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

5-SUBSTITUIERTE PYRIMIDINDERIVATE EINES KONFORMATIONSFESTGELEGTEN NUKLEOSIDANALOGEN

DERIVES DE PYRIMIDINE 5-SUBSTITUEE D'ANALOGUES DE NUCLEOSIDES A BLOCAGE CONFORMATIONNEL

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(56) References cited:
**WO-A-95/08541 WO-A-98/05662**

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EP 1 305 296 B9

## Description

**[0001]** 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.

**[0002]** 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 need for effective anti-viral and anti-cancer agents.

**[0003]** 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.

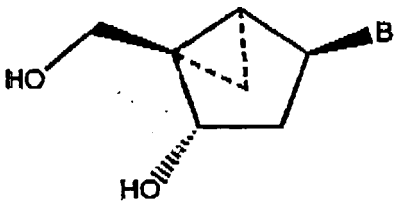
**[0004]** Marquez et al., J. Med. Chem. 39:3739 (1996) describe purine and pyrimidine nucleoside analogs having natural bases attached to a bicyclo[3.1.0]hexane pseudosugar template. Grignet-Debrus et al., Cancer Gene Therapy 7:215 (2000) describe modified bases, particularly 5-substitated uracils, 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 bicycle[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.

**[0005]** WO 95/08541-A discloses conformationally locked 4',6'-cyclopropanc-fused carbocyclic nucleoside analogues prepared by condensing a cyclopropane-fused carbocyclic allylic alcohol with substituted purine or pyrimidine bases, then modifying the condensation product to produce the adenosine, guanosine, cytidine, thymidine and uracil nucleoside analogues. The compounds are disclosed as therapeutically useful as antimetabolites, or in the preparation of antimetabolic agents.

**[0006]** WO 98/05662-A discloses a method for the treatment of herpes virus infection by administering an effective virus-inhibiting amount of a cyclopropanated carbocyclic 2'-deoxynucleoside to an individual in need thereof. The nucleoside analogs are disclosed as effective against herpes simplex virus types 1 and 2, Epstein-Barr virus and human cytomegalovirus.

**[0007]** 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.

**[0008]** 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues have the formula:




**[0009]** B is a 5-substituted derivative of uracil and the 5-substituent is a member of the group consisting of I, $CH=CH_2$, CH=CHF, CH=CHCl, CH=CHBr and CH=CHI.

**[0010]** 5-substituted pyrimidine derivatives of conformationally locked nucleoside analogues may be tested for antiviral activity by a variety of methods known in the art.

**[0011]** One assay is the cytopathogenic effect inhibition assay. Another assay is the virus plaque reduction assay.

**[0012]** 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 $2.5 \times 10^4$ 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% $CO_2$-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 $C0_2$ 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.

**[0013]** 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 μg/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.

**[0014]** 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.

**[0015]** 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-substitutent 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, CH=CH-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.

**[0016]** Tables 1, 2, and 3 demonstrate antiviral activity of 5-substituted uracils (Br, I, CH=CH-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-substitutuent 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.

**[0017]** Thus, the invention also relates to the use of the compounds as defined in the annexed claims for the manufacture of a medicament for the treatment of a viral infection in an individual in need thereof. The use of the invention typically comprises mixing said compound 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 Cytomegalovinis (CMV).

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

| Compound | HSV-1 (HFF)[a] CPE inhib. $BC_{50}$[b] (μg/ml) | HSV-1 (HFF) CPE inhib. $CC_{50}$[c] (μg/ml) | SI[d] | 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 | 0 | 3.3 | >100 | 30.3 | | | | | | |
| (N)-MCdBrU (**3**) reference example | 0.39 | >100 | >256 | 0.70 | >100 | >142 | 0.03 | >100 | 3333 | 0.12 | >L00 | 833 |
| (N)-MCBVDU **(5)** | 2.2 | >100 | >45 | >100 | >100 | 0 | | | | | | |
| ACV[e] (control) | 0.5 | | | | | | | | | | | |

[a]HFF = human foreskin fibroblast;
[b]$EC_{50}$ = inhibitory concentration required to reduce virus induced cytopathogenic effect (CPE) or virus plaques by 50%;
[c]$CC_{50}$ = cytotoxic concentration that produces 50% of cell growth; [d]SI = selectivity index ($CC_{50}/EC_{50}$);
[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 $EC_{50}$ (μg/ml) | 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)** reference example | 0.64 | >100 | >156 | 2.6 | >100 | 38.5 | 11.5 | >100 | 8.7 | 3.4 | >100 | 29.4 |

[a]HFF = human foreskin fibroblast;
[b]$BC_{50}$ = inhibitory concentration required to reduce virus induced cytopathogenic effect (CPE) or virus plaques by 50%;
[c]$CC_{50}$ = cytotoxic concentration that produces 50% of cell growth; [d]SI = selectivity index ($CC_{50}/EC_{50}$);
[e] acyclovir

Table 3. Antiviral activity against VZV

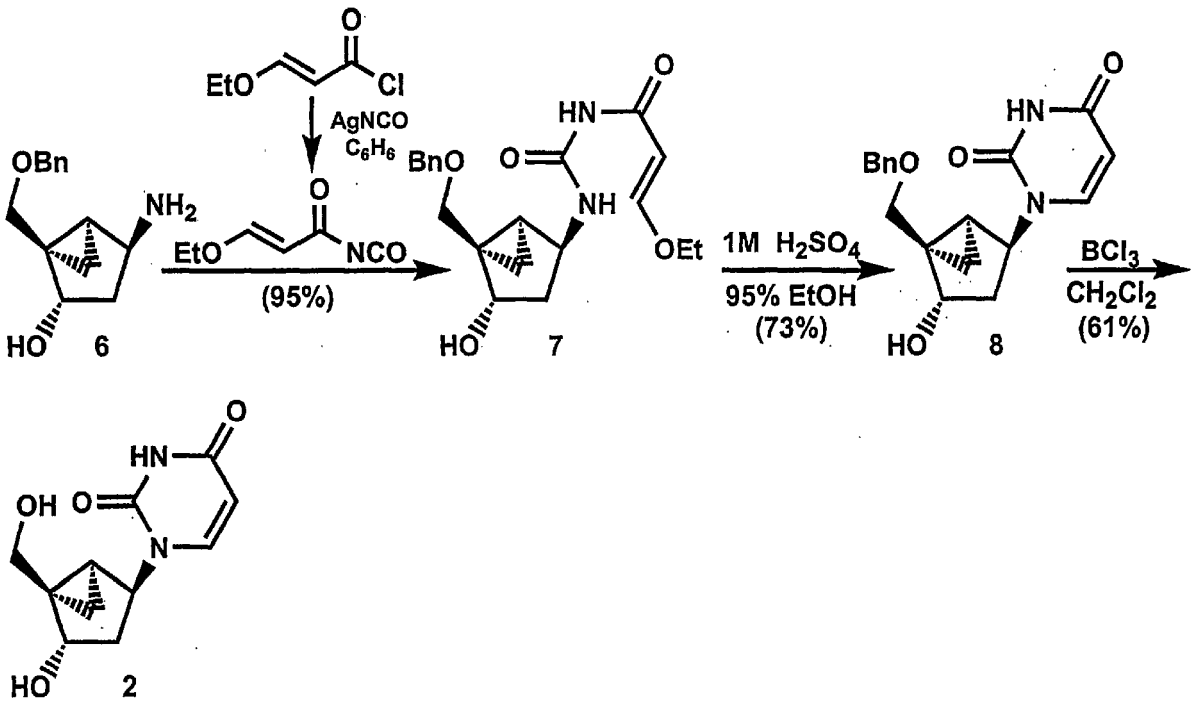
| Compound | Antiviral activity (Plaque reduction) $EC_{50}$ (μM)[a] | | | | Cytotoxicity (μM) | |
|---|---|---|---|---|---|---|
| | TK+ VZV | | TK-VZV | | Cell Morphology (MCC)[b] | $CC_{50}$[c] |
| | YS strain | OKA strain | 07/1 strain | YS/R strain | | |
| (N)-MCdBrU **(3)** reference example | 0.03 | 0.89 | 107 | 37 | >200 | >200 |
| (N)-MCdIU **(4)** | 012 | 0.16 | >5 | 3 | >5 | >200 |
| (N)-MCBVDU **(5)** | 0.007 | 0.005 | >5 | >5 | >5 | >200 |
| ACV (control) | 2.4 | 2.4 | 30 | 19 | >200 | 488 |
| BVDU (control) | 0.008 | 0.005 | >150 | >150 | >150 | >400 |

[a]$EC_{50}$ = inhibitory concentration required to reduce virus plaques by 50%;
[b]MCC = minimum cytotoxic concentration that causes microscopically detectable alteration of cell morphology;
[c]$CC_{50}$ = cytotoxic concentration that produces 50% of cell growth.

**Methods of Synthesis**

**Scheme 1**

**[0018]** Scheme 1 illustrates how to synthesize a bicyclo[3.1.0]hexane uracil nucleoside **(2)** starting with a bicyclo[3.1.0] hexane template **(6).**



Compound **7**

**[0019]** 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**

**[0020]** 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, $\alpha]_D$ =+59(C,0.16,MeOH)) was obtained by silica gel flash chromatography using EtOAc.

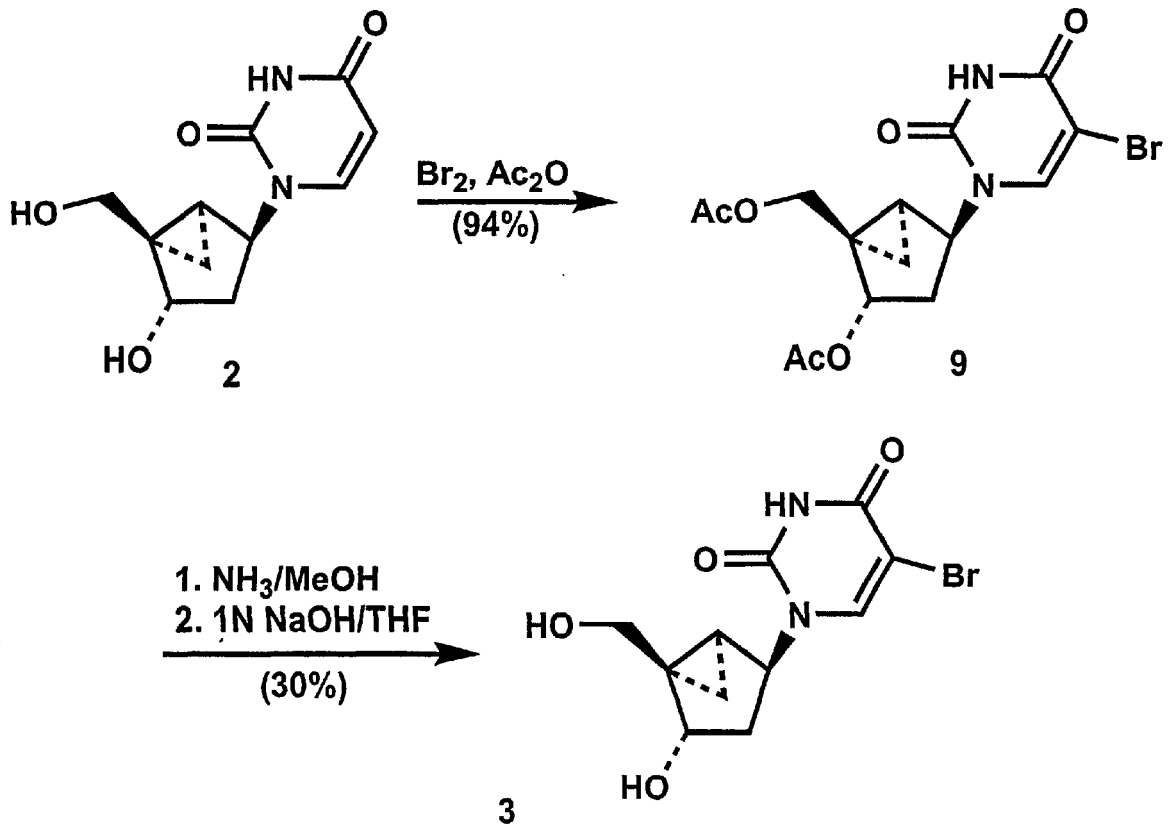
| Analyzed for $C_{18}H_{20}N_2O_4 \cdot 0.25H_2O$ | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | Mw 332.88 | |
| Calc: | C | 64.95; | H | 6.21; | N | 8.42 |
| Found: | C | 65.12; | H | 6.33; | N | 8.20 |
| | | 65.02 | | 6.27 | | 8.14 |

Compound **2**

**[0021]** To **8** (0.164g, 0.5 mmole) in $CH_2Cl_2$ (30mL) cooled to -78°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).

**Scheme 2**

**[0022]** Scheme 2 illustrates how to synthesize a Bromine (Br) 5-substituted uracil nucleoside **(3)** (reference example) starting with the bicyclo[3.1.0]hexane uracil nucleoside **(2)**.




Compound **9**

**[0023]** **2** (0.073g, 0.31 mmole) was dissolved in $Ac_2O$ (2mL) with warming and cooled to RT under Ar. $Br_2$ (0.02 mL, 0.33 mmole) was added dropwise, the reaction stirred at RT for 0.5h and stored in the refrigerator overnight. The $Ac_2O$ 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.

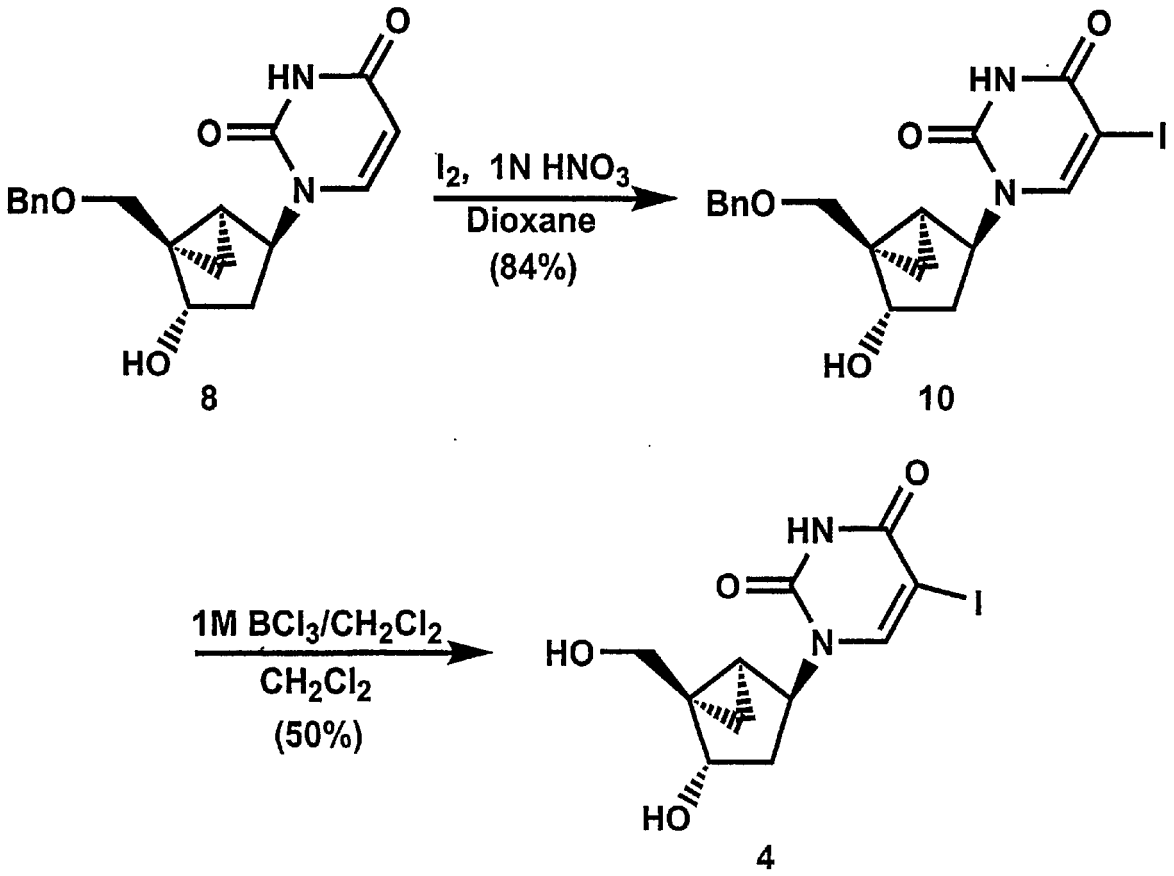
Reference Compound **3**

**[0024]** **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 $NH_3$/MeOH ($R_F$ 0.32, 15% MeOH/$CHCl_3$) 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, $\alpha]_D$ =+18.2 (C, 0.11, MeOH), $MH^+$ 317/319, 30%) was obtained by silica gel flash chromatography using a step gradient of $CFiCl_3$ and 10% MeOH/$CHCl_3$.

| | | | | | | |
|---|---|---|---|---|---|---|
| Analyzed for $C_{11}H_{13}N_2O_4Br$ | | 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**

**[0025]** Scheme 3A illustrates how to synthesize a Iodine (1) 5-substituted uracil nucleoside **(4)** starting with the bicyclo [3.1.0]hexane uracil nucleoside (2).




Compound **10**

**[0026]** A stirred solution of **8** (0.164g, 0.5 mmole), $I_2$ (0.254g, 1.0 mmole) and 1N $HNO_3$ (0.5mL) in dioxane (5mL) was heated at 100°C for 1h. Concentrated in vacuo and reconcentrated with ethanol (3 x5mL) 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.

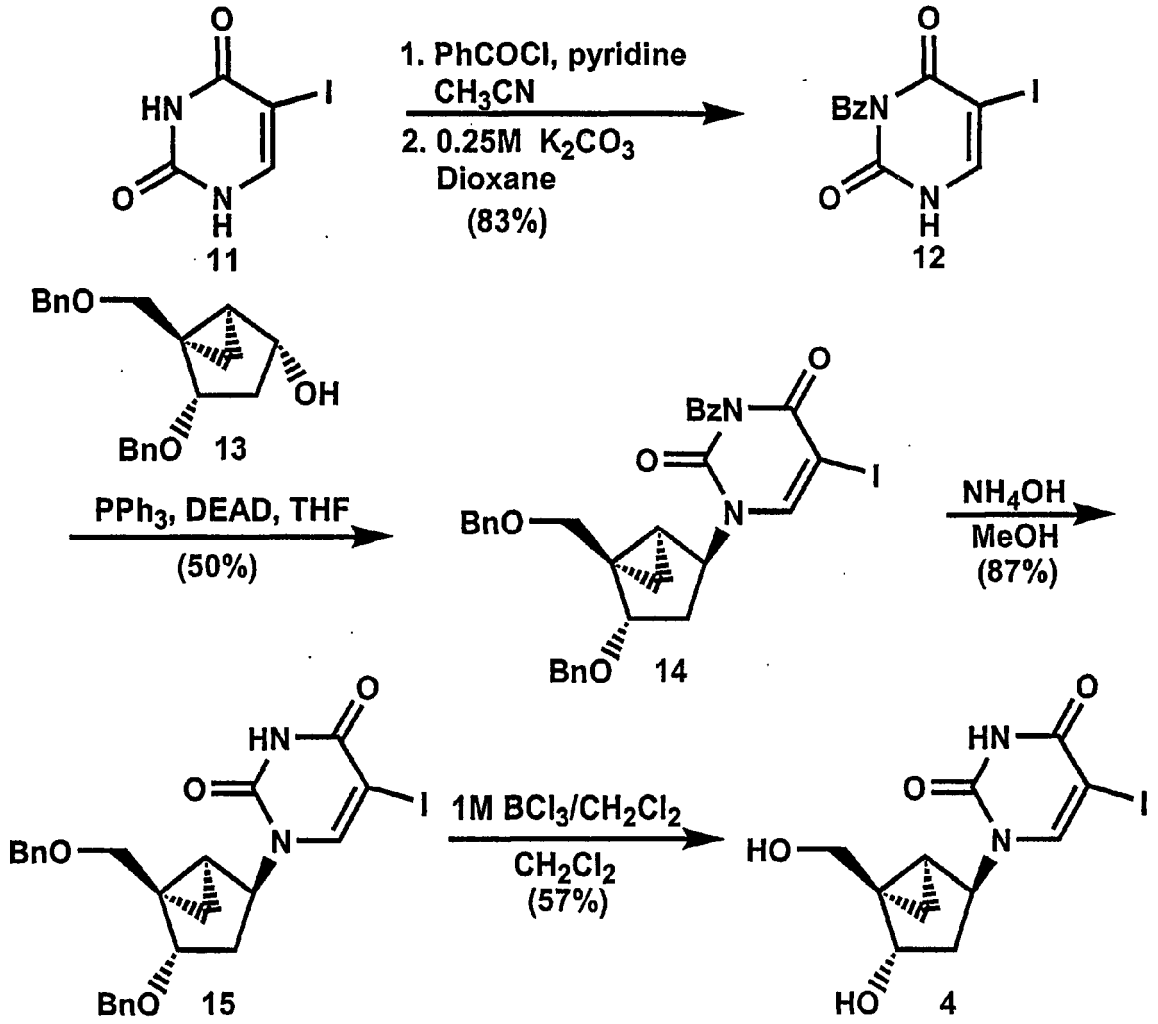
| | | | | | | |
|---|---|---|---|---|---|---|
| Analyzed for $C_{18}H_{19}N_2O_4I \cdot 0.5\ H_2O$ | | | | | Mw 463.28 | |
| Calc: | C | 46.67; | H | 4.35; | N | 6.06 |
| Found: | C | 46.24; | H | 4.22; | N | 5.89 |
| | | 46.33 | | 4.18 | | 5.92 |

Compound **4**

**[0027]** To a solution of **10** (0.072g, 0.16mmole) in $CH_2Cl_2$ (10 mL) cooled in dry ice/acetone was added 1M $BCl_3/CH_2Cl_2$ (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 $CHCl_3$ and 5% MeOH/$CHCl_3$ gave a colorless residue which, on treatment with $Et_2O$ gave **4** (0.028g, 50%, mp 229-230°C, $\alpha]_D$ =1.5 (C, 0.13, MeOH) $MH^+$ 365).

**Scheme 3B**

**[0028]** 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**

**[0029]** 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 acetonitrite (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 $K_2CO_3$ (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**

**[0030]** 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 $Ph_3P$ (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 $C_{32}H_{29}N_20_5I$ | | | | 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**

**[0031]** **14** (0.110g, 0.17 mmole), conc. $NH_4$ 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 $C_{25}H_{25}N_2O_4I$ | | | | Mw 544.39 | | |
| Calc: | C | 55.16; | H | 4.63; | N | 5.16 |
| Found: | C | 55.18; | H | 4.62; | N | 5.19 |

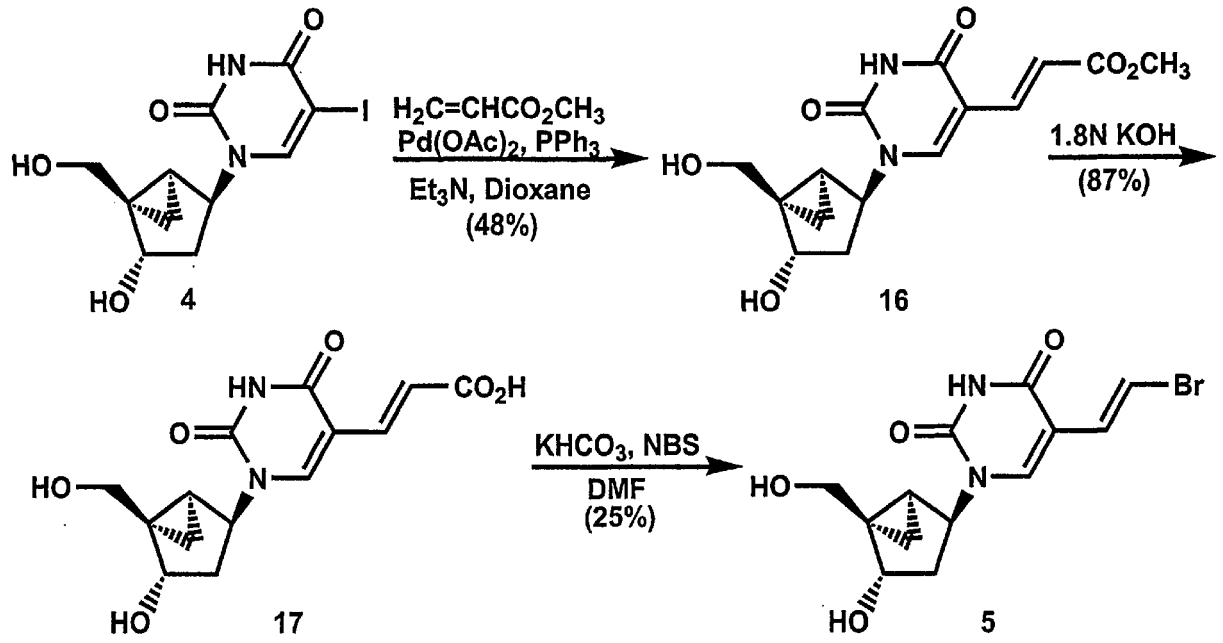
Compound **4**

**[0032]** To a solution of **15** (0.031g, 0.057 mmole) in $CH_2Cl_2$ (10 mL) cooled in a dry ice/acetone bath was added 1.2 mL 1M $BCl_3$ / $CH_2Cl_2$ and the reaction stirred cold for 1 h. MeOH (3 mL) was added and the reaction concentrated in vacuo. Reconcentrated 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 $CHCl_3$ and 5% MeOH/$CHCl_3$ and then recrystallization from MeOH/$CHCl_3$.

| | | | | | | |
|---|---|---|---|---|---|---|
| Analyzed for $C_{11}H_{13}N_2O_4I \cdot 01H_2O$ | | | | Mw 365.94 | | |
| 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**

**[0033]** 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**

**[0034]** Pd II$(OAc)_2$ (0.0055g, 0.025 mmole) and $Ph_3P$ (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. $Et_3N$ (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 $CHCl_3$, 5% MeOH/$CHCl_3$ and 10% MeOH/$CHCl_3$ 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/$CHCl_3$), $\alpha]_D$ = -2.4 [c, 2.3, MeOH]).

| | | | | | | |
|---|---|---|---|---|---|---|
| Analyzed for $C_{15}H_{18}N_20_60\cdot25H_2O$ | | | | | 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**

**[0035]** **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**

**[0036]** To a fixture of **17** (0.12g, 0.41 mmole) in DMF (2.5 mL) was added $KHCO_3$ (0.118g, 1.18, 1.18 mmole) and then dropwise a solution ofNBS (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 $[\alpha]_D$ = -23[c, 0.13, MeOH], $MH^+$ 343/345).

| | | | | | | |
|---|---|---|---|---|---|---|
| Analyzed for $C_{13}H_{15}N_2O_4Br \cdot 0.5 H_2O$ | | | | | Mw 352.19 | |
| 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**

**[0037]** 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).

**[0038]** 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.

**[0039]** 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 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.

**[0040]** 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.

**[0041]** 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 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.

**[0042]** 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.

**[0043]** 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 vehicle is employed.

**[0044]** 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.

**[0045]** 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 freon.

**[0046]** 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.

**[0047]** The dosage of the compositions according to this invention generally is 10mg/kg/day to 50 mg/kg/day, when

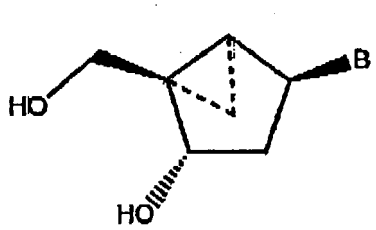
administered to patients, e.g., humans, to treat viral infections analogously to the known agent acyclovir.

**[0048]** 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.

**[0049]** 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.

## Claims

**1.** A 5-substituted pyrimidine derivative of a conformationally locked nucleoside analogue having the formula:




wherein B is a 5-substituted derivative of uracil, wherein the 5-substituent is I, $CH=CH_2$, CH=CHF, CH=CHCl, CH=CHBr or CH=CHI.

**2.** The nucleoside analogue of claim 1 which is Iodine 5-substituted uracil nucleoside.

**3.** The nucleoside analogue of claim 1 which is Bromovinyl 5-substituted uracil nucleoside.

**4.** A method of testing the nucleoside analogue of any of claims 1-3 for antiviral activity comprising the step of measuring its effect in a cytopathogenic effect inhibition assay.

**5.** A method of testing the nucleoside analogue of any of claims 1-3 for antiviral activity comprising the step of measuring its effect in a virus plaque reduction assay.

**6.** A method of making the nucleoside analogue of any of claims 1-3 comprising the step of combining a 5-substitafed pyrimidine moiety with a bicyclo[3.1.0]hexane template.

**7.** A method of making the nucleoside analogue of any of claims 1-3 comprising the step of proceeding via Mitsunobu coupling with a 5-substituted base.

**8.** Use of a compound of any of claims 1-3 in a pharmaceutically acceptable carrier for the manufacture of a medicament for treating viral infections in an individual in need thereof.

**9.** The use of claim 8 wherein said viral infection is selected from the group consisting of Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), and Cytomegalovirus (CMV).

**10.** The use of claim 8 wherein the administering step of the medicament is parenteral, enteral, topical, or sustained or directed release.

**11.** The use of claim 8 wherein the effective amount of the compound is about 100 mg to 500 mg per unit dosage.

**12.** Use of a compound of any of claims 1-3 in a pharmacentically acceptable carrier for the manufacture of a medicament for terminating DNA-chain elongation in the cells of an individual in need thereof.

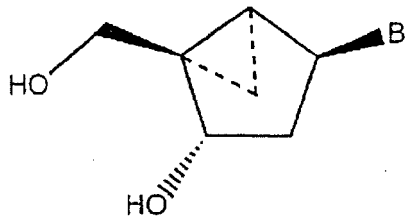
**13.** The use of claim 12 wherein said cells are modified to express a Herpes simplex virus thymidine kinase.

**14.** A pharmaceutical composition comprising the compound of any of claims 1-3 in a pharmaceutically acceptable excipient.

**15.** A pack comprising the compound of any of claims 1-3 in unit dosage form.

**Patentansprüche**

**1.** 5-substituiertes Pyrimidinderivat eines konformations-festgelegten Nucleosidanalogen mit der Fonnel:




wobei B ein 5-substituiertes Uracilderivat ist, wobei der 5-Substituent für I, $CH=CH_2$, CH=CHF, CH=CHCl, CH=CHBr oder CH=CHI steht.

**2.** Nucleosidanalog gemäß Anspruch 1, nämlich Jod-5-substituiertes Uracilnucleosid.

**3.** Nucleosidanalog gemäß Anspruch 1, nämlich Bromvinyl-5-substituiertes Uracilnucleosid.

**4.** Verfahren zum Testen des Nucleosidanalogs gemäß einem der Ansprüche 1 bis 3 auf antivirale Aktivität, umfassend den Schritt des Messens seiner Wirkung in einer cytopathogenen Effekt-Verzögerungsanalyse.

**5.** Verfahren zum Testen des Nucleosidanalogs gemäß einem der Ansprüche 1 bis 3 auf antivirale Aktivität, umfassend den Schritt des Messens seiner Wirkung in einer Virusplaque-Reduktionsanalyse.

**6.** Verfahren zur Herstellung des Nucleosidanalogs gemäß einem der Ansprüche 1 bis 3, umfassend den Schritt des Kombinierens einer 5-substituierten Pyrimidinkomponente mit einem Bicyclo[3.1.0]hexan-Template.

**7.** Verfahren zur Herstellung des Nucleosidanalogs gemäß einem der Ansprüche 1 bis 3, umfassend den Schritt des Vorgehens über eine Mitsunobu-Kopplung mit einer 5-substituierten Base.

**8.** Verwendung einer Verbindung gemäß einem der Ansprüche 1 bis 3 in einem pharmazeutisch verträglichen Träger zur Herstellung eines Medikaments zur Behandlung von Virusinfektionen bei einem Individuum, das dieser bedarf.

**9.** Verwendung gemäß Anspruch 8, wobei die Virusinfektion ausgewählt ist aus Herpes Simplex-Virus (HSV), Varicella Zoster-Virus (VZV), Epstein Barr-Virus (EBV) und Cytomegalovirus (CMV).

**10.** Verwendung gemäß Anspruch 8, wobei der Schritt des Verabreichens des Medikaments parenteral, enteral, topisch oder mit Freisetzung mit konstanter Geschwindigkeit bzw. mit direkter Freisetzung von statten geht.

**11.** Verwendung gemäß Anspruch 8, wobei die wirksame Menge der Verbindung etwa 100 mg bis 500 mg pro Dosierungseinheit beträgt.

**12.** Verwendung einer Verbindung gemäß einem der Ansprüche 1 bis 3 in einem pharmazeutisch verträglichen Träger zur Herstellung eines Medikaments zum Beenden von DNA-Ketten-Ausdehnung in den Zellen eines Individuums, das dieser Behandlung bedarf.

**13.** Verwendung gemäß Anspruch 12, wobei die Zellen derart modifiziert sind, dass sie eine Herpes Simplex-Virus-

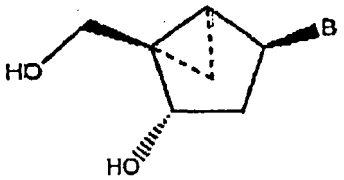
Thymidinkinase exprimieren.

**14.** Arzneimittel, umfassend die Verbindung gemäß einem der Ansprüche 1 bis 3 in einem pharmazeutisch verträglichen Exzipienten.

**15.** Packung, umfassend die Verbindung gemäß einem der Ansprüche 1 bis 3 in Einheitsdosisform.

**Revendications**

**1.** Dérivé de pyrimidine 5-substitué d'un analogue de nucléoside à conformation bloquée ayant la formule :




dans laquelle B est un dérivé 5-substitué d'uracile, dans lequel le 5-substituant est I, $CH=CH_2$, CH=CHF, CH=CHCl, CH=CHBr ou CH=CHI.

**2.** Analogue de nucléoside selon la revendication 1, qui est le nucléoside uracile 5-substitué par de l'iode.

**3.** Analogue de nucléoside selon la revendication 1, qui est le nucléoside uracile 5-substitué par un bromovinyle.

**4.** Procédé pour tester l'analogue de nucléoside selon l'une quelconque des revendications 1 à 3 quant à l'activité antivirale, comprenant l'étape consistant à mesurer son effet dans un essai d'inhibition d'effet cytopathogène.

**5.** Procédé pour tester l'analogue de nucléoside selon l'une quelconque des revendications 1 à 3 quant à l'activité antivirale, comprenant l'étape consistant à mesurer son effet dans un essai de réduction de plaque virale.

**6.** Procédé de préparation de l'analogue de nucléoside selon l'une quelconque des revendications 1 à 3, comprenant l'étape consistant à combiner un fragment pyrimidine 5-substitué avec une matrice de bicyclo[3.1.0]hexane.

**7.** Procédé de préparation de l'analogue de nucléoside selon l'une quelconque des revendications 1 à 3, comprenant l'étape consistant à procéder par couplage de Mitsunobu avec une base 5-substituée.

**8.** Utilisation d'un composé selon l'une quelconque des revendications 1 à 3 dans un support pharmaceutiquement acceptable pour la fabrication d'un médicament pour traiter des infections virales chez un individu en ayant besoin.

**9.** Utilisation selon la revendication 8, dans laquelle ladite infection virale est choisie dans le groupe constitué par le virus de l'herpès simplex (HSV), le virus varicelle-zona (VZV), le virus d'Epstein Barr (EBV) et le cytomégalovirus (CMV).

**10.** Utilisation selon la revendication 8, dans laquelle l'étape d'administration du médicament est parentérale, entérale, topique, ou à libération prolongée ou dirigée.

**11.** Utilisation selon la revendication 8, dans laquelle la quantité efficace du composé est d'environ 100 mg à 500 mg par dosage unitaire.

**12.** Utilisation d'un composé selon l'une quelconque des revendications 1 à 3 dans un support pharmaceutiquement acceptable pour la fabrication d'un médicament pour mettre fin à un allongement de chaîne d'ADN dans les cellules d'un individu en ayant besoin.

**13.** Utilisation selon la revendication 12, dans laquelle lesdites cellules sont modifiées pour exprimer une thymidine kinase du virus de l'herpès simplex.

**14.** Composition pharmaceutique comprenant le composé selon l'une quelconque des revendications 1 à 3 dans un excipient pharmaceutiquement acceptable.

**15.** Conditionnement comprenant le composé selon l'une quelconque des revendications 1 à 3 sous forme de dosage unitaire.

## REFERENCES CITED IN THE DESCRIPTION

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- **Herdewijn, P. et al.** *J. Med. Chem.,* 1985, vol. 28, 550-555 **[0034]**