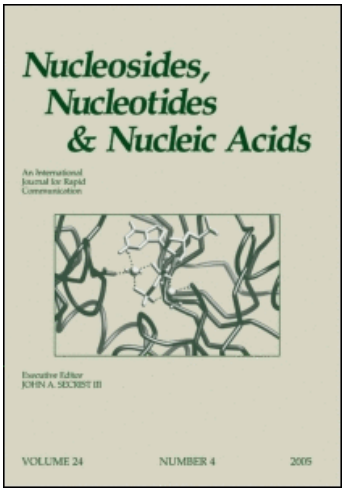

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Shigetada Kozai[a]; Tomoyo Fuzikawa[a]; Keisuke Harumoto[a]; Tokumi Maruyama[a]
[a] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan

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A New Method for the Synthesis of 2′-*O*-Benzyladenosine Using Mitsunobu Reaction

Shigetada Kozai, Tomoyo Fuzikawa, Keisuke Harumoto, and Tokumi Maruyama*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan

ABSTRACT

A new method to introduce a benzyl group onto the 2′-OH of purine ribonucleoside is described. Thus, 6-chloropurine 3′-*O*-benzoylriboside and its 5′-*O*-trityl congener were condensed with benzyl alcohol using the Mitsunobu reaction to give the 2′-*O*-benzyl derivative. The yields were varied from 4.6 to 62.9% depending on the solvent used. The product was converted to adenosine, indicating that the stereochemistry at C-2′ is retained.

Antisense oligonucleotides have emerged as antiviral agents and for other therapeutic purposes.[1,2] Therefore, several methods for the synthesis of the 2′-*O*-alkyl ribonucleoside have been intensely investigated due to the importance of these monomers in this field. However, introduction of an alkyl group at the 2′-hydroxyl group has been a challenging issue for nucleoside chemists since the 3′- and

*Correspondence: Tokumi Maruyama, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 7708514, Japan.

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5′-hydoroxyl groups should be protected prior to alkylation.[3] An attempted direct reaction of adenosine with phenyldiazomethane gave 2′-*O*-benzyladenosine in only 27% yield.[4] Also, Moffatt et al. reported the preparation of the 2′- and 3′-*O*-monoalkylated ribonucleosides using 2′,3′-*O*-(dibutylstannylene)-nucleosides as an intermediate.[5] An efficient protecting group for the specific reaction onto the 2′-hydroxyl group was established by 3′,5′-*O*-(tetra-isopropyldisiloxane-1,3-diyl) (TIPS) ribonucleosides.[6] In a large scale, however, it is difficult to use this protecting group because of the cost for reagent. Recently, direct 2′-*O*-alkylation of the protected cytidine was achieved with alkyl halide in the presence of 1.5–2.5 equivalents of silver oxide.[7] A drawback to this method is the use of silver which is thought to be a causative substance of pollution, and there has been no report regarding purine nucleosides.

The Mitsunobu reaction is a universal method to condense the acid and alcohol accompanied with inversion of the configuration of the alcoholic hydroxyl group.[8] One exceptional case is a sterically hindered sugar, 1,2:5,6-di-*O*-isopropylidene-D-*gluco*-furanose,[9] in which resistance to S_N2 displacement was reported. Also, intramolecular ethereal bond formation was reported in the trans-diol sugar.[8] Wentwarth and Janda reported the displacement of arabinoside to 2′-*O*-benzylated riboside using the Mitsunobu reaction with benzyl alcohol.[10] This method could be an alternative approach to obtain the 2′-*O*-alkylated ribonucleoside (Chart 1). However, it takes several steps to prepare arabinoside.

This background prompted us to develop a method to introduce an alkyl group onto the purine ribonucleoside. In this report the Mitsunobu reaction of the 6-chloropurine 3′-*O*-benzylriboside with benzyl alcohol is described.

6-Chloropurine 3′-*O*-benzoylriboside (**1a**) and its 5′-*O*-trityl congener (**1b**) were prepared by the method as described in an earlier report.[11] Then, compound **1b** was subjected to the reaction with benzyl alcohol (4 eq.) in the presence of *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide [TMAD], 1,1′-azobis-(*N*,*N*-dimethylformamide)[12] and triphenylphosphine (TPP) in a solvent. The reaction was monitored by high-performance liquid chromatography (HPLC), and the results are presented in Table 1. In an aprotic polar solvent such as *N*,*N*-dimethylformamide (DMF), the peak of the 2′-*O*-benzyl congener (**2b**) appeared in low yield. Also, the reaction in tetrahydrofuran (THF) gave **2b** in 14% yield. In spite of this result, a similar reaction performed in 1,4-dioxane gave **2b** in 45% yield. This discrepancy is interesting since the effects of both solvents are thought to be similar. The best result was obtained when the reaction was carried out in benzene, in which conversion was estimated to be 62.5% yield (Table 1). After work-up of the solution, **2b** was obtained in 57% yield.

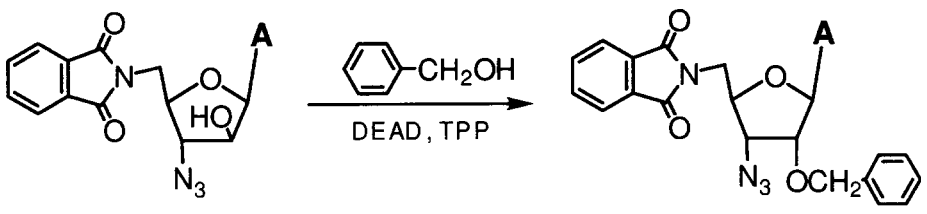

Chart 1.

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Table 1. The Mitsunobu reaction of **1b** with benzyl alcohol (10 eq.) in solvent.

Mitsunobu reagent	Reagent	Solvent	**1b** remain (%)	**2b** yields (%)
4 eq TMAD	4 eq TPP	THF	0.6	14.4
4 eq TMAD	4 eq TPP	DMF	0.3	4.6
4 eq TMAD	4 eq TPP	1,4-dioxane	2.9	45.1
4 eq TMAD	4 eq TPP	Benzene	0.5	62.5
4 eq TMAD	4 eq TPP	Benzene : hexane (1 : 2)	1.3	59.7
4 eq DIAD	4 eq TPP	1,4-dioxane	43.7	17.7
4 eq TMAD	TPP, $(NH_4)_2SO_4$	1,4-dioxane	1.0	45.0

An attempt to change TMAD to diisopropyl azodicarboxylate (DIAD) decreased the yield of **2b** to 17.7% in the 1,4-dioxane solvent system. To evaluate the role of the 5′-*O*-protecting group, 3′-*O*-benzoylriboside **1a** was subjected to a similar reaction and the results are presented in Table 2. It appeared that the 5′-*O*-protection did not benefit the condensation and, as a whole, the yields were even improved as demonstrated in the case of 1,4-dioxane. The reaction mechanism could be explained as follows. It is well known that 2′-OH is more acidic than 5′-OH. Therefore, the initial reaction of **1** with *N*-phosphonium salt gives an intermediary benzyloxyphosphonium ion, which receives a nucleophilic displacement of the 2′-alkoxide anion of **1a**,**b**. The other route that form an intermediary 2′-*O*-phosphinio-riboside is considered to be unfavourable since the β-site of this intermediate is tight, therefore, less accessible to nucleophilic displacement of benzyloxy anion. Compound **2b** was treated with NH_3 in MeOH to afford 2′-*O*-benzyladenosine (**3**),[13] which showed nuclear Overhauser effect (NOE) between H2′ and H3′ in the two-dimensional NOE (NOESY) spectrum as shown in Fig. 1. Thus, the configuration of **3** was identified as a 2′(*R*) -riboside structure. A part of the product was converted to the adenosine **4**, for which the measured data were identical with those of the authentic sample. To substantiate the efficacy of this method, compound **1b** was condensed with 3,4,5-trimethoxybenzyl alcohol to give 2′-*O*-(3,4,5-trimethoxybenzyl) derivative **5** in 56% yield. However, an attempt to condense with 2,4-dimethoxybenzyl alcohol

Table 2. The Mitsunobu reaction of **1a** with benzyl alcohol (10 eq.) in solvent.

Mitsunobu reagent	Reagent	Solvent	**1a** remain (%)	**2a** yields (%)
4 eq TMAD	4 eq TPP	THF	5.2	25.5
4 eq TMAD	4 eq TPP	DMF	7.5	0.4
4 eq TMAD	4 eq TPP	1,4-dioxane	4.7	61.0
4 eq TMAD	4 eq TPP	$CHCl_3$	0	61.6
4 eq TMAD	4 eq TPP	Benzene	0	60.9
4 eq TMAD	4 eq TPP	Benzene : 1,4-dioxane (1 : 1)	1.2	62.9
4 eq TMAD	4 eq TPP	Benzene : hexane (1 : 2)	3.6	55.8
4 eq TMAD	4 eq TBP	1,4-dioxane	3.9	62.6
4 eq DIAD	4 eq TPP	1,4-dioxane	54.7	26.6

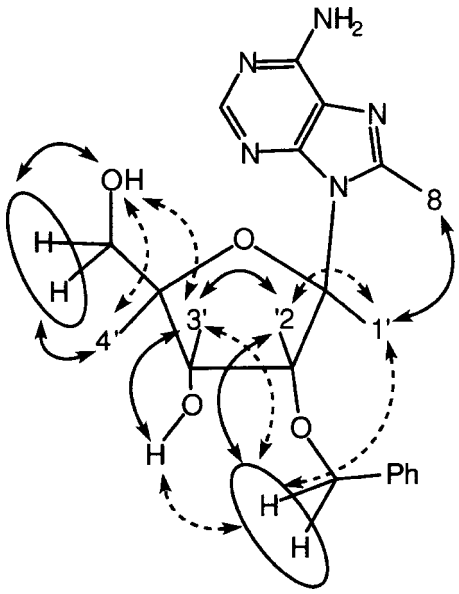

Figure 1. NOESY correlation of compound **3** in DMSO-d_6.

was unsuccessful, suggesting the methoxy group at the ortho-position disturbs the approach of the anion species (Chart 2).

In conclusion, a new method to introduce a benzyl group at 2′-OH of purine ribonucleoside using the Mitsunobu reaction was developed. The acidity of the 2′-OH group of **1** is increased by electron-withdrawing groups, 6-chloropurine and

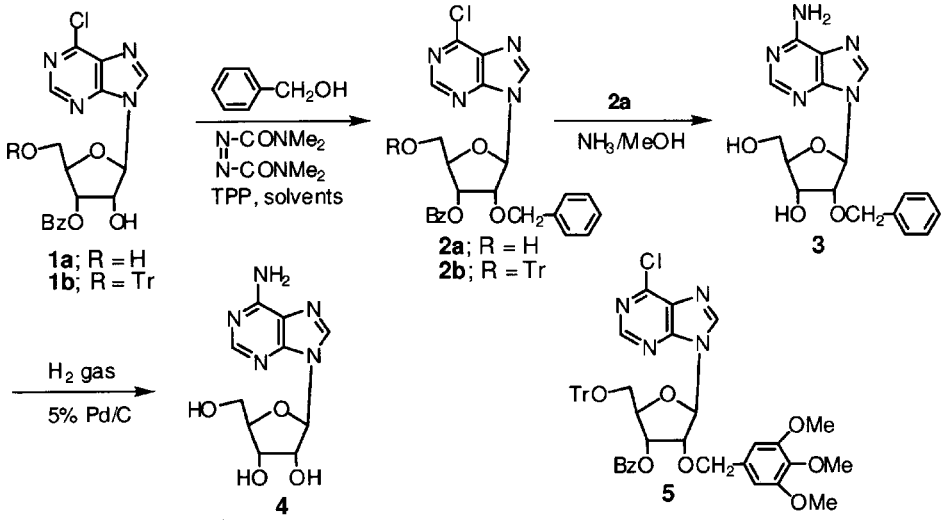

Chart 2.

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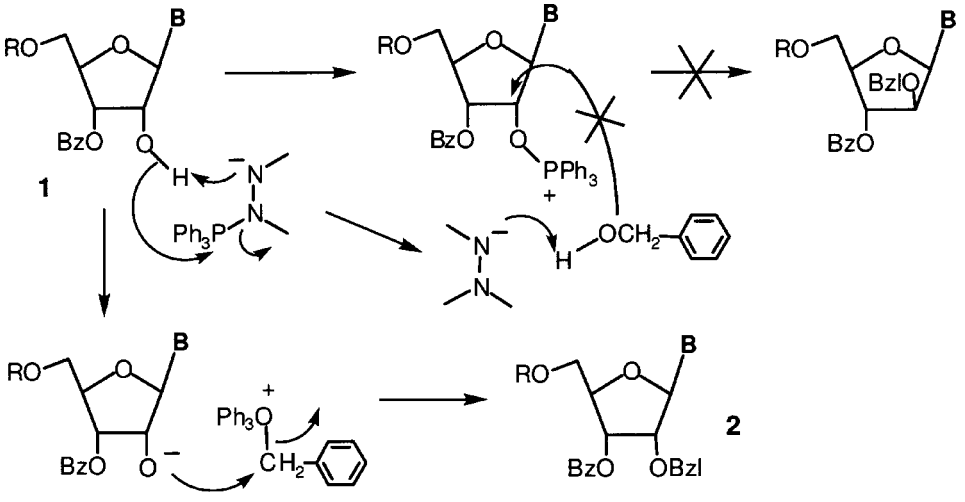
3′-*O*-benzyol groups. These groups also evolve steric hindrance in 2′-OH; therefore the stereochemistry of sugars is retained.[9] This method could be an alternative way to obtain the 2′-*O*-benzylnucleosides.

EXPERIMENTAL

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low-resolution mass spectra were obtained on a Shimadzu-LKB 9000B mass spectrometer in the direct-inlet mode. High-resolution mass spectra were obtained on a JMS AX-500 spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on either Varian UNITY 200 (200 MHz) or Varian UNITY 600 (600 MHz) in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with silica gel 60 containing fluorescent indicator F_{254} were used for thin-layer chromatography and silica gel 60 (Merck 7734, 60–200 mesh) was employed for column chromatography.

High-Performance Liquid Chromatography

The apparatus for high performance liquid chromatography (HPLC) were CCPD pump (Toso Co.) and SPD-M10A photo diode array UV-VIS detector (Shimadzu Co.). The HPLC conditions were as follows: the columns, connection with Cosmosil Guard Column 5C18-MS (4.6 × 10 mm, Nacalai Tesque INC.) and Cosmosil Packed Column 5C18-MS (4.6 × 150 mm, Nacalai Tesque Inc.); eluent, 10 mM phosphoric acid-MeOH (2 : 3) for **2a**, and (1 : 4) for **2b**; flow rate, 1 mL/min; column temperature, 50°C. The weight of compounds were calculated by the absolute calibration method from the peak areas, and the yields were converted to percentages.



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9-(3-*O*-Benzoyl-2-*O*-benzyl-β-D-ribofuranosyl)-6-chloropurine (2a). To a mixture of 6-chloro-9-(3-*O*-benzoyl-β-D-ribofuranosyl) purine (**1a**, 390 mg, 1 mmol) and benzyl alcohol (1.04 mL, 10 mmol) in 1,4-dioxane (25 mL) were added triphenylphosphine (1.05 g, 4 mmol) and TMAD (668 mg, 4 mmol). The solution was stirred at 50 °C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.2 × 34 cm) using 25–50% AcOEt in hexane (1.4 l) to give **12a** as white crystals (231 mg, 48%), mp 167.5–169.5°C. *Anal* Calcd for $C_{24}H_{21}ClN_4O_5$: C, 59.94; H, 4.40; N, 11.65. Found: C, 59.97; H, 4.49; N, 11.60. MS *m/z*: 450, 452 (M^+-CH_2O). UV λ_{max} (MeOH) nm: 265. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.55 (1H, s, H8), 8.15–8.16 (2H, m, two of Ph), 8.10 (1H, s, H2), 7.63–7.66 (1H, m, one of Ph), 7.51–7.54 (2H, m, two of Ph), 6.84–6.94 (5H, m, Ph), 5.91 (1H, d, J = 8.2 Hz, H1′), 5.90 (1H, d, J = 5.2 Hz, H3′), 5.55 (1H, br s, 5′OH), 4.89–4.91 (1H, m, H2′), 4.55–4.57 (2H, m, H4′ and one of CH_2), 4.29 (1H, d, J = 12.1 Hz, one of CH_2), 3.97–4.00 (1H, m, H5′a), 3.86–3.88 (1H, m, H5′b).

9-(3-*O*-Benzoyl-2-*O*-benzyl-5-*O*-trityl-β-D-ribofuranosyl)-6-chloropurine (2b). To a mixture of 6-chloro-9-(3-*O*-benzoyl-5-*O*-trityl-β-D-ribofuranosyl)purine (**1b**, 633 mg, 1 mmol) and benzyl alcohol (1.04 mL, 10 mmol) in 1,4-dioxane (25 mL) were added triphenylphosphine (1.05 mg, 4 mmol) and TMAD (688 mg, 4 mmol). The solution was stirred at 50°C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.5 × 45 cm) using 20–25% AcOEt in hexane (1.4 l) to give a caramel (411 mg, 57%). MS *m/z*: 479, 481 (M^+-Tr). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.10, 8.51 (each 1H, s, H2, H8), 8.11–8.13 (2H, m, two of C_6H_5CO), 7.62–7.65 (1H, m, one of C_6H_5CO), 7.48–7.52 (2H, m, two of C_6H_5CO), 7.22–7.44 (ca 15H, m, Tr), 6.95–7.11 (5H, m, Ph), 6.14 (1H, d, J = 6.6 Hz, H1′), 5.75 (1H, dd, J = 3.0, 5.2 Hz, H3′), 5.04 (1H, dd, J = 5.5, 6.6 Hz, H2′), 4.63 (1H, d, J = 12.4 Hz, one of $C\underline{H}_2C_6H_5$), 4.54–4.56 (1H, m, H4′), 4.45 (1H, d, J = 12.4 Hz, one of $C\underline{H}_2C_6H_5$), 3.56 (1H, dd, J = 3.8, 10.7 Hz, H5′a), 3.49 (1H, dd, J = 4.1, 10.4 Hz, H5′b).

2′-*O*-Benzyladenosine (3). A solution of **12a** (250 mg, 0.52 mmol) in MeOH (10 mL) saturated with ammonia at 0°C was heated in a steel bomb at 120°C overnight, then ice-cooled. The solution was concentrated to a small volume and chromatographed over a column of silica gel G (2.7 × 33 cm) using a 0–33% EtOH in $CHCl_3$. Evaporation of the fraction and crystallization from CCl_4 and $CHCl_3$ gave white crystals (173 mg, 93%). mp 150–151.5°C. (lit.[4] 147–150°C). *Anal* Calcd for $C_{17}H_{19}N_5O_4$ 0.7 H_2O: C, 55.19; H, 5.56; N, 18.93. Found: C, 55.09; H, 5.65; N, 18.94. MS *m/z*: 327 (M^+-CH_2O). UV λ_{max} (MeOH) nm: 261. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 8.33 (1H, s, H8), 8.09 (1H, s, H2), 7.35 (2H, br s, NH_2), 7.15–7.22 (5H, m, Ph), 6.07 (1H, d, J = 6.3 Hz, H1′), 5.46 (1H, dd, J = 4.4, 7.4 Hz, 5′OH), 5.35 (1H, d, J = 5.2 Hz, 3′OH), 4.57 (2H, dd, J = 12.1, 12.5 Hz, CH_2), 4.55 (1H, dd, J = 4.9, 6.3 Hz, H2′), 4.37 (1H, dt, J = 3.0, 4.9 Hz, H3′), 4.03–4.04 (1H, m, H4′), 3.67–3.69 (1H, m, H5′a), 3.55–3.59 (1H, m, H5′b).

Adenosine (4). A suspension of **13** (60 mg, 0.17 mmol) and palladium on activated carbon (10%, 60 mg) in EtOH (20 mL) was stirred vigorously under H_2

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atmosphere at 50°C overnight. The catalyst was removed and the filtrate was concentrated to a small volume to give white crystals (45 mg, quantitative), which were identical in all respects with an authentic sample. MS m/z: 267 (M^+). UV λ_{max} (MeOH) nm:260.5. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 8.41 (1H, s, H8), 8.20 (1H, s, H2), 5.90 (1H, d, $J = 5.9$ Hz, H1′), 4.60 (1H, t, $J = 5.1$ Hz, H2′), 4.16 (1H, dd, $J = 3.3$, 7.0 Hz, H3′), 3.99–4.00 (1H, m, H4′), 3.53–3.72 (1H, m, H5′).

6-Chloro-9-[2-*O*-(3,4,5-trimethoxy)benzyl-3-*O*-benzoyl-5-*O*-trityl-β-D-ribofuranosyl]purine (5). To a mixture of 6-chloro-9-(3-*O*-benzoyl-5-*O*-trityl-β-D-ribofuranosyl) purine (1.89 g, 3 mmol) and 2,3,4-trimethoxybenzyl alcohol (4.8 mL, 30 mmol) in benzene (75 mL) were added triphenylphosphine (3.15 g, 12 mmol) and TMAD (2.06 g, 12 mmol). The solution was stirred at 50°C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G to give a caramel (1.36 g, 56%). FAB-MS m/z: 835 ($M^+ + Na - H$). UV λ_{max} (MeOH) nm: 265. ^{1}H-NMR (600 MHz, $CDCl_3$)δ: 8.61 (1H, s, H8), 8.25 (1H, s, H2), 7.22–8.13 (ca 22H, m, Tr, Bz, Ph), 6.26 (1H, d, $J = 6.0$ Hz, H1′), 5.79 (1H, dd, $J = 3.3$, 5.2 Hz, H3′), 5.15 (1H, dd, $J = 5.5$, 6.0 Hz, H2′), 4.45–4.59 (3H, m, H4′, CH_2), 3.87 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.52–3.59 (2H, m, H5′).

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