The mixture was evaporated under reduced pressure (0.2 mmHg) and the residue purified by flash chromatography (25% EtOAc–light petroleum) to give the purine **21** (91 mg, 0.174 mmol, 91%) as a colourless gum; R_f 0.54 (50% EtOAc–light petroleum); $[\alpha]_D^{26.5}$ +0.31 (*c* 1.8, $CHCl_3$); λ_{max}(EtOH)/nm 247; δ_H($CDCl_3$; 250 MHz) 8.40 (1 H, s, 2-H or 8-H), 7.88 (1 H, s, 8-H or 2-H), 5.30 (1 H, ddd, *J* 54, 10 and 7.5, 6′-H), 5.03–4.83 (2 H, m, 3′-H and 1′-H), 4.15 (3 H, s, OCH_3); 3.98 (2 H, m, OCH_2), 2.56–2.41 (2 H, m, 2′α-H and 4′-H), 2.15 (1 H, m, 2′β-H) and 1.14–0.98 (28 H, m, 4 × Pr^i); δ_C($CDCl_3$; 62.9 MHz) 161.21 (C), 151.77 (CH), 151.00 (C), 141.94 (CH), 122.80 (C), 93.94 (d, *J* 192, C-6′), 66.44 (d, *J* 10, CH), 58.71 (d, *J* 23, CH), 57.28 (C-5′), 54.07 (CH_3), 53.80 (d, *J* 17, CH), 36.63 (d, *J* 5, C-2′), 17.44–16.98 (8 × CH_3), 13.32 (CH), 13.26 (CH), 12.81 (CH) and 12.57 (CH) [Found: M + H$^+$ (CI, NH_3), 525.2729. $C_{24}H_{41}FN_4O_4Si_2$ requires M + H$^+$, 525.2729].

(−)-(1′S,3′S,4′R,6′S)-9-(6′-*Fluoro*-3′-*hydroxy*-5′-*hydroxymethylcyclopentylamino*)*adenine* **2**.—The methoxypyrimidine **21** (60 mg, 0.114 mmol) was dissolved in liquid ammonia (10 cm^3) and the solution sealed in a PTFE lined, stainless steel Parr bomb. The bomb was allowed to equilibrate to room temp., after which it was placed in an oven at 60 °C for 5 days. The bomb was removed from the oven and allowed to cool to room temp., after which the pressure was released. The ammonia was allowed to evaporate under atmospheric pressure and the residue purified by flash chromatography (EtOAc) to give the protected purine as a colourless gum (34 mg, 0.067 mmol, 58%); R_f 0.25 (EtOAc); $[\alpha]_D^{27}$ +1.25 (*c* 0.60, $CDCl_3$); λ_{max}(EtOH)/nm 260; δ_H($CDCl_3$; 250 MHz) 8.21 (1 H, s, 2-H or 8-H), 7.79 (1 H, s, 8-H or 2-H), 6.08 (2 H, br s, NH_2), 5.31 (1 H, ddd, *J* 55, 10 and 6.5, 6′-H), 5.00–4.80 (2 H, m, 1′-H and 3′-H), 4.01 (2 H, m, OCH_2), 2.63–2.10 (3 H, m, 2 × 2′-H and 4′-H) and 1.20–1.01 (28 H, m, 4 × Pr^i); δ_C($CDCl_3$; 62.9 MHz) 155.71 (C), 152.67 (CH), 149.63 (C), 140.21 (CH), 120.55 (C), 94.08 (d, *J* 191, C-6′), 66.59 (d, *J* 10, CH), 58.60 (d, *J* 23, CH), 57.51 (C-5′), 53.87 (d, *J* 16, CH), 36.73 (d, *J* 4, C-2′), 17.48–17.02 (8 × CH_3), 13.35 (CH), 13.30 (CH), 12.83 (CH) and 12.59 (CH) (Found: M + H$^+$ (EI), 510.2732. $C_{23}H_{40}FN_5O_3Si_2$ requires M + H$^+$, 510.2732].

A solution of this silyl derivative (24.7 mg, 0.048 mmol) in dry THF (5 cm^3) was stirred at room temp. A solution of tetrabutylammonium fluoride in THF (1 mol dm^{-3}; 120 mm^3, 0.120 mmol) was added and the mixture stirred for 12 h. The solvent was evaporated under reduced pressure and the residue purified by flash chromatography (20% MeOH–$CHCl_3$) to give the title compound **2** as a colourless gum (12 mg, 0.449 mmol; 93%); R_f 0.33 (15% MeOH–EtOAc); $[\alpha]_D^{26}$ −47 (*c* 0.170, H_2O); λ_{max}(H_2O)/nm 261; δ_H(CD_3OD) 8.21 (1 H, s, 8-H or 2-H), 7.90 (1 H, s, 2-H or 8-H), 5.36 (1 H, ddd, *J* 53.7, 7 and 7, 6′-H), 5.27 (1 H, m, 1′-H), 4.31 (1 H, m, 3′-H), 3.82 (2 H, d, OCH_2), 3.25 (1 H, m, 2′α-H), 2.62 (1 H, m, 4′-H) and 2.28 (1 H, m, 2′β-H) [Found: M + H$^+$ (CI, NH_3), 268.1210. $C_{11}H_{14}FN_5O_2$ requires M + H$^+$, 268.1210].

(+)-(1′S,3′S,4′R,6′S)-1-[4′-(4,4′-*Dimethoxytrityloxymethyl*)-6′-*fluoro*-3′-*hydroxycyclopentyl*]*thymine* **22**.—The diol **1** (88 mg, 0.341 mmol) was dissolved in dry pyridine containing activated powdered molecular sieves. The mixture was stirred at room temp. for 10 min, then 4,4′-dimethoxytrityl chloride (147 mg, 0.434 mmol) was added in one portion. The mixture was stirred for 12 h and methanol (1 cm^3) was then added. After 10 min the colourless suspension was filtered through a Celite pad and the solvent evaporated from the filtrate under reduced pressure. Purification of the residue by rapid flash chromatography (75% EtOAc–light petroleum) gave the title compound **22** (146 mg, 0.260 mmol; 76%) that was precipitated from hexane (−78 °C) to free it from a trace of 4,4′-dimethoxytrityl methyl ether to give the pure compound **22**; R_f 0.50 (EtOAc); m.p. 115–117 °C softens, 123–125 °C melts (hexane); $[\alpha]_D^{25}$ +6.43 (*c* 0.41, CH_2Cl_2); λ_{max}(EtOH)/nm 234 and 273; ν_{max}(CH_2Cl_2)/cm^{-1} 1702 (C=O) and 1689 (C=O); δ_H(CD_3OD; 250 MHz) 7.47–6.79 (14 H, m, 2 × C_6H_4, 1 × C_6H_5 and 6-H), 5.20 (1 H, ddd, *J* 55.5, 7 and 7, 6′-H), 4.88 (1 H, dddd, *J* 20, 10, 10 and 7, 1′-H), 4.30 (1 H, ddd, *J* 11, 7 and 5, 3′-H), 3.76 (6 H, s, 2 × OCH_3), 3.39–3.27 (2 H, m, OCH_2), 2.40–1.97 (3 H, m, 4′-H and 2 × 2′-H) and 1.82 (3 H, d, *J* 1, 5-CH_3); δ_C(CD_3OD; 62.9 MHz) 164.40 (C), 158.64 (C), 150.99 (C), 144.88 (C), 139.29 (CH), 136.04 (C), 135.92 (C), 130.09 (CH), 128.09 (CH), 127.91 (CH), 126.90 (CH), 113.32 (CH), 111.04 (C), 95.81 (d, *J* 188, C-6′), 86.51 (C), 70.45 (d, *J* 6, CH), 63.28 (d, *J* 24, CH), 61.82 (CH_2), 52.66 (d, *J* 17, CH), 36.18 (CH_2) and 12.22 (CH_3); δ_F(CD_3OD; 235 MHz) −21.94 (adt, *J* 55.5 and 21) [Found: M$^+$ (FAB, NOBA), 560.2323. $C_{32}H_{33}FN_2O_6$ requires M, 560.2323].

(1′S,3′S,4′R,6′S)-1-{3′-[2-*Cyanoethyl*(*diisopropylamino*)*phosphinoyloxy*]4′-(4,4′-*dimethoxytrityloxymethyl*)-6′-*fluorocyclopentyl*}*thymine* **23**.—The trityl alcohol **22** (142 mg, 0.253 mmol) was dissolved in dry dichloromethane (1 cm^3) at room temp.

and dry diisopropylethylamine (110 mm^3, 0.608 mmol) was added to the solution. Chlorodiisopropylamino-β-cyanoethyl phosphoramidite (64 mm^3, 0.287 mmol) was added dropwise over 5 min. The resultant solution was stirred for 0.75 h after which time saturated aqueous sodium carbonate (10 cm^3) was added. The mixture was extracted with dichloromethane (4 × 10 cm^3) and the extract dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by rapid flash chromatography (33% EtOAc–light petroleum containing 2% triethylamine) to give the title compounds **23** as a mixture of diastereoisomers (153 mg, 0.201 mmol, 79%) that were precipitated from hexane (−78 °C) and gave a white solid foam on repeated flask evacuation. Pure samples of each diastereoisomer were obtained by careful flash chromatography (33% EtOAc–light petroleum containing 2% triethylamine) for individual characterization.

Data for diastereoisomer a. R_f 0.38 (50% EtOAc–light petroleum); $[\alpha]_D^{27.5}$ +2.57 (*c* 1.17, CH_2Cl_2); λ_{max}(EtOH)/nm 234 and 272; ν_{max}/cm^{-1} 2249m (CN) and 1685 (C=O); δ_H(acid free $CDCl_3$; 250 MHz) 8.08 (1 H, br s, NH), 7.53–6.79 (13 H, m, 2 × C_6H_4 and C_6H_5), 7.00 (1 H, s, 6-H), 5.22 (1 H, ddd, *J* 55, 6.5 and 6.5, 6′-H), 4.96 (1 H, m, 1′-H), 4.44 (1 H, ddd, *J* 10, 10 and 5, 3′-H), 3.81 (6 H, s, 2 × OCH_3), 3.72–3.51 [4 H, m, 2 × $(CH_3)_2CH$ and OCH_2], 3.35 (2 H, d, *J* 4.5, 2 × 5′-H), 2.61 (2 H, t, *J* 6.5, CH_2CN), 2.52–2.21 (3 H, m, 2 × 2′-H and 4′-H) and 1.24–1.05 (15 H, m, 5 × CH_3); δ_F(acid free $CDCl_3$; 235 MHz) −22.98 (ddd, *J* 55, 22 and 22); δ_P(acid free $CDCl_3$; 101 MHz) 149.28 (s) [Found: M + H^+ (FAB, NOBA), 761.3479. $C_{41}H_{50}FN_4O_7P$ requires *M* + H^+, 761.3479).

Data for diastereoisomer b. R_f 0.32 (50% EtOAc–light petroleum); $[\alpha]_D^{28}$ −15.9 (*c* 0.60, CH_2Cl_2); λ_{max} and ν_{max} identical to diastereoisomer a; δ_H(acid free $CDCl_3$; 250 MHz) 8.66 (1 H, br s, NH), 7.53–6.79 (13 H, m, 2 × C_6H_4 and C_6H_5), 7.00 (1 H, s, 6-H), 5.27 (1 H, ddd, *J* 55, 7 and 7, 6′-H), 4.90 (1 H, m, 1′-H), 4.51 (1 H, ddd, *J* 10, 10 and 5.5, 3′-H), 3.79 (6 H, s, 2 × OCH_3), 3.72–3.50 [4 H, m, 2 × $(CH_3)_2CH$ and OCH_2], 3.38 (2 H, d, *J* 4.5, 2 × 5′-H), 2.48 (2 H, t, *J* 6.5, CH_2CN), 2.44–2.18 (3 H, m, 2 × 2′-H and 4′-H) and 1.32–1.03 (15 H, m, 5 × CH_3); δ_F(acid free $CDCl_3$; 235 MHz) −23.10 (ddd, *J* 55, 22 and 22); δ_P(acid free $CDCl_3$; 101 MHz) 148.75 (s) [Found: M + H^+ (FAB, NOBA), 761.3479. $C_{41}H_{50}FN_4O_7P$ requires *M* + H^+, 761.3479].

General Procedures for Oligonucleotide Synthesis.—The oligonucleotides **24**–**32** were synthesized on a Biosearch Cyclone DNA Synthesiser using standard protocols on a 0.2 μmol scale. All reagents were of DNA quality unless otherwise stated and were used without further purification. Water refers to Milli-Q grade water. All aqueous solutions were stored at 4 °C to prevent microbial growth.

The oligonucleotides were removed from the columns and deprotected in the following manner. A disposable plastic syringe was fitted to each end of the synthesis column and concentrated ammonium hydroxide (1 cm^3) was passed back and forth through the column five times. The tube was left at room temp. for 45 min, whereupon the ammonia solution was again agitated. The column was left for a further 45 min after which the solution was pumped through it for a further five times and then deposited in a screw cap vial. The vial was sealed and heated for 5 h at 55 °C. The sample was cooled to room temp. and evaporated under reduced pressure to yield the crude oligonucleotide.

The oligonucleotide was purified by polyacrylamide gel electrophoresis under urea (7 mol dm^{-3}) denaturation. Urea (420.4 g), acrylamide (190.0 g), bisacrylamide (10.0 g) and 10X TBE buffer (100 cm^3) were mixed and the total volume of solution adjusted to 1000 cm^3 with water. The solution was filtered and an aliquot (40 cm^3) removed from this stock solution for each gel required. The aliquot was thoroughly degassed under reduced pressure for 3 min (until bubbling had ceased) and to this solution was added fresh ammonium persulfate (10% w/v solution in water; 8 mm^3). *N,N,N′,N′*-Tetramethylethylenediamine (40 mm^3) was added carefully and the gel then poured as rapidly as possible into the vertical electrophoresis apparatus.

The gels were pre-electrophoresed for at least 30 min prior to loading. The sample (*ca.* 6% of a 0.2 μmol synthesis) was dissolved in loading buffer and heat denatured (90 °C) for 10 min prior to rapid cooling in an ice bath. The solution of oligonucleotide was loaded into the gel wells and the gels subjected to electrophoresis at 15 mA per gel.

The gel was removed from the glass plates, wrapped in cling film and laid on a TLC plate. The band was observed under a UV lamp due to the fluorescent quenching; the slowest moving major band being the required band. The band was excised from the gel with a scalpel and the slice ground to a powder with a glass rod. A few drops of ammonium hydrogen carbonate (0.1 mol dm^{-3}) were added and the mixture ground for a further 2 min. The mixture was diluted with ammonium hydrogen carbonate (0.1 mol dm^{-3}; 0.5 cm^3), incubated for 10 min at 50 °C and then at 30 °C in an orbital shaker for 16 h.

The supernatant was decanted, the solid washed with a little aqueous ammonium hydrogen carbonate (0.1 mol dm^{-3}) and the combined supernatants passed down a Sephadex G-25 column (NAP-10 columns, Pharmacia), eluting with sodium phosphate buffer (0.01 mol dm^{-3}; pH 6.8). Fractions (500 mm^3) were taken and monitored by UV (λ_{max} 260 nm) for activity. The first eluting band was lyophilized to give the salt free oligonucleotide.

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References

1 S. M. Roberts, *Chem. Brit.*, 1991, 518.
2 A. D. Borthwick, B. E. Kirk, K. Biggadike, A. M. Exall, S. Butt, S. M. Roberts, D. J. Knight, J. A. V. Coates and D. M. Ryan, *J. Med. Chem.*, 1991, **34**, 907 and references therein.
3 C. T. Evans, S. M. Roberts, K. A. Shoberu and A. G. Sutherland, *J. Chem. Soc., Perkin Trans. 1*, 1992, 589; D. M. Coe, D. C. Orr, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans 1*, 1991, 3378 and references therein.
4 D. M. Coe, D. M. Parry, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2373.
5 R. M. Highcock, H. Hilpert, P. L. Myers, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1127 and references therein.
6 Z. Grudzinski and S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1767.
7 *cf.* R. Franz, *J. Fluorine Chem.*, 1980, **15**, 423.
8 *cf.* Z. Grudzinski, S. M. Roberts, C. Howard and R. F. Newton, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1182; see also P. Hamley, A. B. Holmes, D. R. Marshall and J. W. M. Mackinnon, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1793.
9 G. M. Loudon, A. S. Radhakrishna, M. R. Almond, J. K. Blodgett and R. H. Boutin, *J. Org. Chem.*, 1984, **49**, 4272; R. H. Boutin and G. M. Loudon, *J. Org. Chem.*, 1984, **49**, 4277.
10 I. M. Lazbin and G. F. Koser, *J. Org. Chem.*, 1986, **51**, 2669; R. M. Moriarty, R. K. Vaid and G. M. Koser, *Synlett.*, 1990, 365.
11 G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 1958, 153; Y. F. Shealy and C. A. O'Dell, *J. Heterocycl. Chem.*, 1976, **13**, 1041.
12 H. Schaller, G. Weiman, B. Lerch and H. G. Khorana, *J. Am. Chem.*

Soc., 1963, **85**, 3821; N. D. Sinha, J. Biernat and H. Köster, *Tetrahedron Lett.*, 1983, **24**, 5843; N. D. Sinha, J. Biernat, J. McManus and H. Köster, *Nucleic Acids Res.*, 1984, **12**, 4539.
13 M. Perbost, M. Lucas, C. Chavis, A. Pompon, H. Baumgartner, B. Rayner, H. Griengl and J. L. Imbach, *Biochem. Biophys. Res. Commun.*, 1989, **165**, 742.
14 T. Maniatis, A. Jeffrey and H. van de Sande, *Biochemistry*, 1975, **14**, 3787; J. C. Pinder, D. Z. Staynov and W. A. Gratzer, *Biochemistry*, 1974, **13**, 5373.

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