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Synthesis of 4′-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl Pyrimidines

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The 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosylpyrimidines were prepared from D-glucose through properly protected 4′-*C*-formyl-D-*ribo*-ribofuranose as the key intermediate, and preliminary biological tests against some viruses and tumor cells showed that the compounds were not active.

Key words: 4′-*C*-substituted nucleosides; ethynyl group

Considerable attention has been focused on *branched-chain sugar nucleosides* because of their biological importance.[1-5] Most modifications of the sugar moiety have been focused on the 2′, 3′ and 5′ positions because of the easy chemical modification. For example, Matsuda and his co-workers have recently reported the synthesis of 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl) cytosine (**A**) and its remarkable growth-inhibitory activity against various human solid tumor cells.[6] However, only a few examples of 4′-branched nucleosides have been reported.[7-10] We have developed a versatile method for the synthesis of 4′-branched nucleosides starting from D-glucose and have reported the very strong growth-inhibitory activity of 4′-*C*-methyl-2′-deoxycytidine (**B**) against leukemic cells.[11,12] As part of our study on the synthesis and biological activity of 4′-*C*-branched nucleosides, we describe in this paper the synthesis of 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosylpyrimidines and preliminary biological tests against some viruses and tumor cells.

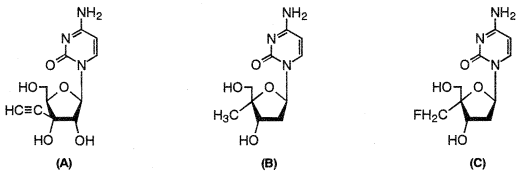

Fig. 1. Structures of Representative Biologically Active Branched-chain Sugar Nucleosides A, B and C.

Materials and Methods

General methods. Melting point (mp) data were obtained with Shibata melting apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with a JEOL JNMEX-400 spectrometer at 21–23°C in $CDCl_3$, using tetramethylsilane as an internal standard. IR spectra were recorded with a Shimazu FTIR-8100M spectrometer, while mass spectra were recorded with a Hitachi M-80B spectrometer at 70 eV. Specific rotation was measured with a Jasco DIP-360 at 589 nm. Merck silica gel Art. 9385 was used for column chromatography, and Merck silica gel Art. 5554 for analytical thin-layer chromatography.

1, 2:5, 6-Di-O-isopropylidene-3-O-p-methoxybenzyl-α-D-allofuranose (1). To a solution of 1, 2:5, 6-di-*O*-isopropylidene-α-D-allofuranose[18] (10 g, 38.4 mmol) in dry dimethylformamide (120 ml) was added while stirring 60% sodium hydride in oil (1.84 g, 46 mmol) at 0°C. After 30 min, *p*-methoxybenzyl chloride (6.25 ml, 46 mmol) was added dropwise to the solution. After stirring for 4 h at room temperature, ethanol (10 ml) was added dropwise to the mixture. The reaction mixture was then partitioned between ethyl acetate and water. The ethyl acetate layer was dried over magnesium sulfate and evaporated to give a crude syrup. This syrup was purified by silica gel column chromatography, using a mixture of hexane and ethyl acetate (2:1, v/v), to give **1** (13.4 g, 92%), mp 73–75°C. $[\alpha]_D$+106.6° (c 1.02, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.33–6.88 (4H, m, Ph), 5.75 (1H, d, *H*-1, $J_{1,2}$=3.67), 4.70, 4.53 (2H, 2d, CH_2Ph, J=11.23), 4.55 (1H, t, *H*-2, $J_{1,2}$=3.67, $J_{2,3}$=4.40), 4.40–4.34 (1H, m, *H*-5), 4.13 (1H, dd, *H*-4, $J_{3,4}$=8.79, $J_{4,5}$=2.93), 3.87 (1H, dd, *H*-3, $J_{2,3}$=4.40, $J_{3,4}$=8.79), 3.81 (3H, s, OCH_3), 1.58, 1.39, 1.37, 1.35 (3Hx4, 4s, acetonide). *Anal.* Found: C, 63.18; H, 7.45%. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42%.

1,2-O-Isopropylidene-3-O-p-methoxybenzyl-α-D-allofuranose (2). To a solution of **1** (12.3 g, 4 mmol) in acetic acid (210 ml) was added water (90 ml), and the solution was stirred for 21 h at room temperature. The solution was condensed *in vacuo*, and the resulting residue was coevaporated with toluene (50 ml). The residual syrup was subjected to silica gel column chromatography. Elution with a mixture of chloroform and methanol (30:1, v/v) gave **2** (9.57 g, 87%), mp 70–72°C, $[\alpha]_D$+107.8° (c 1.03, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.32–7.26 (2H, m, Ph), 6.91–6.86 (2H, m, Ph), 5.76 (1H, d, *H*-1, $J_{1,2}$=3.66), 4.71, 4.49 (2H, 2d, CH_2Ph, J=10.99), 4.59 (1H, t, *H*-2, $J_{1,2}$=3.66, $J_{2,3}$=4.40), 4.09 (1H, dd, *H*-4, $J_{3,4}$=8.79, $J_{4,5}$=3.17), 4.01–3.97 (1H, m, *H*-5), 3.90 (1H, dd, *H*-3, $J_{2,3}$=4.40, $J_{3,4}$=8.79), 3.80

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Abbreviations: PDC, pyridinium dichromate; DDQ, 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone; NOE, nuclear Overhauser effect

(3H, s, OCH_3), 3.74–3.61 (2H, m, *H*-6, 6′), 2.47 (2H, br. s, 5-O*H*, 6-O*H*), 1.59, 1.36 (3Hx2, 2s, acetonide). *Anal.* Found: C, 60.14; H, 7.14%. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11%.

4-C-Hydroxymethyl-1,2-O-isopropylidene-3-O-p-methoxybenzyl-α-D-ribo-pentofuranose (3). To a solution of **2** (15.7 g, 46.1 mmol) in water (140 ml) was added a solution of sodium periodate (10.85 g, 50.7 mmol) in water (140 ml). After the solution had been stirred for 1.5 h at room temperature, ethylene glycol (2.5 ml, 46 mmol) was added, and the solution was stirred for 5 min more, before being extracted with chloroform. The chloroform solution was dried over magnesium sulfate and evaporated under reduced pressure to afford a syrupy aldehyde. This aldehyde was used without purification for the next step. To a solution of the aldehyde and formaldehyde (37%, 40.8 ml) in a mixture of water and tetrahydrofuran (1:1, v/v, 200 ml) was added a 1N sodium hydroxide solution (92.2 ml) while stirring at 0°C, before the mixture was stirred for 46 h at room temperature and then extracted with chloroform. The chloroform solution was dried over magnesium sulfate and evaporated under reduced pressure to give a syrupy residue. This syrup was purified by silica gel column chromatography with a mixture of chloroform and methanol (20:1, v/v) as the eluent to give **3** (13.5 g, 86%), mp 143.5–145.5°C, $[\alpha]_D$ +79.7° (c 1.07, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.32–7.26 (2H, m, Ph), 6.92–6.87 (2H, m, Ph), 5.76 (1H, d, *H*-1, $J_{1,2}$=3.90), 4.74, 4.50 (2H, 2d, CH_2Ph, J=11.23), 4.62 (1H, dd, *H*-2, $J_{1,2}$=3.90, $J_{2,3}$=5.13), 4.19 (1H, d, *H*-3, $J_{2,3}$=5.13), 3.90 (2H, s, 5-CH_2), 3.81 (3H, s, OCH_3), 3.77, 3.55 (1Hx2, 2d, 5′-CH_2, J=12.20), 2.14 (2H, b. s, 5-O*H*, 6-O*H*), 1.63, 1.33 (3Hx2, 2s, acetonide). *Anal.* Found: C, 60.20; H, 7.24%. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11%.

4-C-Hydroxymethyl-1,2-O-isopropylidene-3,5-di-O-p-methoxybenzyl-α-D-ribo-pentofuranose (4). To a solution of **3** (10.0 g, 29.4 mmol) in dry dimethylformamide (100 ml) was added while stirring at −20°C sodium hydride (60% in oil, 1.41 g, 32.3 mmol). After the mixture had been stirred at −20°C for 30 min, a solution of *p*-methoxybenzyl chloride (4.37 ml, 32.3 mmol) in dry dimethylformamide (10 ml) was added dropwise to the mixture. The resulting mixture was stirred for 6 h at −20°C. Ethanol (10 ml) was added at −20°C, and the mixture was stirred for 5 min. The mixture was condensed under reduced pressure, and the resulting residue was partitioned between ethyl acetate and water. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure to give a syrup. This syrup was subjected to silica gel column chromatography with a mixture of hexane and ethyl acetate (3:1, v/v) to give three compounds. 1st-eluting *4-C-hydroxymethyl-1,2-O-isopropylidene-3,5,5′-tri-O-p-methoxybenzyl-α-D-ribo-pentofuranose (4b*; 2.48 g, 15%*)*, syrup, $[\alpha]_D$ +33.3° (c 1.35, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.25–6.81 (12H, m, Ph), 5.74 (1H, d, *H*-1, $J_{1,2}$=3.9), 4.61–4.45 (1Hx2, 2d, CH_2Ph, J=11.72), 4.58, 4.45 (1Hx2, 2d, CH_2Ph, J=11.96), 4.54 (1H, dd, *H*-2, $J_{1,2}$=3.91, $J_{2,3}$=5.13), 4.46, 4.36 (1Hx2, 2d, CH_2Ph, J=11.72), 4.18 (1H, d, *H*-3, $J_{2,3}$=5.3), 3.93, 3.47 (1Hx2, 2d, CH_2, J=10.50), 3.68, 3.66 (1Hx2, d, CH_2, J=10.99), 3.78 (9H, s, OCH_3), 1.50, 1.31 (3Hx2, 2s, acetonide). *Anal.* Found: C, 68.43; H, 7.10%. Calcd. for $C_{33}H_{40}O_9$: C, 68.26; H, 6.94%. 2nd-eluting *4-C-hydroxymethyl-1,2-O-isopropylidene-3,5-di-O-p-methoxybenzyl-α-D-ribo-pentofuranose (4*; 8.93 g, 66%*)*, mp 65–68°C, $[\alpha]_D$ +72.6° (c 0.95, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.22–6.84 (8H, m, Ph), 5.77 (1H, d, *H*-1, $J_{1,2}$=3.90), 4.69, 4.42 (1Hx2, 2d, CH_2Ph, J=11.47), 4.60 (1H, dd, *H*-2, $J_{1,2}$=3.90, $J_{2,3}$=5.12), 4.48, 4.37 (1Hx2, 2d, CH_2Ph, J=11.72), 4.22 (1H, d, *H*-3, $J_{2,3}$=5.12), 3.89, 3.80 (1Hx2, 2d, H-5′, J=11.72), 3.80 (3Hx2, 2s, OCH_3x2), 3.56, 3.48 (1Hx2, 2d, *H*-5, J=11.50), 2.41 (1H, dd, 5′-O*H*), 1.62, 1.34 (3Hx2, 2s, acetonide). *Anal.* Found: C, 65.16; H, 6.93%. Calcd. for $C_{25}H_{32}O_8$: C, 65.20; H, 7.00%. 3rd-eluting *4-C-p-methoxybenzyloxymethyl-1,2-O-isopropylidene-3-O-p-methoxybenzyl-α-D-ribo-pentofuranose (4a*; 0.91 g, 7%*)*, $[\alpha]_D$+26.3° (c 0.98, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.29–6.84 (8H, m, Ph), 5.73 (1H, d, *H*-1, $J_{1,2}$=3.91), 4.66, 4.56, 4.53, 4.46 (2Hx2, CH_2Phx2, J=11.72), 4.55 (1H, dd, *H*-2, $J_{1,2}$=3.91, $J_{2,3}$=5.13), 4.10 (1H, d, *H*-3, $J_{2,3}$=5.13), 4.01, 3.71 (1Hx2, 2d, CH_2, J=10.74), 3.91, 3.40 (2H, d, *H*-5, J=11.96), 3.81, 3.80 (3Hx2, 2s, OCH_3x2), 1.94 (1H, dd, 5-OH), 1.51, 1.31 (3Hx2, 2s). *Anal.* Found: C, 65.21; H, 6.70%. Calcd. for $C_{25}H_{32}O_8$: C, 65.20; H, 7.00%.

4-C-Formyl-1,2-O-isopropylidene-3,5-di-O-p-methoxybenzyl-α-D-ribo-pentofuranose (5). To a solution of **4** (8.55 g, 18.6 mmol) in dimethylformamide (100 ml) were added 4A molecular sieves (0.5 g), and the mixture was stirred for 5 min. To the solution was then added pyridinium dichromate (PDC, 20.95 g, 55.3 mmol), and the mixture was stirred for 10 h at 40°C. The mixture was filtered through Celite, and the filtrate was condensed under reduced pressure to give a syrup. This syrup was dissolved in ethyl acetate (100 ml), washed with water and dried over magnesium sulfate. After filtering away the magnesium sulfate, ethyl acetate was removed under reduced pressure to afford crystalline **5** (6.45 g, 76%), mp 107.5–109.5°C, $[\alpha]_D$+24.3° (c 1.03, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 9.89 (1H, s, C*H*O), 7.26–7.15 (4H, m, Ph), 6.88–6.84 (4H, m, Ph), 5.82 (1H, d, $J_{1,2}$=3.30), 4.55 (1H, dd, *H*-2, $J_{1,2}$=3.30, $J_{2,3}$=4.40), 4.62, 4.52 (1Hx2, 2d, CH_2Ph, J=12.09), 4.46, 4.38 (1Hx2, 2d, CH_2Ph, J=11.55), 4.32 (1H, d, *H*-3, $J_{2,3}$=4.39), 3.80, 3.79 (3Hx2, 2s, OCH_3x2), 3.62, 3.57 (1Hx2, 2d, *H*-5, J=10.63), 1.59, 1.33 (3Hx2, 2s, acetonide). *Anal.* Found C, 65.40; H, 6.66%. Calcd. for $C_{25}H_{30}O_8$: C, 65.49; H, 6.59%.

4-C-(2,2-Dibromoethenyl)-1,2-O-isopropylidene-3,5-di-O-p-methoxybenzyl-α-D-ribo-pentofuranose (6). To a solution of **5** (10.5 g, 22.9 mmol) in dichloromethane (200 ml) were added carbon tetrabromide (15.19 g, 45.8 mmol) and triphenylphosphine (24.03 g, 91.6 mmol) at 0°C, and the mixture was stirred for 30 min at room tem-

perature. To this mixture was added triethylamine (19.2 ml, 13.74 mmol), and mixture was stirred for 5 min at room temperature. The reaction mixture was poured into hexane (1000 ml) while stirring, and the precipitated by-products were removed by filtration. The filtration was condensed under reduced pressure to afford a crude crystalline mass (27.0 g). This was purified by silica gel column chromatography with chloroform to give **6** as a syrup (13.3 g, 95%). $[\alpha]_D+10.5°$ (c 1.05, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.27–7.17 (4H, m, Ph), 7.09 (1H, s, $Br_2C=CH$-), 6.89–6.85 (4H, m, Ph), 5.75 (1H, d, *H*-1, $J_{1,2}=3.85$), 4.62, 4.54, 4.51, 4.35 (1Hx4, 2d, CH_2Ph, $J=11.81$), 4.49 (1H, t, *H*-2, $J=4.12$), 4.16 (1H, d, *H*-3, $J_{2,3}=4.67$), 3.80, 3.79 (3Hx2, 2s, OCH_3x2), 3.79, 3.35 (1Hx2, 2d, *H*-5, $J=11.26$), 1.57, 1.29 (3Hx2, s, acetonide).

4-C-Ethynyl-1,2-O-isopropylidene-3,5-di-O-p-methoxybenzyl-α-D-ribo-pentofuranose (7). To a solution of **6** (4.58 g, 7.46 mmol) in tetrahydrofuran (60 ml) was added *n*-butyl lithium (10.25 ml of 1.6 M in *n*-hexane, 16.4 mmol) at −78°C under a nitrogen atmosphere, and the mixture was stirred for 2 h at −78°C. Water (25 ml) was then added dropwise to the mixture, and the solution was stirred for 5 min and raised to room temperature. Ethyl acetate (300 ml) was added to the mixture before washing with water. The organic layer was dried over magnesium sulfate and condensed under reduced pressure to afford a syrupy residue. This syrup was purified by silica gel column chromatography with a mixture of hexane and ethyl acetate (4:1, v/v) to afford **7** (3.12 g, 92%) as a syrup. $[\alpha]_D+21.0°$ (c 1.07, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.33–6.83 (8H, m, Ph), 5.69 (1H, d, *H*-1, $J_{1,2}=3.85$), 4.68, 4.61 (1Hx2, 2d, CH_2Ph, $J=12.22$), 4.50 (1H, t, *H*-2, $J=4.26$), 4.47, 4.37 (1Hx2, 2d, CH_2Ph, $J=11.68$), 4.12 (1H, d, *H*-3, $J_{2,3}=4.40$), 3.80 (6H, s, OCH_3x2), 3.67, 3.50 (1Hx2, 2d, *H*-5, *H*-5′, $J=11.54$), 2.63 (1H, s, ethynyl), 1.72, 1.32 (3Hx2, 2s, acetonide).

4-C-Ethynyl-1,2-O-isopropylidene-α-D-ribo-pentofuranose (8). A mixture of **7** (2.89 g, 6.36 mmol), 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ, 4.33 g, 19 mmol), dichloromethane (100 ml) and water (20 ml) was stirred for 24 h at room temperature. Chloroform (200 ml) was added to the mixture before washing with water. The organic layer was dried over magnesium sulfate and condensed under reduced pressure. The residue was subjected to silica gel column chromatography. Elution with a mixture of hexane and ethyl acetate (1:1, v/v) gave crystalline **8** (428 mg, 32%), $[\alpha]_D+3.74°$ (c 0.99, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 5.83 (1H, d, *H*-1, $J_{1,2}=4.12$), 4.65 (1H, dd, *H*-2, $J_{1,2}=4.12$, $J_{2,3}=5.22$), 4.30 (1H, dd, *H*-3, $J_{2,3}=5.22$), 3.85, 3.66 (1Hx2, 2d, *H*-5, *H*-5′, $J=12.09$), 2.82 (1H, d, 3-O*H*), 2.71 (1H, s, ethynyl), 1.99 (1H, dd, 5-O*H*), 1.68, 1.36 (3Hx2, 2s, acetonide). *Anal.* Found: C, 55.95; H, 6.75%. Calcd. for $C_{10}H_{14}O_5$: C, 56.07; H, 6.59%.

3,5-Di-O-benzoyl-4-C-ethynyl-1,2-O-isopropylidene-α-D-ribo-pentofuranose (9). A mixture of **7** (1.20 g, 2.64 mmol), 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (1.80 g, 7 mmol), dichloromethane (45 ml) and methanol (5 ml) was stirred for 24 h at room temperature and then evaporated under reduced pressure to give a crude crystalline mass (4.40 g). To a solution of this residue in pyridine (50 ml) was added benzoyl chloride (3.07 ml, 26.40 mmol) at 0°C, and the mixture was stirred for 2 h at room temperature. To the mixture was added an ice-water mixture (5 ml), before evaporating under reduced pressure and coevaporating the residue with toluene. The residue was dissolved in ethyl acetate (50 ml), washed with water and dried over magnesium sulfate. The magnesium sulfate was filtered off, and the ethyl acetate was evaporated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, with a mixture of hexane and ethyl acetate (9:1, v/v) as the eluent, to give **9** as a syrup (0.80 g, 72%), $[\alpha]_D+68.4°$ (c 0.99, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.18–7.99 (4H, m, Ph), 7.64–7.34 (6H, m, Ph), 5.96 (1H, d, *H*-1, $J_{1,2}=3.85$), 5.31 (1H, d, *H*-3, $J_{2,3}=5.22$), 5.06 (1H, dd, *H*-2, $J_{1,2}=3.85$, $J_{2,3}=5.22$), 4.67, 4.45 (1Hx2, 2d, *H*-5, *H*-5′, $J=11.81$), 2.77 (1H, s, ethynyl), 1.71, 1.34 (3Hx2, 2s, acetonide). *Anal.* Found: C, 68.36; H, 5.34%. Calcd. for $C_{24}H_{22}O_7$: C, 68.24; H, 5.25%.

1,2-Di-O-acetyl-3,5-di-O-benzoyl-4-C-ethynyl-α-D-ribo-pentofuranose (11). To a solution of **9** (3.66 g, 8.66 mmol) in acetic acid (50 ml) was added water (40 ml) and trifluoroacetic acid (10 ml), and the resulting solution was stirred for 18 h at room temperature before being evaporated under reduced pressure to afford a syrup. After this syrup had been coevaporated with toluene, the syrup was dissolved in pyridine (100 ml). To the solution was added acetic anhydride (8.19 ml, 86 mmol) at 0°C, and the mixture was stirred for 4 h at room temperature. To the mixture was then added water (10 ml), and the solution was evaporated under reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, using a mixture of hexane and ethyl acetate (5:1, v/v) as the eluent, to give **11** (3.08 g, α:β=12:88, 76%). ^{1}H-NMR ($CDCl_3$) δ for the α isomer: 6.54 (1H, d, *H*-1, $J_{1,2}=4.67$), 5.90 (1H, d, *H*-3, $J_{2,3}=6.60$), 5.50 (1H, dd, *H*-2, $J_{1,2}=4.67$, $J_{2,3}=6.60$); δ for the β isomer: 6.36 (1H, d, *H*-1, $J_{1,2}=0.55$), 5.92 (1H, d, *H*-3, $J_{2,3}=4.95$), 5.57 (1H, dd, *H*-2, $J_{1,2}=0.55$, $J_{2,3}=4.95$). HRMS: found, 467.1338; calcd. for $C_{25}H_{23}O_9$; 467.1341.

2′-O-Acetyl-3′,5′-di-O-benzoyl-4′-C-ethynyl-β-D-ribo-pentofuranosyluracil (12). A mixture of **11** (440 mg, 943 μmol), uracil (264 mg, 2.2 mmol) and bis(trimethylsilyl)acetamide (1.63 ml, 6.2 mmol) in dichloroethane was stirred under reflux for 1 h and then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (0.24 ml, 1.2 mmol) was added to the mixture and the mixture was stirred under reflux for 24 h before being cooled to room temperature. Ice-cold saturated aqueous sodium hydrogen carbonate (10 ml) was added, and the mixture was extracted with chloroform (3 × 10 ml). The organic layer was successively washed with saturated

aqueous sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml), and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography, using hexane and ethyl acetate (1:1, v/v) as the eluent, to give **12** as a foam (393 mg, 80%), $[\alpha]_D$ −39.4° (c 1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.16 (1H, s, N*H*), 8.13–8.08 (4H, m, Ph), 7.66–7.45 (6H, m, Ph), 7.20 (1H, d, *H*-6, $J_{5,6}$=8.06), 6.18 (1H, d, *H*-1′, $J_{1',2'}$=5.37), 5.91 (1H, d, *H*-3′, $J_{2',3'}$=6.84), 5.59–5.56 (2H, m, *H*-2′, H-5), 4.87, 4.59 (1Hx2, 2d, *H*-5′a, *H*-5′b, J=12.21), 2.77 (1H, s, ethynyl), 2.06 (3H, s, Ac). *Anal.* Found: C, 62.38; H, 4.38; N, 5.58%. Calcd. for $C_{27}H_{22}N_2O_9$: C, 62.55; H, 4.28; N, 5.40%.

2′-O-Acetyl-3′,5′-di-O-benzoyl-4′-C-ethynyl-β-D-ribo-pentofuranosylthymine (13). A mixture of **11** (732 mg, 1.57 mmol), thymine (495 mg, 3.9 mmol) and bis(trimethylsilyl)acetamide (2.72 ml, 11 mmol) in dichloroethane (40 ml) was stirred under reflux for 1 h and then cooled to room temperature. Trimethylsilyl trifluoromethanesulfonate (0.39 ml, 2.0 mmol) was added to the mixture, which was stirred at reflux for 24 h and then cooled to room temperature. Ice-cold aqueous sodium hydrogen carbonate (10 ml) was added to the mixture, before it was extracted with chloroform (3 × 10 ml). The organic layer was successively washed with saturated sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml) and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography, using a mixture of hexane and ethyl acetate (2:1, v/v) as the eluent, to give **13** (600 mg, 72%) as a foam, $[\alpha]_D$ −61.5° (c 1.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.15–8.11 (5H, m, Ph), 8.09 (1H, s, N*H*), 7.68–7.46 (5H, m, Ph), 6.98 (1H, d, *H*-6, $J_{6,Me}$=1.24), 6.30 (1H, d, *H*-1′, $J_{1',2'}$=6.04), 5.93 (1H, d, *H*-3′, $J_{2',3'}$=6.59), 5.57 (1H, dd, *H*-2′, $J_{1',2'}$=6.04, $J_{2',3'}$=6.59), 4.92, 4.56 (1Hx2, 2d, *H*-5′a, *H*-5′b, J=12.09), 2.75 (1H, s, ethynyl), 2.05 (3H, s, Ac), 1.59 (3H, d, 6-C*H*$_3$, $J_{6,Me}$=1.24). *Anal.* Found: C, 62.44; H, 4.77; N, 5.39%. Calcd. for $C_{28}H_{24}N_2O_9$. 1/4H_2O: C, 62.62; H, 4.60; N, 5.22%.

4′-C-Ethynyluridine (14). A solution of **12** (550 mg, 1.06 mmol) in sat. methanolic ammonia (30 ml) was stirred for 17 h at room temperature and then evaporated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, using a mixture of chloroform and methanol (7:1, v/v) as the eluent, to give crystalline **14** (240 mg, 84%), mp 120–122°C, $[\alpha]_D$+2.48° (c 1.01, H_2O). UV λ_{max} (MeOH) nm (ε): 261 (10100). ^{1}H- NMR (DMSO-d_6) δ: 11.15 (1H, s, N*H*), 7.71 (1H, d, *H*-6, $J_{5,6}$=8.06), 5.86 (1H, d, *H*-1′, $J_{1',2'}$=5.86), 5.66 (1H, d, *H*-5, $J_{5,6}$=8.06), 5.41 (1H, t, 5′-O*H*), 4.14 (1H, dd, *H*-2′, $J_{1',2'}$=5.86, $J_{2',3'}$=6.10), 4.07 (1H, d, *H*-3′, $J_{2',3'}$=6.10), 3.58, 3.55 (1Hx2, 2d, *H*-5′a, *H*-5′b, $J_{5'a,5'b}$=11.96), 3.33 (1H, s, ethynyl). *Anal.* Found: C, 48.12; H, 4.51; N, 10.01%. Calcd. for $C_{11}H_{12}N_2O_6$. 1/4H_2O: C, 48.41; H, 4.62; N, 10.27%.

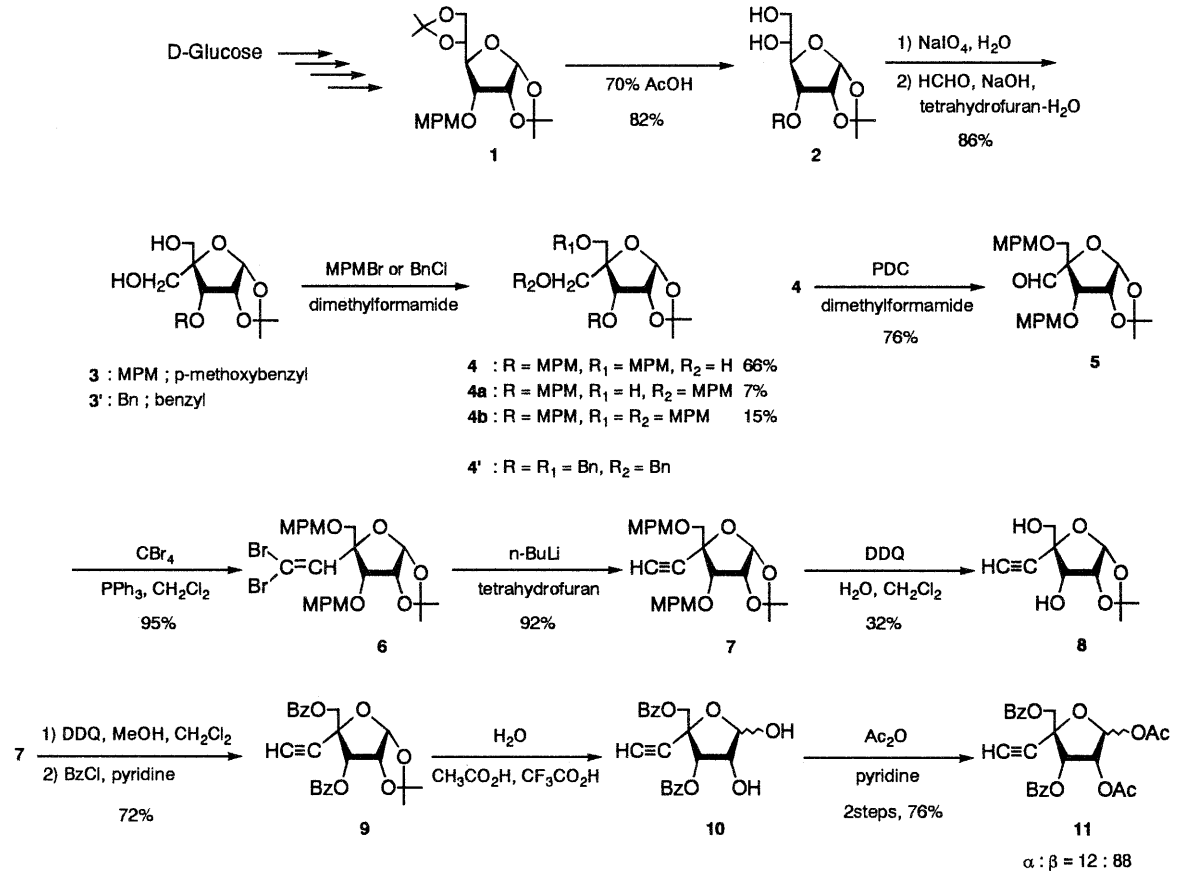
4′-C-Ethynyl-β-D-ribo-pentofuranosylthymine (15). A solution of **13** (600 mg, 1.13 mmol) in sat. methanolic ammonia (30 ml) was stirred for 16 h at room temperature and then evaporated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, using a mixture of chloroform and methanol (10:1, v/v), to give **15** (260 mg, 82%) as a foam, $[\alpha]_D$+10.3° (c 0.50, H_2O). UV λ_{max} (MeOH) nm (ε): 266 (9650). ^{1}H-NMR (DMSO-d_6) δ: 11.22 (1H, s, N*H*), 7.60 (1H, d, *H*-6, $J_{6,Me}$=1.22), 5.90 (1H, d, *H*-1′, $J_{1',2'}$=6.10), 5.40 (1H, t, 5′-O*H*), 5.22, 5.07 (1Hx2, 2d, 2′-O*H*, 3′-O*H*), 4.16 (1H, dd, *H*-2′, $J_{1',2'}$=6.10, $J_{2',3'}$=5.80), 4.10 (1H, t, *H*-3′, $J_{2',3'}$=5.80), 3.62, 3.57 (1Hx2, 2d, *H*-5′a, *H*-5′b, $J_{5'a,5'b}$=11.90), 3.42 (1H, s, ethynyl), 1.59 (3H, Me, $J_{6,Me}$=0.92). *Anal.* Found: C, 50.23; H, 5.32; N, 9.57%. Calcd. for $C_{12}H_{14}N_2O_6$. 1/4H_2O: C, 50.26; H, 5.10; N, 9.77%.

4′-C-Ethynylcytidine (17). To a solution of **12** (620 mg, 1.2 mmol) in pyridine (30 ml) was added 4-chlorophenyl dichlorophosphate (0.48 ml, 3.0 mmol) at 0°C during 2 min. To the mixture was then added 1, 2, 4-triazole (734 mg, 12 mmol), and the mixture was stirred for 72 h at room temperature. The solvent was removed under reduced pressure. The residual syrup was purified by silica gel column chromatography, using a mixture of hexane, ethyl acetate and 2-propanol (10:10:1, v/v/v), to give a triazole derivative (**16**) as a syrup. A solution of this syrup (850 mg) in a mixture of ammonium hydroxide and dioxane (1:3, v/v, 40 ml) was stirred for 2 h, and then the solvent was removed under reduced pressure to afford a syrup. This syrup was dissolved in sat. methanolic ammonia (30 ml) and stirred for 18 h at room temperature. The solvent was removed under reduced pressure to give a syrup (560 mg). This syrup was purified by silica gel column chromatography to afford crystalline **17** (160 mg, 50%), mp 217–220°C, $[\alpha]_D$+18.7° (c 0.47, H_2O). UV λ_{max} (MeOH) nm (ε): 273 (10700). ^{1}H-NMR (DMSO-d_6) δ: 7.68 (1H, d, *H*-6, $J_{5,6}$=7.32), 7.08 (2H, s, N*H*$_2$), 5.91 (1H, d, *H*-1′, $J_{1',2'}$=4.64), 5.66 (1H, d, $J_{5,6}$=8.06), 5.34 (1H, t, 5′-O*H*), 5.15, 4.95 (1Hx2, 2d, 2′-O*H*, 3′-O*H*), 4.08–4.11 (2H, m, *H*-2′, *H*-3′), 3.61, 3.55 (1Hx2, 2d, *H*-5′a, *H*-5′b, $J_{5'a,5'b}$=11.96), 3.40 (1H, s, ethynyl). Found: C, 49.06; H, 5.09; N, 15.30%. Calcd. for $C_{11}H_{13}N_3O_5 \cdot 1/8H_2O$: C, 49.03; H, 4.96; N, 15.59%.

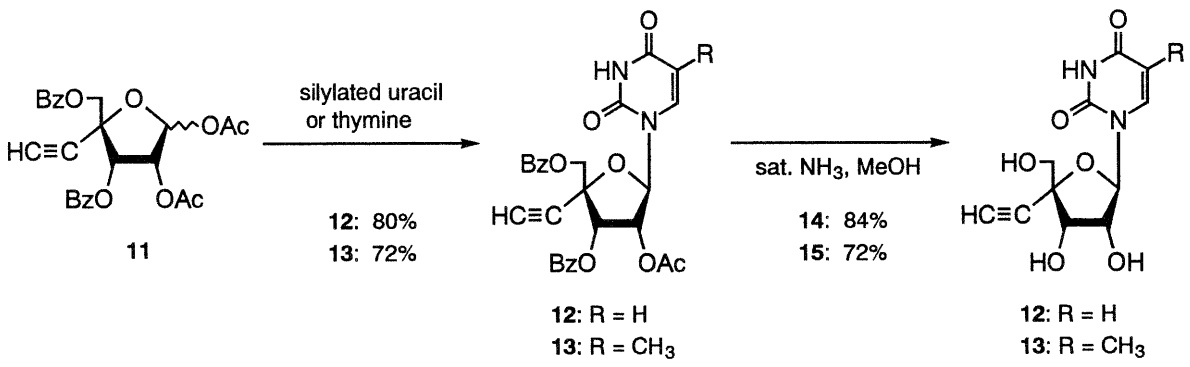
Results and Discussion

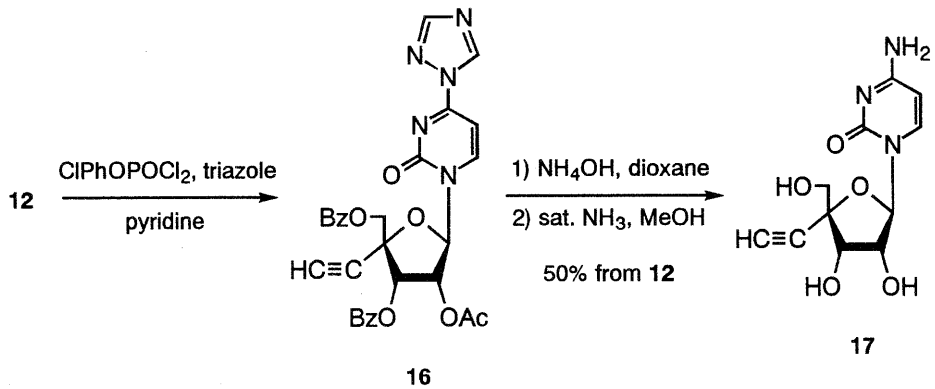
We had previously used 3, 5-di-*O*-benzyl-4-*C*-hydroxymethyl-1, 2-*O*-isopropylidene-α-D-*ribo*-pentofuranose **4′** for the synthesis of both **B** and 4′-*C*-fluoromethyl-2′-deoxycytidine **C**.[13] However, we used here 4-*C*-hydroxymethyl-1, 2-*O*-isopropylidene-3, 5-di-*O*-*p*-methoxybenzyl-α-D-*ribo*-pentofuranose **4** as an intermediate because it was anticipated that de-*O*-protection of the *p*-methoxybenzyl group in the presence of an ethynyl group would be much easier than that of the benzyl group (Scheme 1).

The selective *p*-methoxybenzylation of 4-*C*-hydroxymethyl-1, 2-*O*-isopropylidene-3-*O*-p-methoxybenzyl-α-D-*ribo*-pentofuranose (**3**), which had been prepared



Scheme 1. Synthetic Pathway for the 4′-*C*-Ethynyl-D-*ribo*-pentofuranosyl Glycosyl Donor.





Scheme 2. Synthesis of 4′-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl Pyrimidines.

from D-glucose by a similar procedure[7] to that used for the synthesis of corresponding 3-*O*-benzyl derivative **3′**, with a limited amount of *p*-methoxybenzyl chloride and sodium hydride in dimethylformamide at −20°C gave a mixture of 6-*O*-*p*-methoxybenzyl derivative **4a** (7%) and 5, 6-di-*O*-*p*-methoxybenzyl derivative **4b** (15%), as well as desired 5-*O*-*p*-methoxybenzyl derivative **4** (66%). The structure of the major product was tentatively assigned as **4**, because **4′** was the major product of the reaction of **3′** with benzyl chloride under similar conditions. The assignment was later confirmed by nuclear Overhauser effect (NOE) experiments with both the 4-*C*-formyl and 4-*C*-ethynyl derivatives. Oxidation of **4** with pyridinium dichromate in dimethylformamide gave 4-*C*-formyl derivative **5** in a 76% yield. Aldehyde **5** could be a versatile intermediate for the synthesis of various 4′-*C*-substituted β-D-*ribo*-pentofuranosyl nucleosides. Here, **5** was converted to ethynyl derivative **7** by Corey's protocol.[14] Thus, **5** was reacted with carbon tetrabromide in the presence of triphenylphosphine to give syrupy vinyl dibromide **6** in a 95% yield. **6** was treated with *n*-butyl lithium to afford 3, 5-di-*O*-*p*-methoxybenzyl-4-*C*-ethynyl-1, 2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (**7**) in a 92% yield. The 4-*C*-substituted D-*ribo* structures of **4**, **5** and **7** were confirmed by NOE experiments with **5** and **7**. Irradiation at the *endo* methyl of the 1, 2-*O*-isopropylidene group of **5** and **7** enhance the signals of the aldehyde proton and the ethynyl proton, respectively, to show the *cis* stereochemistry of these groups on the furanose ring (Fig. 2). At this stage of synthesis, the 3- and 5-*O*-*p*-methoxybenzyl groups were removed, and the hydroxyl groups were benzoylated, because it was anticipated that oxidative removal of the *p*-methoxybenzyl group at any later stage of the synthesis would be cumbersome due to the difficulty of isolating the deprotected more hydrophilic product from the reaction mixture. Treatment of **7** with 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone in a mixture of dichloromethane and water gave expected 3, 5-dihydroxy product **8** in a moderate yield judged by thin-layer chromatography. However, 8 could be obtained in only a 32% yield because of the difficulty of isolating **8** from the reaction mixture. Therefore, the oxidative de-*O*-*p*-methoxybenzylation of **7** was conducted in a mixture of dichloromethane and methanol[15] with 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone, and after evaporating the solvent, the whole residue was benzoylated without isolating **8**, and desired 3, 5-di-*O*-benzoyl derivative **9** was obtained in a 72% yield. The isopropylidene group of **9** was removed in aqueous acetic acid, and reducing sugar **10** was acetylated without purification to afford 1, 2-di-*O*-acetyl-3, 5-di-*O*-benzoyl-4-*C*-ethynyl-D-*ribo*-pentofuranose **11** as an anomeric mixture (α: β=12:88) in a 76% yield. In the ^{1}H-NMR analysis of **11**, the signal of an anomeric proton with the larger $J_{1,2}$ value was assigned to that of the a isomer ($J_{1,2}$ for the α isomer=4.67 Hz, $J_{1,2}$ for the β isomer=0.55 Hz). The condensation of **11** with silylated uracil or thymine in the presence of trimethylsilyl trifluoromethanesulfonate as a catalyst in 1, 2-dichloroethane needed refluxing for 24 h to complete the condensation to give 4′-*C*-ethynyl uridine (**12**) and 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosyl thymine (**13**) in 80% and 72% yields, respectively. In this reaction, the α isomer was not detected. Since the condensation of 1, 2-di-*O*-acetyl-3, 5-di-*O*-benzoyl-4-*C*-methyl-D-*ribo*-pentofuranose with silylated uracil and thymine proceeded at room temperature for 2 h,[11] the ethynyl group at the 4-position of **11** seems to have deactivated its anomeric position. The anomeric configurations of **12** and **13** can both be expected to be β by the Vorbruggen protocol[15] that affords the nucleosides with 1′, 2′-*trans* stereochemistry when the furanoses have a neighboring participating acyloxy group at the 2-position. However, **12** and **13** had relatively large H-1′ and H-2′ coupling constants of 5.4 and 6.0 Hz, respectively, which might be assigned to an α-configuration according to the ^{1}H-NMR empirical rule for assigning the anomeric configuration of *ribo*furanosyl nucleosides.[16] Although β-selective *N*-glycosylation and similar relatively large $^3J_{H\text{-}1',H\text{-}2'}$ values have been reported for 4′-*C*-hydroxymethyl-[7] and 4′-*C*-methyl-β-D-*ribo*-pentofuranosyl[11] nucleosides, the β-stereochemistry of **12** and **13** was confirmed by the results of NOE experiments (Fig. 2). Both **12** and **13** showed clear NOEs between H-6 and H-2′, and between H-6 and H-5′. These clear NOE contacts indicated the β-stereochemistry of these nucleosides and the predominant *anti*-conformation of the uracil and thymine bases.[10] The large $^3J_{H\text{-}1',H\text{-}2'}$ value indicated more distortion of the conformation of the furanose rings of 4′-ethynyl-*ribo*-nucleosides than of *ribo*furanosyl nucleosides, as was the case with other 4′-*C*-substituted *ribo*-pentofuranosylnucleosides.[7,11] Deacylation of **12** and **13** gave 4′-*C*-ethynyluridine **14** and 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosylthymine **15**, respectively, in good yields. Since the condensation of **11** with silylated *N*-acetylcytosine did not proceed at all, 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosylcytosine (**17**) was prepared from **12** by Reese's methodology[18] through 1, 2, 4-triazole derivative **16** in a 50% yield. A preliminary biological test on these 4′-*C*-ethynylnucleosides showed that they were not active against HSV-1, HSV-2, CCRF-HSB-2, and KB cells.

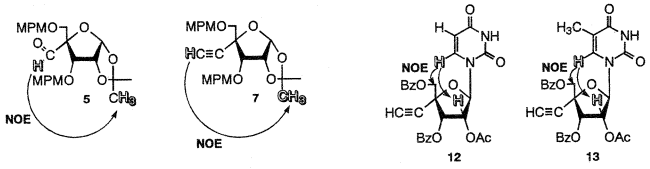


Fig. 2. NOE Contacts to Confirm the Stereochemistry.

Conclusions

We prepared 4′-*C*-ethynyl-β-D-*ribo*-pentofuranosylpyrimidines in moderate yields from D-glucose. However, their preliminary biological evaluation showed that they were not active against viruses and tumor cells, as was the case with 4′-*C*-methyl-β-D-*ribo*-pentofuranosylnucleosides.[10] The preparation of purine derivatives, 2′-deoxy and arabinofuranosyl derivatives is underway in our laboratory.

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