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Antiviral Activity of Cyclopentenyl Nucleosides Against Orthopox Viruses (Smallpox, Monkeypox and Cowpox)

C. K. Chu,a,* Y. H. Jin,a R. O. Bakerb and J. Hugginsb

a Department of Pharmaceutical and Biomedical Science, College of Pharmacy, The University of Georgia, Athens, GA 30602, USA
b US Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD 21702, USA

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Abstract—An improved method for the synthesis of enantiomerically pure D-cyclopentenyl nucleosides has been accomplished and their antiviral activity against orthopox viruses have been evaluated. The key intermediate, L-cyclopent-2-enone 13 was prepared from D-ribose using a ring closing metathesis reaction in eight steps. Among the synthesized nucleosides, the adenine 2 (Neplanocin A), cytosine 14, and 5-F-cytosine 15 analogues exhibited potent anti-orthopox virus activity, including smallpox virus.

Due to their interesting biological activity, carbocyclic nucleosides have received much attention in recent years. Various synthetic methodologies have been reported not only for naturally occurring carbocyclic nucleosides, such as (-)-aristeromycin 1 and neplanocin A 2 but also for synthetic analogues, including abacavir 3 and carbovir 4, which demonstrated potent anti-HIV activity (Fig. 1). Neplanocin A 2, an unsaturated analogue of (-)-aristeromycin 1, has potent antiviral activity as well as cytotoxicity. The antiviral effect may be due to the inhibition of S-adenosylhomocysteine (AdoHcy) hydrolase. AdoHcy is a potential feedback inhibitor of cellular transmethylation using S-adenosyl-l-methionine as a methyl donor, which is essential for mRNA maturation. Therefore, inhibition of AdoHcy hydrolase would provide broad-spectrum of antiviral activity for DNA as well as RNA viruses. Although neplanocin A 2 itself is not an effective antiviral agent due to its cytotoxicity to the host cell, it is a lead compound for the discovery of structurally related chemotherapeutic agents. Therefore, a number of neplanocin A analogues have been synthesized and evaluated as potential antiviral and antitumor agents.

Previously, we have developed a synthetic method for enantiomerically pure D- and L-cyclopentenyl and cyclopentyl nucleosides, including (+)-aristeromycin and neplanocin A, using L-cyclopent-2-enone 13 as the key intermediate. Optically pure starting material, such as D-ribose, D-mannose and D-ribonolactone, have been used for the synthesis of L-cyclopent-2-enone 13. However, the synthesis of 13 from these carbohydrates has been problematic due to low and inconsistent yield. Some of the reactions are very sensitive to the reaction conditions, such as moisture and temperature, resulting in low yield. Therefore, an efficient synthetic method for the key intermediate, such as D-cyclopent-2-enone 13, is highly desirable in order to carry out studies for structural–activity relationships of optically pure carbocyclic nucleosides. Recently, we have developed an efficient and practical methodology for the synthesis of D-cyclopent-2-enone using ring closure metathesis (RCM)

Figure 1. Biologically active natural and synthetic carbocyclic nucleosides cyclopentenyl nucleosides as reported in this article.
reaction from D-ribose in excellent overall yield in preparative scale. Therefore, our efforts were directed toward the synthesis of the L-cyclopent-2-enone 13 as the key intermediate for the synthesis of D-cyclopentenyl nucleosides as reported in this article.

Smallpox (Variola virus), which has only human as the natural host, has been responsible for worldwide epidemics. Smallpox is highly contagious virus with a case fatality rate of 30% in unvaccinated individuals. Under the massive vaccination program by World Health Organization (WHO), it has been more than 22 years since the last case of smallpox was confirmed, and WHO finally declared in 1980 that the global eradication had been achieved. Smallpox is a particularly dangerous biological threat because of its clinical and epidemiological properties, and no clinically proven antiviral treatment exists at this time. The recent increasing concern of biological terrorism affects all segments of the population and the growing concern of smallpox virus as a prime bioterrorism agent prompted us to search for antiviral agents to prevent and to treat the smallpox virus.

In this communication, we describe an improved synthesis of the key intermediate L-cyclopenten-2-one 13 for D-cyclopentenyl nucleosides and their potent anti-orthopox virus (smallpox, monkeypox, cowpox and vaccinia virus) activity.

Chemistry

The protected D-ribose 5 was readily available from D-ribose in two steps, which underwent Wittig reaction using NaH, DMSO and methyltriphenylphosphonium bromide in THF to give olefin 6 in 91% yield (Scheme 1). The carbonyl compound 7 was obtained by oxidation of the secondary hydroxyl group. Several oxidation conditions were investigated to optimize the yield. Among them, Moffatt oxidation with dicyclohexyl carbodiimide, DMSO, pyridine and trifluoroacetic acid in toluene gave the best result to afford ketone 7 in 75% yield. To introduce another olefin moiety, Grignard reaction was carried out with vinylmagnesium bromide to provide inseparable mixture of diene 8 in 80% yield. This underwent the ring-closing metathesis (RCM) reaction using 5% Grubbs catalyst at refluxing temperature in anhydrous CH2Cl2 to provide z- and β-cyclopentanol 9 and 10 (9:10 ratio = 10:1) in 88% yield. The z- and β-configurations were assigned by 1H NMR and NOE experiments. The removal of primary hydroxyl protecting group using 1 M solution of tetra-n-butylammonium fluoride in THF to form a vicinal diol 11 and 12, followed by an oxidative cleavage with sodium periodate to afford the key intermediate cyclopentenone 13 in 95% yield. The prepared cyclopentenone 13 was converted to adenine (Neplanocin A) 2, cytosine 14, 5-Fluoro-cytosine 15, and inosine 16 analogues as we previously reported.

Antiviral Activity

The synthesized nucleosides were evaluated for their antiviral activity against smallpox, monkeypox, cowpox, and vaccinia viruses as well as their cytotoxicity in Vero and MK2 cells. The results are summarized in Table 1. It was found that adenine 2 (Neplanocin A), cytosine 14, and 5-fluoro-cytosine 15 analogues exhibited...
In summary, we have developed an improved synthesis of 1-cyclopentenone I3 using the ring closure metathesis reaction which was applied to synthesize several unsatu-

rated carbocyclic nucleosides, and it was found that adenine, cytosine and 5-F-cytosine derivatives possess potent anti-viral activity against orthopox viruses, including smallpox virus.

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Table 1. Antiviral activity and cytotoxicity against orthopox viruses of d-cyclopentenyl nucleosides in MK2 and Vero cells

| Compd | Cell | Virus | Activity EC50 (µg/mL) | Cytotoxicity IC50 (µg/mL) | TIh |
|-------|------|-------|------------------------|--------------------------|-----|
| Adenine 2 | MK2 | 7124 | 0.10 | 23 | 230 |
| | | BSH 10 | 0.10 | 36 | 360 |
| | | CPX | 100 | 100 | 1.0 |
| | | MPX | 0.26 | 42 | 161 |
| | | VAC | 2.62 | 31 | 12 |
| Vero 1 | 7124 | 0.03 | 50 | 1667 |
| | | BSH | 0.14 | 10 | 71 |
| | | CPX | >100 | 20 | <0.2 |
| | | MPX | 0.21 | 57 | 271 |
| | | VAC | >100 | >100 | 1.0 |
| Cytosine 14 | MK2 | 7124 | 0.08 | 44 | 550 |
| | | BSH | 0.03 | 3 | 100 |
| | | CPX | 0.06 | 10 | >166 |
| | | MPX | 0.1 | 21 | 210 |
| | | VAC | 0.12 | 36 | 300 |
| Vero 1 | 7124 | <0.05 | >100 | >2000 |
| | | BSH | >100 | 30 | 0.3 |
| | | CPX | 0.05 | 1 | >20 |
| | | MPX | <0.05 | 4 | >80 |
| | | VAC | 0.04 | 40 | 1000 |
| 5-F-Cytosine 15 | MK2 | 7124 | 1.73 | >100 | >58 |
| | | BSH | 0.51 | >100 | >196 |
| | | CPX | 0.43 | 70 | 162 |
| | | MPX | 0.61 | >100 | >164 |
| | | VAC | 0.53 | 100 | 189 |
| Vero 1 | 7124 | 2.63 | 39 | 15 |
| | | BSH | 1.17 | 100 | 86 |
| | | CPX | 1.35 | >100 | >74 |
| | | MPX | 2.05 | 100 | 49 |
| | | VAC | 1.46 | >100 | >69 |
| Inosine 16 | MK2 | 7124 | >100 | >100 | — |
| | | BSH | >100 | >100 | — |
| | | CPX | >100 | >100 | — |
| | | MPX | >100 | >100 | — |
| | | VAC | >100 | >100 | — |
| Vero 1 | 7124 | >100 | >100 | — |
| | | BSH | >100 | >100 | — |
| | | CPX | >100 | >100 | — |
| | | MPX | >100 | >100 | — |
| | | VAC | >100 | >100 | — |

*LLC-MK2 (ATCC CRL 7).

1bVero 76 (ATCC CRL 1587).

2bSmallpox virus.

3bCowpox virus.

4bMonkeypox virus.

5bVaccinia virus.

6bTI=IC50/EC50.

potent anti-smallpox, -cowpox, -monkeypox, and -vaccinia virus activity.

These preliminary studies against orthopoxviruses showed that d-cyclopentenyl nucleoside analogues, particularly adenine, cytosine and 5-F-cytosine derivatives, possess potent anti-smallpox virus activities. Therefore we plan to further investigate the structure–activity relationships as well as biochemical studies of these carbocyclic nucleosides.

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