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Note

Synthesis of lactosyl phosphate diester derivatives of nucleosides

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Abstract

Derivatives of the lactosyl phosphate diester and the lactosyl thiophosphate diester of 1-(β-D-arabinofuranosyl)cytosine (Ara-C) and 9-(β-D-arabinofuranosyl)adenine (Ara-A) were synthesized by condensation of 3-hydroxypropyl lactoside and a protected Ara-C or Ara-A via H-phosphonate methodology and phosphoramidite methodology, respectively. © 1997 Elsevier Science Ltd.

Keywords: Lactosyl phosphate diester; Sugar nucleotide; H-phosphonate methodology; Phosphoramidite methodology

There are many carbohydrate receptors on the cell surface. For example, the asialoglycoprotein receptor, which exists on the surface of hepatocytes, can bind with terminal galactose residues [1]. To improve the selectivity of some bioactive compounds, several glycosyl phosphate triesters of nucleosides have been synthesized as lipophilic, macrophage-targeted carriers [2–5]. Nishikawa et al. developed a new carrier system for hepatic targeting and found that glycosylated *O*-carboxymethyldextrans are carriers with a high affinity for liver parenchymal or nonparenchymal cells without any affinity for other tissues [6]. We have reported the synthesis of galactosyl phosphate diester derivatives of Ara-C and Ara-A as a site-directing moiety towards glycosyl-binding proteins. It was found that the galactosyl phosphate diesters of Ara-C were active in the human cytomegalovirus (HCMV) assay. The IC_{50} of some compounds for anti-HCMV activity was 0.1–0.2 μM [7]. To develop this work, we present here the synthesis of lactosyl phosphate diester derivatives of Ara-C and Ara-A.

The target compounds **7a**, **7b** and **10a**, **10b** were synthesized according to Schemes 1 and 2. Formation of lactosyl phosphate diesters of Ara-C and Ara-A (**7a** and **7b**) was accomplished by H-phosphonate methodology (Scheme 1) [4]. A solution of hepta-*O*-acetyl-lactosyl bromide (**1**) [8] in benzene–$MeNO_2$ (1:1) was slowly added to an excess of 1,3-propanediol in the presence of $Hg(CN)_2$ and $CaSO_4$, and stirred for 5 days at room temperature, then purified by chromatography on silica gel with 20:1 CH_2Cl_2–MeOH to give 3-hydroxypropyl hepta-*O*-acetyl-β-lactoside (**2**) in 80.5% yield. Compound **2** was identified as the β anomer by ^1H NMR [δ 4.41 ($H_{1'}$, d), $J_{1',2'}$ 7.8 Hz]. Compound **2** was condensed with the protected nucleoside 5′-hydrogenphosphonate (**4a** or **4b**), in the presence of pivaloyl chloride as an activating agent in a mixture of dry pyridine and acetonitrile

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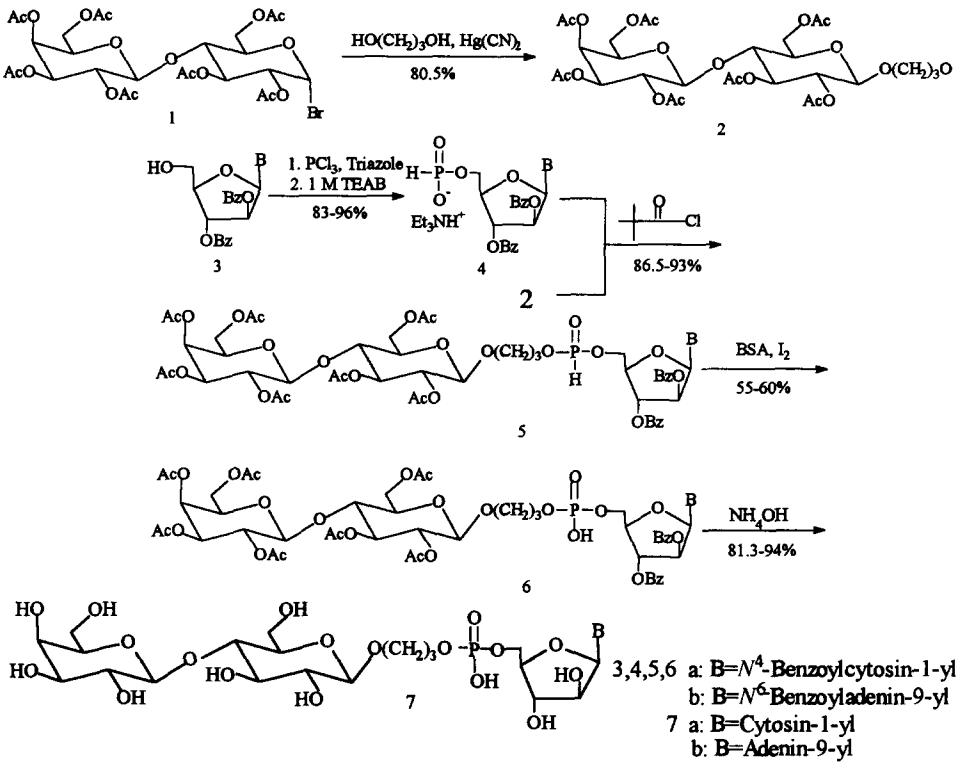
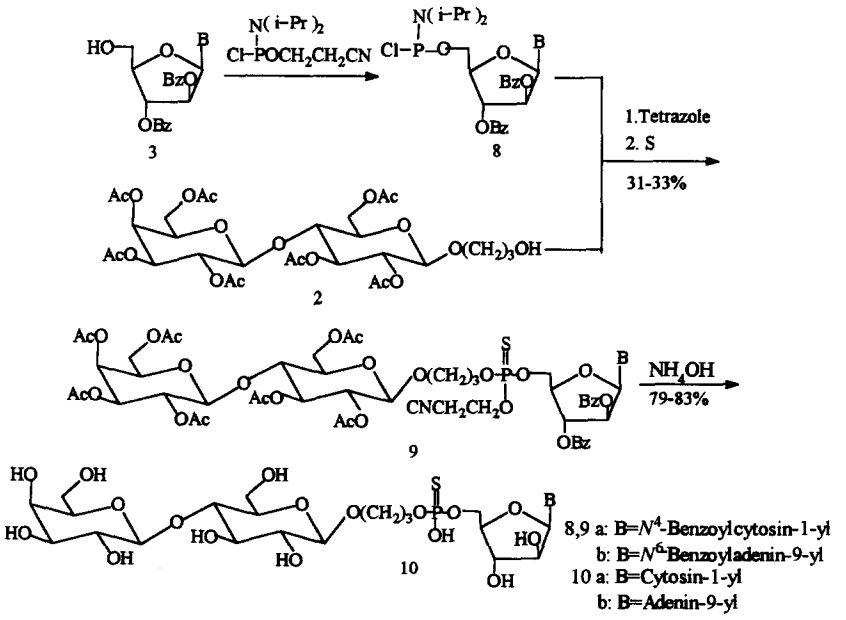


Scheme 1.

(1:1) for 5 min at room temperature. The product was purified by chromatography on silica gel with 20:1 CH_2Cl_2–MeOH to give the hydrogenphosphonate derivatives **5a** and **5b** in 93 and 87% yields, respectively. The ^{31}P NMR spectra of **5a** and **5b** showed that they were mixtures of diasteroisomers in 1:1 ratio, and a P–H coupling constant of ~ 700 Hz was also observed. The intermediates **5a** and **5b** were oxidized by iodine in the presence of bis(trimethylsilyl)acetamide (BSA) and triethylamine, and purified on a column of reverse-phase C_{18} silica gel by elution with 1:10 MeOH–H_2O to give the phosphate diesters **6a** and **6b** in yields of 60 and 55%, respectively. Lactosyl thiophosphate diesters **9a** or **9b** were



Scheme 2.

synthesized by the phosphoramidite methodology reported previously (Scheme 2) [6]. Deprotection of **6a**, **6b**, **9a** and **9b** was performed with concd. NH_4OH for 24 h at 50 °C, followed by purification with Sephadex G-15 chromatography using water as eluant and lyophilization, to afford **7a**, **7b**, **10a** and **10b** in yields of 80–100%.

The tissue distribution of **7a** was studied in mice by using the nonspecific-position ^{3}H-labelled compound. After intravenous injection of ^{3}H-labelled **7a** into mice, radioactivity in the liver tissue was higher than other organs except kidney, and liver had the highest radioactivity after two h. The preliminary results showed that **7a** has a higher affinity to liver cell than Ara-C. Detailed biological activities will be reported elsewhere.

1. Experimental

General methods.—All evaporations were conducted in a rotary evaporator under diminished pressure. TLC was conducted on Silica Gel F_{254} by developing with 5:1 $CHCl_3$–acetone. Column chromatography was performed on silica gel (200–300 mesh, purchased from Qing Dao Chemical Company, China). ^{1}H NMR spectra were recorded with FX-90Q and VXR 300 spectrometers, with Me_4Si as the internal standard. ^{31}P NMR spectra were recorded with VXR 300 and VXR 200 spectrometers, with 85% H_3PO_4 as the external standard. A ZAB-HS source was used for fast-atom bombardment (FAB) mass spectra. UV spectra were recorded with a DU-7 spectrophotometer. Optical rotations were determined on a Perkin–Elmer 243B polarimeter at ambient temperature (20 ± 1 °C). Microanalyses were obtained using a Perkin–Elmer 240c elemental analyser.

3 - Hydroxypropyl hepta - O - acetyl - β - lactoside (**2**).—1,3-Propanediol (1.2 g, 16 mmol) was dissolved in $MeNO_2$ (20 mL) and benzene (20 mL) containing $Hg(CN)_2$ (2 g) and anhydrous $CaSO_4$ (4 g), and a solution of hepta-*O*-acetyl-β-lactosyl bromide **1** (6 g, 8 mmol) in $MeNO_2$ (7 mL) and benzene (7 mL) was slowly added at room temperature. The mixture was stirred for 5 days and filtered. The filtrate was washed successively with 10% KI, satd $NaHCO_3$ solution and water. The solution was dried (Na_2SO_4), and purified by chromatography on silica gel with 20:1 CH_2Cl_2–MeOH to give 4.8 g (80.5%) of **2** as a white powder, mp 79–81 °C; ^{1}H NMR ($CDCl_3$): δ 1.65–1.75 (m, 2 H, $-CH_2-$), 1.85–2.05 (5s, 21 H, CH_3CO-), 2.7–3.0 (broad, 1 H, –OH), 3.5–3.6 (m, 4 H, $-CH_2-$), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1); FABMS: *m/z* 695 ($[M + H]^+$). Anal. Calcd for $C_{29}H_{42}O_{19}$: C, 50.14; H, 6.09. Found: C, 50.33; H, 6.11.

N^4 - Benzoyl - 1 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)cytosine 5' - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] phosphonate (**5a**).—Pivaloyl chloride (95 μL, 0.75 mmol) was added to the solution of **2** (70 mg, 0.1 mmol) and **4a** (145 mg, 0.2 mmol) in anhydrous pyridine (7 mL) and MeCN (7 mL). The solution was stirred for 5 min at room temperature and hydrolyzed with a small amount of water. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with satd. aq. $NaHCO_3$ and water. After drying (Na_2SO_4) and evaporation, the residue was chromatographed on a column of silica gel and eluted with 20:1 CH_2Cl_2–MeOH, to give 120 mg (93%) of **5a** as a white powder; UV, λ_{max}^{MeOH} 233, 261 nm; ^{31}P NMR ($CDCl_3$): δ 8.88 (J_{PH} 719 Hz) and 10.17 (J_{PH} 715 Hz); ^{1}H NMR ($CDCl_3$): δ 1.80–1.94 (m, 2 H, $-CH_2-CH_2-CH_2-$), 1.95–2.15 (7s, 21 H, CH_3CO-), 3.5–3.6 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.55–6.65 (m, 1 H, H-1'), 7.3–8.2 (m, 16 H, Bz, H-6), 8.75 (d, 1 H, *J* 7.2 Hz, H-5); FABMS: *m/z* 1296 ($[M + H]^+$).

N^6 - Benzoyl - 9 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)adenine 5' - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] phosphonate (**5b**).—Compound **5b** was prepared from **2** (1.17 g, 1.68 mmol) and **4b** (1.04 g, 1.4 mmol) by the method described for **5a**; 1.6 g (86.5%) of the product was obtained as a white powder; UV, λ_{max}^{MeOH} 232, 278 nm; ^{31}P NMR ($CDCl_3$): δ 9.05 (J_{PH} 710 Hz) and 10.07 (J_{PH} 709 Hz); ^{1}H NMR ($CDCl_3$): δ 1.80–1.95 (m, 2 H, $-CH_2-CH_2-CH_2-$), 1.96–2.19 (7s, 21 H, CH_3CO-), 3.5–3.6 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.85–6.90 (m, 1 H, H-1'), 7.30–8.09 (m, 15 H, Bz), 8.10 (s, 1 H, H-8), 8.13 (s, 1 H, H-2); FABMS: *m/z* 1320 ($[M + H]^+$).

N^4 - Benzoyl - 1 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)cytosine 5' - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] phosphate (**6a**).—Compound **5a** (1 g, 0.77 mmol) was dissolved in a solution Et_3N (350 μL) in dry tetrahydrofuran (16 mL), and then bis(trimethylsilyl)acetamide (1.9 mL, 24.6 mmol) was added at room temperature. After 10 min, iodine (1.35 g) was added. The mixture was stirred for 4 h and evaporated. The residue was purified on a column of reverse-phase C_{18} silica gel and eluted with 1:10 MeOH–H_2O to give 600 mg (60%) of **6a** as a syrup; UV, λ_{max}^{MeOH} 233, 262 nm; ^{31}P NMR ($CDCl_3$): δ 0.96; ^{1}H NMR ($CDCl_3$): δ 1.85–1.95 (m, 2 H,

$-CH_2-CH_2-CH_2-$), 1.95–2.18 (5s, 21 H, CH_3CO-), 3.5–3.6 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.55 (d, 1 H, *J* 3.8 Hz, H-1′), 7.3–8.0 (m, 16 H, Bz, H-6), 8.40 (d, 1 H, *J* 7.8 Hz, H-5); FABMS: *m/z* 1313 ($[M + 2]^+$).

N^6 - Benzoyl - 9 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)adenine 5′ - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] phosphate (**6b**).—Compound **6b** was prepared from **5b** (0.5 g, 0.38 mmol) by the method described for **6a**; 280 mg (55%) of the product was obtained as a syrup; UV, λ_{max}^{MeOH} 232, 277 nm; ^{31}P NMR ($CDCl_3$): δ 0.46; ^1H NMR ($CDCl_3$): δ 1.85–1.95 (m, 2 H, $-CH_2-CH_2-CH_2-$), 1.96–2.15 (5s, 21 H, CH_3CO-), 3.5–3.6 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.85 (d, 1 H, *J* 3.8 Hz, H-1′), 7.3–8.0 (m, 15 H, Bz), 8.08 (s, 1 H, H-8), 8.10 (s, 1 H, H-2); FABMS: *m/z* 1336 ($[M + H]^+$).

1 - β - D - Arabinofuranosylcytosine 5′ - [(prop - 3 - yl β - lactoside) - 1 - yl] phosphate (**7a**).—Compound **6a** (60 mg, 0.046 mmol) was dissolved in concd. NH_4OH (25 mL), and the solution was heated at 60 °C in a sealed tube for 2 days. The solution was evaporated to remove NH_3. The residue was extracted with $CHCl_3$ then dissolved in water and purified on a column of Sephadex G-15 using water as eluant. After freeze drying, **7a** (30 mg, 94%) was obtained as a white powder; UV, λ_{max}^{MeOH} 272 nm; $[\alpha]_D^{20}$ +30° (*c*, 1.47, MeOH); ^{31}P NMR (D_2O): 0.06; ^1H NMR (D_2O): 1.7–1.8 (m, 2 H, $-CH_2-CH_2-CH_2-$), 3.00–3.15 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 5.86 (d, 1 H, *J* 7.8 Hz, H-5), 5.98 (d, 1 H, *J* 3.3 Hz, H-1′), 7.69 (d, 1 H, *J* 7.8 Hz, H-6); FABMS: *m/z* 706 ($[M + H]^+$). HRMS, Calcd for $C_{24}H_{41}N_3O_{19}P$: 706.2072. Found: 706.2072 ($[M + H]^+$).

9 - β - D - Arabinofuranosyladenine 5′ - [(prop - 3 - yl β - lactoside) - 1 - yl] phosphate (**7b**).—Compound **7b** was prepared from **6b** (30 mg, 0.022 mmol) by the method described for **7a**; 13 mg (81.3%) of the product was obtained as a white powder; UV, λ_{max}^{MeOH} 262 nm; $[\alpha]_D^{20}$ +12.3° (*c*, 1.87, CH_3OH); ^{31}P NMR (D_2O): δ 0.51; ^1H NMR (D_2O): δ 1.7–1.8 (m, 2 H, $-CH_2-CH_2-CH_2-$), 3.00–3.15 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.21 (d, 1 H, *J* 5.7 Hz, H-1′), 8.02 (s, 1 H, H-8), 8.21 (s, 1 H, H-2); FABMS: *m/z* 730 ($[M + H]^+$). HRMS, Calcd for $C_{25}H_{41}N_5O_{18}P$: 730.2184. Found: 730.2198 ($[M + H]^+$).

N^4 - Benzoyl - 1 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)cytosine 5′ - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] 2 - cyanoethyl thiophosphate (**9a**).—Compound **3a** (278 mg, 0.5 mmol) was dissolved in a solution of ethyldiisopropylamine (174 μL) in CH_2Cl_2 (5 mL), and then 2-cyanoethyl *N,N*-diisopropylaminochlorophosphoramidite (167 μL) was added at room temperature under nitrogen. After 15 min, the mixture was diluted with CH_2Cl_2 (15 mL) and washed with 10% $NaHCO_3$ and water. The solution was dried (Na_2SO_4) and evaporated under reduced pressure. The residue and **2** (333 mg, 0.48 mmol) were dissolved in CH_2Cl_2 (5 mL), and a solution of tetrazole (80 mg) in MeCN (5 mL) was added. The mixture was stirred at room temperature for 30 min and added to a mixture of sulfur (200 mg) in toluene (2 mL). After stirring for 2 h, the mixture was diluted with CH_2Cl_2 (15 mL) and washed with water and satd. NaCl. After drying and removal of the solvent, the residue was purified by chromatography on silica gel in 10:1 $CHCl_3$–acetone as the eluant, to give 230 mg (33%) of **9a** as a white powder; UV, λ_{max}^{MeOH} 235, 265 nm; ^{31}P NMR ($CDCl_3$): δ 69.03, 69.17; ^1H NMR ($CDCl_3$): δ 1.25 (m, 2 H, $-CH_2-CH_2-CH_2-$), 1.96–2.15 (5s, 21 H, CH_3CO-), 2.73 (t, 2 H, *J* 6.2 Hz, $-CH_2CN$), 3.5–3.6 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.60–6.65 (m, 1 H, H-1′), 7.4–8.0 (m, 16 H, Bz, H-6), 8.10 (d, 1 H, *J* 8.4 Hz, H-5); FABMS: *m/z* 1382 ($[M + 2]^+$).

N^6 - Benzoyl - 9 - (2, 3 - di - O - benzoyl - β - D - arabinofuranosyl)adenine 5′ - [(prop - 3 - yl hepta - O - acetyl - β - lactoside) - 1 - yl] 2 - cyanoethyl thiophosphate (**9b**).—Compound **9b** was prepared from **3b** (145 mg, 0.25 mmol) and **2** (167 mg, 0.24 mmol) by the method described for **9a**; 110 mg (31%) of the product was obtained as a white powder; UV, λ_{max}^{MeOH} 231, 279 nm; ^{31}P NMR ($CDCl_3$): δ 68.66, 68.87; ^1H NMR ($CDCl_3$): δ 1.25 (m, 2 H, $-CH_2-CH_2-CH_2-$), 1.96–2.15 (5s, 21 H, CH_3CO-), 2.82 (t, 2 H, *J* 6.2 Hz, $-CH_2CN$), 3.55–3.65 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.65–6.70 (m, 1 H, H-1′), 7.25–8.03 (m, 15 H, Bz), 8.27 (s, 1 H, H-8), 8.83 (s, 1 H, H-2); FABMS: *m/z* 1406 ($[M + 2]^+$).

1 - β - D - Arabinofuranosylcytosine 5′ - [(prop - 3 - yl β - lactoside) - 1 - yl] thiophosphate (**10a**).—Compound **10a** was prepared from **9a** (150 mg, 0.11 mmol) by the method described for **7a**; 65 mg (83%) of the product was obtained as a white powder; UV, λ_{max}^{MeOH} 272 nm; $[\alpha]_D^{20}$ +38.9° (*c*, 1.47, CH_3OH); ^{31}P NMR (D_2O): δ 56.46, 56.57; ^1H NMR (D_2O): δ 1.9–2.0 (m, 2 H, $-CH_2-CH_2-CH_2-$), 2.9–3.3 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 6.07 (d, 1 H, *J* 5.7 Hz, H-5), 6.22 (d, 1 H, *J* 3.9 Hz, H-1′), 7.03 (d, 1 H, *J* 5.7 Hz, H-6); FABMS: *m/z* 722 ($[M + H]^+$). HRMS, Calcd for $C_{24}H_{41}N_3O_{18}PS$: 722.1843. Found: 722.1840 ($[M + H]^+$).

9 - β - D - Arabinofuranosyladenine 5′ - [(prop - 3 - yl β - lactoside) - 1 - yl] thiophosphate (**10b**).—Compound

10b was prepared from **9b** (50 mg, 0.036 mmol) by the method described for **7a**; 21 mg (79%) of the product was obtained as a white powder; UV, λ_{max}^{MeOH} 260 nm; $[\alpha]_D^{20}$ −3.5° (*c*, 0.87, CH_3OH); ^{31}P NMR (D_2O): δ 56.35, 56.50; ^1H NMR (D_2O): δ 1.65–1.80 (m, 2 H, $-CH_2-CH_2-CH_2-$), 3.0–3.2 (m, 4 H, $-O-CH_2-CH_2-CH_2-O-$), 5.94 (d, 1 H, *J* 3.6 Hz, H-1′), 8.04 (s, 1 H, H-8), 8.33 (s, 1 H, H-2); FABMS: *m/z* 746 ($[M + H]^+$). HRMS, Calcd for $C_{25}H_{41}N_5O_{17}PS$: 746.1956. Found: 746.1949 ($[M + H]^+$).

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