Regio- and Stereoselective Synthesis of Carbocyclic 2',3'-Dideoxy-3'-fluoro Nucleosides as Potential Antiviral Agents

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The synthesis and antiviral activity of racemic carbocyclic 2',3'-dideoxy-3'-fluoro nucleosides are reported. Carbo cyclic 2',3'-dideoxy-3'-fluoro nucleosides were obtained from the 3-fluoro cyclopentane derivative 4, which was prepared by two methods. The S-dep-protection of the hydroxyl group of (±)-(1β,2α,3β,4β)-4-acetamido-2-fluoro-3-hydroxyxycyclopentymethyl acetate (1) with Ph3P-I2 followed by tin hydride reduction afforded the 3-fluoroamino alcohol derivative 3. Alternatively, the protected fluoroamino alcohol 3 was prepared by regio- and stereoselective bromo-fluorination of cis-4β-acetamidocyclopent-2-enemethyl acetate (5) with hydrogen fluoride-pyridine/N-homosuccinimide followed by tin hydride reduction to remove the bromine atom. Carbocyclic 2',3'-dideoxy-3'-fluoroguanosine (14) thus obtained was moderately active against herpes simplex virus in vitro.

Keywords: bromo-fluorination; carbocyclic nucleoside; fluoronucleoside; tin hydride reduction; antiviral agent; herpes simplex virus

Nucleoside analogues have been extensively investigated in the search for effective antiviral agents. There has been considerable interest in carbocyclic nucleosides, since the replacement of the oxygen atom of the furanosine ring with methylene is a drastic change in terms of stereo-electronic effect on the nucleoside molecule and greater metabolic stability.13 Much effort has been made to synthesize carbocyclic nucleoside analogues and also to modify the cyclopentane ring by introduction of an electronegative fluorine substituent.13 The synthesis of a carbocyclic analogue of 3'-deoxy-3'-fluorothymidine was an important target, since it is the most potent anti-human immunodeficiency virus (anti-HIV) agent among fluorinated nucleosides so far synthesized.13 However, only a limited amount of work has been done on the synthesis of 2',3'-dideoxy-3'-fluoro analogues.44

Independently, we have studied the stereo- and regioselective introduction of a fluorine atom onto a carbocyclic sugar moiety. In this report, we describe the synthesis of 2',3'-dideoxy-3'-fluorocarbocyclic nucleosides using two methods, and we report on the antiviral activity of the products.51

Chemistry For the synthesis of 3'-fluoro carbocyclic nucleoside analogues, we required the fluorocyclopentylamine (4) (Chart 1). The successful introduction of a fluorine atom into the carbocyclic ring by stereoselective epoxidation of cis-4β-acetamidocyclopent-2-enemethyl acetate (5)60 followed by regioselective ring opening with hydrogen fluoride-pyridine to give the protected fluoro alcohol (1) with required stereochemistry has been described in an earlier report.51 However, reduction of the phenylthionocarbonate7 of the 2'-hydroxyl group with tin hydride gave a complex mixture. After several attempts, replacement of the hydroxyl group by hydrogen atom to afford 3 was achieved by the substitution of the hydroxyl group with iodide using Ph3P-I2 to give the cis-fluoro

![Chemical Diagram]

Chart 1

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iodide (2) in poor yield, followed by reduction with 
Bu$_3$SnH/azobisisobutyronitrile (AIBN).\textsuperscript{9,10}

A different stereochemistry of the leaving group adjac-
et to the fluorine atom might be expected to improve
the overall yield in the synthesis of the fluorinated
deoxyribonucleic acid moiety, since the electronic effect
of the fluorine atom is expected to affect the substitution
reaction. To explore this possibility, we examined bromo-
fluorination\textsuperscript{11} of cis-4β-acetamidocyclopent-2-enemethyl
acetate (5) to give the trans-bromo-fluoride. Bromo-
fluorination of 5 with hydrogen fluoride-pyridine/N-
acrylamide was regio- and stereoselective due to the
syn-directing allylic amide group,\textsuperscript{6} giving the ex-
pected trans-fluoro amide (6) and the rearranged fluoro-
acetate (7) in the ratio of 74:26 (65% yield) in tetrahy-
drofuran as a solvent. When the reaction was carried out
in ether as a less polar solvent, the desired trans-
fluoro amide was obtained exclusively in 77% yield. As
expected, the bromine atom was smoothly reduced with
Bu$_3$SnH/AIBN to provide 3 in 88% yield. Finally,
deprotection of the acetyl group gave the fluorinated
carboxylic sugar moiety (4) in good yield.

The synthetic routes (Chart 2) to the fluorinated uridine,
thymidine and cytidine analogues (8--10) from the
fluorocyclopentylamine (4) were based on Shealy and
O'dell's procedure.\textsuperscript{12} Treatment of 4 with 3-ethoxyac-
ryloyl isocyanate in N,N-dimethylformamide at 0 °C gave
an intermediate acryloylurea, which was cyclized under
reflux in 2 N hydrochloric acid to afford the fluorinated

\begin{align*}
\text{Chart 2} \\
4 \hspace{1cm} a) \text{ EtOCH=CHCONCO} \\
\hspace{1cm} b) \text{ H}^+ \\
\hspace{1cm} c) \text{ EtOCH=C(Me)CONCO} \\
\hspace{1cm} d) \text{ Ac}_2\text{O, DMAP} \\
\hspace{1cm} e) \text{ mesitylenesulfonyl chloride, Et}_3\text{N} \\
\hspace{1cm} f) \text{ sat. NH}_3\text{–MeOH}
\end{align*}

\begin{align*}
\text{Chart 3} \\
\text{a) 5-amino-4,6-dichloropyrimidine, b) HCl(OEt)$_2$, H}^+ \\
\text{c)aq. NH}_3, \text{d) 2-amino-4,6-dichloropyrimidine,} \\
\text{e) p-chlorobenzene diazonium chloride, then Zn AcOH. f) 2 N HCl}
\end{align*}
uracil (8). The corresponding carbocyclic 3'α-fluorothymidine (9) was prepared from 4 in a similar manner to that used for the synthesis of 8. The cytidine analogue was obtained from the fluorinated uridine (8) according to the reported procedure.13

On the other hand, the amine (4) was condensed with 5-amino-4,6-dichloropyrimidine14 and the resulting pyrimidine (11) was treated with triethyl orthoformate in the presence of concentrated hydrochloric acid to give the 6-chloropurine. Reaction of the 6-chloropurine with ammonia gave carbocyclic 2',3'-dideoxy-3'-fluoroadenosine (12) as illustrated in Chart 3.

The amine (4) was coupled with 2-amino-4,6-dichloropyrimidine to afford the diamine (13). Reaction of 13 with p-chlorobenzene diazonium chloride followed by reduction of the intermediate diazo compound gave the triamine, which was cyclized with triethyl orthoformate and then treated with 2N hydrochloric acid to afford the desired fluoroguanosine (14).

**Biological Results**

The carbocyclic 2',3'-dideoxy-3'-fluoro nucleosides 8, 9, 10, 12, and 14 prepared in this study were tested in vitro for activity against herpes simplex virus type 1 (HSV-1), influenza virus type A, adenovirus type 3 and vesicular stomatitis virus (VSV). The assay method of Munoz et al.15 using crystal violet for staining viable cells was used to evaluate the activity of these compounds against the cytopathic effect of the viruses. The potency of each compound was evaluated in terms of the efficacy against the viruses at the concentration of 20 μg/ml. The cytotoxicity of each compound was also determined at the concentration of 100 μg/ml. The results obtained are summarized in Table I.

Of the five carbocyclic nucleoside analogues tested, carbocyclic 2',3'-dideoxy-3'-fluoroguanosine (14) showed a marked activity against HSV-1 at 20 μg/ml, and carbocyclic 2',3'-dideoxy-3'-fluoroadenosine (12) was slightly active against adenovirus type 3, but the other compounds 8, 9, and 10 showed no activity against any virus at 20 μg/ml. Compound 14 was slightly cytotoxic to Vero cells, but showed no cytotoxicity towards MDCK and HeLa S3 cells.

The potency of carbocyclic 2',3'-dideoxy-3'-fluoroguanosine (14) was further tested against herpes simplex virus type 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), and human cytomegalovirus (HCMV) in HEL cells by plaque reduction assays. The results are given in Table II.

## Table I. Antiviral and Anticellular Activity of Carbocyclic 2',3'-Dideoxy-3'-fluoro Nucleosides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition % of cytopathic effect of viruses at 20 μg/ml dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Inhibition % of cytotoxicity at 100 μg/ml dose&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-1 (KOS)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Influenza A</td>
</tr>
<tr>
<td>8</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>9</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>14</td>
<td>75–100</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by the assay method of Munoz et al.<sup>15</sup> using crystal violet for staining viable Vero (HSV-1), MDCK (influenza A), or HeLa S3 (adeno 3 and VSV) cells or uninfected cells.  
<sup>b</sup> The strain is given in parentheses.

## Table II. Antiherpes and Anticellular Activity of Carbocyclic 2',3'-Dideoxy-3'-fluoroguanosine

<table>
<thead>
<tr>
<th>Virus or cell</th>
<th>ID&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 (KOS)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7</td>
</tr>
<tr>
<td>HSV-2 (UW268)</td>
<td>1.0</td>
</tr>
<tr>
<td>VZV (CaQu)</td>
<td>1.1</td>
</tr>
<tr>
<td>HCMV (AD169)</td>
<td>1.5</td>
</tr>
<tr>
<td>HEL cells</td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by plaque reduction assays in HEL cells or cell proliferation assays in uninfected cells.  
<sup>b</sup> The strain is given in parentheses.

II. Adenine arabinoside (Ara-A) and acyclovir (ACV) were used as positive controls in this experiment.

Compound 14 was the most active against HSV-1, with ID<sub>50</sub> values of 2.7 μg/ml, but was less active against HSV-2, VZV and HCMV, with ID<sub>50</sub> values of 10.0, 12.1 and 15.0 μg/ml, respectively. The activity against HSV-1 was similar to that of Ara-A, but about 10 times less than that of ACV. The cytotoxicity of 14 towards HEL cells was quite high (<15.0 μg/ml), and was comparable to that of Ara-A.

## Experimental

1H-NMR and 13C-NMR spectra were taken on a JEOL JNM-FX-90Q spectrometer. References were tetramethylsilane for 1H spectra and CF<sub>3</sub>Cl for 13C spectra. All chemical shifts were recorded from the references. IR spectra were measured on a JASCO IR-810 spectrophotometer. High-resolution mass spectra (high-resolution MS) were recorded on a JEOL SX-102A mass spectrometer. Products were purified by column chromatography on silica gel (Merck, 0.063–0.200 mm or 0.040–0.063 mm).

(±)-(1β,2α,3β,4β)-4-Acetamido-2-fluoro-3-(iodocyclopentyl)methyl Acetate (2) Triphenylphosphine (1.45 g, 1.8 mmol), iodine (0.94 g, 3.7 mmol) and imidazole (0.36 g, 5.5 mmol) were added to a solution of (+)-(1β,2α,3β,4β)-4-acetamido-2-fluoro-3-(hydroxycyclopentyl)methyl acetate (1) (0.43 g, 1.6 mmol) in toluene (37 ml), and the mixture was heated under reflux. After 4 h, the mixture was poured into saturated aqueous sodium hydrogen carbonate solution and extracted with chloroform. The combined extracts, dried with magnesium sulfate, were evaporated to dryness in vacuo. Purification by column chromatography gave 2 (oil, 55 mg, 9% yield). 1H-NMR (CDCl<sub>3</sub>): δ: 1.20–2.60 (3H, m), 1.93 (3H, s), 2.03 (3H, s), 4.00–5.50 (5H, m), 13C-NMR (CDCl<sub>3</sub>): δ: 160.0 ppm (ddd, J = 51.8, 27.3, 6.6 Hz).

(±)-(1β,2α,3β,4β)-4-Acetamido-3-bromo-2-(fluorocyclopentyl)methyl Acetate (6) Hydrogen fluoride-pyridine (70%) (1 ml) was added dropwise to a solution of 3-bromosuccinimide (218 mg, 1.2 mmol) in ether (1 ml) at 0 °C, and then a solution of cis-4β-acetamidocyclopentenylmethyl acetate (5) (243 mg, 1.2 mmol) in ether (1 ml) was further added dropwise at 0 °C. The mixture was stirred at the same temperature for 30 min and at room temperature for 2 h, then poured into saturated...
aqueous sodium hydrogen carbonate solution and extracted with chloroform. The combined extracts were dried with magnesium sulfate, and evaporated to dryness in vacuo. Purification by column chromatography (chloroform: methanol = 50:1) gave 6 (oil, 280 mg, 77% yield). 1H-NMR (CDCl₃): δ = 1.3—2.8 (3H, m), 2.03 (2H, 3H, s), 4.2—5.5 (SH, m), 5.9 (H, br). 13C-NMR (CDCl₃): δ = 154.0 ppm (d, δ = 149.6, J = 13.7 Hz, IR (CDCl₃), 150, 1520 cm⁻¹).

When the reaction was carried out in tetrahydrofuran, 6 was obtained in 48% yield accompanied with the rearranged product (7) in 17% yield.²⁻¹

²⁻¹ 1H-NMR (CDCl₃): δ = 1.5—1.8 (3H, m), 2.03 (3H, s), 2.08 (3H, s), 3.4—3.7 (2H, m), 3.9—4.9 (3H, m), 5.9—6.1 (1H, m). 13C-NMR (CDCl₃): δ = -218.5 ppm (d, δ = 147.4, J = 28.1, 11.0 Hz).

±(1S,2S,4R)-4-Acetamido-2-fluorocyclopentane-1-carboxylic acid methyl ester (3a) Methylamine (40 mg, 0.60 mmol) in 3 mL of ethanol was added to the solution of 1 (139 mg, 0.98 mmol) in N,N-dimethylformamide (5 mL) at 0°C over a period of 5 min. After 10 min, all the volatiles were evaporated off under reduced pressure at 30°C. The residue was taken up in 2N hydrochloric acid (10 mL) and heated under reflux for 20 min. After cooling to 0°C, the solution was neutralized with 2N sodium hydroxide and the solvent was evaporated off. Purification by silica gel column chromatography afforded 4 (fine powder, 150 mg, 67% yield). 1H-NMR (CDCl₃): δ = 1.6—3.0 (3H, m), 3.8—4.0 (2H, m), 4.9—5.8 (2H, m), 6.10 (1H, d, δ = 7.9 Hz, 8.0 (1H, d, δ = 7.9 Hz). 13C-NMR (CDCl₃): δ = -112.9 (m). High-resolution MS (FAB) m/z: Caled for C₂₁H₂₁NF₂O₂: 329.0989. Found: 329.0985. Anal. Calcd for C₂₁H₂₁NF₂O₂: C = 52.63; H = 4.74; N = 0.85; S = 12.47. Found: C = 52.33; H = 4.74; N = 0.85; S = 12.47.

±(1S,2S,4R)-3-Fluoro-4-hydroxy(cyclopropenyl)cyclopentanone (7)³⁻¹ 3-Fluoro-2-propenyl isocyanate (0.46 mmol) in benzene, 2.5 mL, 1.0 M soln was added to a solution of 4 (130 mg, 0.98 mmol) in N,N-dimethylformamide (5 mL) at 0°C over a period of 5 min. After 10 min, all the volatiles were evaporated off under reduced pressure at 30°C. The residue was taken up in 2N hydrochloric acid (10 mL) and heated under reflux for 20 min. After cooling to 0°C, the solution was neutralized with 2N sodium hydroxide and the solvent was evaporated off. Purification by silica gel column chromatography afforded 8 (fine powder, 150 mg, 67% yield). 1H-NMR (CDCl₃): δ = 1.6—3.0 (3H, m), 3.8—4.0 (2H, m), 4.9—5.8 (2H, m), 6.10 (1H, d, δ = 7.9 Hz, 8.0 (1H, d, δ = 7.9 Hz). 13C-NMR (CDCl₃): δ = -112.9 (m). High-resolution MS (FAB) m/z: Caled for C₂₁H₂₁NF₂O₂: 329.0989. Found: 329.0985. Anal. Calcd for C₂₁H₂₁NF₂O₂: C = 52.63; H = 4.74; N = 0.85; S = 12.47. Found: C = 52.33; H = 4.74; N = 0.85; S = 12.47.

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References and Notes
10) On the other hand, the 2'-hydroxy group of carbocyclic 3'-deoxy-3'-fluoroadenosine was replaced by a hydrogen atom by the following procedure: selective protection of 6-NH$_2$ and 5'-OH group, transformation of 2'-OH group to phenylthionocarbonate, reduction with Bu$_2$SnH/AlBN, and deprotection in 21% overall yield (see Experimental).
16) The unprecedented rearrangement might proceed via migration of the C-5 acetoxyl group to C-3, activated by bromonium ion, followed by the attack of fluorine atom at C-5.