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CONFORMATIONNEL

(54) Title: 5-SUBSTITUTED PYRIMIDINE DERIVATIVES OF CONFORMATIONALLY LOCKED NUCLEOSIDE
ANALOGUES

(57) Abrégé/Abstract:

The present invention relates to 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues and to the use of these derivatives as anti-viral and anti-cancer agents.

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(54) Title: 5-SUBSTITUTED PYRIMIDINE DERIVATIVES OF CONFORMATIONALLY LOCKED NUCLEOSIDE ANALOGUES

(57) Abstract: The present invention relates to 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues and to the use of these derivatives as anti-viral and anti-cancer agents.

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5-SUBSTITUTED PYRIMIDINE DERIVATIVES OF CONFORMATIONALLY LOCKED NUCLEOSIDE ANALOGUES

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Field of the Invention

The present invention relates to 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues and to the use of these derivatives as anti-viral and anti-cancer agents.

Background of the Invention

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It has been recognized that incorporating modified nonfunctional analogues of DNA substituents during replication is an effective method for terminating DNA replication and in turn preventing generation of viable progeny. Recent studies have demonstrated that modified and synthetic riboses and nitrogenous bases have antiviral activity against varying viral genera depending on the modification. There is a constant

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need for effective anti-viral and anti-cancer agents.

Summary of the Invention

The present invention provides 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues and the use of these derivatives as anti-viral and anti-cancer agents.

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Detailed Description of the Invention

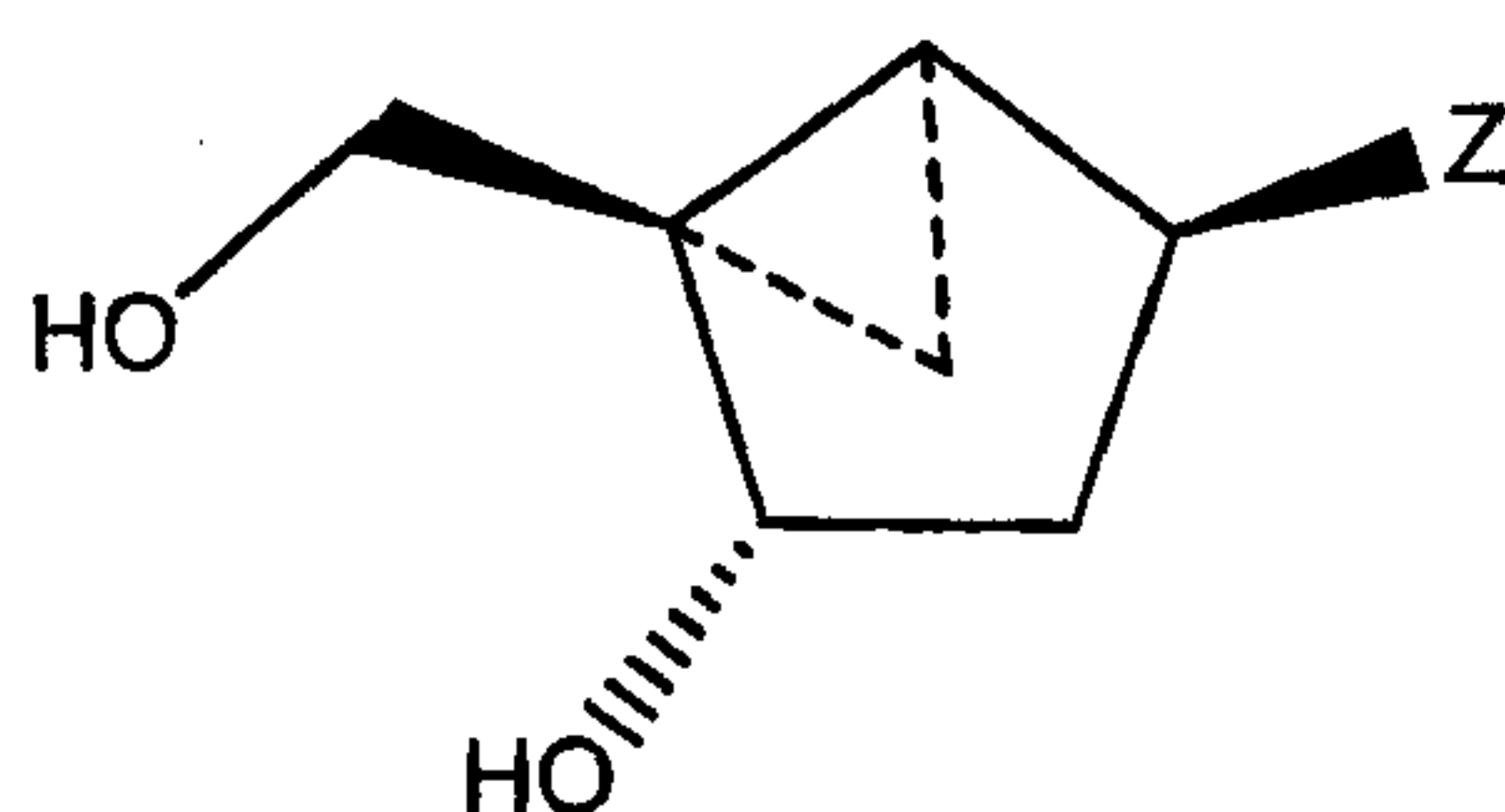
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Marquez et al., J. Med. Chem. 39:3739 (1996) describe purine and pyrimidine nucleoside analogs having natural bases attached to a bicyclo[3.1.0]hexane pseudosugar template. Grignet-Debrus et al., Cancer Gene Therapy 7:215 (2000) describe modified bases, particularly 5-substituted uracils, which are characteristic moieties of antiviral nucleosides when attached to common sugars. The substitution of the modified bases of Grignet-Debrus et al. for the natural bases of Marquez et al. produces pyrimidine analogs that are completely surprising, because the incorporation of these known bases to a bicyclo[3.1.0]hexane template provides antivirally active compounds that are potent

like their nucleoside counterparts and the carbocyclic analogues with a plain cyclopentane ring.

The present invention is directed to 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues, and to methods of using these derivatives as anti-viral and anti-cancer agents. The present invention is also directed to methods of making 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues.

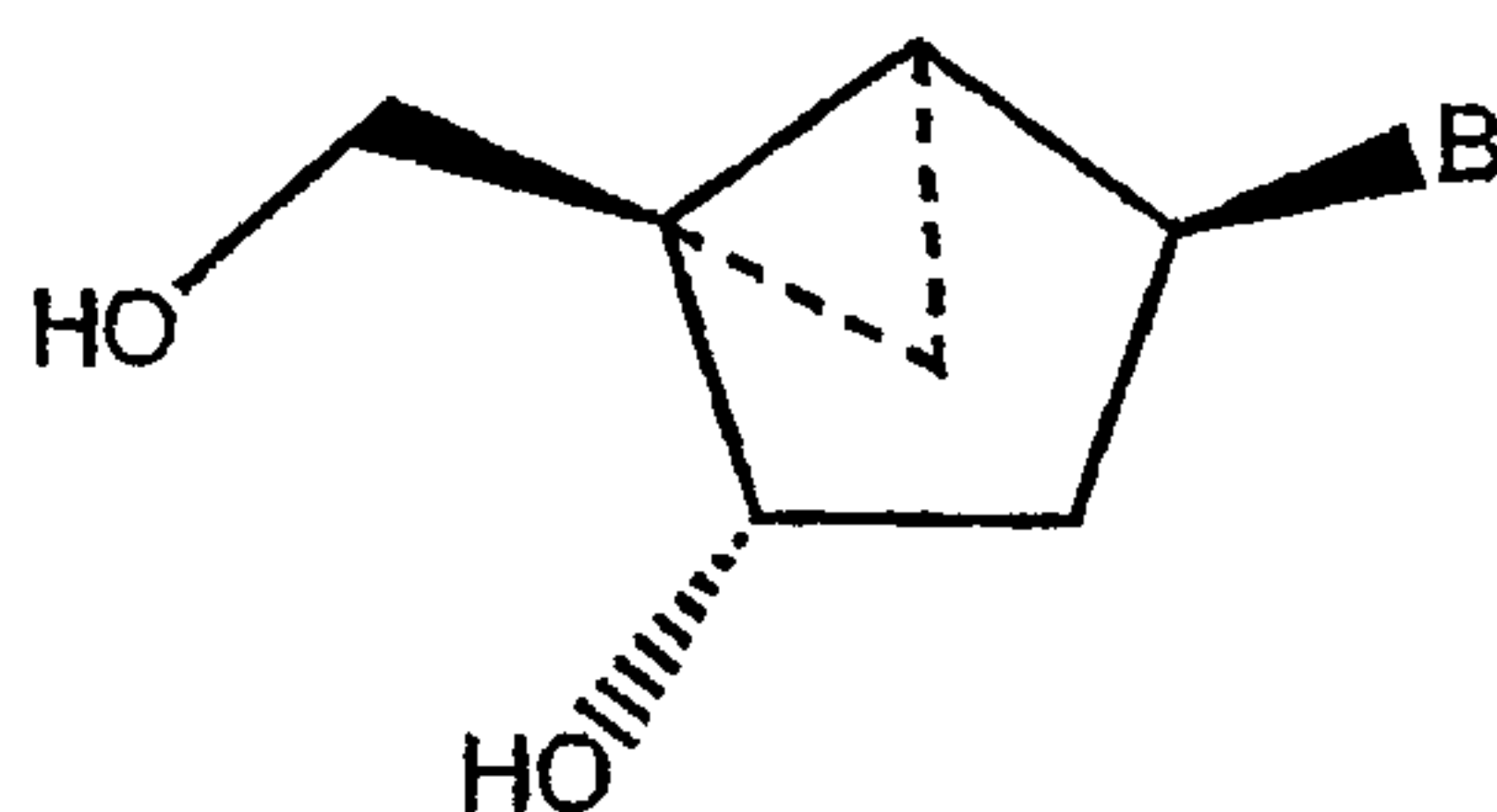
5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues have the formula:



In one embodiment, Z is a 5-substituted cytosin-1-yl and the 5-substituent is F, Cl, Br, I, CH=CH₂, C≡CH, C≡CCH₃, CH=CHF, CH=CHCl, CH=CHBr, or CH=CHI.

In another embodiment, Z is a 5-substituted uracil-1-yl and the 5-substituent is F, Cl, Br, I, CH=CH₂, C≡CH, C≡CCH₃, CH=CHF, CH=CHCl, CH=CHBr, or CH=CHI.

5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues have the formula:



Where B is a 5-substituted derivative of uracil, except when the 5-substituent is methyl, and cytosine. The 5-substituent is understood to be a halogen, alkyl, alkene, or alkyne group. In preferred embodiments, the 5-substituent is F, Cl, Br, I, CH_2CH_3 , $\text{CH}=\text{CH}_2$, $\text{C}\equiv\text{CH}$, $\text{C}\equiv\text{CCH}_3$, $\text{CH}=\text{CHF}$, $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ or $\text{CH}=\text{CHI}$.

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In one embodiment, B is a 5-substituted derivative of uracil, except when the 5-substituent is methyl, and the 5-substituent is a member of the group consisting of F, Cl, Br, I, CH_2CH_3 , $\text{CH}=\text{CH}_2$, $\text{C}\equiv\text{CH}$, $\text{C}\equiv\text{CCH}_3$, $\text{CH}=\text{CHF}$, $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ and $\text{CH}=\text{CHI}$.

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In an another embodiment, B is a 5-substituted derivative of cytosine, and the 5-substituent is a member of the group consisting of F, Cl, Br, I, CH_2CH_3 , $\text{CH}=\text{CH}_2$, $\text{C}\equiv\text{CH}$, $\text{C}\equiv\text{CCH}_3$, $\text{CH}=\text{CHF}$, $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$ and $\text{CH}=\text{CHI}$.

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It is to be understood that the symbols B and Z are used interchangeably throughout the entire application.

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5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues may be tested for antiviral activity by a variety of methods known in the art.

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One assay is the cytopathogenic effect inhibition assay. Another assay is the virus plaque reduction assay.

The cytopathogenic effect inhibition assay proceeds generally as follows. Marquez et al., J. Med. Chem. 39:3739 (1996). Low-passage (3-10) human foreskin fibroblast (HFF) cells are trypsinized, counted, and seeded into 96-well tissue culture plates at a cell concentration of 2.5×10^4 cells in 0.1 mL of minimal essential media (MEM) supplemented with 10% fetal bovine serum media (FBS). The cells are then incubated for 24 h at 37 °C in a 5% CO₂-95% air, 90% humidified atmosphere. The media are then removed, and 100 µL of MEM containing 2% FBS is added to all but the first row. In the first row, 125 µL of media containing the experimental compound are added in triplicate wells. Media alone are added to both cell and virus control wells. The compound in the first row of wells is then diluted serially 1:5 throughout the remaining wells by transferring 25 µL using the Cetus Liquid Handling Machine. The plates are then incubated for 1 h, and 100 µL of the appropriate virus concentration is added to each well, excluding cell control wells, which receive 100 µL of MEM. The viral concentration utilized is 1000 PFU/well. The plates are then incubated at 37 °C in a CO₂ incubator for 3 days. After the incubation period, media are aspirated and the cells stained with a 0.1% crystal violet solution for 30 min. The stain is then removed, and the plates are rinsed with tap water until all excess stain is removed. The plates are allowed to dry for 24 h and then read on a BioTek Multiplate Autoreader.

The virus plaque reduction assay proceeds generally as follows. Marquez et al., J. Med. Chem. 39:3739 (1996). On the date of the assay, the drug is made up at 2 times the desired concentration in 2 x MEM and then serially diluted 1:5 in 2 x MEM to give six concentrations of drug. The drug concentrations utilized are usually 200 down to 0.06 µg/mL. The virus to be used is diluted in MEM containing 10% FBS to a desired concentration which will give 20-30 plaques/well. The media are then aspirated from the wells, and 0.2 mL of virus is added to each well in duplicate with 0.2 mL of media being added to drug toxicity wells. The plates are then incubated for 1 hr with shaking every 15 min. After the incubation period, an equal amount of 1% agarose is added to an equal volume of each drug dilution. This gives a final drug concentration beginning

with 100 and ending with 0.03 $\mu\text{g/mL}$ and a final agarose overlay concentration of 0.5%. The drug agarose mixture is applied to each well in 2 mL volume, and the plates are incubated for 3 days, after which the cells are stained with a 1.5 solution of neutral red. At the end of the 4-6 h incubation period, the stain is aspirated, and plaques are counted using a stereomicroscope at 10 x magnification.

The 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues have been found to possess valuable pharmacological properties. They have an anti-viral effect. This effect can be demonstrated using the cytopathogenic effect inhibition assay and the virus plaque reduction assay.

The conformationally locked (North)-methanocarbathymine is a potent and selective antiherpes agent, 30 times more potent than acyclovir against Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2) in the plaque reduction assay. Since the 5-substituent in pyrimidine nucleosides is a modulator of antiherpes activity, such as in the very effective antiviral compound bromovinyluridine (BVDU), we decided to explore a set of substituents of 5-substituted uracils (Br, I, $\text{CH}=\text{CH}-\text{Br}$) on this new class of carbocyclic nucleosides built on a bicyclo[3.1.0]hexane template. The series was limited only to the conformationally locked North analogues since the South conformational antipode of methanocarbathymine was found to be inactive. The syntheses of these compounds can proceed linearly from the corresponding carbocyclic amine via the uracil analogue or by a convergent approach via Mitsunobu coupling with the 5-substituted base.

Tables 1, 2, and 3 demonstrate antiviral activity of 5-substituted uracils (Br, I, $\text{CH}=\text{CH}-\text{Br}$) attached to a bicyclo[3.1.0]hexane template relative to the corresponding known active controls using the cytopathogenic effect inhibition assay and the virus plaque reduction assay. These results predict that combining any 5-substituted uracil moiety, not just when the 5-substituent is methyl, with a bicyclo[3.1.0]hexane pseudosugar will result in compounds with antiviral activity. These results additionally predict that the combination of any 5-substituted pyrimidine moiety with a bicyclo[3.1.0]hexane template will also will result in compounds with antiviral activity.

Thus, the invention also relates to a method for the treatment of a viral infection, which comprises administering to a patient having a viral infection a therapeutically effective amount of an anti-viral composition comprising at least one of a 5-substituted conformationally locked nucleoside analogue. The method of the invention typically
5 comprises mixing the analogue with a pharmaceutically acceptable carrier to facilitate the administration of the anti-viral composition. Preferably, the anti-viral compositions are used to treat Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), and Cytomegalovirus (CMV).

Table 1. Antiviral activity against herpes viruses HSV-1 and HSV-2

Compound	HSV-1 (HFF) ^a CPE inhib. EC ₅₀ ^b (μg/ml)	HSV-1 (HFF) CPE inhib. CC ₅₀ ^c (μg/ml)	HSV-1 (HFF) CPE inhib. EC ₅₀ ^c (μg/ml)	HSV-2 (HFF) CPE inhib. EC ₅₀ (μg/ml)	HSV-2 (HFF) CPE inhib. CC ₅₀ (μg/ml)	SI	HSV-1 (HFF) plaque red'n EC ₅₀ (μg/ml)	HSV-1 (HFF) plaque red'n CC ₅₀ (μg/ml)	SI	HSV-2 (HFF) plaque red'n EC ₅₀ (μg/ml)	HSV-2 (HFF) plaque red'n CC ₅₀ (μg/ml)	SI
(N)- MCdIU (4)	>100	>100	>100	3.3	>100	30.3						
(N)- MCdBrU (3)	0.39	>100	>256	0.70	>100	>142	0.03	>100	3333	0.12	>100	833
(N)- MCBVDU (5)	2.2	>100	>45	>100	>100	0						
ACV ^e (control)	0.5											

^aHFF = human foreskin fibroblast;
^bEC₅₀ = inhibitory concentration required to reduce virus induced cytopathogenic effect (CPE) or virus plaques by 50%;
^cCC₅₀ = cytotoxic concentration that produces 50% of cell growth; ^dSI = selectivity index (CC₅₀/EC₅₀);
^e acyclovir

Table 2. Antiviral activity against vaccinia and pox

Compound	Vaccinia (HFF) ^a CPE inhib. EC ₅₀ ^b (µg/ml)	Vaccinia (HFF) CPE inhib. CC ₅₀ ^c (µg/ml)	SI ^d	Vaccinia (HFF) Plaque red'n CC ₅₀ (µg/ml)	SI	Cowpox (HFF) CPE inhib. EC ₅₀ (µg/ml)	Cowpox (HFF) CPE inhib. CC ₅₀ (µg/ml)	SI	Cowpox (HFF) Plaque red'n EC ₅₀ (µg/ml)	Cowpox (HFF) Plaque red'n CC ₅₀ (µg/ml)	SI
(N)- MCdBrU (3)	0.64	>100	>156	2.6	>100	38.5	11.5	8.7	3.4	>100	29.4

^aHFF = human foreskin fibroblast;
^bEC₅₀ = inhibitory concentration required to reduce virus induced cytopathogenic effect (CPE) or virus plaques by 50%;
^cCC₅₀ = cytotoxic concentration that produces 50% of cell growth; ^dSI = selectivity index (CC₅₀/EC₅₀);
^e acyclovir

Table 3. Antiviral activity against VZV

Compound	Antiviral activity (Plaque reduction) EC ₅₀ (μM) ^a			Cytotoxicity (μM)		
	TK+ VZV		TK- VZV	Cell Morphology (MCC) ^b	CC ₅₀ ^c	
	YS strain	OKA strain				
(N)-MCdBrU (3)	0.03	0.89	07/1 strain 107	>200	>200	
(N)-MCdIU (4)	0.12	0.16	>5	>5	>200	
(N)-MCBVDU (5)	0.007	0.005	>5	>5	>200	
ACV (control)	2.4	2.4	30	>200	488	
BVDU (control)	0.008	0.005	>150	>150	>400	

^aEC₅₀ = inhibitory concentration required to reduce virus plaques by 50%;
^bMCC = minimum cytotoxic concentration that causes microscopically detectable alteration of cell morphology;
^cCC₅₀ = cytotoxic concentration that produces 50% of cell growth.

The invention also relates to a method of making a nucleoside analogue comprising the steps of: reacting a bicyclo[3.1.0]hexane template with (E)-3-ethoxyacryloyl isocyanate to yield a first intermediate; and reacting the first intermediate with H_2SO_4 in ethanol, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with Br_2 in AcO_2 to yield a second intermediate; and reacting the second intermediate with NH_3 in methanol, followed by NaOH in tetrahydrofuran, whereby the nucleoside analogue is obtained.

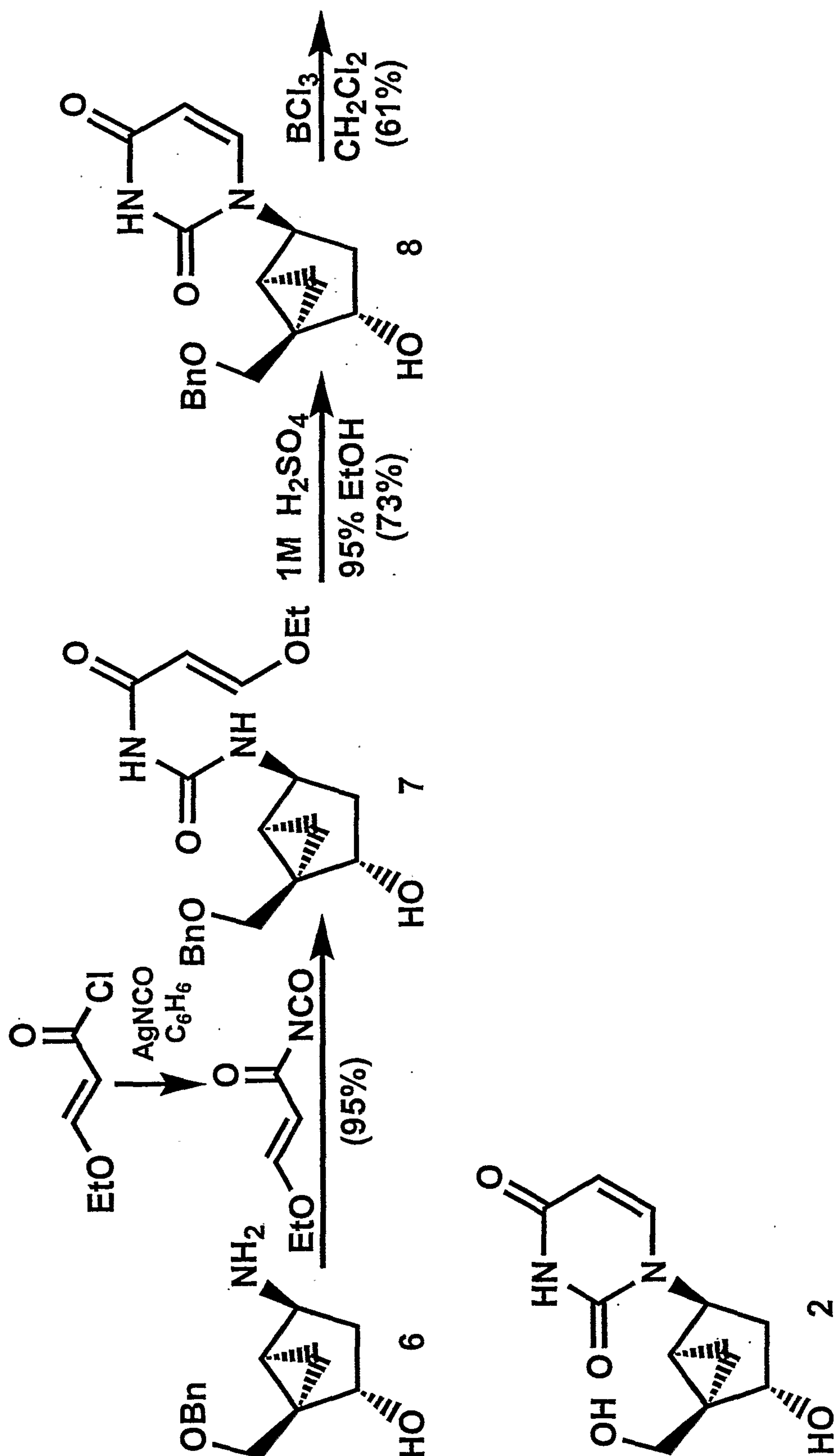
The invention further relates to a method of making a nucleoside analogue comprising the steps of: reacting 5-iodo-3-phenylpyrimidine-2,4 (1H,3H)-dione with diethyl azodicarboxylate and PPh_3 in a solution of tetrahydrofuran, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with NH_4OH in methanol, whereby a first intermediate is obtained; and reacting the first intermediate with BCl_3 in methylene chloride, whereby the nucleoside analogue is obtained. In an embodiment, the first intermediate may be reacted with BCl_3 in methylene chloride to form a second intermediate, whereby the second intermediate may be converted to the nucleoside analogue obtained.

The invention also relates to a method of making a nucleoside analogue comprising the steps of: reacting 5-iodo-3-benzoylpyrimidine-2,4 (1H,3H)-dione with diethyl azodicarboxylate and PPh_3 in a solution of tetrahydrofuran, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with NH_4OH in methanol, whereby a first intermediate is obtained; and reacting the first intermediate with BCl_3 in methylene chloride, whereby the nucleoside analogue is obtained. In an embodiment, the first intermediate may be reacted with BCl_3 in methylene chloride whereby a second intermediate is obtained, and the method may further comprise the steps of: reacting the second intermediate with methacrylate in the presence of $\text{PdII}(\text{Oac})_2$, PPh_3 , NEt_3 and dioxane, whereby a third intermediate is obtained; hydrolyzing the third intermediate with KOH , whereby a fourth intermediate is obtained; and reacting the fourth intermediate with NBS in the presence of KHCO_3 and DMF , whereby the nucleoside analogue is obtained.

Methods of Synthesis

Scheme 1

Scheme 1 illustrates how to synthesize a bicyclo[3.1.0]hexane uracil nucleoside
5 (2) starting with a bicyclo[3.1.0]hexane template (6).



Compound 7

To 3-ethoxy acrylchloride (2.41g, 0.018m) in 50mL benzene was added AgNCO (6.0g, 0.036m) which had been dried for 2h at 100°C under vacuum. The mixture was refluxed under Ar for 0.75h. Cooled to RT. 40mL of the organic supernatant was added dropwise to a solution of **6** (2.08g, 0.009m) in DMF (50mL) which had been cooled in an ice/salt bath under Ar. Reaction stirred overnight as bath warmed to RT. Concentrated in vacuo. **7** (3.19g, yellow "glass", 95%) was obtained by silica gel flash chromatography using 50% EtOAc/hexane and EtOAc. A small portion of **7** was purified for analysis

Analyzed for $C_{20}H_{26}N_2O_5 \cdot 0.25H_2O$ Mw 378.94

Calc: C 63.39; H 7.05; N 7.39

Found: C 63.56; H 6.93; N 7.50

63.50 7.00 7.48

Compound 8

To **7** (3.19g, 0.085m) dissolved in 95% EtOH (100mL) was added 1M H_2SO_4 (100mL) and the reaction refluxed for 1h. The EtOH was removed, the resulting mixture neutralized with 2N NaOH to pH7, and extracted with chloroform (3 x 100mL). The combined extracts were washed 2 x 100mL saturated $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo. **8** (2.02g, 73% colorless foam, $\alpha_D^{25} = +59(C, 0.16, MeOH)$) was obtained by silica gel flash chromatography using EtOAc.

Analyzed for $C_{18}H_{20}N_2O_4 \cdot 0.25H_2O$ Mw 332.88

Calc: C 64.95; H 6.21; N 8.42

Found: C 65.12; H 6.33; N 8.20

65.02 6.27 8.14

Compound 2

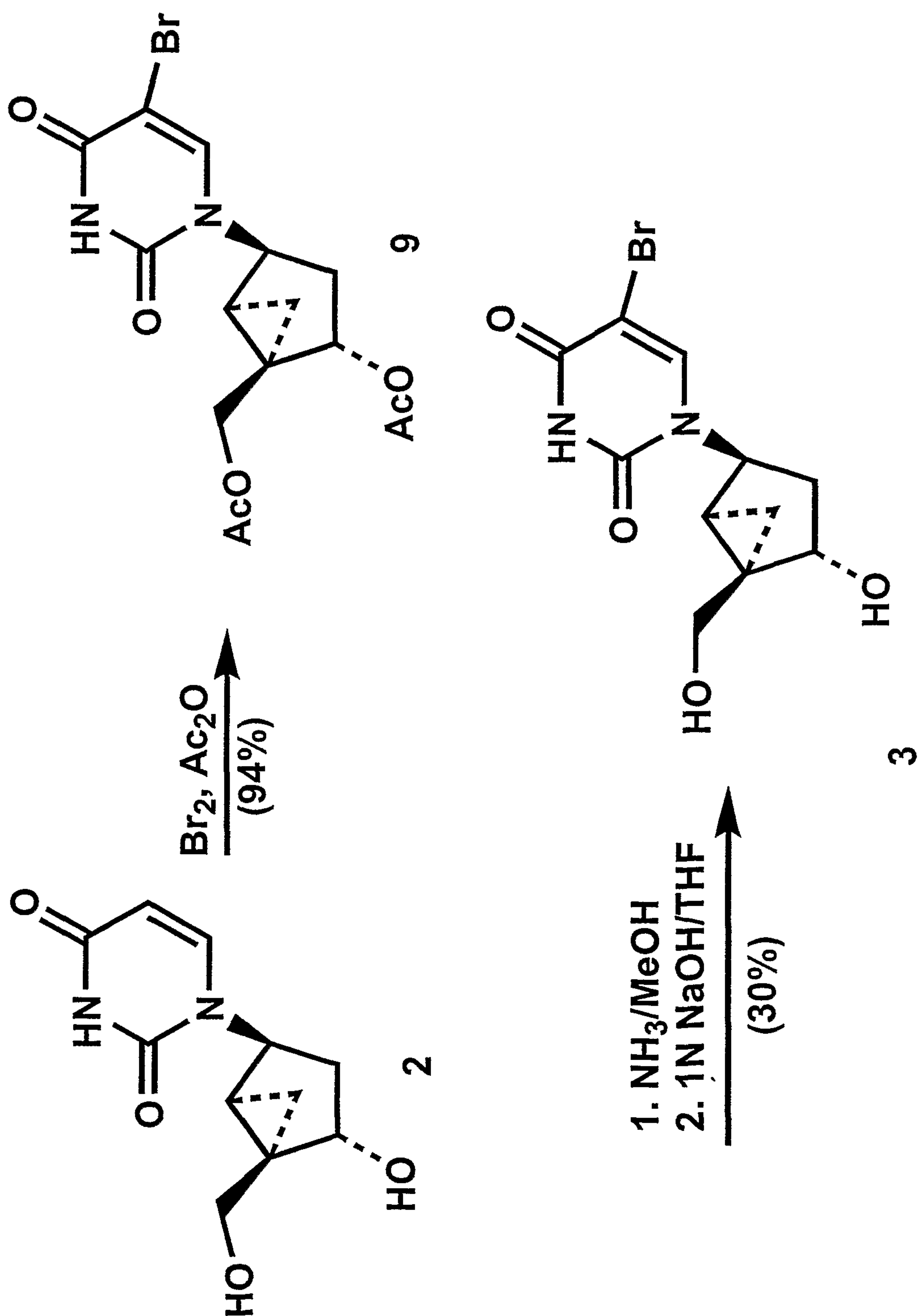
To **8** (0.164g, 0.5 mmole) in CH_2Cl_2 (30mL) cooled to $-78^\circ C$ was added 1M BCl_3 / CH_2Cl_2 and the reaction stirred cold under Ar for 1h. MeOH (5mL) added, the reaction concentrated in vacuo and reconcentrated with methanol (2 x 5mL). **2** (0.073g,

white solid) was obtained by silica gel flash chromatography using CHCl_3 and 10% MeOH / CHCl_3

Note: this is a known compound (US Patent 5,840,728).

5 **Scheme 2**

Scheme 2 illustrates how to synthesize a Bromine (Br) 5-substituted uracil nucleoside (3) starting with the bicyclo[3.1.0]hexane uracil nucleoside (2).



Compound 9

2 (0.073g, 0.31 mmole) was dissolved in Ac₂O (2mL) with warming and cooled to RT under Ar. Br₂ (0.02 mL, 0.33 mmole) was added dropwise, the reaction stirred at RT for 0.5h and stored in the refrigerator overnight. The Ac₂O was removed in vacuo and reconcentrated with toluene (3 x 5mL). Treatment of the orange residue with water gave 0.062g white solid and 0.050g orange semisolid both containing crude 9. Reasonably pure 9 (0.117g, colorless foam, R_F 0.86 (EtOAc, MH⁺ 401/403, 94%)) was obtained by silica gel flash chromatography using 50% EtOAc/hexane and EtOAc.

Compound 3

9 (0.111g, 0.277mmole) was dissolved in 3mL of saturated ammonia in methanol, kept at RT overnight and then heated at 50°C for another 24h. The solid obtained by removal of the NH₃/MeOH (R_F 0.32, 15% MeOH/CHCl₃) was found by NMR to contain one acetate group. To this solid dissolved in THF (2.5mL) was added 1N NaOH (0.2mL) and the reaction stirred at RT. Acetic acid (20μL) was added and the reaction concentrated in vacuo. Crude 3 was obtained by C-18 reverse phase flash chromatography using water and methanol. Pure 3 (0.026g, mp 218-219°C, α_D²⁰ = +18.2 (C, 0.11, MeOH), MH⁺ 317/319, 30%) was obtained by silica gel flash chromatography using a step gradient of CHCl₃ and 10% MeOH/CHCl₃.

Analyzed for C₁₁ H₁₃ N₂ O₄ Br Mw 317.14

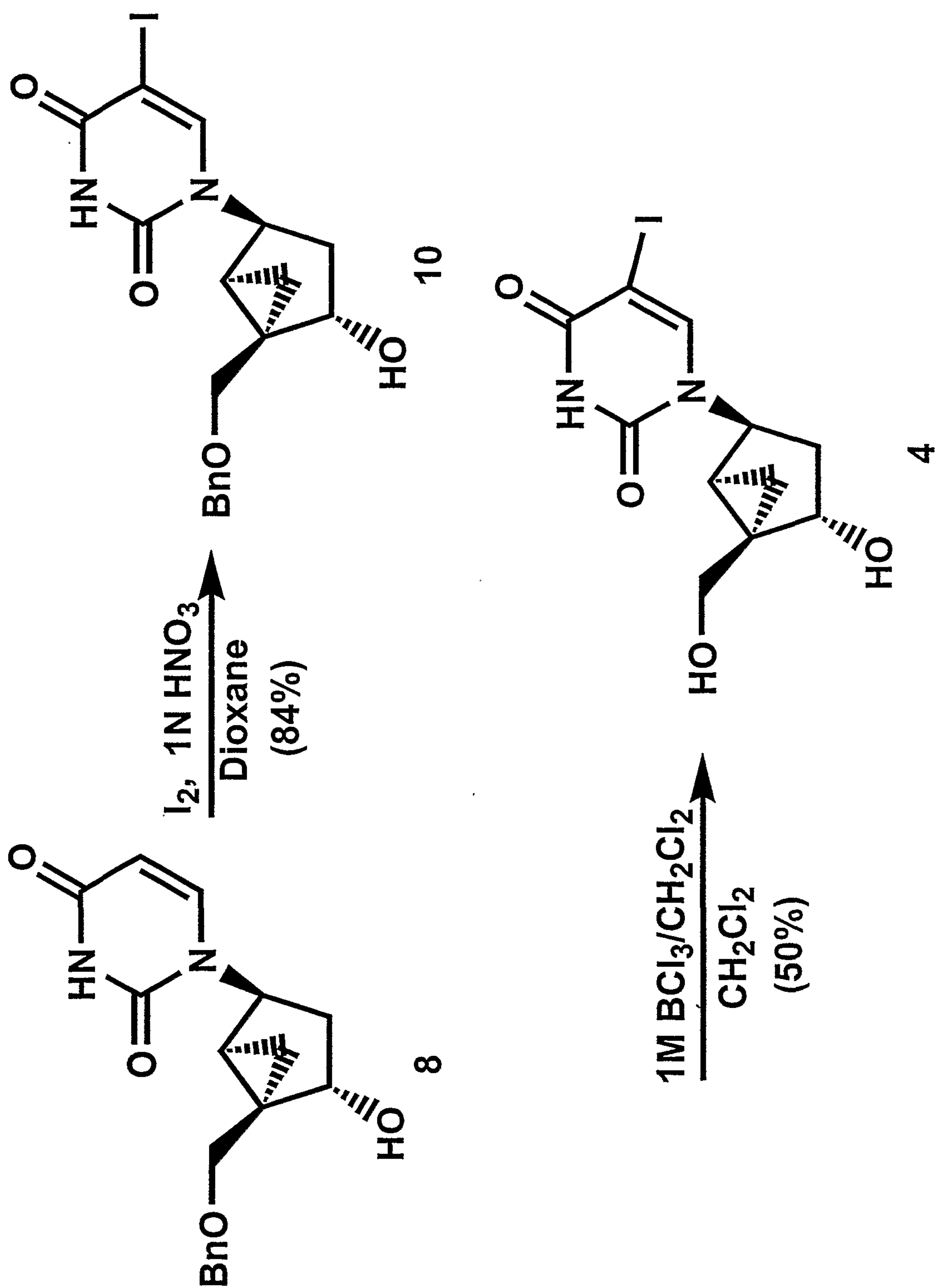
Calc: C 41.66; H 4.13; N 8.83

Found: C 42.14; H 4.22; N 8.54

42.19 4.31 8.57

Scheme 3A

Scheme 3A illustrates how to synthesize a Iodine (I) 5-substituted uracil nucleoside (4) starting with the bicyclo[3.1.0]hexane uracil nucleoside (2).



Compound 10

A stirred solution of **8** (0.164g, 0.5 mmole), I₂ (0.254g, 1.0 mmole) and 1N HNO₃ (0.5mL) in dioxane (5mL) was heated at 100°C for 1h. Concentrated in vacuo and reconcentrated with ethanol (3 x 5mL) and chloroform (3 x 10mL). **10** (0.191g off-white solid, 84%, mp 85-88°C, R_F 0.6 (EtOAc)) was obtained by silica gel flash chromatography using a step gradient of 50% EtOAc/hexane and EtOAc.

Analyzed for C₁₈ H₁₉ N₂ O₄ I • 0.5 H₂O Mw 463.28

Calc: C 46.67; H 4.35; N 6.06

10 Found: C 46.24; H 4.22; N 5.89

46.33 4.18 5.92

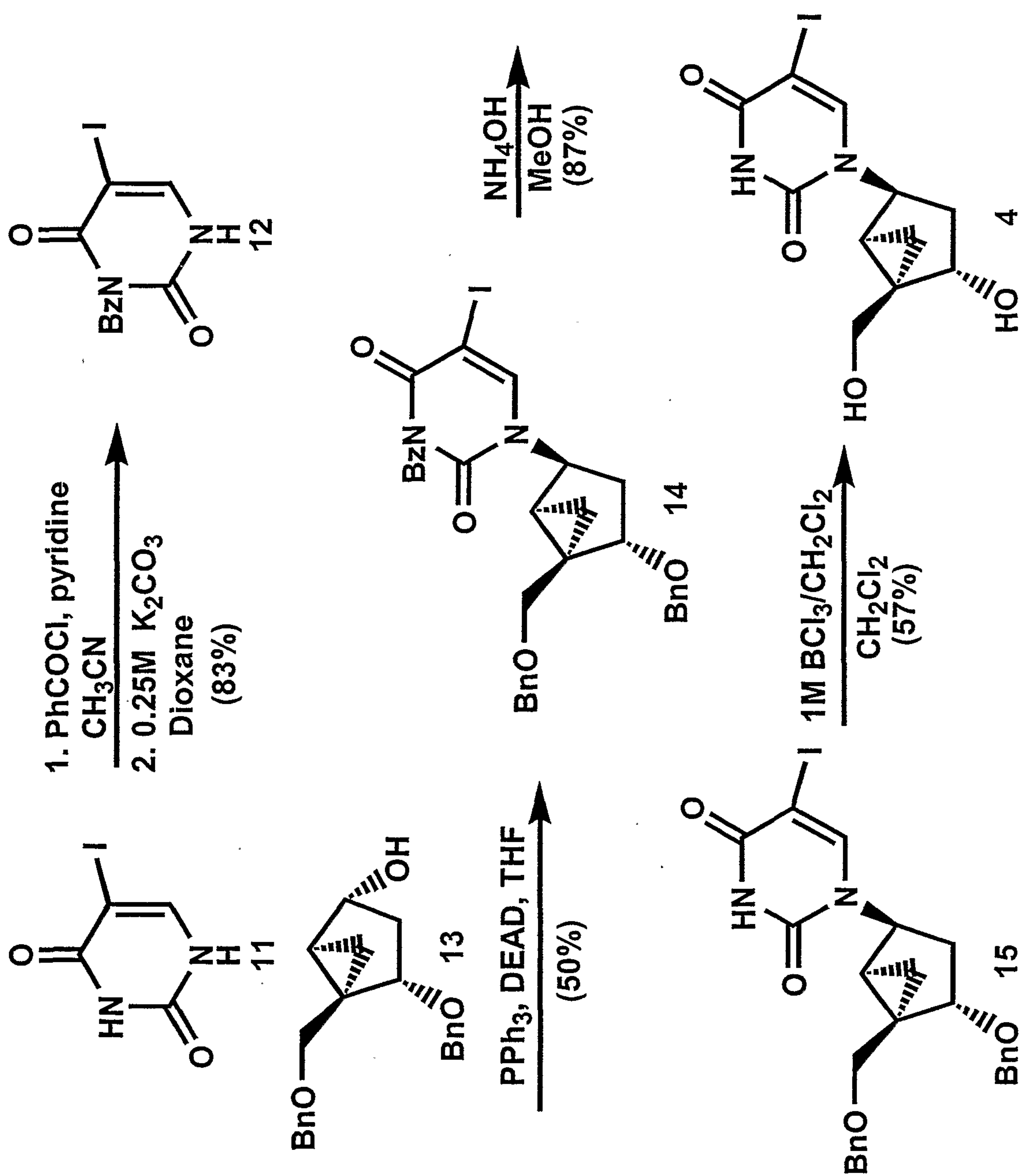
Compound 4

To a solution of **10** (0.072g, 0.16mmole) in CH₂Cl₂ (10 mL) cooled in dry ice/acetone was added 1M BCl₃/CH₂Cl₂ (1.6 mL). After 1h methanol (3 mL) was added to the cold reaction. The reaction was concentrated in vacuo and reconcentrated with methanol (3 x 5mL). Purification by silica gel slash chromatography using CHCl₃ and 5% MeOH/CHCl₃ gave a colorless residue which, on treatment with Et₂O gave **4** (0.028g, 50%, mp 229-230°C, α_D = -1.5 (C, 0.13, MeOH) MH⁺ 365).

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Scheme 3B

Scheme 3B illustrates how to synthesize a Iodine (I) 5-substituted uracil nucleoside (**4**) via Mitsunobu coupling with the 5-substituted base (**11**).



Compound 12

Benzoyl chloride (1.0 mL, 0.009m) was added dropwise to a stirred suspension of **11** (0.95g, 0.004m) in pyridine (4 mL) and acetonitrile (10 mL) under Ar at RT for 4 days. The reaction was concentrated in vacuo and reconcentrated with toluene (3 x 20 mL). Dioxane (15 mL) and 0.25m K₂CO₃ (15 mL) were added and the reaction stirred at RT for 2h. The dioxane was removed in vacuo and the mixture was diluted with water (20 mL). The solid was isolated and recrystallized from 95% ethanol to give **12** (1.13g, 84%, mp 205-207°C).

Compound 14

A solution of DEAD (0.52 mL, 3.31 mmole) in THF (20mL) was added dropwise to a stirred solution of **12** (1.34 g, 3.92 mmole), **13** (0.536g, 165 mmole) and Ph₃P (0.866g, 3.31 mmole) in THF (50mL) under Ar. The reaction was stirred overnight at RT. Concentrated in vacuo. Crude **14** was isolated by silica gel flash chromatography using a step gradient of hexane and 25% EtOAc/hexane. Pure **14** (0.117g, 11%) was obtained by silica gel flash chromatography using a step gradient of hexane, 25% and 30% EtOAc/hexane as a white solid /glass. R_f 0.77(50% EtAc/hexane).

Analyzed for C₃₂H₂₉N₂O₅ I Mw 648.50

Calc: C 59.27; H 4.51; N 4.32

Found: C 59.33; H 4.59; N 4.31

Compound 15

14 (0.110g, 0.17 mmole), conc. NH₄ OH (1mL), and MeOH (14 mL) were stirred for 1h at RT. Concentrated in vacuo. **15** (0.080g, 87%) a white solid was obtained by silica gel flash chromatography using a step gradient of hexane, 25% and 50% EtOAc/hexane.

Analyzed for C₂₅H₂₅N₂O₄ I Mw 544.39

Calc: C 55.16; H 4.63; N 5.16

Found: C 55.18; H 4.62; N 5.19

Compound 4

To a solution of **15** (0.031g, 0.057 mmole) in CH₂Cl₂ (10 mL) cooled in a dry ice/acetone bath was added 1.2 mL 1M BC1₃ / CH₂Cl₂ and the reaction stirred cold for 1 h. MeOH (3 mL) was added and the reaction concentrated in vacuo. Reconcentrated
5 three times with methanol (5mL). **4** (0.012g 57% mp 226-227°C, $\alpha_D = -3$ [c,0.1, MeOH]) was obtained by silica gel flash chromatography using a step gradient of CHCl₃ and 5% MeOH/CHCl₃ and then recrystallization from MeOH/CHCl₃.

Analyzed for C₁₁ H₁₃ N₂ O₄ I·0.1H₂O Mw 365.94

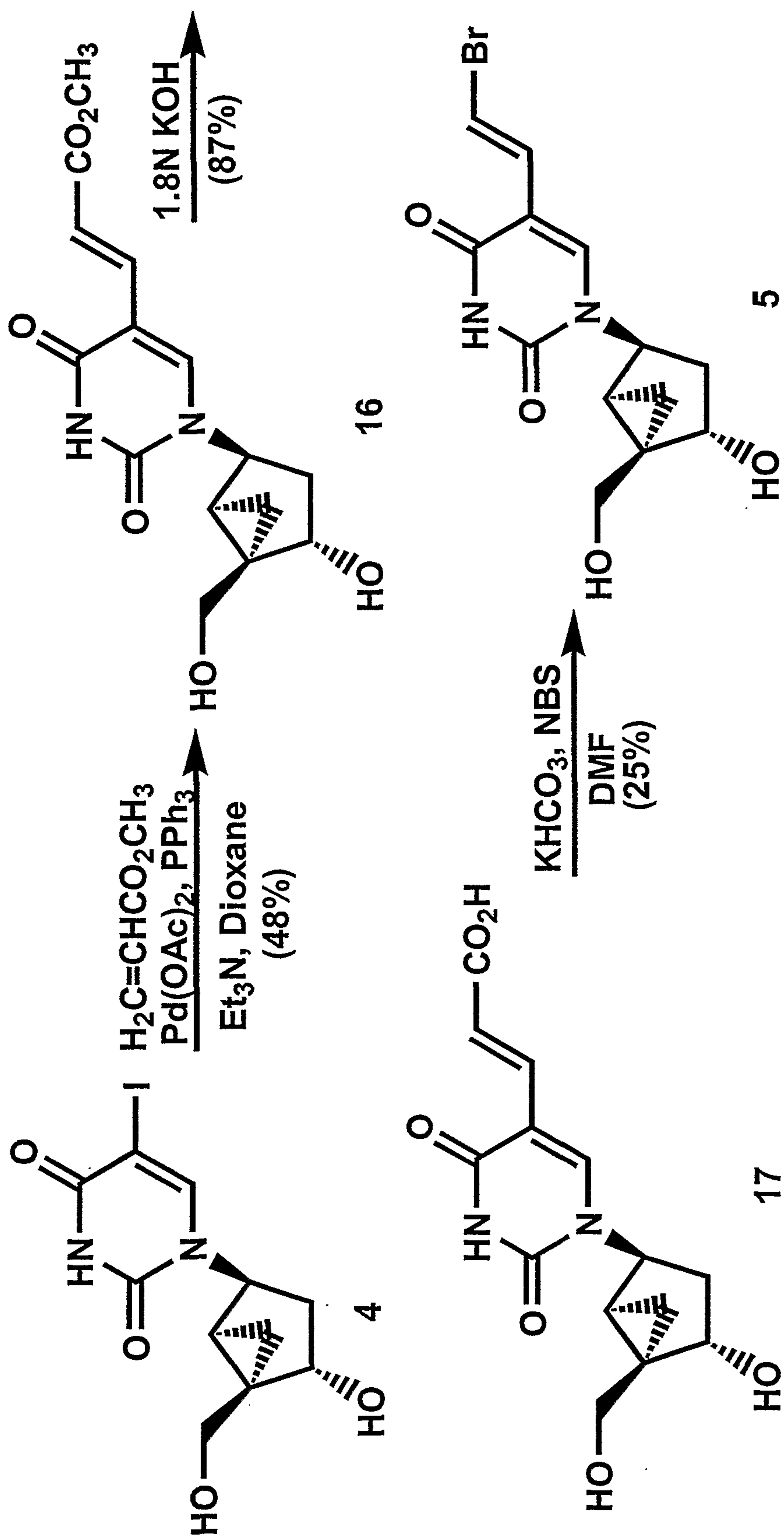
10 Calc: C 36.10; H 3.65; N 7.66

Found: C 36.08; H 3.74; N 7.44

36.13 3.69 7.37

Scheme 4

15 Scheme 4 illustrates how to synthesize a Bromovinyl (CH=CH-Br) 5-substituted uracil nucleoside (**5**) starting with the bicyclo[3.1.0]hexane uracil nucleoside (**2**).



Compound 16

Pd II(OAc)₂ (0.0055g, 0.025 mmole) and Ph₃P (0.013g, 0.050 mmole) were dissolved in dioxane (1 mL) in a vial fitted with a teflon lined cap. Let stand for 10 min during which time the solution turned red. Et₃N (0.055 mL, 0.40 mmole), then methylacrylate (0.110 mL, 1.24 mm), then 4 (0.089g, 0.247 mmole), and finally dioxane (2mL) were added, the vial sealed and heated at 78°C for 4h. Reaction repeated on same scale. The two reactions were combined and concentrated in vacuo. Initial purification by silica gel flash chromatography using a step gradient of CHCl₃, 5% MeOH/CHCl₃ and 10% MeOH/CHCl₃ separated the unreacted 4 from product 16. Using the same chromatography conditions unreacted 4 (0.054g, mp 224-226) was recovered. Treatment of the crude product with EtOAc (2mL) gave 16 (0.076g, 48%) white crystals, mp 240-241°C. $R_F = 0.54$ (15% MeOH/CHCl₃), $[\alpha]_D = -2.4$ [c, 2.3, MeOH]).

Analyzed for C₁₅ H₁₈ N₂ O₆ 0.25 H₂O Mw 326.32

Calc: C 55.21; H 5.56; N 8.58

Found: C 55.14; H 5.69; N 8.50

55.15 5.60 8.53

Ref: Herdewijn, P. et al, J. Med. Chem. 1985, 28, 550-555.

Compound 17

16 (0.165g, 0.512mmole) was stirred in 1.8N KOH (2 mL) overnight. The reaction was acidified with HCl (conc) to pH2 and the solid was isolated by vacuum filtration. The filtrate was concentrated in vacuo and the residue triturated with MeOH. The supernatant was concentrated in vacuo. The combined solids were dissolved in boiling methanol, filtered, and the filtrate concentrated in vacuo to give 17 (0.137g, white solid, 87%, mp 240°C (dec). Used with no further purification.

Compound 5

To a mixture of 17 (0.12g, 0.41 mmole) in DMF (2.5 mL) was added KHCO₃ (0.118g, 1.18, 1.18 mmole) and then dropwise a solution of NBS (0.072g, 0.41 mmole)

in DMF (1 mL). Stirred at RT for 2.5 h. Insolubles were removed by filtration and the filtrate concentrated in vacuo. Crude **5** was isolated by silica gel flash chromatography using a step gradient of CHCl_3 , 5% MeOH/ CHCl_3 and 10% MeOH/ CHCl_3 . **5** was purified further by silica gel flash chromatography using a step gradient of CH_2Cl_2 , 5% i-PrOH/ CH_2Cl_2 and 10% i-PrOH/ CH_2Cl_2 and finally by reverse phase C-18 silica gel flash chromatography using water and 20% MeOH/water to give pure **5** (0.035g, 25% off-white solid, mp 120-122°C [α]_D = -23[c, 0.13, MeOH], MH^+ 343/345).

Analyzed for $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_4\text{Br}\cdot 0.5\text{H}_2\text{O}$ Mw 352.19

10 Calc: C 44.33; H 4.58; N 7.95

Found: C 44.47; H 4.29; N 7.76

44.40; 4.36 7.78

Method of Using

15 The 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues have been found to possess valuable pharmacological properties. They have an anti-viral effect. This effect can be demonstrated using the cytopathogenic effect inhibition assay and the virus plaque reduction assay (Tables 1, 2, and 3).

20 Thus, these analogues can be used to treat viral infections, specifically infections of DNA viruses including members of the Herpesviridae family, especially Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), and Cytomegalovirus (CMV) as well as members of the Poxviridae family.

25 In addition, the analogues can be used in cancer gene therapy. Gene-mediated prodrug activation is a therapeutic approach for the treatment of cancer. It relies on the transfer into tumor cells of a "suicide" gene that encodes an enzyme which, unlike the cellular enzymes, is able to convert a nontoxic prodrug into a toxic metabolite. The most widely investigated system combines the thymidine kinase (tk)-encoding gene of herpes simplex virus (HSV) and ganciclovir (GCV), a nucleoside analogue. The enzyme converts the prodrug into a metabolite that is incorporated into the DNA of
30 dividing cells, which leads to termination of DNA-chain elongation, resulting in death

of the cell. We contemplate the replacement of GCV by 5-substituted conformationally locked nucleoside analogues in the field of suicide gene therapy.

5 The pharmacologically active compounds of this invention can be processed in accordance with conventional methods of galenic pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans.

10 The compounds of this invention can be employed in admixture with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application, which do not deleteriously react with the active compounds. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical
15 preparations can be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. They can also be combined where desired with other active agents, *e.g.*, vitamins.

20 For parenteral application, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules. A syrup, elixir, or the like can be used wherein a sweetened
25 vehicle is employed.

Sustained or directed release compositions can be formulated, *e.g.*, by inclusion in liposomes or incorporation into an epidermal patch with a suitable carrier, for

example DMSO. It is also possible to freeze-dry these compounds and use the lyophilizates obtained, for example, for the preparation of products for injection.

For topical application, there are employed as non-sprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., a FreonTM.

Generally, the compounds of this invention are dispensed in unit dosage form comprising 100 mg to 500 mg in a pharmaceutically acceptable carrier per unit dosage. They are incorporated in topical formulations in concentrations of about 2 % to 10% by weight.

The dosage of the compositions according to this invention generally is 10mg/kg/day to 50 mg/kg/day, when administered to patients, e.g., humans, to treat viral infections analogously to the known agent acyclovir.

It will be appreciate that the actual preferred amounts of active compound in a specific case will vary according to the specific compound being utilized, the compositions formulated, the mode of application, and the particular situs and organism being treated. Dosages for a given host can be determined using conventional considerations, e.g., by customary comparison of the differential activities of the subject compounds and of a known agent, e.g., by means of an appropriate, conventional pharmacological protocol.

The compositions may, if desired, be presented in a pack or dispenser device, which may contain one or more unit dosage forms containing the active ingredient. The

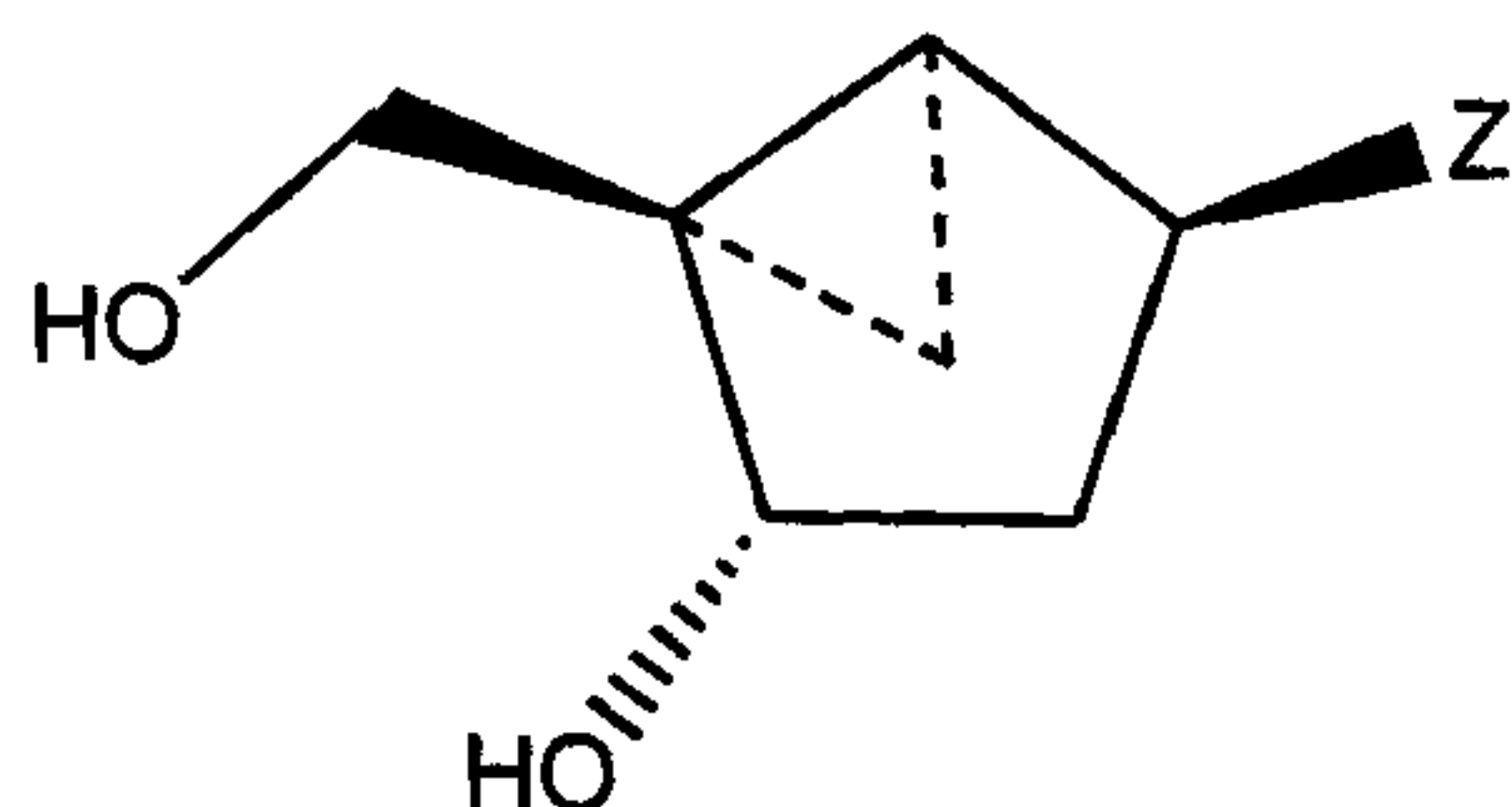
pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

5 Having now fully described the invention, it will be understood to those of ordinary skill in the art that the same can be performed with a wide and equivalent range of conditions, formulations, and other parameters without affecting the scope of the invention or any embodiment thereof.

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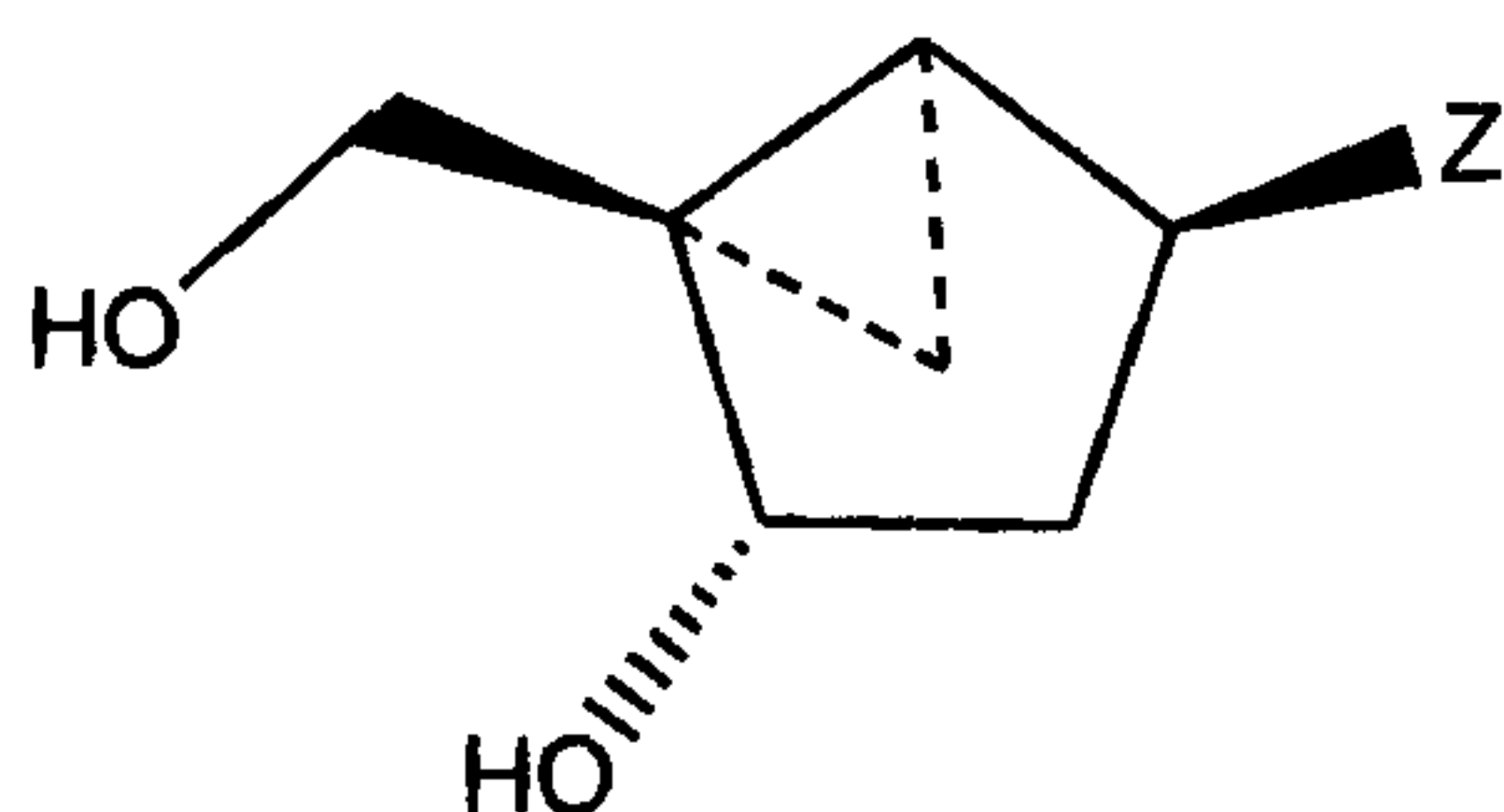
WHAT IS CLAIMED IS:

1. A conformationally locked nucleoside analogue having the formula:



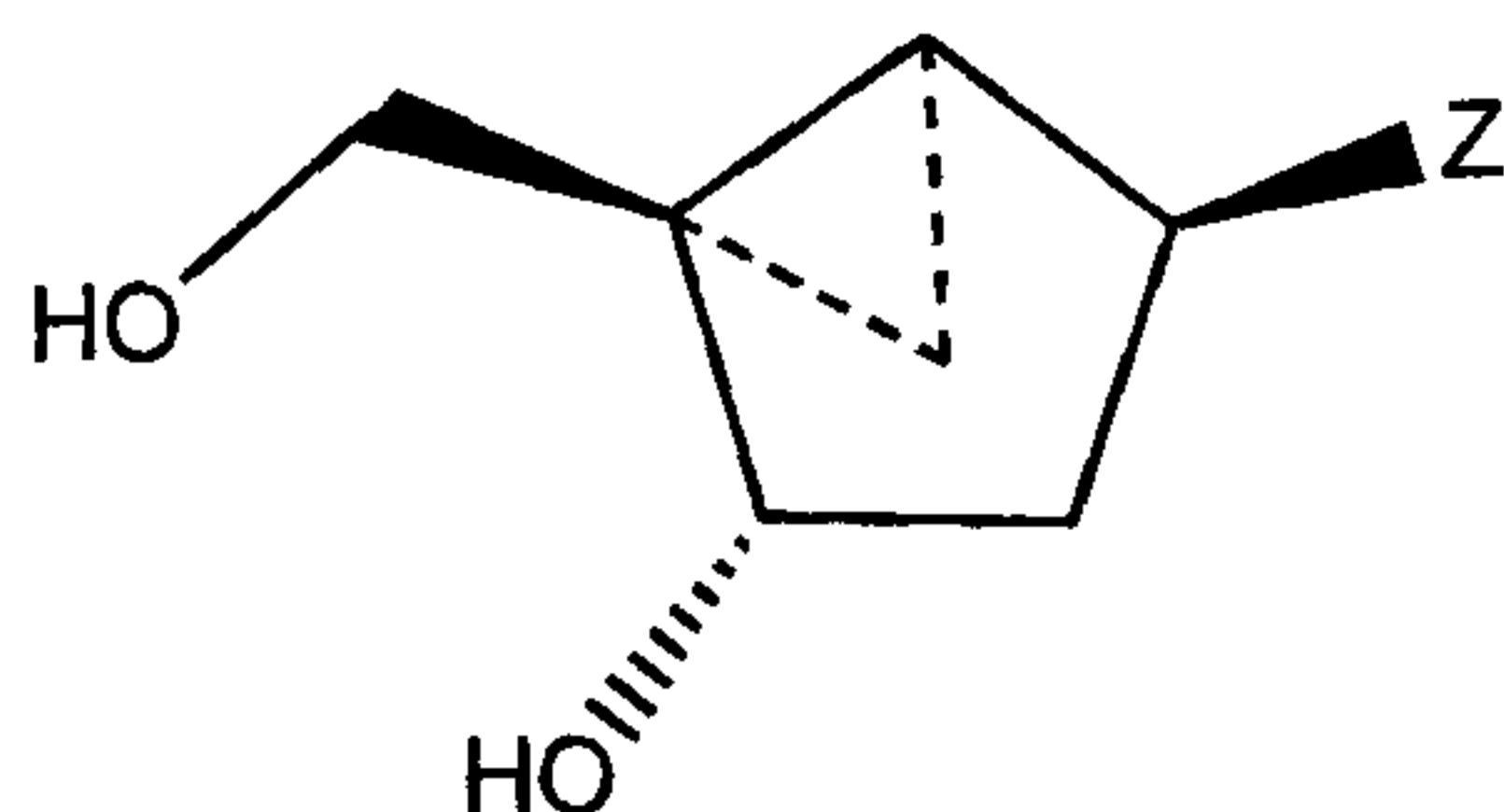
wherein Z is a 5-substituted cytosin-1-yl and the 5-substituent is F, Cl, Br, I, CH=CH₂, C≡CH, C≡CCH₃, CH=CHF, CH=CHCl, CH=CHBr, or CH=CHI.

2. A conformationally locked nucleoside analogue having the formula:



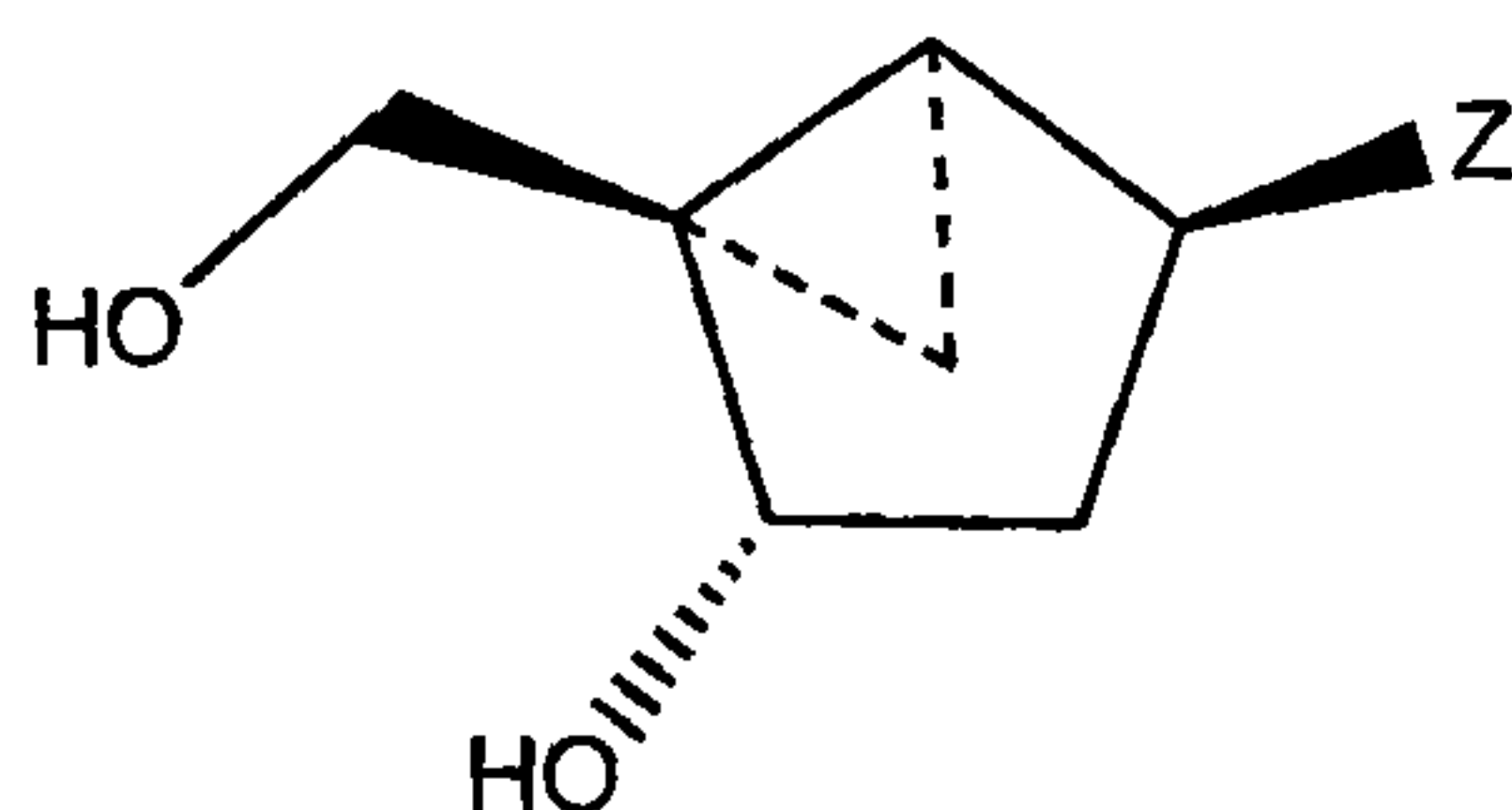
wherein Z is a 5-substituted uracil-1-yl and the 5-substituent is F, Cl, Br, I, CH=CH₂, C≡CH, C≡CCH₃, CH=CHF, CH=CHCl, CH=CHBr, or CH=CHI.

3. A conformationally locked nucleoside analogue having the formula:



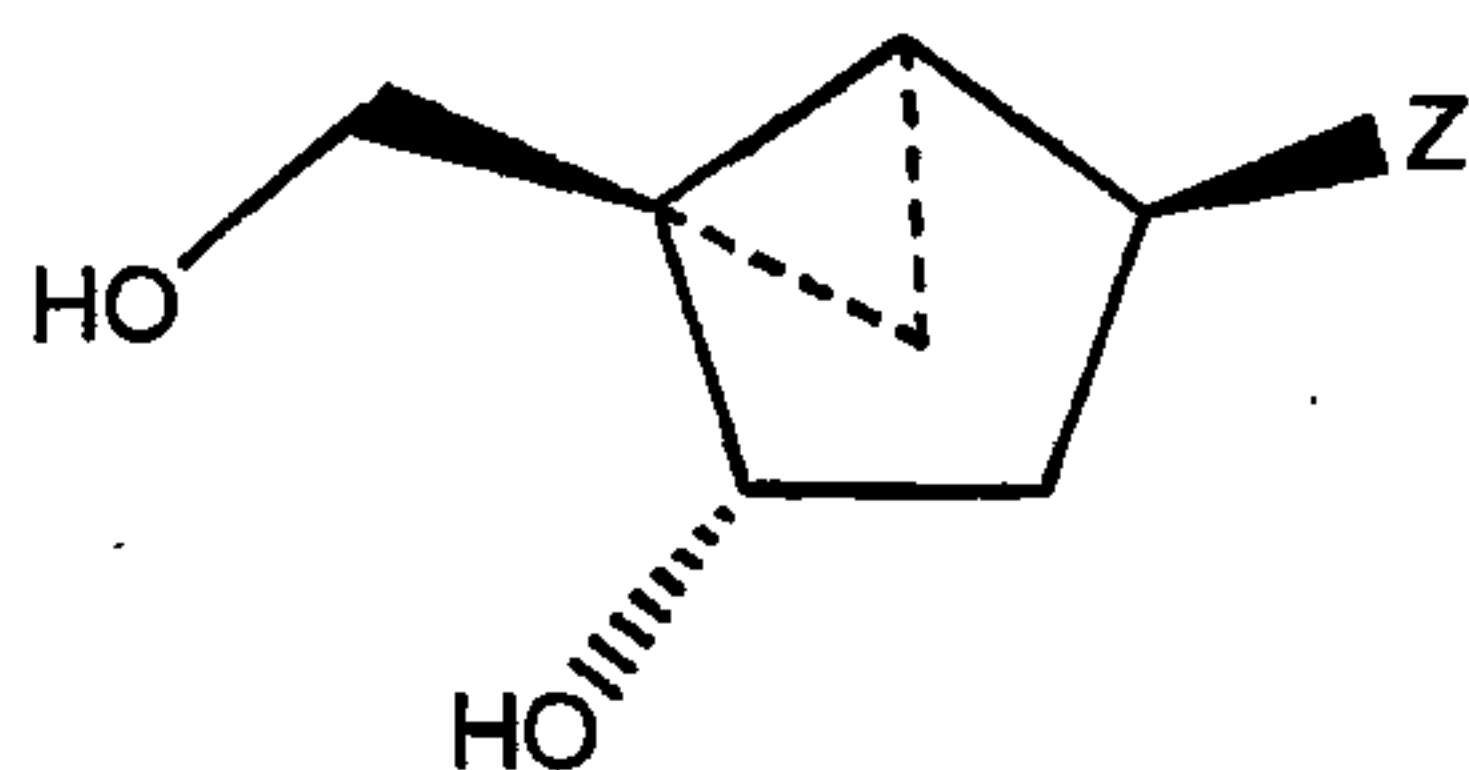
wherein Z is uracil-1-yl having a 5-substituent, wherein the 5-substituent is bromine.

4. A conformationally locked nucleoside analogue having the formula:



wherein Z is uracil-1-yl having a 5-substituent, wherein the 5-substituent is iodine.

5. A conformationally locked nucleoside analogue having the formula:



wherein Z is uracil-1-yl having a 5-substituent, wherein the 5-substituent is bromovinyl.

6. A method of testing the nucleoside analogue as defined in any one of claims 1 to 5 for antiviral activity, the method comprising the step of measuring an effect of the nucleoside analogue in a cytopathogenic effect inhibition assay.
7. A method of testing the nucleoside analogue as defined in any one of claims 1 to 5 for antiviral activity, the method comprising the step of measuring an effect of the nucleoside analogue in a virus plaque reduction assay.
8. Use of an effective amount of the nucleoside analogue as defined in any one of claims 1 to 5 for the treatment of a viral infection in an individual.
9. The use of claim 8 wherein the viral infection is Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), or Cytomegalovirus (CMV).
10. The use of claim 8 wherein the viral infection is Herpes simplex virus HSV-1 or Herpes simplex virus HSV-2.
11. The use of any one of claims 8 to 10 wherein the nucleoside analogue is for parenteral, enteral, topical, or sustained or directed release administration.
12. The use of any one of claims 8 to 11 wherein the effective amount is about 100 mg to 500 mg per unit dosage.
13. Use of an effective amount of the nucleoside analogue as defined in any one of claims 1 to 5 for the termination of DNA-chain elongation in a cell of an individual.
14. The use of claim 13 wherein the cell is modified to express a Herpes simplex virus thymidine kinase.
15. A pharmaceutical composition comprising the nucleoside analogue as defined in any one of claims 1 to 5 and a pharmaceutically acceptable excipient.
16. The pharmaceutical composition of claim 15 for the treatment of a viral infection in an individual.

17. The pharmaceutical composition of claim 16 wherein the viral infection is Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), or Cytomegalovirus (CMV).

18. The pharmaceutical composition of claim 16 wherein the viral infection is Herpes simplex virus HSV-1 or Herpes simplex virus HSV-2.

19. The pharmaceutical composition of any one of claims 15 to 18 which is for parenteral, enteral, topical, or sustained or directed release administration.

20. The pharmaceutical composition of any one of claims 15 to 19 wherein the nucleoside analogue is present in an amount of about 100 mg to 500 mg per unit dosage.

21. The pharmaceutical composition of claim 15 for the termination of DNA-chain elongation in a cell of an individual.

22. The pharmaceutical composition of claim 21 wherein the cell is modified to express a Herpes simplex virus thymidine kinase.

23. A pack comprising the nucleoside analogue as defined in any one of claims 1 to 5 in unit dosage form, together with instructions for the use thereof in the treatment of a viral infection.

24. A method of making a nucleoside analogue comprising the steps of: reacting 5-iodo-3-phenylpyrimidine-2,4 (1H,3H)-dione with diethyl azodicarboxylate and PPh_3 in a solution of tetrahydrofuran, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with NH_4OH in methanol, whereby a first intermediate is obtained; and reacting the first intermediate with BCl_3 in methylene chloride to form the nucleoside analogue as defined in claim 2 where the 5-substituent is I.

25. A method of making the nucleoside analogue as defined in claim 3 comprising the steps of: reacting a bicyclo[3.1.0]hexane template with (E)-3-ethoxyacryloyl isocyanate to yield a first intermediate; and reacting the first intermediate with H_2SO_4 in ethanol, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with Br_2 in AcO_2 to yield a second intermediate; and reacting the second intermediate with NH_3 in methanol, followed by NaOH in tetrahydrofuran, whereby the nucleoside analogue as defined in claim 3 is obtained.

26. A method of making the nucleoside analogue as defined in claim 4 comprising the steps of: reacting 5-iodo-3-benzoylpyrimidine-2,4 (1H,3H)-dione with

diethyl azodicarboxylate and PPh_3 in a solution of tetrahydrofuran, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with NH_4OH in methanol, whereby a first intermediate is obtained; and reacting the first intermediate with BCl_3 in methylene chloride, whereby the nucleoside analogue as defined in claim 4 is obtained.

27. A method of making the nucleoside analogue as defined in claim 5 comprising the steps of: reacting 5-iodo-3-benzoylpyrimidine-2,4 (1H,3H)-dione with diethyl azodicarboxylate and PPh_3 in a solution of tetrahydrofuran, whereby a bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate is obtained; reacting the bicyclo[3.1.0]hexane pyrimidine nucleoside intermediate with NH_4OH in methanol, whereby a first intermediate is obtained; reacting the first intermediate with BCl_3 in methylene chloride whereby a second intermediate is obtained; reacting the second intermediate with methacrylate in the presence of $\text{PdII}(\text{OAc})_2$, PPh_3 , NEt_3 and dioxane, whereby a third intermediate is obtained; hydrolyzing the third intermediate with KOH , whereby a fourth intermediate is obtained; and reacting the fourth intermediate with NBS in the presence of KHCO_3 and DMF, whereby the nucleoside analogue as defined in claim 5 is obtained.

28. The method of claim 24, further comprising converting the nucleoside analogue where the 5-substituent is I to the nucleoside analogue of claim 2, where the 5-substituent is F, Cl, Br, $\text{CH}=\text{CH}_2$, $\text{C}\equiv\text{CH}$, $\text{C}\equiv\text{CCH}_3$, $\text{CH}=\text{CHF}$, $\text{CH}=\text{CHCl}$, $\text{CH}=\text{CHBr}$, or $\text{CH}=\text{CHI}$.