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Preliminary Communication

Synthesis of 4′-*C*-Ethynyl-β-D-*arabino*- and 4′-*C*-Ethynyl-2′-deoxy-β-D-*ribo*-pentofuranosyl Pyrimidines, and Their Biological Evaluation

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4′-*C*-Ethynyl-β-D-*arabino*-pentofuranosyl thymine (14) and cytosine (16), and 4′-*C*-ethynyl-2′-deoxy-β-D-*ribo*-pentofuranosyl thymine (25) and cytosine (27) were synthesized by properly protected 4′-*C*-hydroxy-methyl-3,5-di-*O*-benzyl-α-D-*ribo*-pentofuranose (1) from D-glucose. Among them, 2′-deoxy derivatives 25 and 27 exhibited antiviral activity, while cytidine derivatives 16 and 27 inhibited the growth of neoplastic cells.

Key words: 4′-*C*-substituted nucleoside; ethynyl group; arabinosyl pyrimidine; 2′-deoxyribosyl pyrimidine; biological activity

A wide variety of sugar-modified nucleosides have been reported as antitumor or antiviral agents.[1–6] Among them, there are not many examples of 4′-substituted nucleosides, mainly due to difficult synthesis in spite of their excellent biological activity.

We have previously reported the synthesis of 4′-*C*-methyl-2′-deoxy-D-*ribo*-pentofuranosyl cytosine (**A**) and 4′-*C*-methyl-D-*arabino*-pentofuranosyl cytosine (**B**) and their remarkable anti-HIV and antileukemic activity, in sharp contrast to the corresponding D-*ribo*-pentofuranosyl counterparts which lack these activities.[2,3]

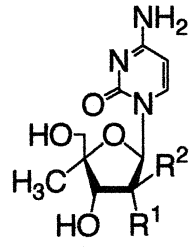
Very recently, we have reported the synthesis of 4′-*C*-ethynyl-D-*ribo*-pentofuranosyl pyrimidines (**C**) which showed no antiviral or antitumor activity.[5] Furthermore, Matsuda *et al.* have reported the regioselective synthesis of 4′-α-branched nucleosides and that 4′-*C*-ethynyl-2′-deoxy-thymidine had potent antiviral activity.[6]

In this paper, we describe the synthesis of 4′-*C*-ethynyl-*arabino*-pentofuranosyl pyrimidines (**D**) and 4′-*C*-ethynyl-2′-deoxy-*ribo*-pentofuranosy pyrimidines (**E**) which can be expected to act as inhibitors of DNA metabolism.

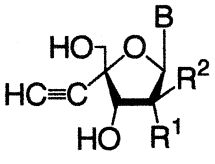
We selected 4-*C*-hydroxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (**1**)[2] as the starting material for the synthesis of the 2′-deoxy and *arabino* analog of 4′-*C*-ethynyl nucleosides because of the ease of transformation at the 2′-position. Synthesis of 4-*C*-ethynyl-3,5-di-*O*-benzyl-1,2-di-*O*-acetyl-D-*ribo*-pento-furanose (**6**), and its condensation with thymine or uracil were performed by a modified method of the one previously described[5] (Scheme 1).

The syntheses of 4′-*C*-ethynyl-β-D-*arabino*-furanosyl thymine (**14**) and cytosine (**16**) are outlined in scheme 2. The acetyl groups in nucleosides **8** and **9** were hydrolysed with 0.1 N sodium hydroxide in water-methanol. The resulting 2′-α-hydroxyl group was methane-sulfonylated and inverted to 2′-β-orientation by treating with 0.6 N sodium hydroxide in water-tetrahydrofuran involving an O^2,2′-cyclonucleoside to give **10** and **11** in 91% and 73% yields, respectively. Deprotection of the 3′- and 5′–*O*-benzyl groups in **10** and **11** by boron tribromide[4] in dichloromethane and subsequent acetylation of the resulting hydroxyl groups afforded **12** and **13** in 91% and 94% yields, respectively. 4′-*C*-Ethynyl-β-D-*arabino*-pentofuranosyl thymine (**14**) was obtained by treating **12** with sodium methoxide in methanol in an 80% yield. On the other hand, 4′-*C*-ethynyl-β-D-*arabino*-pentofuranosyl cytosine (**16**) was synthesized from **13** by Reese's protocol[7] through 4-triazolouridine derivative **15** in a 75% yield.

In deoxygenation of the 2′-hydroxyl group of a ribonucleoside, a radical reduction treatment of the 2′-halo or thionocarbonate of the ribonucleoside with tri-*n*-butyltin hydride has generally been utilized.[8,9] In this reaction, however, tributyltin radical is known to add to the terminal alkyne.[10] Thus, we planned to protect the terminal alkyne in **4** by a triethylsilyl group. Treatment of **4** with *n*-butyl lithium in tetrahydrofuran and then



A: $R^1 = R^2 = H$
B: R^1 =H, R^2 = OH



C: B = uracil, thymine or cytosine
R^1 = OH, R^2 = H
D: B = thymine or cytosine
R^1 = H, R^2 = OH
E: B = thymine or cytosine
$R^1 = R^2 = H$

Fig. 1. Structures of the 4′-Substituted Nucleosides.

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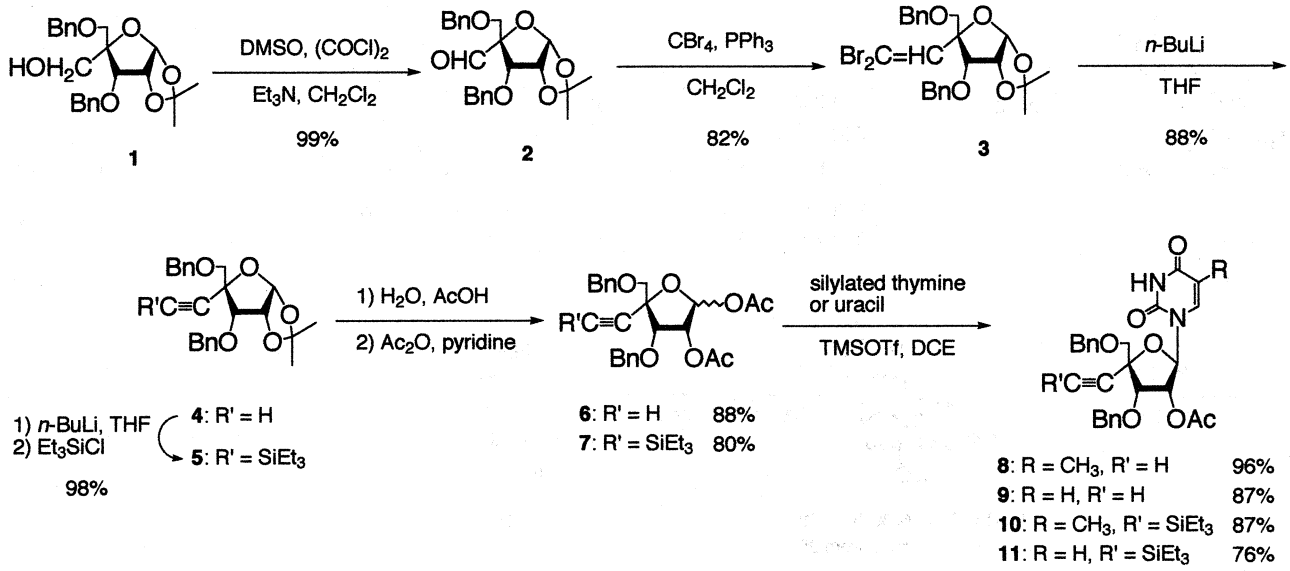
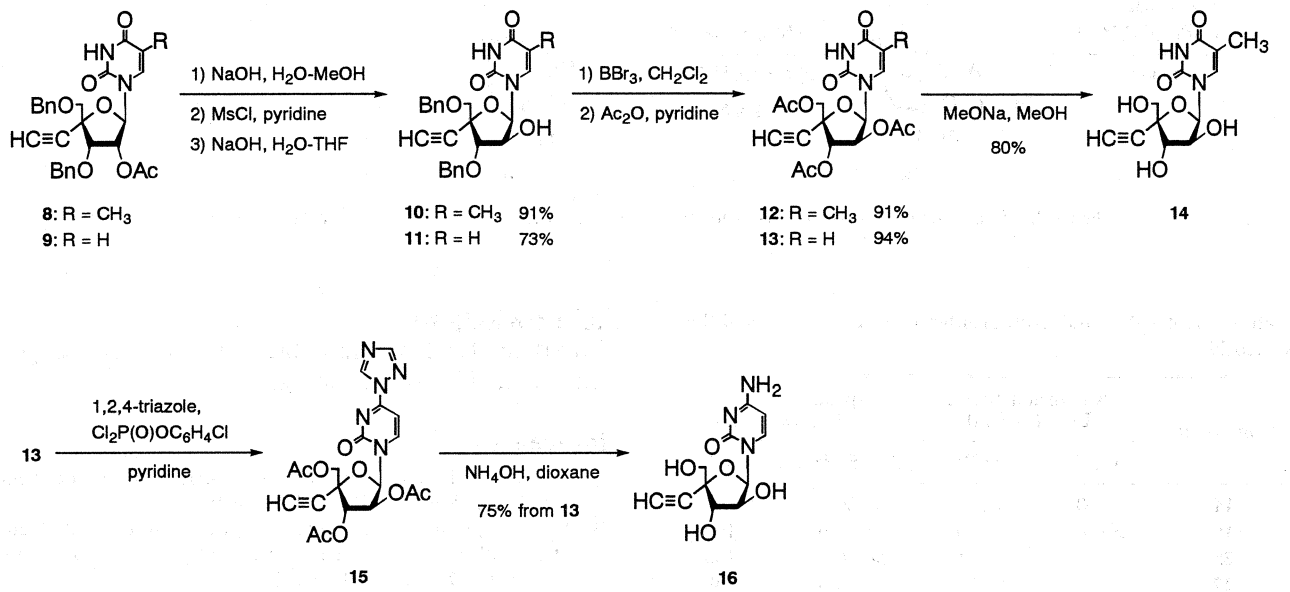
Abbreviations: HSV, herpes simplex virus; ara C, 1-(β-D-*arabino*-pentofuranosyl)cytosine



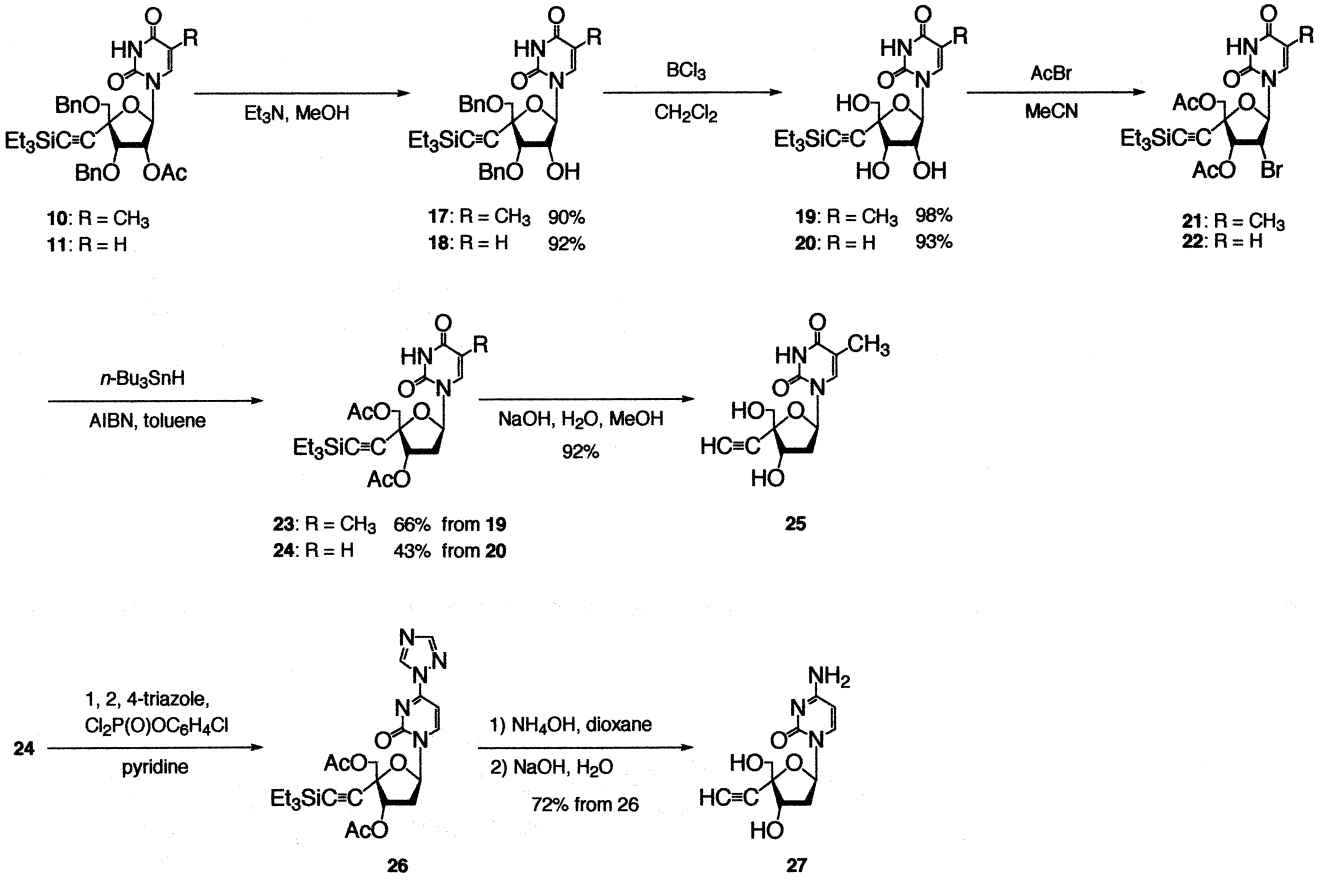
Scheme 1. Synthetic Pathway to the 4′-*C*-Ethynyl-D-*ribo*-pentofuranosyl Glycosyl Donor and Its Glycosylation with Thymine or Uracil.



Scheme 2. Synthesis of 4′-*C*-Ethynyl-D-*arabino*-pentofuranosyl Thymine (**14**) and Cytosine (**16**).

chlorotriethylsilane afforded ribose derivative **5** bearing an ethynyl group protected with the triethylsilyl group in a 98% yield. **5** was converted to **7** by a similar procedure to that described for **6**, and **7** was condensed with silylated thymine and uracil. The synthetic route to 4′-*C*-ethynyl-2′-deoxy-β-D-*ribo*-furanosyl thymine (**25**) and cytosine (**27**) is shown in scheme 4. **10** and **11** were deacetylated in methanol containing 5% triethylamine in 90% and 92% yields, respectively. Debenzylation of **17** and **18** by boron trichloride in dichloromethane gave **19** and **20** in 98% and 93% yields, respectively. Treatment of **19** and **20** with acetyl bromide in acetonitrile afforded 3′,5′-di-*O*-acetyl-2′-bromo-2′-deoxy pyrimidine nucleosides **21** and **22**, these being used for deoxygenation without further purification. **21** and **22** were reduced to 2′-deoxy derivatives **23** and **24** by radical reduction with tri-*n*-butyltin hydride in toluene in the presence of 2,2′-azobis(isobutyro-nitrile) at 85°C without any tributyltin radical addition to the terminal alkyne in 66% and 43% yields from **19** and **20**, respectively. 4′-*C*-Ethynylthymidine (**25**) was obtained in a 92% yield by deacetylating of **23**, and 4′-*C*-ethynyl-2′-deoxycytidine (**27**) was obtained by a similar method to that described for **16** from **24** in a 72% yield.

Synthesized 4′-*C*-ethynylnucleosies **14**, **16**, **25** and **27** were evaluated for their antivirul and anti-proliferative activities (Table 1). Deoxy derivatives **25** and **27** both showed antiviral activity against the herpes simplex virus (HSV) whose EC_{50} values were 1.23 against HSV-1 and HSV-2 for **25**, and 33 against HSV-1 for **27**, but



Scheme 3. Synthesis of 4′-*C*-Ethynyl-2′-deoxy-D-*ribo*-pentofuranosyl Thymine (**25**) and Cytosine (**27**).

Table 1. Anti-HSV and Anti-proliferative Activity of 4′-Ethynyl Nucleosides

Compound	Antiviral activity, EC_{50} (μg/ml)		Anti-proliferative activity, IC_{50} (μg/ml)	
	HSV-1	HSV-2	CCRF-HSB-2	KB
14	>100	>100	>100	>100
16	>100	>100	3.0	>100
25	1.23	1.23	>100	>100
27	33	>100	0.87	48
ara C	ND	ND	0.030	0.30

arabino derivatives **14** and **16** were inactive. In the evaluation of the anti-proliferative activity of the ethynyl nucleosides, cytosine derivatives **16** and **27** inhibited the growth of human lukemia cells (CCRF-HSB-2) with IC_{50} values of 0.87 and 3.0, respectively, while the growth-inhibitory activity against pharyngeal carcinoma (KB) was weak or not effective.

In conclusion, we synthesized 4′-*C*-ethynyl-β-D-*arabino*-pentofuranosyl thymine (**14**) and cytosine (**16**), and 4′-*C*-ethynyl-2′-deoxy-β-D-*ribo*-pentofuranosyl thymine (**25**) and cytosine (**27**). It was found that the 2′-deoxy derivatives and cytidine derivatives exhibited antiviral and antineoplastic activity, respectively. Syntheses of *arabino* and 2′-deoxy analogues of the 4′-*C*-ethynyl purine nucleosides are in progress.

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