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Preparation and antiviral properties of new acyclic, achiral nucleoside analogues: 1- or 9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]nucleobases and 1- or 9-[2,3-dihydroxy-2-(hydroxymethyl)-propyl]nucleobases

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Acyclic, achiral nucleoside derivatives **1b**–**e** of adenine, cytosine, 5-methylcytosine, and guanine, containing a 3-hydroxy-2-(hydroxymethyl)prop-1-enyl group on *N*-1 or *N*-9, have been prepared analogously to the previously described thymine derivative **1a**. In contrast to the adenine and guanine derivatives, the cytosine derivative **9** was unstable, and was obtained in a low yield due to side reactions. These include cleavage of the propenyl group from the base, and the formation of a bicyclic compound. The thymine derivative, although stable under neutral conditions, likewise underwent a reversible cyclization reaction (Michael addition) in the presence of acids or bases. The 5-methylcytosine derivative was stable under neutral and basic conditions. Four other nucleoside derivatives **26a**–**d** containing a 2,3-dihydroxy-2-(hydroxymethyl)propyl group on *N*-1 or *N*-9, three of which are new, have likewise been prepared. All compounds were evaluated as antiviral agents against HIV-1 and HSV-1 but were devoid of antiviral activity.

Introduction

Nucleoside analogues, as well as modified oligonucleotides, have been widely used in recent years to regulate viral growth and cellular gene expression.[1] A host of analogues have been prepared and evaluated in order to obtain compounds with improved stability towards enzymatic cleavage, better cell membrane penetration, and higher and more selective binding to their substrates, when compared with their natural counterparts. In the antiviral field, successful nucleoside analogues have often been found accidentally, since it is difficult to design compounds that are sufficiently selective towards viral enzymes. Although many are close analogues of nucleosides, *e.g.* the HIV drug 3′-azido-2′,3′dideoxythymidine (AZT),[2] others are acyclic analogues, *e.g.* the herpes virus drug acyclovir.[3] In the antisense and antigene field, downregulation of gene expression follows more recognized patterns, such as established rules for efficient Watson–Crick binding and base stacking, a good knowledge of the factors which lead to nuclease stability, and the importance of preorganization. In the antisense field, the first successfully modified oligonucleotides were conservatively modified compounds like phosphorothioates and methylphosphonates, but in recent years many highly modified oligonucleotides with improved binding properties have been developed, *e.g.* PNA,[4] LNA,[5] and anhydrohexitol oligonucleotides.[6]

Recently we prepared a new acyclic, achiral nucleoside analogue, 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine (Fig. 1, **1a**).[7] This analogue is quite different from a normal thymine nucleoside, but molecular models and geometry calculations indicate that it could be a good nucleoside mimic.[8] However, preliminary binding studies of oligonucleotides containing one or two molecules of **1a** towards complementary DNA and RNA strands showed a reduced binding (ΔT_m −2 to −6.5 °C per single introduced nucleotide modification).[9] In order to investigate the potential of this type of nucleoside analogue in more detail we decided to prepare and study four other nucleobase analogues. This paper describes an improved synthesis of **1a**, the synthesis of the cytosine analogue **1b**, the 5-methylcytosine analogue **1c**, the adenine analogue **1d**, the guanine analogue **1e**, the synthesis of some related trihydroxyalkylnucleosides **26a**–**d**, and some of their antiviral properties.

1a B = thymine
1b B = cytosine
1c B = 5-methylcytosine
1d B = adenine
1e B = guanine

26a B = thymine
26b B = cytosine
26c B = adenine
26d B = guanine

Fig. 1

Results and discussion

Preparation of 1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]-pyrimidines

The previously described epoxide route to the thymine derivative **1a**[7] is shown in Scheme 1. This route gave **1a** in a reasonable yield, but the elimination step was found to give highly variable yields of **5a** depending on the quality of the base, potassium *tert*-butoxide. When potassium *tert*-butoxide was very dry, the yield of **5a** was approximately 40% after purification. However, samples of potassium *tert*-butoxide that had absorbed water gave no **5a** but only the isomeric alkenes **6**. Contrary to our first report,[7] **6** could not be isomerized to **5a** by treatment with strong bases. Better results have now been obtained using sodium *tert*-amyloxide, freshly prepared from

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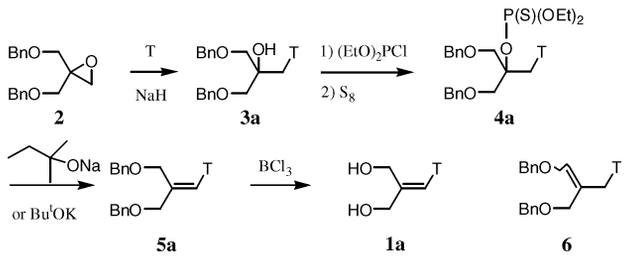
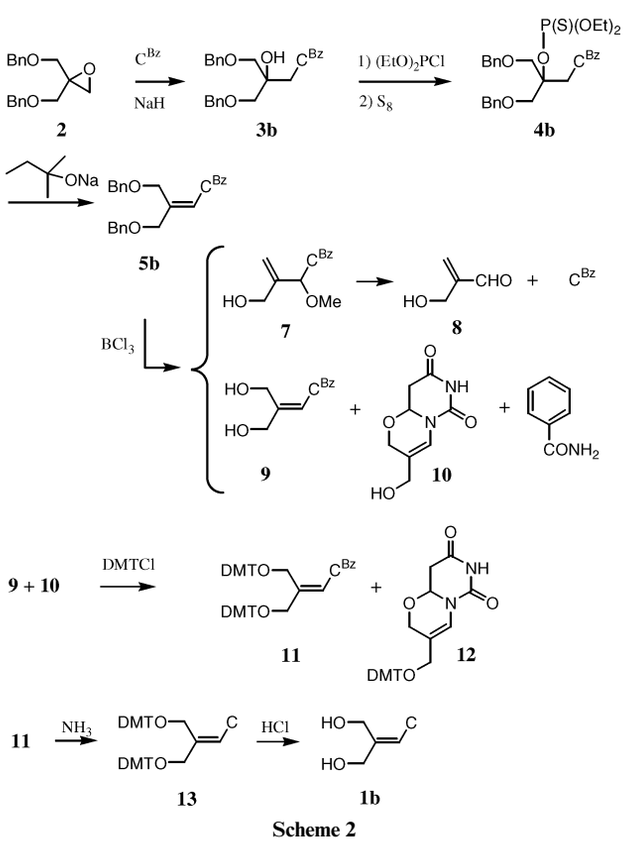


Scheme 1

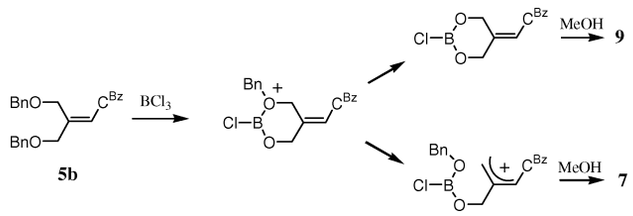
anhydrous *tert*-amyl alcohol and NaH in dry toluene, instead of potassium *tert*-butoxide. This base gave **5a** reproducibly in a 50–55% yield after purification. The last step, removal of the benzyl groups, was also improved. **1a** Was found to be somewhat unstable in the strongly acidic solution produced by methanolysis of excess boron trichloride. However, it was obtained in a reproducible yield of *ca*. 65% after purification when the reaction mixture was concentrated and neutralized quickly, as described in the Experimental section.

A similar synthesis of the cytosine derivative **1b** was attempted from *N*-4-benzoylcytosine[10] (C^{Bz}, Scheme 2). The preparation of **3b**, **4b**, and **5b** proceeded just as well as for the analogous thymine derivatives. However, when the thymine procedure (6 mol eq. of BCl_3 in CH_2Cl_2, 4 h at −78 °C) to convert **5b** to **9** was attempted, only C^{Bz} was isolated from a mixture of products that only contained small amounts of **9**. Under modified conditions (10 mol eq. of BCl_3 in CH_2Cl_2, 1 h at 0 °C), **9** could be obtained in a yield of approximately 35% together with a mixture of several other compounds. The crude mixture was partitioned between $CHCl_3$ and H_2O, and **9** and an unexpected compound **10** (see below for the structure elucidation of **10**) were isolated from the aq. phase as a mixture (3 : 1 according to NMR), from which only **10** could be crystallized in a pure state. However, after conversion to the 4,4′-dimethoxytrityl (DMT) derivatives **11** and **12** the mixture could be separated by chromatography, and deprotection of **11** produced **1b** as the hydrochloride. Of the other products **7** and benzamide were isolated from of the $CHCl_3$ phase by chromatographic separation.



Scheme 2

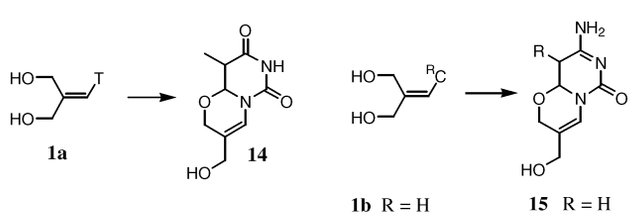
The structure of **7** was ascertained by ^{1}H NMR and MS, and by its slow hydrolysis to C^{Bz} and presumably **8**.[11] Thus the ^{1}H NMR spectrum (DMSO-d_6) of **7** showed two vinylic proton signals, one primary alcohol, and one methoxy group. Since the compound is an aminal it should hydrolyze easily to the aldehyde **8** and C^{Bz} as was observed. The formation of **7** from **5b** and BCl_3 can be rationalized as follows (Scheme 3). Removal of one benzyl group from **5b** gives an intermediate that can react in two different ways, to give either the cyclic boron ester which leads to **9** or a stabilized carbocation which leads to **7** after quenching with MeOH. Apparently the reaction proceeds mainly *via* the stabilized carbocation at −78 °C, whereas at 0 °C a substantial part of the reaction occurs *via* the cyclic ester to give **9**. However, the *N*-4 unprotected analogue of **5b** reacted with BCl_3 at 0 °C solely *via* the cyclic ester to give **1b** and **10** (data not shown).



Scheme 3

The structure of **10** was determined by ^{1}H NMR, MS, and elemental analysis. Characteristic NMR signals are one OH signal only, the ABX system for the 5- and 6-protons of the perhydropyrimidine ring, the AB system for the CH_2 group in the other ring, and the multiplet signal for the C*H*$_2$OH. The absence of aromatic signals and the presence of only one NH signal shows that the benzamide group has been hydrolysed to an oxo group, *i.e.*, that the compound is a reduced uracil derivative. The bicyclic system is undoubtedly formed by a Michael addition of one of the OH groups of **9** to the 5,6-double bond of cytosine; whether the 4-NHBz group is removed before or after the ring closure is not known.

The Michael addition leading to bicyclic compounds like **10** to our knowledge has no close equivalents in the literature, but conjugate addition of good nucleophiles to the 5,6-double bond of cytosine or uracil derivatives is well-known.[12] Compounds **1a** and **1b** both undergo a similar Michael addition reaction to give **14** and **15**, respectively (Scheme 4). The rate of cyclization, however, is very different for **1a** and **1b**·**HCl**.

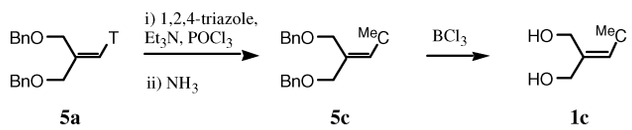


Scheme 4

Thus, **1a** is very stable in solution under neutral conditions, but both acids and bases catalyze the formation of **14**. Triethylamine, but not pyridine, gave, after a few hours in MeOH at rt, an equilibrium mixture of **1a** and **14** from which **14** could be precipitated and characterized. *p*-Toluenesulfonic acid in MeOH likewise catalyzed the reaction, although more slowly, and the reaction was less clean. In contrast **1b** (as its hydrochloride) is unstable in solution and was found to partly cyclize to **15** (and/or the corresponding uracil derivative **10**) during an overnight recording of its ^{13}C NMR spectrum in CD_3OD. Apparently the 5-CH_3 group of thymine protects the double

bond from the Michael addition of weak nucleophiles sufficiently to make **1a** stable under neutral or near neutral conditions. Based on these observations we decided to prepare the 5-methylcytosine (^{Me}C) derivative **1c**.

The 5-methylcytosine derivative **1c** was prepared in two steps from the thymine derivative **5a** (Scheme 5). The 4-oxo group of **5a** was transformed to an amino group using standard methods[13] to give **5c**, which could be debenzylated with BCl_3 to give **1c** in 70% yield. Unlike **1a** and **1b.HCl**, **1c** was stable in solution under both neutral and basic conditions. However, the *N*-4-benzoyl derivative of **1c** was prone to cyclization (data not shown).



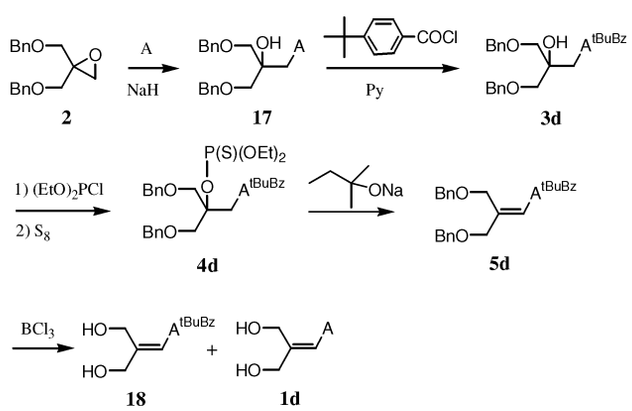
5a **5c** **1c**

Scheme 5

At present we do not fully understand the factors which govern the tendency of **1a**–**c** and their derivatives to cyclize. A more thorough investigation of this interesting reaction is under way.

Preparation of 9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]-purines

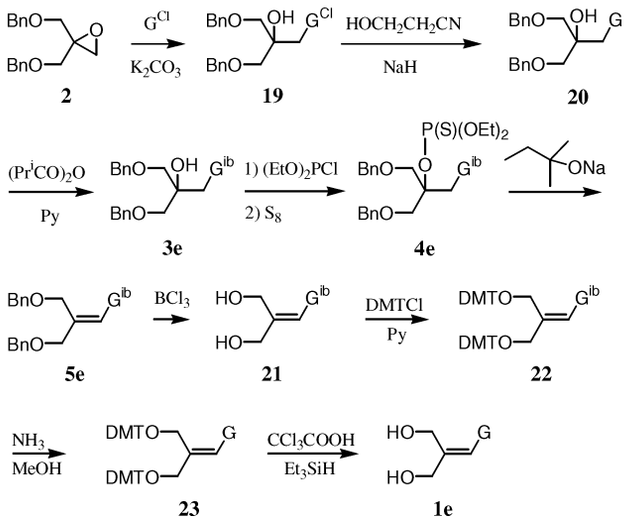
The procedure developed for thymine worked well for adenine (Scheme 6). When **2** was treated with adenine the main product was **17** together with the *N*-7 isomer that was easily removed by column chromatography. Purified **17** was protected on *N*-6 with a 4-*tert*-benzoyl group to give **3d**; this protection group gave nucleosides that were more soluble than when a benzoyl group was employed. The remaining steps proceeded satisfactorily to give a mixture of **18** and the *N*-6 unprotected product **1d** that were separated by column chromatography.



2 **17** **3d** **4d** **5d** **18** **1d**

Scheme 6

The guanine derivative **1e** was prepared from **2** and 2-amino-6-chloropurine (Scheme 7). This reaction gave **19** without discernible amounts of the *N*-7 isomer, whereas *N*-2-isobutyryl-guanine and **2** gave a mixture of the *N*-7 and the *N*-9 isomer with the former dominating. **19** was transformed to the guanine derivative **20** with 3-hydroxypropionitrile and NaH[14] and the product protected at *N*-2 with isobutyric anhydride to give **3e**. Phosphitylation and oxidation with sulfur to **4e** proceeded as usual, but the elimination to **5e** was at first troublesome. When **4e** was dried by coevaporation with pyridine, traces of pyridine gave rise to a mixture of **5e** and the regioisomeric alkenes (analogous to **6**, Scheme 1). Since these alkenes were difficult to separate it was important to obtain a highly regioselective elimination. Fortunately it was possible to remove traces of pyridine from **4e** by co-evaporation with toluene and thereby obtain pure **5e**. Removal of the benzyl groups to give **21** proceeded well with BCl_3, however, the product was difficult to purify due to its high polarity. Crude **21** was therefore transformed to the bis-dimethoxytrityl derivative **22** which after removal of the isobutyryl group gave **23**. From **23** the unprotected **1e** could be isolated by precipitation from a CH_2Cl_2 solution upon addition of CCl_3COOH and Et_3SiH.[15] **1e** Has previously been described in a Japanese patent without characterization, apart from ^{1}H NMR.[16]

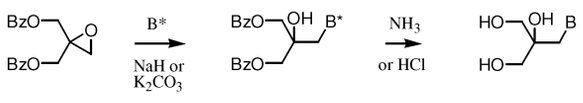


2 **19** **20** **3e** **4e** **5e** **21** **22** **23** **1e**

Scheme 7

Preparation of 1- or 9-[2,3-dihydroxy-2-(hydroxymethyl)propyl]-nucleobases

The trihydroxyalkyl nucleobase derivatives **26a**–**d** (Scheme 8) could be prepared from the dibenzyl protected epoxide **2**, but a simpler route was developed from the dibenzoyl protected epoxide **24** (Scheme 8). The epoxide **24** has been reported as a by-product previously without proper characterization.[17] We prepared it in two steps starting from 3-chloro-2-(chloromethyl)propene in an overall yield of 70%. The reactions of **24** with an excess of thymine, *N*-4-benzoylcytosine, *N*-6-benzoyladenine, or 2-amino-6-chloropurine and a base predominantly gave the *N*-1 or *N*-9 substituted products **25a**–**d** together with small amounts (0–10%) of regioisomers or dialkylated products. The alkylations to give **25a**–**b** were shown to have occurred at *N*-1 by NOE, and **25c**–**d** were shown to be the *N*-9 alkylated products by comparison of their ^{13}C and ^{1}H NMR data with those of known *N*-9 alkylated compounds.[18,19] Treatment with ammonia removed the benzoyl groups of **25a**–**c** to give **26a**–**c**, and **25d** was converted to **26d** with hydrochloric acid. The adenine compound **26c** has been prepared previously by another route.[20]



24 **25a-d** **26a-d**

25a B* = thymine
25b B* = *N*-4-benzoylcytosine
25c B* = *N*-6-benzoyladenine
25d B* = 2-amino-6-chloropurine

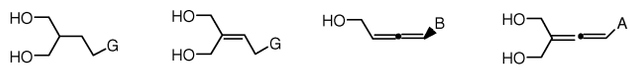
26a B = thymine
26b B = cytosine
26c B = adenine
26d B = guanine

Scheme 8

Antiviral activity

Compounds **1a**, **1c**–**e**, and **26a**–**d** were tested against HIV-1 in MT4 cell cultures infected with wildtype HIV-1 (strain IIIB), and against HSV-1 in vero cells, as described earlier.[21,22] All compounds were devoid of any activity at 100 μM and showed little or no cytotoxicity. The lack of activity might be due to the

relatively short distance between the hydroxy groups and the nucleobases in **1** and **26**. Thus analogues with one additional carbon atom are active against a variety of viruses, *e.g.*, penciclovir **27** (Fig. 2) is active against HSV-1, HSV-2, and VZV,[1a] 9-[4-hydroxy-3-(hydroxymethyl)-2-butenyl]guanine **28** (but not the adenine analogue) is active against HSV-2,[23] and R-Adenallene **29** and R-Cytallene **30** (but not the guanine or thymine analogues) are active against HIV-1.[1a] However, the closely related **31** is devoid of antiviral activity.[24]



27 **28** **29** B = A, **30** B = C **31**

Fig. 2

Conclusion

An epoxide route, previously developed for the thymine DNA analogue **1a**, has been shown to be useful for the preparation of similar DNA analogues **1d** and **1e** derived from adenine and guanine. In the case of cytosine the last synthetic step was low yielding due to several side reactions, and the analogue **1b** as the hydrochloride was unstable in solution. However, the 5-methylcytosine derivative **1c** could be made from the thymine derivative **5a** and was found to be stable. An unexpected propensity of the pyrimidine derivatives to undergo an intramolecular Michael addition to bicyclic compounds **10** and **14**–**16** was discovered. The compounds **1a**, **1c**–**1e**, and some related saturated derivatives **26a**–**d**, were evaluated as antiviral agents against HIV-1 and HSV-1, but were found to be without activity at 100 μM. Work is in progress to build **1c**–**1e** into oligodeoxyribonucleotides in order to evaluate their potential as modifying units in antisense oligonucleotides.

Experimental

2,2-Bis(benzyloxymethyl)oxirane (**2**),[7] 1-[3-benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]thymine (**3a**),[7] 1-[3-benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]-thymine (**4a**),[7] and *N*-4-benzoylcytosine,[10] were prepared according to literature procedures. Other chemicals were 97–99% pure from Aldrich, Fluka, Sigma, or Merck, unless otherwise stated. Solvents were HPLC grade from LABSCAN, of which CH_2Cl_2, DMF, pyridine, *tert*-amyl alcohol and toluene were dried over molecular sieves (4 Å from Grace Davison) and THF freshly distilled from Na-benzophenone to a water content below 20 ppm, measured on a Metrohm 652 KF-coulometer. TLC was run on Merck 5554 silica 60 aluminium sheets, LC on either Merck 9385 silica 60 (0.040–0.063 mm) for normal gravity and flash chromatography, or Merck 15111 silica 60 (0.015–0.040 mm) for dry column vacuum chromatography.[25] NMR spectra (reference tetramethylsilane for δ_H and δ_C, external 85% H_3PO_4 for δ_P, *J* values are given in Hz) were run on a Varian Mercury 300 MHz spectrometer, and FAB MS data obtained on a JEOL HX 110/110 mass spectrometer with m-NBA as the matrix.

1-[3-Benzyloxy-2-(benzyloxymethyl)prop-1-enyl]thymine 5a

To dry *tert*-amyl alcohol (3.50 g, 40 mmol) in dry toluene (40 ml) was added NaH (60% in oil, 1.60 g, 40 mmol) and the mixture was stirred at rt for 1 h. A solution of 1-[3-benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]-thymine (**4a**) (4.50 g, 8.0 mmol, dried by co-evaporation with dry toluene) in dry toluene (40 ml) was added, and the mixture stirred at rt for 3 days. The mixture was quenched with 4 M aq. HCl (20 ml) and the solvents removed *in vacuo*. Purification by flash column chromatography, eluted with CH_2Cl_2–EtOAc 3 : 2 v/v, followed by crystallisation from EtOAc–heptane, gave **5a** (1.66 g, 53%) as colourless crystals, mp 92–94 °C (lit.[7] mp 92–94 °C).

1-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]thymine 1a

To a stirred solution of **5a** (1.18 g, 3.0 mmol) in dry CH_2Cl_2 (90 ml) at −78 °C under N_2 was added dropwise BCl_3 (1 M in CH_2Cl_2, 18.0 ml, 18 mmol) during 5 min. Stirring was continued at −78 °C for 4 h, followed by dropwise addition of MeOH–CH_2Cl_2 (1 : 1 v/v, 25 ml) at −78 °C. The cooling bath was removed and the solvents quickly removed *in vacuo* to give a residue that was immediately dissolved in MeOH–CH_2Cl_2 (1 : 2 v/v, 45 ml), and solid $NaHCO_3$ (approx. 200 mg) was added with stirring in small portions until the solution was neutral on wet pH paper. The solids were removed by filtration and the filtrate concentrated *in vacuo* to give a brown residue that was dissolved in a mixture of hexane (40 ml) and water (15 ml). The aqueous phase was extracted with hexane (2 × 30 ml) followed by concentration of the aqueous phase *in vacuo*. The residue was purified by normal gravity column chromatography, eluted with EtOAc–MeOH 9 : 1 v/v, to give pure **1a** (0.41 g, 64%) as colourless crystals, mp 166–168 °C (lit.[7] mp 166–169 °C).

N-4-Benzoyl-1-[3-benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]cytosine 3b

NaH (60% in oil, 3.6 g, 90 mmol) was added under N_2 to a suspension of *N*-4-benzoylcytosine (19.4 g, 90 mmol) in dry DMF (600 ml) and the mixture stirred for 2 h at rt. Then **2** (12.8 g, 45 mmol) was added and the mixture heated to 110 °C for 24 h. After cooling sat. aq. NH_4Cl (250 ml) and H_2O (100 ml) were added and the mixture stirred for 15 min. The precipitate was removed by filtration and washed with CH_2Cl_2 (6 × 100 ml). The filtrate was extracted with EtOAc (650 ml + 4 × 250 ml) and the combined organic phases wre dried (Na_2SO_4) followed by evaporation *in vacuo.* The residue was suspended in EtOAc (300 ml) and filtered through silica gel. The silica gel was washed extensively with EtOAc and the filtrate evaporated *in vacuo* to pale yellow crystals that were recrystallized from EtOAc–heptane to give pure **3b** (15.4 g, 68%) as colourless crystals, mp 143–144.5 °C. NMR ($CDCl_3$): δ_H 9.1 (1H, br s, NH), 7.94 (2H, d, *J* 7, Ar),7.71 (1H, d, *J* 7, H-6), 7.60 (1H, t, *J* 7, Ar), 7.50 (2H, d, *J* 7, Ar), 7.40–7.25 (11H, m, Ar + H-5), 4.51 (4H, s, PhCH_2), 4.45 (1H, s, OH), 4.20 (2H, s, NCH_2), 3.51 (4H, AB system, Δ 14.7 Hz, J_{AB} 9.7, BnOCH_2). δ_C 166.5, 162.1, 157.0, 150.7, 137.4, 132.9, 132.7, 128.7, 128.1, 128.0, 127.5, 127.4, 96.3, 74.3, 73.3, 71.9, 54.0. FAB$^+$MS: 500.2 (M + H^+ calc. 500.2) (Found: C, 69.7; H, 5.95; N, 8.5. Calc. for $C_{29}H_{29}N_3O_5$: C, 69.7; H, 5.85; N, 8.4%).

N-4-Benzoyl-1-[3-benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]cytosine 4b

To a stirred solution of **3b** (5.00 g, 10.0 mmol) in dry pyridine (50 ml) under N_2 at rt was added dropwise diethyl phosphorochloridite (1.65 ml, 11.5 mmol). After 45 min S_8 (0.40 g, 12.5 mmol S) was added, and the mixture was stirred for 1.5 h, followed by evaporation *in vacuo*. The residue was dissolved in CH_2Cl_2 (250 ml) and the solution extracted with sat. aq. $NaHCO_3$ (2 × 100 ml), brine (100 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was crystallized from EtOAc–hexane to give **4b** (4.05 g, 62%) as colourless crystals, mp 102–103 °C. NMR ($CDCl_3$): δ_H 8.5 (1H, br s, NH), 8.0 (3H, m, Ar + H-6), 7.65 (1H, t, *J* 7, Ar), 7.55 (2H, d, *J* 7, Ar), 7.35–7.20 (11H, m, Ar + H-5), 4.51 (2H, s, NCH_2), 4.49 (4H, AB system, Δ 20.1 Hz, J_{AB} 11.4, PhCH_2), 4.14–4.02 (6H, m, Et + BnOCH_A), 3.82 (2H, d, J_{AB} 10.3, BnOCH_B), 1.25 (6H, t, *J* 6.9, Et). δ_C 161.9, 150.2 br, 137.6, 133.1, 129.0 128.2, 127.8, 127.7, 127.5, 86.2 (d, J_{PC} 9), 73.5, 69.9, 64.5 (d, J_{PC} 6), 51.8, 15.8 (d, J_{PC} 8). δ_P 59.2.

FAB$^+$ MS: 652.5 (M + H$^+$ calc. 652.2)(Found: C, 60.6; H, 5.9; N 6.4; S, 4.8. Calc. for $C_{33}H_{38}N_3O_7PS$: C, 60.8; H, 5.9; N, 6.45; S, 4.9%).

N-4-Benzoyl-1-[3-benzyloxy-2-(benzyloxymethyl)prop-1-enyl]-cytosine 5b

To a stirred solution of dry *tert*-amyl alcohol (0.80 ml, 6 mmol) in dry toluene (30 ml) under N_2 at rt was added NaH (60% in oil, 0.24 g, 6.0 mmol). After stirring for 1 h **4b** (0.78 g, 1.2 mmol) was added, and the stirring was continued for 4 h at rt, followed by neutralization with 2 M aq. HCl at 0 °C. $CHCl_3$ (90 ml) was added, and the mixture washed with sat. aq. $NaHCO_3$ (3 × 50 ml) and brine (50 ml). The organic phase was dried ($MgSO_4$), and the solvent removed *in vacuo* to give light yellow crystals. Purification by flash column chromatography, eluted with CH_2Cl_2–MeOH 97 : 3 v/v, gave **5b** (0.302 g, 52%) as colourless crystals, mp 153–154 °C. NMR ($CDCl_3$): δ_H 8.9 (1H, br, NH), 7.93 (2H, d, *J* 7, Ar), 7.78 (1H, d, *J* 7.3, H-6), 7.60 (1H, t, *J* 7, Ar), 7.50 (2H, d, *J* 7, Ar), 7.45–7.25 (11H, m, Ar + H-5), 6.94 (1H, br t, N–CH=C), 4.57 and 4.49 (2 × 2H, 2 × s, PhCH_2), 4.19 (2H, d, *J* 1.2, BnOCH_2), 4.02 (2H, s, BnOCH_2). FAB$^+$ MS: 482.1 (M + H$^+$ calc. 482.2) (Found: C, 71.7; H, 5.6; N, 8.7. Calc. for $C_{29}H_{27}N_3O_4$: C, 72.3; H, 5.65; N, 8.7%).

N-4-Benzoyl-1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]cytosine 9, 6,8-dioxo-3-(hydroxymethyl)-7,8,9,9a-tetrahydro-2*H*,6*H*-pyrimido[6,1-b][1.3]oxazine 10, and *N*-4-benzoyl-1-(3-hydroxy-1-methoxy-2-methylenepropyl)cytosine 7

To a stirred solution of **5b** (0.748 g, 1.55 mmol) in dry CH_2Cl_2 (30 ml) under N_2 at 0 °C was added dropwise BCl_3 (1 M in CH_2Cl_2, 15 ml, 15 mmol). After stirring for 1 h at 0 °C, MeOH–CH_2Cl_2 (1 : 1 v/v, 5 ml) was added, and the solvents removed *in vacuo*. The residue was dissolved in MeOH–CH_2Cl_2 (1 : 1 v/v, 20 ml) and solid $NaHCO_3$ was added to reach pH 6–7. The solids were removed by filtration and washed with MeOH–CH_2Cl_2 (1 : 1 v/v, 25 ml). The combined filtrates were concentrated *in vacuo*, and the residue partitioned between H_2O (25 ml) and $CHCl_3$ (25 ml). Evaporation of the aqueous phase gave a mixture of **9** and **10** as colourless crystals (0.200 g, *ca*. 3 : 1 estimated from ^{1}H NMR, *ca*. 35% **9** and 12% **10**).

9: NMR (DMSO-d_6): δ_H 11.28 (1H, br, NH), 8.01 (3H, d, *J* 7, Ar + H-6), 7.63 (1H, t, *J* 7, Ar), 7.52 (2H, t, *J* 7, Ar), 7.32 (1H, d, *J* 7, H-5), 6.66 (1H, br, N–CH=C), 5.11 (1H, t, *J* 4, OH), 5.04 (1H, br t, OH), 4.15 (2H, d, *J* 3.5, CH_2C=CH), 3.96 (2H, d, *J* 4, CH_2C=CH).

10: An analytical sample was obtained by recrystallization of the mixture of **9** and **10** from 2-PrOH–MeOH–ether, mp 165–166 °C. NMR (DMSO-d_6): δ_H 10.63 (1H, br, NH), 6.98 (1H, s, N–CH=C), 5.12 (1H, X of ABX system, J_{AX} 5.9, J_{BX} 9.4, O–CH–N), 4.86 (1H, t, *J* 5.5, OH), 4.25 (2H, AB system, Δ 34.3 Hz, J_{AB} 15.8, OCH_2 (ring)), 3.93 (2H, m, CH_2OH), 2.83 (2H, AB of ABX system, Δ 20.3 Hz, J_{AB} 16.7, J_{AX} 5.9, J_{BX} 9.4, CH_2C=O). FAB$^+$ MS: 199.0 (M + H$^+$ calc. 199.1) (Found: C, 47.8; H, 4.95; N, 13.8. Calc. for $C_8H_{10}N_2O_4$: C, 48.5; H, 5.1; N, 14.15%).

The $CHCl_3$ phase was dried ($MgSO_4$), concentrated, and the residue separated by normal gravity column chromatography, eluted with CH_2Cl_2–MeOH (95 : 5 v/v), to give **9** (trace), **10** (5%), benzamide (16.5%) and **7** (20%).

7: NMR (DMSO-d_6): δ_H 11.31 (1H, br, NH), 7.99 (2H, d, *J* 7, Ar), 7.87 (1H, d, *J* 7, H-6), 7.62 (1H, t, *J* 7, Ar), 7.51 (2H, t, *J* 7, Ar), 7.38 (1H, d, *J* 7, H-5), 6.16 (1H, s, NC*H*–OMe), 5.34 and 5.16 (2 × 1H, 2 × d, *J* 1.2 and 1.2, C=CH_2), 4.98 (1H, t, *J* 5.5, OH), 3.90 (2H, m, CH_2–OH), 3.30 (3H, s, O–CH_3). FAB$^+$ MS: 316.0 (M + H$^+$ calc. 316.1). On standing in $CDCl_3$ solution *N*-4-benzoylcytosine precipitated, and a new set of signals, assigned the structure **8** appeared in the NMR spectrum of **7**. **8**: NMR ($CDCl_3$): δ_H 9.62 (1H, s, HC=O), 6.49 and 6.13 (2 × 1H, 2 × s, C=CH_2), 4.39 (2H, s, CH_2–OH).

N-4-Benzoyl-1-[3-dimethoxytrityloxy-2-(dimethoxytrityloxymethyl)prop-1-enyl]cytosine 11 and 3-(dimethoxytrityloxymethyl)-6,8-dioxo-7,8,9,9a-tetrahydro-2*H*,6*H*-pyrimido[6,1-*b*][1.3]oxazine 12

To a solution of **9** and **10** (0.087 g, *ca*. 3 : 1) in dry pyridine (2 ml) under N_2 was added dimethoxytrityl chloride (0.39 g, 1.15 mmol), and the mixture was stirred for 1.5 h at rt followed by concentration *in vacuo*. The residue was partitioned between CH_2Cl_2 (10 ml) and H_2O (10 ml), and the organic phase was extracted with sat. aq. $NaHCO_3$ (3 × 5 ml), brine (5 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification of the residue by normal gravity column chromatography, eluted with EtOAc–hexane 3 : 2 v/v, gave pure **12** (0.020 g, 0.04 mmol, 14%), R_f 0.39 (EtOAc–hexane 3 : 2 v/v), then pure **11** (0.120 g, 0.13 mmol, 46%) as colourless crystals, R_f 0.22 (EtOAc–hexane 3 : 2 v/v). **11**: NMR ($CDCl_3$): δ_H 8.6 (1H, br, NH), 7.90 (2H, d, *J* 7, Ar), 7.65–7.15 (23H, m, Ar + H-5 + H-6), 7.04 (1H, br, N–CH=C), 6.85 (4H, d, *J* 8.8, Ar), 6.75 (4H, d, *J* 8.8, Ar), 3.93 (2H, d, *J* 1.2, CH_2C=CH), 3.79 and 3.73 (2 × 6H, 2 × s, OCH_3), 3.59 (2H, s, CH_2C=CH). FAB$^+$ MS: 906.6 (M + H$^+$ calc. 906.4). **12**: NMR ($CDCl_3$): δ_H 7.84 (1H, br, NH), 7.4–7.2 (9H, m, Ar), 7.07 (1H, br s, N–CH=C), 6.84 (4H, d, *J* 9.1, Ar), 5.05 (1H, X of ABX system, J_{AX} 5.3, J_{BX} 10.3, O–CH–N), 4.35 (2H, br AB system, Δ 19.3 Hz, J_{AB} 16.0, OCH_2 (ring)), 3.80 (6H, s, OCH_3), 3.64 (2H, AB system, Δ 8.8 Hz, J_{AB} 11.7, DMTOCH_2), 2.91 (2H, AB of ABX system, Δ 61.2 Hz, J_{AB} 17.0, J_{AX} 5.3, J_{BX} 10.3, CH_2C=O).

1-[3-dimethoxytrityloxy-2-(dimethoxytrityloxymethyl)prop-1-enyl]cytosine 13

To a solution of **11** (0.12 g, 0.13 mmol) in 2-PrOH (3 ml + a small amount of CH_2Cl_2) was added conc. aq. NH_3 (3 ml), and the solution was stirred overnight at rt, followed by concentration *in vacuo*. The residue (light yellow crystals) was used without purification. NMR ($CDCl_3$, selected signals): δ_H 7.11 (1H, d, *J* 7.3, H-6), 6.95 (1H, s, N–CH=C), 5.28 (1H, d, *J* 7.3, H-5), 3.87 (2H, s, CH_2C=CH), 3.78 and 3.76 (2 × 6H, 2 × s, OCH_3), 3.61 (2H, s, CH_2C=CH). FAB$^+$ MS: 802.5 (M + H$^+$ calc. 802.3).

1-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]cytosine 1b hydrochloride

To crude **13** (0.058 g, containing *ca*.0.063 mmol of **13**) in CH_2Cl_2 (4 ml) at rt was added dropwise HCl (4 M in dioxane) until **13** had disappeared (TLC). Ether (1 ml) was added and the precipitate isolated by centrifugation and washed with CH_2Cl_2 and ether to give pure **1b** as the hydrochloride (0.014 g, 0.06 mmol, *ca*. 95%) as colourless crystals. NMR (CD_3OD): δ_H 7.89 (1H, d, *J* 7.7, H-6), 6.61 (1H, br t, N–CH=C), 6.10 (1H, d, *J* 7.7, H-5), 4.26 (2H, d, *J* 1.5, CH_2C=CH), 4.14 (2H, s, CH_2C=CH). FAB$^+$ MS: 198.1 (M + H$^+$ calc. 198.1).

6,8-Dioxo-3-(hydroxymethyl)-9-methyl-7,8,9,9a-tetrahydro-2*H*,6*H*-pyrimido[6,1-*b*][1.3]oxazine 14

To a solution of **1a** (0.054 g, 0.25 mmol) in MeOH (2 ml) was added triethylamine (0.10 ml, 0.7 mmol). After stirring at rt overnight the precipitate was isolated by filtration, washed with MeOH (0.5 ml) and dried to give practically pure **14** (0.018 g, 33%) as colourless crystals. The MeOH solution contained a *ca*. 55 : 45 mol% mixture of **1a** and **14** (according to ^{1}H NMR in DMSO-d_6 after removal of MeOH). An analytical sample of **14** was obtained by recrystallization from 2-PrOH, mp 197–198 °C. NMR (DMSO-d_6): δ_H 10.61 (1H, s, NH), 6.98 (1H, s, N–CH=C), 4.85 (1H, t, *J* 5.5, OH), 4.79 (1H, d, *J* 9.9, O–CH–N), 4.28 (2H, AB system, Δ 18.4 Hz, J_{AB} 15.7, OCH_2 (ring)), 3.98–3.86 (2H, m, CH_2OH), 2.81 (1H, dq, *J* 9.9 and 6.9, C*H*CH_3), 1.19 (3H, d, *J* 6.9, CH_3). δ_c 169.9, 148.0, 120.0, 117.3, 83.7, 65.1, 59.8, 39.9, 10.4. FAB$^+$ MS: 213.1 (M + H$^+$ calc.

213.1) (Found: C, 51.2; H, 5.9; N, 12.9. Calc. for $C_9H_{12}N_2O_4$: C, 50.9; H, 5.7; N, 13.2%).

1-[3-Benzyloxy-2-(benzyloxymethyl)prop-1-enyl]-5-methylcytosine 5c

To a stirred solution of **5a** (0.988 g, 2.52 mmol) in CH_3CN (50 ml) under N_2 at 0 °C was added Et_3N (3.5 ml, 25 mmol), 1,2,4-triazole (1.76 g, 25.4 mmol), and $POCl_3$ (0.48 ml, 5.2 mmol). Stirring was continued at 0 °C for 15 min and then at rt for 4 h, the mixture was then poured into sat. aq. $NaHCO_3$ (50 ml) and ice, and extracted with EtOAc (3 × 25 ml). The organic phase was extracted with brine (100 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in CH_3CN (50 ml) and 25% aq. ammonia (50 ml) was added. After stirring for 4 h at rt, brine (50 ml) was added and the mixture was extracted with EtOAc (3 × 25 ml). The organic phase was dried (Na_2SO_4), and the solvents removed *in vacuo*. The solid residue was crystallized from EtOAc to give pure **5c** (0.848 g, 86%) as colourless crystals, mp 150–151 °C. NMR (DMSO-d_6) δ_H 7.42 (1H, br. s, NH), 7.38–7.25 (11H, m, Ph + H-6), 6.92 (1H, br s, NH), 6.75 (1H, s, N–CH=C), 4.50 and 4.41 (2 × 2H, 2 × s, PhCH_2), 4.12 and 3.97 (2 × 2H, 2 × s, BnOCH_2), 1.78 (3H, s, CH_3). δ_C 165.7, 154.6, 142.1, 138.1, 137.7, 128.6, 128.1, 128.1, 127.9, 127.6, 127.5, 127.4, 127.4, 100.9, 71.7, 71.1, 69.4, 64.0, 12.9. Fab^+ MS: 392.0 (M + H^+ calc. 392.2) (Found C, 70.0; H, 6.5; N, 10.7. Calc. for $C_{23}H_{25}N_3O_3$ + 1/4H_2O: C, 69.8; H, 6.5; N, 10.7%).

1-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]-5-methylcytosine 1c

To a stirred solution of **5c** (0.254 g, 0.65 mmol) in dry CH_2Cl_2 (8 ml) at −78 °C under N_2 was added dropwise BCl_3 (1 M in CH_2Cl_2, 4.0 ml, 4.0 mmol) during 15 min. Stirring was continued at −78 °C for 4 h, followed by dropwise addition of MeOH–CH_2Cl_2 (1 : 1 v/v, 6 ml) at −78 °C. The cooling bath was removed and the solvents quickly removed *in vacuo* to give a residue that was immediately dissolved in MeOH–CH_2Cl_2 (1 : 2 v/v, 9 ml), and solid $NaHCO_3$ was added with stirring in small portions until the pH reached *ca*. 5 on wet pH paper. The solids were removed by centrifugation and the filtrate concentrated *in vacuo* to give a brown residue that was dissolved in a mixture of heptane (4 ml) and water (15 ml). The aqueous phase was extracted with heptane (2 × 4 ml) followed by concentration of the aq. phase *in vacuo* to give **1c** as the hydrochloride. This was dissolved in hot MeOH (3 ml) and a saturated solution of NaOH in MeOH was added until pH reached 9–10. After standing overnight at −18 °C the crystals were isolated by centrifugation to give pure **1c** (0.096 g, 70%) as colourless crystals, mp 229–230 °C (dec.). NMR (D_2O) δ_H 7.33 (1H, s, H-6), 6.60 (1H, s, N–CH=C), 4.31 and 4.15 (2 × 2H, 2 × s, CH_2–C=CH), 1.98 (3H, s, CH_3). δ_C 166.6, 157.8, 143.6, 138.4, 125.4, 104.4, 61.0, 56.1, 12.2. Fab^+ MS: 212.1 (M + H^+ calc. 212.1) (Found: C, 51.1; H, 6.35; N, 18.7. Calc. for $C_9H_{13}N_3O_3$ + 1/4CH_3OH: C, 50.8; H, 6.4; N, 19.2%).

9-[3-Benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]adenine 17

Dry adenine (12.5 g, 92.5 mmol) was dissolved in dry DMF (400 ml) under N_2 and NaH (60% in oil, 2.0 g, 50 mmol) added. After stirring for 0.5 h **2** (13.9 g, 54 mmol) was added, and the solution stirred at 110 °C for 3 days. The solution was neutralized with sat. aq. NH_4Cl (100 ml), diluted with H_2O (500 ml), and extracted with $CHCl_3$ (4 × 250 ml). The combined organic phases were extracted with brine (2 × 300 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give a yellow oil. Purification by flash column chromatography, eluted with CH_2Cl_2–MeOH 95 : 5 v/v, followed by crystallization from EtOAc–heptane, gave pure **17** as colourless crystals (13.8 g, 60%), mp 176.5–178 °C, R_f 0.24 (EtOAc–MeOH–Et_3N 90 : 8 : 2 v/v/v). NMR ($CDCl_3$): δ_H 8.32 (1H, s, H-2), 7.78 (1H, s, H-8), 7.36–7.23 (10H, m, Ar), 6.02 (2H, br s, NH_2), 4.47 (4H, s, PhCH_2), 4.40 (2H, s, NCH_2), 3.43 (4H, AB system, Δ 7.8 Hz, J 9.3, BnOCH_2). δ_C 155.6, 152.9, 150.8, 142.4, 137.7, 128.6, 128.0, 127.9, 119.3, 74.1, 73.8, 71.8, 48.2. FAB^+ MS: 420.1 (M + H^+ calc. 420.2) (Found: C, 65.8; H, 5.9; N, 16.5. Calc. for $C_{23}H_{25}N_5O_3$: C, 65.9; H, 6.0; N, 16.7%).

N-6-(4-*tert*-Butylbenzoyl)-9-[3-benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]adenine 3d

To a stirred solution of dry **17** (13.7 g, 32.7 mmol) in dry pyridine (200 ml) at rt was added dropwise 4-*tert*-butylbenzoyl chloride (27.7 g, 141 mmol). After 2 h at rt the solution was poured into a mixture of sat. aq. $NaHCO_3$ (25 ml) and H_2O (225 ml), and the product extracted with EtOAc (3 × 300 ml). To convert di- or tribenzoylated byproducts to **3d** the EtOAc solution was concentrated *in vacuo* and the residue dissolved in a mixture of pyridine (325 ml), MeOH (150 ml), H_2O (25 ml) and NaOH (20 g). After 0.5 h at rt NH_4Cl (32 g) was added and solvents were removed *in vacuo*. The residue was treated with $CHCl_3$ (300 ml) and the suspension extracted with H_2O (2 × 200 ml). The organic phase was dried (Na_2SO_4), the solvent removed *in vacuo*, and the residue purified by flash column chromatography, eluted with CH_2Cl_2–MeOH 97.5 : 2.5 v/v, to give **3d** (13.9 g, 73%). A sample was crystallized from EtOAc–heptane to give colourless crystals, mp 114.5–116 °C, R_f 0.57 (EtOAc–MeOH–Et_3N 90 : 8 : 2 v/v/v). NMR ($CDCl_3$): δ_H 9.05 (1H, br s, NH), 8.79 (1H, s, H-2), 7.97 (2H, d, J 8.5, Ar), 7.94 (1H, s, H-8), 7.53 (2H, d, J 8.5, Ar), 7.36–7.20 (10H, m, Ar), 4.49–4.40 (6H, m, PhCH_2 + NCH_2), 3.44 (4H, s, BnOCH_2), 1.36 (9H, s, *tert*-butyl). δ_C 164.5, 156.6, 152.8, 152.6, 149.6, 144.7, 137.5, 131.0, 128.6, 128.1, 127.9, 127.9, 125.9, 122.4, 73.9, 73.8, 71.7, 47.9, 35.3, 31.3. FAB^+ MS: 579.9 (M + H^+ calc. 580.3) (Found: C, 70.05; H, 6.4; N, 12.05. Calc. for $C_{34}H_{37}N_5O_4$: C, 70.45; H, 6.4; N, 12.1%).

N-6-(4-*tert*-Butylbenzoyl)-9-[3-benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]adenine 4d

To a stirred solution of dry **3d** (2.89 g, 5 mmol) in dry pyridine (20 ml) at rt under N_2 was added dropwise diethyl phosphorochloridite (0.94 g, 6.0 mmol). After 2 h at rt S_8 (0.211 g, 6.6 mmol S) was added and the mixture stirred for another 2 h. Pyridine was removed *in vacuo* and the residue in CH_2Cl_2 (50 ml) extracted with sat. aq. $NaHCO_3$ (2 × 25 ml) and brine (25 ml). The organic phase was dried (Na_2SO_4), the solvent removed *in vacuo*, and the residue purified by flash column chromatography, eluted with EtOAc–hexane 1 : 1 v/v, to give **4d** (3.15 g, 86%). A sample was crystallized from EtOAc–heptane to give colourless crystals, mp 81–83 °C, R_f 0.59 (EtOAc–heptane 8 : 2 v/v). NMR ($CDCl_3$): δ_H 9.12 (1H, s, NH), 8.76 (1H, s, H-2), 8.25 (1H, s, H-8), 7.96 (2H, d, J 8.6, Ar), 7.51 (2H, d, J 8.6, Ar), 7.34–7.24 (10H, m, Ar), 4.80 (2H, s, NCH_2), 4.46 (4H, AB system, Δ 10.8 Hz, J 11.6, PhCH_2), 4.07–3.95 (4H, m, $POCH_2$), 3.86 (4H, AB system, Δ 13.2 Hz, J 10.3, BnOCH_2), 1.35 (9H, s, *tert*-butyl), 1.18 (6H, t, J 7.0, $POCCH_3$). δ_C 164.4, 156.4, 152.8, 152.6, 149.4, 144.4, 137.3, 130.9, 128.4, 127.9, 127.8, 127.7, 125.8, 122.4, 85.4 (d, J_{PC} 9), 73.5, 69.4, 64.5 (d, J_{PC} 6), 45.9, 35.1, 31.1, 15.8 (d, J_{PC} 8). δ_P 59.3. FAB^+ MS: 732.9 (M + H^+ calc. 732.3) (Found: C, 63.2; H, 6.5; N, 8.9. Calc. for $C_{38}H_{46}N_5O_6PS$: C, 62.4; H, 6.3; N, 9.6%).

N-6-(4-*tert*-Butylbenzoyl)-9-[3-benzyloxy-2-(benzyloxymethyl)-prop-1-enyl]adenine 5d

4d (0.800 g, 1.09 mmol) Was dried by co-evaporation with dry toluene and dissolved in dry toluene (10 ml) under N_2. Dry *tert*-amyl alcohol (1.2 g, 14 mmol) and NaH (60% in oil, 0.20 g, 5 mmol) were added and the mixture stirred for 3 days at rt.

Volatiles were removed *in vacuo* and the residue suspended in CH_2Cl_2 (50 ml) and washed with sat. aq. $NaHCO_3$ (2 × 25 ml) and brine (25 ml). The organic phase was dried (Na_2SO_4), the solvent removed *in vacuo*, and the residue purified by flash column chromatography, eluted with CH_2Cl_2–EtOAc 3 : 2 v/v, to give **5d** (0.740 g, 95%). A sample was crystallized from EtOAc–heptane to give colourless crystals, mp 108.5–109.5 °C, R_f 0.61 (CH_2Cl_2–EtOAc 3 : 2 v/v). NMR ($CDCl_3$): δ_H 9.31 (1H, s, NH), 8.76 (1H, s, H-2), 8.30 (1H, s, H-8), 7.97 (2H, d, *J* 8.4, Ar), 7.50 (2H, d, *J* 8.4, Ar), 7.36–7.23 (10H, m, Ar), 7.21 (1H, s, N–CH=C), 4.60 (2H, s, PhCH_2), 4.50 (2H, s, PhCH_2), 4.27 (2H, d, *J* 1.2, BnOCH_2), 4.11 (2H, s, BnOCH_2), 1.34 (9H, s, *tert*-butyl). δ_C 164.6, 156.5, 153.0, 152.0, 149.8, 142.8, 137.6, 137.1, 132.2, 130.7, 128.5, 128.0, 127.9, 125.8, 122.7, 120.2, 73.3, 72.8, 70.3, 64.9, 35.1, 31.1. FAB^+ MS: 562.5 (M + H^+ calc. 562.3) (Found: C, 72.0; H, 6.2; N, 12.4. Calc. for $C_{34}H_{35}N_5O_3$: C, 72.7; H, 6.3; N, 12.5%).

N-6-(4-*tert*-Butylbenzoyl)-9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]adenine 18 and 9-[3-hydroxy-2-(hydroxymethyl)prop-1-enyl]adenine 1d

To a stirred solution of **5d** (2.70 g, 4.81 mmol) in dry CH_2Cl_2 (100 ml) at −78 °C under N_2 was added dropwise BCl_3 (1 M in CH_2Cl_2, 28 ml, 28 mmol) during 10 min. Stirring was continued at −78 °C for 4 h, followed by dropwise addition of MeOH–CH_2Cl_2 (1 : 1 v/v, 50 ml) at −78 °C. The cooling bath was removed and solid $NaHCO_3$ (4.5 g) was added with stirring. Volatiles were removed *in vacuo* and the solid extracted with hexane (2 × 50 ml), followed by dry pyridine (2 × 100 ml). The pyridine solution was evaporated, and the residue purified by normal gravity column chromatography, eluted with toluene–MeOH–Et_3N 79 : 20 : 1 v/v/v, to give **18** (0.37 g, 20%) and **1d** (0.27 g, 25%). Both compounds contained some salts and were further purified by recrystallization. **18**, mp 235–236 °C (from MeOH), R_f 0.33 (toluene–MeOH–Et_3N 79 : 20 : 1 v/v/v). NMR (DMSO-d_6): δ_H 11.12 (1H, s, NH), 8.75 (1H, s, H-2), 8.54 (1H, s, H-8), 8.00 (2H, d, *J* 8.5, Ar), 7.57 (2H, d, *J* 8.5, Ar), 7.02 (1H, s, N–CH=C), 5.25 (1H, t, *J* 5.4, OH), 5.13 (1H, t, *J* 5.4, OH), 4.27 (2H, d, *J* 5.4, CH_2), 4.05 (2H, d, *J* 5.4, CH_2), 1.34 (9H, s, *tert*-butyl). δ_C 165.34, 155.37, 152.10, 151.82, 150.36, 143.85, 139.27, 130.61, 128.35, 125.22, 124.70, 115.51, 60.87, 56.32, 34.77, 30.88. FAB^+ MS: 382.3 (M + H^+ calc. 382.2) (Found: C, 62.6; H, 6.0; N, 18.2. Calc. for $C_{20}H_{23}N_5O_3$: C, 63.0; H, 6.1; N, 18.4%). **1d**, mp 222–223 °C (from H_2O), R_f 0.13 (toluene–MeOH–Et_3N 79 : 20 : 1 v/v/v). NMR (DMSO-d_6): δ_H 8.23 (1H, s, H-8), 8.16 (1H, s, H-2), 7.32 (2H, br s, NH_2), 6.91 (1H, br s, N–CH=C), 5.17 and 5.15 (2 × 1H, two overlapping t, *J* 5.5, 2 × OH), 4.22 (2H, dd, *J* 5.5 and 1.6, CH_2), 4.01 (2H, d, *J* 5.5, CH_2). δ_C 156.1, 152.9, 149.4, 140.2, 137.6, 118.1, 116.2, 61.0, 56.2. FAB^+ MS: 222.1 (M + H^+ calc. 222.2) (Found: C, 48.4; H, 5.1; N, 31.2. Calc. for $C_9H_{11}N_5O_2$ + 0.1 H_2O: C, 48.5; H, 5.1; N, 31.4%).

2-Amino-9-[3-benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]-6-chloropurine 19

To a solution of 2-amino-6-chloropurine (6.78 g, 40.0 mmol) in dry DMF (400 ml) was added **2** (13.6 g, 48 mmol) and K_2CO_3 (0.60 g, 4.0 mmol), and the mixture stirred with heating to 110 °C under N_2 for 8 h. The solvent was removed *in vacuo*, and the residue purified by dry column vacuum chromatography (0–100% EtOAc in heptane, 10% increments) to give **19** (12.1 g, 67%) as colourless crystals, R_f 0.35 (EtOAc–heptane 9 : 1 v/v), pure according to ^1H NMR. A sample was recrystallized from heptane–toluene, mp 101.5–103 °C. NMR ($CDCl_3$): δ_H 7.74 (1H, s, H-8), 7.30–7.15 (10H, m, Ph), 4.98 (2H, s, NH_2), 4.41 (4H, s, CH_2Ph), 4.22 (2H, s, CH_2N), 3.53 (1H, s, OH), 3.34 (4H, AB system, Δ 12.5 Hz, J_{AB} 9.5, BnOCH_2). δ_C 158.8, 154.2, 151.0, 143.8, 137.2, 128.2, 127.7, 127.5, 124.5, 73.6, 73.4, 71.2, 47.2. FAB^+MS: 454.3 (M + H^+ calc. 454.2) (Found: C, 60.8; H, 5.2; N, 15.3. Calc. for $C_{23}H_{24}ClN_5O_3$: C, 60.9; H, 5.3; N, 15.4%).

9-[3-Benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]guanine 20

To a solution of 3-hydroxypropionitrile (8.2 ml, 120 mmol) in dry THF (1000 ml) under N_2 was added NaH (60% in mineral oil, 5.9 g, 147 mmol). After stirring for 30 min a suspension of **19** (12.1 g, 26.7 mmol) in dry THF (100 ml) was added, and the mixture stirred for 4 h at rt. Sat. aq. NH_4Cl (250 ml) was added, the solvents removed *in vacuo*, and EtOAc (200 ml) and H_2O (400 ml) added to give a precipitate that was isolated, washed with EtOAc (100 ml) and H_2O (40 ml) and dried *in vacuo* to give **20** (9.9 g, 85%) as a colourless solid, mp 192–194 °C, R_f 0.12 (EtOAc–MeOH 9 : 1 v/v). NMR (DMSO-d_6, sparingly soluble): δ_H 10.66 (1H, s, NH), 7.56 (1H, s, H-8), 7.34–7.23 (10H, m, Ph), 6.55 (2H, br s, NH_2), 5.29 (1H, br s, OH), 4.44 (4H, s, PhCH_2), 4.06 (2H, s, CH_2N), 3.30 (4H, s, BnOCH_2). FAB^+MS: 436.2 (M + H^+ calc. 436.2) (Found: C, 59.9; H, 5.9; N, 15.6. Calc. for $C_{23}H_{24}ClN_5O_3 \cdot 1H_2O$: C, 59.7; H, 6.1; N, 15.1%).

9-[3-Benzyloxy-2-(benzyloxymethyl)-2-hydroxypropyl]-*N*-2-isobutyrylguanine 3e

To a solution of **20** (4.61 g, 10.6 mmol) in dry pyridine (500 ml) was added isobutyric anhydride (5.0 ml, 30 mmol). The solution was heated under N_2 to 50 °C for 28 days when according to TLC **20** was consumed. The reaction was quenched with H_2O (50 ml) and the solvents removed *in vacuo*. To the residue was added EtOAc (200 ml), H_2O (100ml) and sat. aq. $NaHCO_3$ (100 ml), and the resulting precipitate was washed with H_2O (30 ml) and dried *in vacuo* to give **3e** (3.64 g, 68%) as a colourless solid. A sample was recrystallized from $CHCl_3$–heptane, mp 204–206 °C, TLC R_f 0.38 (EtOAc–MeOH 9 : 1 v/v). NMR (DMSO-d_6): δ_H 12.04 (1H, s, NH), 11.60 (1H, s, NH); 7.85 (1H, s, H-8), 7.32–7.26 (10H, m, Ph), 5.32 (1H, br s, OH), 4.44 (4H, s, PhCH_2), 4.18 (2H, s, CH_2N), 3.35 (4H, m, BnOCH_2); 2.79 (1H, septet, *J* 6.5, CH_3CH), 1.11 (6H, d, *J* 6.5, CH_3CH). δ_C 179.9, 154.7, 149.1, 147.4, 140.3, 137.9, 128.0, 127.4, 127.3, 119.2, 72.8, 72.6, 71.6, 47.1, 34.6, 18.9. FAB^+MS: 506.1 (M + H^+ calc. 506.2).

9-[3-Benzyloxy-2-(benzyloxymethyl)-2-(diethoxythiophosphoryloxy)propyl]-*N*-2-isobutyrylguanine 4e

To a stirred solution of **3e** (1.83 g, 3.61 mmol) in dry pyridine (100 ml) under N_2 was added dropwise diethyl phosphorochloridite (0.57 ml, 4.0 mmol). After 30 min S_8 (0.164 g, 4.0 mmol S) was added and the mixture stirred for 1 h. The solvent was removed *in vacuo*, the residue dissolved in $CHCl_3$ (80 ml), the organic phase washed with sat. aq. $NaHCO_3$ (40 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was crystallized from THF–heptane to give **4e** (1.90 g, 80%) as colourless crystals, mp 181–183 °C. NMR ($CDCl_3$): δ_H 11.97 (1H, s, NH), 8.97 (1H, s, NH), 7.86 (1H, s, H-8), 7.33–7.23 (10H, m, Ph), 4.53 (2H, s, CH_2N), 4.46 (4H, s, PhCH_2), 4.05–3.93 (4H, m, CH_3CH_2), 3.79 (4H, AB system, Δ 4.0 Hz, *J* 10.5, BnOCH_2), 2.49 (1H, septet, *J* 7.0, CH_3CH), 1.19–1.13 (12H, m, CH_3CH_2 + CH_3CH). δ_C 178.6, 155.5, 149.1, 147.2, 140.4, 137.5, 128.3, 127.7, 127.5, 127.3, 120.3, 85.4 (d, J_{PC} 9), 73.3, 69.2 (d, J_{PC} 3), 64.6 (d, J_{PC} 6), 45.8 (d, J_{PC} 5), 36.3, 19.1, 15.9 (d, J_{PC} 8). δ_P 59.4. FAB^+MS: 658.2 (M + H^+ calc. 658.2) (Found: C, 56.2; H, 6.1; N, 10.6. Calc. for $C_{31}H_{40}N_5O_7PS$: C, 56.6; H, 6.1; N, 10.7%).

9-[3-Benzyloxy-2-(benzyloxymethyl)prop-1-enyl]-*N*-2-isobutyrylguanine 5e

To dry *tert*-amyl alcohol (0.54 ml, 5.0 mmol) in dry THF (200 ml) under N_2 was added NaH (60% in oil, 0.20 g, 5.0 mmol). After 1 h was added **4e** (0.63 g, 1.00 mmol, dried by co-evaporation with dry pyridine, followed by co-evaporation with dry toluene to remove traces of pyridine) dissolved in dry THF (80 ml). After stirring for 27 h at rt sat. aq. NH_4Cl (10 ml)

and H_2O (25 ml) were added, and the mixture concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (100 ml) and washed with sat. aq. $NaHCO_3$ (2 × 30 ml), and the organic phase dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by dry column vacuum chromatography (0–100% CH_3CN in toluene, 10% increments, then 0–10% MeOH in CH_3CN, 1% increments) to give **5e** (0.48 g, 58%) as a colourless solid. An analytical sample was obtained by recrystallization from THF–heptane, mp 128–130 °C, R_f 0.52 (CH_3CN–toluene 9 : 1 v/v). NMR ($CDCl_3$): δ_H 12.25 (1H, s, NH), 9.95 (1H, s, NH), 7.89 (1H, s, H-8), 7.34–7.18 (10H, m, Ph), 6.83 (1H, s, N–CH=C), 4.48 and 4.42 (2 × 2H, 2 × s, PhCH_2), 4.15 (2H, d, *J* 1, BnOCH_2), 3.98 (2H, s, BnOCH_2), 2.76 (1H, septet, *J* 7, CH_3CH), 1.17 (6H, d, *J* 7, CH_3CH). δ_C 179.2, 155.4, 148.3, 147.8, 138.6, 137.3, 136.8, 131.5, 128.2, 128.1, 127.7, 127.6, 127.4, 120.2, 119.9, 76.9, 76.5, 73.1, 72.6, 70.0, 64.5, 36.0, 18.9. FAB$^+$MS: 488.2 (M + H$^+$ calc. 488.2) (Found: C, 65.0; H, 6.1; N, 14.0. Calc. for $C_{27}H_{29}N_5O_4$ + 1H_2O: C, 65.3; H, 6.1; N, 14.1%).

9-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]-*N*-2-isobutyryl-guanine 21

To a stirred solution of **5e** (0.460 g, 0.94 mmol) in dry CH_2Cl_2 (50 ml) under N_2 at −78 °C was added BCl_3 (1 M in CH_2Cl_2, 5.6 ml, 5.6 mmol). After 3 h at −78 °C a mixture of MeOH and CH_2Cl_2 (1 : 1 v/v, 10 ml) was added, followed by solid $NaHCO_3$ at −78 °C until neutral pH. The mixture was concentrated *in vacuo* and the residue extracted first with heptane (2 × 10 ml), then with 2-PrOH (3 × 5 ml) to give, after evaporation of 2-PrOH, crude **21** (0.29 g, 90%) as a nearly colourless solid. NMR (CD_3OD): δ_H 8.23 (1H, s, H-8), 6.89 (1H, s, N–CH=C), 4.37 (2H, d, *J* 1, HOCH_2), 4.18 (2H, s, HOCH_2), 2.72 (1H, septet, *J* 6.7, CH_3CH), 1.20 (6H, d, *J* 6.7, CH_3CH).

9-[3-Dimethoxytrityloxy-2-(dimethoxytrityloxymethyl)prop-1-enyl]-*N*-2-isobutyrylguanine 22

To a solution of crude **21** (0.28 g, 0.91 mmol) in dry pyridine (50 ml) was added 4,4′-dimethoxytrityl chloride (0.81 g, 2.4 mmol). After 72 h at rt the solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2 (20 ml), washed with sat. aq. $NaHCO_3$ (2 × 10 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by dry column vacuum chromatography (0–100% CH_3CN in toluene, 10% increments) to give **22** (0.46 g, 55%) as a colourless foam, TLC R_f 0.64 (EtOAc–MeOH 9 : 1 v/v). NMR ($CDCl_3$): δ_H 7.64–7.16 (19H, m, H-8 + Ar), 6.91–6.71 (9H, m, N–CH=C + Ar), 4.03 (2H, s, DMTOCH_2), 3.79 and 3.76 (2 × 6H, s, 2 × OCH_3), 3.68 (2H, s, DMTOCH_2), 2.60 (1H, septet, *J* 7.0, CH_3CH); 1.23 (6H, d, *J* 7.0, CH_3CH). FAB$^+$MS: 912.3 (M + H$^+$ calc. 912.4).

9-[3-Hydroxy-2-(hydroxymethyl)prop-1-enyl]guanine 1e

To a solution of **22** (0.050 g, 0.055 mmol) in CH_2Cl_2 (5 ml) was added sat. NH_3 in MeOH (5 ml). After stirring for 48 h the solution was concentrated *in vacuo*, and the residue, **23**, dissolved in CH_2Cl_2 (2 ml). Addition of Cl_3CCOOH in CH_2Cl_2 (0.20 M, 5 ml) and Et_3SiH (0.044 ml, 0.28 mmol) gave a precipitate that was isolated after 2 h and washed with CH_2Cl_2 (4 × 1 ml). The precipitate was dissolved in H_2O (2 ml) and **1e** was precipitated from the aqueous solution by neutralization with 1 M aq. NaOH. The precipitate was washed with H_2O followed by ether to give **1e** (0.009 g, 70%) as colourless crystals, mp. *ca*. 265 °C (dec.). NMR (DMSO-d_6): δ_H 10.63 (1H, s, NH); 7.79 (1H, s, H-8), 6.72 (1H, s, N–CH=C), 6.52 (2H, br s, NH_2), 5.11 and 5.03 (2 × 1H, 2 × t, *J* 5.3 and 5.3, 2 × OH), 4.17 (2H, d, *J* 5.3, HOCH_2), 3.98 (2H, d, *J* 5.3, HOCH_2). δ_C 156.7, 153.8, 151.1, 137.0, 136.7, 116.3, 116.0, 60.9, 56.1. FAB$^-$ MS: 236.1 (M − H$^+$ calc. 236.1) (Found: C, 45.4; H, 4.5; N, 28.8. Calc. for $C_9H_{11}N_5O_3$ + 1H_2O: C, 45.6; H, 4.7; N, 29.5%).

2,2-Bis(benzoyloxymethyl)oxirane 24

A) 3-Benzoyloxy-2-(benzoyloxymethyl)propene. 3-Chloro-2-(chloromethyl)propene (10.04 g, 0.080 mol) was added to a suspension of NaOBz (46.3 g, 0.320 mol) in anhydrous DMF (350 ml). The suspension was refluxed for 4 h under N_2 and then cooled to rt. The brown suspension was filtered and the solid washed with DMF (2 × 50 ml). The solvent was removed *in vacuo* to give a brown oily residue that was dissolved in CH_2Cl_2 (150 ml) and washed with brine (3 × 100 ml). The organic phase was dried (Na_2SO_4), and the solvent removed *in vacuo* to give the crude alkene (25 g). Purification by dry column vacuum chromatography (0–60% EtOAc in heptane, 2% increments) gave pure *3-Benzoyloxy-2-(benzoyloxymethyl)-propene* as a colourless solid (17.48 g, 73%), mp 38–39.5 °C, R_f 0.51 (hexane–CH_2Cl_2–EtOAc 50 : 45 : 5 v/v/v). NMR (DMSO-d_6): δ_H 7.97 (4H, d, *J* 7.4, Ar), 7.64 (2H, t, *J* 7.4, Ar), 7.49 (4H, t, *J* 7.4, Ar), 5.44 (2H, s, C=CH_2), 4.94 (4H, s, CH_2). δ_C 165.3, 139.0, 133.4, 129.5, 129.2, 128.8, 116.6, 64.8. GC-MS: 296.0 (M calc. 296.1) (Found: C, 72.8; H, 5.4. Calc. for $C_{18}H_{16}O_4$: C, 73.0; H, 5.4%).

B) 2,2-Bis(benzoyloxymethyl)oxirane 24. 3-Benzoyloxy-2-(benzoyloxymethyl)propene (5.0 g, 0.017 mol) was dissolved in anhydrous CH_2Cl_2 (50 ml) and *m*-CPBA (70%, 6.25 g, 0.036 mol) was added. The mixture was refluxed overnight, cooled, diluted with CH_2Cl_2 (50 ml) and washed with sat. aq. $NaHCO_3$ (4 × 30 ml), 20% aq. $NaHSO_3$ (2 × 30 ml), sat. aq. $NaHCO_3$ (3 × 30 ml), and brine (30 ml). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo* to afford **24** (5.1 g, 97%) as a colourless solid, mp 60.5–61.5 °C, R_f 0.50 (hexane–CH_2Cl_2–EtOAc 45 : 45 : 10 v/v/v). An analytical sample, crystallized from EtOAc–hexane–ether, had mp 62–63 °C. NMR ($CDCl_3$): δ_H 8.04 (4H, d *J* 8.3, Ar), 7.57 (2H, t, *J* 7.4, Ar), 7.43 (4H, t, *J* 7.4, Ar), 4.51 (4H, AB system, Δ 36 Hz, *J* 12.2, BzOCH_2), 2.94 (2H, s, CH_2(ring)). δ_C 165.9, 133.2, 129.6, 129.3, 128.4, 64.4, 55.5, 49.9. FAB$^+$MS: 313.0 (M + H$^+$ calc. 313.1) (Found; C, 69.1; H, 5.15. Calc. for $C_{18}H_{16}O_5$: C, 69.2; H, 5.2%).

1-[3-Benzoyloxy-2-(benzoyloxymethyl)2-hydroxypropyl]thymine 25a

A mixture of thymine (1.26 g, 10 mmol) and NaH (60% in oil, 0.08 g, 2 mmol) in dry DMF (40 ml) was stirred under N_2 at rt. After 1.5 h **24** (0.625 g, 2.00 mmol) was added, and the mixture was stirred at 110 °C for 24 h. After cooling to 0 °C sat. aq. NH_4Cl (10 ml) was added, followed by $CHCl_3$ (50 ml) and water (60 ml). The aqueous phase was extracted with $CHCl_3$ (2 × 50 ml), and the combined organic phases washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give a light brown oil. Purification by normal gravity column chromatography, eluted with CH_2Cl_2–MeOH 97 : 3 v/v, gave the 1,3-disubstituted thymine derivative (0.090 g, 6%) and **25a** (0.640 g, 73%) as colourless crystals. An analytical sample was obtained by recrystallisation from EtOAc–hexane, mp 177–178 °C. NMR ($CDCl_3$ + DMSO-d_6): δ_H 10.10 (1H, s, NH), 7.93 (4H, d, *J* 8, Ar), 7.47 (2H, t, *J* 8, Ar), 7.33 (4H, t, *J* 8, Ar), 7.18 (1H, q, *J* 1.2, H-6), 5.13 (1H, s, OH), 4.35 (4H, AB system, Δ 13.5 Hz, J_{AB} 11.7, OCH_2), 4.01 (2H, s, NCH_2), 1.73 (3H, d, *J* 1.2, CH_3). FAB$^+$ MS: 439.1 (M + H$^+$ calc. 439.1) (Found: C, 61.85; H, 5.0; N, 6.2. Calc. for $C_{23}H_{22}N_2O_7$ + 0.5 H_2O: C, 61.75; H, 5.2; N, 6.25%).

1-[2,3-Dihydroxy-2-(hydroxymethyl)propyl]thymine 26a

25a (0.140 g, 0.32 mmol) Was dissolved in a mixture of MeOH (2 ml) and aq. NH_3 (32%, 2 ml). After 24 h at rt the solution was concentrated *in vacuo*, H_2O (15 ml) was added, the mixture extracted with $CHCl_3$ (3 × 10 ml), and the aqueous phase was concentrated *in vacuo*. Recrystallization from 2-PrOH gave **26a** (0.053 g, 72%) as colourless crystals, mp 189–192 °C. NMR

(DMSO-d_6): δ_H 11.26 (1H, s, NH), 7.40 (1H, s, H-6), 4.56 (2H, t, 1/2(J_{AX}+J_{BX}) 5.7, OH), 4.53 (1H, s, OH), 3.71 (2H, s, NCH_2), 3.26 (4H, AB of an ABX system appearing as a doublet, OCH_2), 1.74 (3H, s, CH_3). δ_C 164.2, 152.1, 143.0, 107.7, 74.7, 62.9, 49.7, 12.1. FAB^+ MS: 231.0 (M + H^+ calc. 231.1) (Found: C, 46.7; H, 5.95; N, 12.2. Calc. for $C_9H_{14}N_2O_5$: C, 46.95; H, 6.1; N, 12.15%).

N-4-Benzoyl-1-[3-benzoyloxy-2-(benzoyloxymethyl)2-hydroxypropyl]cytosine 25b

A mixture of *N*-4-benzoylcytosine (0.86 g, 4.0 mmol) and NaH (60% in oil, 0.08 g, 2 mmol) in dry DMF (20 ml) was stirred under N_2 at rt. After 1.5 h **24** (0.600 g, 1.90 mmol) was added, and the mixture was stirred at 110 °C for 24 h. After cooling to 0 °C sat. aq. NH_4Cl (10 ml) was added, followed by $CHCl_3$ (50 ml) and H_2O (60 ml). The aqueous phase was extracted with $CHCl_3$ (2 × 50 ml), and the combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give almost pure **25b** (0.740 g, 74%) as colourless crystals. Recrystallization from EtOAc–MeOH gave pure **25b** (0.65 g, 65%), mp 200–201 °C. NMR ($CDCl_3$ + DMSO-d_6): δ_H 9.58 (1H, s, NH), 7.93 (4H, d, *J* 8, Ar), 7.86 (2H, d, *J* 8, Ar), 7.83 (1H, d, *J* 7.3, H-6), 7.52–7.28 (10H, m, Ar + H-5), 5.59 (1H, s, OH), 4.43 (4H, AB system, Δ 18.2 Hz, J_{AB} 11.8, OCH_2), 4.30 (2H, s, NCH_2). FAB^+ MS: 528.3 (M + H^+ calc. 528.2) (Found: C, 65.9; H, 4.65; N, 7.9. Calc. for $C_{29}H_{25}N_3O_7$: C, 66.0; H, 4.8; N, 7.95%).

1-[2,3-Dihydroxy-2-(hydroxymethyl)propyl]cytosine 26b

25b (0.450 g, 0.85 mmol) Was dissolved in a mixture of MeOH (5 ml) and aq. NH_3 (32%, 5 ml). After 24 h at rt the solution was concentrated *in vacuo*, water (15 ml) was added, the mixture extracted with $CHCl_3$ (3 × 10 ml), and the aqueous phase was concentrated *in vacuo*. Recrystallisation from 2-PrOH–H_2O gave **26b** (0.144 g, 73%) as colourless crystals, mp 228–229 °C. NMR (DMSO-d_6): δ_H 7.48 (1H, d, *J* 7.0, H-6), 7.18 and 7.13 (2 × 1H, 2 × br s, NH_2), 5.70 (1H, d, *J* 7.0, H-5), 4.80 (1H, s, OH), 4.79 (2H, t, 1/2(J_{AX}+J_{BX}) 6.2, OH), 3.73 (2H, s, NCH_2), 3.20 (4H, AB of an ABX system, Δ 23.5 Hz, J_{AB} 11.4, J_{AX} 7.0, J_{BX} 5.5, OCH_2). δ_C 166.0, 158.0, 147.7, 93.4, 74.9, 63.1, 51.2. FAB^+ MS: 216.1 (M + H^+ calc. 216.1) (Found: C, 41.2; H, 6.5; N, 17.8. Calc. for $C_8H_{13}N_3O_4$ + H_2O: C, 41.2; H, 6.5; N, 18.0%).

N-6-Benzoyl-9-[3-benzoyloxy-2-(benzoyloxymethyl)2-hydroxypropyl]adenine 25c

A mixture of *N*-6-benzoyladenine (0.96 g, 4.0 mmol) and NaH (60% in oil, 0.08 g, 2 mmol) in dry DMF (20 ml) was stirred under N_2 at rt. After 1.5 h **24** (0.600 g, 1.90 mmol) was added, and the mixture stirred at 110 °C for 24 h. After cooling to 0 °C sat. aq. NH_4Cl (10 ml) was added, followed by $CHCl_3$ (50 ml) and H_2O (60 ml). The aqueous phase was extracted with $CHCl_3$ (2 × 50 ml), and the combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to give a light brown oil. Purification by normal gravity column chromatography, eluted with CH_2Cl_2–MeOH 97 : 3 v/v gave first the *N*-7-isomer (*ca*. 10%) and then **25c** (0.452 g, 43%) as colourless crystals, mp 193–195 °C. NMR ($CDCl_3$): δ_H 9.2 (1H, br, NH), 8.58 (1H, s, H-2), 8.29 (1H, s, H-8), 7.99–7.28 (15H, m, Ar), 5.61 (1H, s, OH), 4.63 (2H, s, NCH_2), 4.37 (4H, AB system, Δ 38.1 Hz, J_{AB} 11.7, OCH_2). FAB^+ MS: 552.3 (M + H^+ calc. 552.2) (Found: C, 64.85; H, 4.45; N, 12.55. Calc. for $C_{30}H_{25}N_5O_6$: C, 65.3; H, 4.55; N, 12.7%).

9-[2,3-Dihydroxy-2-(hydroxymethyl)propyl]adenine 26c

25c (0.160 g, 0.29 mmol) Was dissolved in a mixture of MeOH (5 ml) and aq. NH_3 (32%, 5 ml). After 24 h at rt the solution was concentrated *in vacuo*, H_2O (15 ml) was added, the mixture extracted with $CHCl_3$ (3 × 10 ml), and the aqueous phase was concentrated *in vacuo*. Recrystallization from 2-PrOH–H_2O gave **26c** (0.058 g, 84%) as colourless crystals, mp 220–221 °C (lit.[20] mp 218–220 °C). NMR (DMSO-d_6): δ_H 8.13 (1H, s, H-2), 7.99 (1H, s, H-8), 7.27 (2H, s, NH_2), 4.87 (2H, t, 1/2(J_{AX}+J_{BX}) 6.0, OH), 4.75 (1H, s, OH), 4.18 (2H, s, NCH_2), 3.24 (4H, AB of an ABX system, Δ 14.7 Hz, J_{AB} 11.2, J_{AX} 6.1, J_{BX} 5.9, OCH_2). δ_C 156.0, 152.2, 150.0, 142.2, 118.1, 74.3, 62.9, 46.1. FAB^+ MS: 240.1 (M + H^+ calc. 240.1) (Found: C, 45.3; H, 5.45; N, 29.6. Calc. for $C_9H_{13}N_5O_3$: C, 45.2; H, 5.5; N, 29.3%).

2-Amino-9-[3-benzoyloxy-2-(benzoyloxymethyl)-2-hydroxypropyl]-6-chloropurine 25d

A mixture of 2-amino-6-chloropurine (0.51 g, 3 mmol) and K_2CO_3 (0.04 g, 0.3 mmol) in dry DMF (15 ml) was stirred under N_2 at rt. After 1.5 h **24** (0.600 g, 1.90 mmol) was added, and the mixture was stirred at 105 °C for 6 h. After cooling, the mixture was filtered and evaporated *in vacuo* to give light brown crystals. Purification by normal gravity column chromatography, eluted with CH_2Cl_2–MeOH 95 : 5 v/v, gave first the *N*-7-isomer (0.14 g, 9.7%) and then **25d** (0.93 g, 64%) as colourless crystals, mp 146–148 °C. NMR ($CDCl_3$): δ_H 7.99 (4H, d, *J* 8, Ar), 7.89 (1H, s, H-8) 7.59 (2H, t, *J* 8, Ar), 7.44 (4H, t, *J* 8, Ar), 4.99 (2H, s, NH), 4.76 (1H, br s, OH), 4.46 (2H, s, NCH_2), 4.44 (4H, AB system, Δ 14.7 Hz, J_{AB} 11.8, OCH_2). FAB^+ MS: 482.2 (M + H^+ calc. 482.1) (Found: C, 57.05; H, 4.05; N, 14.2. Calc. for $C_{23}H_{20}ClN_5O_5$: C, 57.35; H, 4.2; N, 14.55%).

9-[2,3-Dihydroxy-2-(hydroxymethyl)propyl]guanine 26d

25d (0.400 g, 0.83 mmol) Was refluxed with aq. HCl (2 M, 15 ml). After 4 h the solution was cooled and extracted with $CHCl_3$ (3 × 10 ml), and the aqueous phase was evaporated *in vacuo* to give the hydrochloride of **26d** as colourless crystals (0.243 g, 100%). The hydrochloride was dissolved in water (10 ml), aq. NaOH (2 M) was added to pH *ca*. 8, where upon **26d** (0.125 g, 59%) precipitated as colourless crystals. An analytical sample was obtained by recrystallization from 2-PrOH–H_2O, mp > 300 °C. NMR (DMSO-d_6): δ_H 10.59 (1H, s, NH), 7.56 (1H, s, H-8), 6.53 (2H, s, NH), 4.75 (2H, t, 1/2(J_{AX}+J_{BX}) 6.1, OH), 4.68 (1H, s, OH), 3.97 (2H, s, NCH_2), 3.22 (4H, AB of an ABX system, Δ 12.6 Hz, J_{AB} 11.4, J_{AX} 6.2, J_{BX} 5.9, OCH_2). δ_C 157.0, 153.5, 151.7, 139.2, 115.9, 74.3, 62.8, 45.9. FAB^+ MS: 256.0 (M + H^+ calc. 256.1) (Found: C, 38.45; H, 5.5; N, 24.7. Calc. for $C_9H_{13}N_5O_4 \cdot 1.5\ H_2O$: C, 38.3; H, 5.7; N, 24.8%).

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