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Introduction of a Benzoyl Group onto Riboside in Aqueous Solution: One-Step Synthesis of 6-Chloropurine 2',3'-di-*O*-benzoylriboside

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Abstract: A benzoyl group was introduced onto the 3'-hydroxyl group of 6-chloropurine riboside by treatment with benzoylating agents in the presence of an organic or inorganic base in aqueous solution, in which further reaction gave 6-chloropurine 2',3'-di-*O*-benzoylriboside. © 1999 Elsevier Science Ltd. All rights reserved.

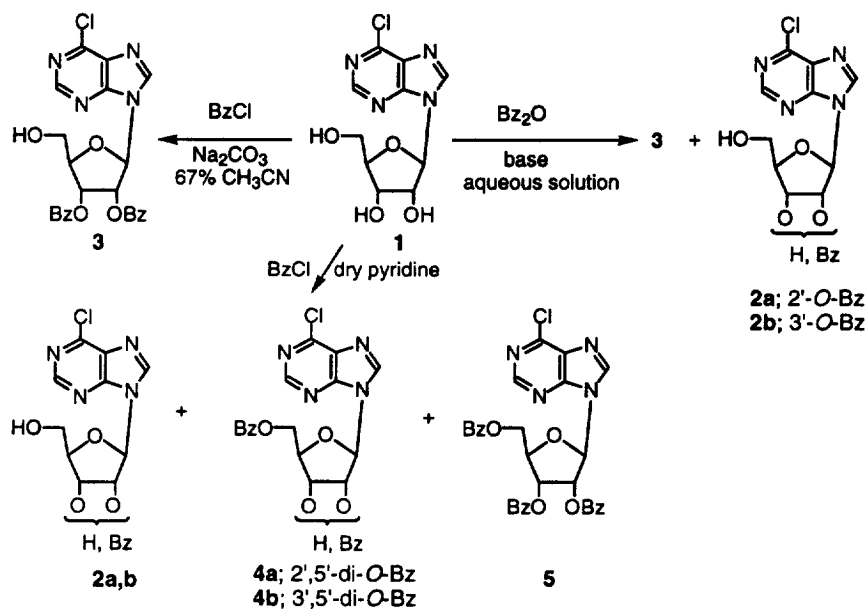
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A convenient method to prepare the 2',3',5'-tri-*O*-acyl analog is to react the ribonucleoside with acid anhydride or chloride in pyridine.¹ However, selective introduction of a benzoyl group to one hydroxyl group appeared difficult. One exception was benzoylation of the 2',3'-di-*O*-*n*-butylstannylene complex, which is an intermediate to prepare a mixture of 2'- and/or 3'-*O*-benzoyl riboside.² The drawback to this method is the use of stannum which is thought to be a causative substance of pollution. That prompted us to develop a new pollution-free method to obtain the 3'-*O*-benzoate. In the case of 2',3'-cis diol, protection with acetal was popular.¹ Also, 2',3'-di-*O*-acyl protection has been employed for acid labile substances. However, preparation of 2',3'-di-*O*-acylnucleosides involves a 3-step process.³ Although Singh *et al.* recently reported an enzymatic 5' selective deacylation of 2',3',5'-tri-*O*-acylriboside to give the 2',3'-di-*O*-acylnucleosides,⁴ the disadvantage of this method is the cost involved in large-scale synthesis. That also prompted us to prepare the 2',3'-di-*O*-benzoate directly from the corresponding riboside.

In this report we describe the benzoylation of 6-chloropurine riboside **1** in aqueous CH₃CN solution in the presence of a base to form 2'- and/or 3'-*O*-benzoylate **2a,b** and 2',3'-di-*O*-benzoate **3**.

6-Chloropurine riboside **1**⁵ was treated with 1.5 equivalents of benzoic anhydride in the presence of an organic or inorganic base in 50-67% aqueous solution of CH₃CN, and a part of the solution was immediately analyzed on HPLC. The conversion yields are highly dependent on the base used as shown in Table 1. It became clear that benzoylation did not proceed in the presence of pyridine in aqueous solution. However, when 1.5 eq of tri-*n*-butylamine was employed as a base, the monobenzoates **2a,b** were observed on HPLC in 59% conversion yields. The structures of **2a,b** were determined as a mixture of the 2'- **2a** and 3'-*O*-benzoate

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Scheme 1

2b by comparison with authentic samples.⁷ Exchange of tri-*n*-butylamine to triethylamine improved the yield up to 69%. A secondary amine was also effective as a base and gave the best yield when diisopropylamine was used. Next, an inorganic base was explored under similar conditions. A strong alkali such as NaOH or Na₂CO₃ effected the reaction. However, no benzoate was observed on HPLC when sodium acetate was selected as a base. These data explain the importance of the basicity of amines. Since the acidity of 2'-OH of ribonucleoside has been reported,⁸ removal of the proton from the 2'-OH could be the initial step in the benzylation. It is supposed that pyridine and sodium acetate are too weak to remove this proton. It is noteworthy that Na₂HPO₄ buffer (pH 8) is effective as a base. Di-*O*-benzoate was also observed in this reaction as a minor product on HPLC. This product was separated by silica gel chromatography and crystallized from MeOH to give **3** as white crystals.⁹ The ¹H-NMR spectrum of **3** revealed that the signals of both H2' and H3' appeared relatively high-field (6.36-6.39, 6.07 ppm) and a proton exchangeable with deuterium oxide was identified as 5'-OH, indicating that the benzoyl groups were introduced at the 2',3'*cis*-diol of the ribose. This evidence suggests that the 2',3'-di-*O*-benzoate could be obtainable directly from ribonucleoside. Thus, **1** (5.0 mmol) was treated with benzoyl chloride (6 eq) in the presence of Na₂CO₃ (12 eq) in 67% CH₃CN (150 ml) and the solution was treated as mentioned above to give **3** as white crystals in 81% yield. Since hydrolysis of the tri-*O*-benzoate **5** was not observed under the reaction conditions, the mechanism to form **3** could be explained as a preferential acylation at the 2',3'-*cis* diol of **1** in aqueous solution. The present method is most convenient and practical for large-scale synthesis of the 2',3'-di-*O*-benzoate. Also, **1** was subjected to benzylation under anhydrous conditions. Thus, **1** (2 mmol) was reacted with benzoyl chloride (1.7 eq) in dry pyridine (12 ml) at 0°C for 15 min, and after work-up and separation, three fractions were obtained. The first fraction was determined as the tri-*O*-benzoate **5**¹⁰ (9%) and the third fraction was a mixture of the monobenzoate **2a,b**⁷ (20%). The second fraction obtained in 42% yield was proved to be a mixture of the 2',5'-di-*O*-benzoate

Table 1. Reaction of 6-Chloropurine Riboside **1** (143 mg, 0.5 mmol) with Bz₂O in the Presence of a Base in Aqueous Solution of CH₃CN

| Run | Solv. (30 ml) | Amine (1.5 eq) | Bz ₂ O/eq. | Temp. | 3'Bz/% | 2'Bz/% | 2'+3'/% | 2',3'Bz/% |
|-----|----------------------------|--|-----------------------|-------|--------|--------|---------|-----------|
| 1 | 50% CH ₃ CN aq. | Bu ₃ N | 1.0 | r. t. | 37.9 | 19.9 | 57.7 | 5.7 |
| 2 | 67% CH ₃ CN aq. | Bu ₃ N | 1.5 | r. t. | 39.1 | 20.2 | 59.3 | 5.0 |
| 3 | 67% CH ₃ CN aq. | Py. | 1.5 | r. t. | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | 67% CH ₃ CN aq. | Et ₃ N | 1.5 | r. t. | 45.1 | 23.5 | 68.5 | 9.5 |
| 5 | 67% CH ₃ CN aq. | (iPr) ₂ NH | 1.5 | r. t. | 46.4 | 27.4 | 71.1 | 9.9 |
| 6 | 67% CH ₃ CN aq. | NMM | 1.5 | r. t. | 7.1 | 4.3 | 11.4 | 0.1 |
| 7 | 67% CH ₃ CN aq. | NH ₃ | 1.5 | r. t. | 0.3 | 0.1 | 0.4 | 0.0 |
| 8 | 67% CH ₃ CN aq. | NaOH | 1.5 | r. t. | 30.1 | 18.0 | 48.1 | 11.1 |
| 9 | 67% CH ₃ CN aq. | Na ₂ CO ₃ (1.5) | 1.5 | r. t. | 27.2 | 12.4 | 39.6 | 6.1 |
| 10 | 67% CH ₃ CN aq. | Na ₂ CO ₃ (3.0) | 1.5 | r. t. | 43.0 | 24.9 | 67.9 | 20.5 |
| 11 | 50% CH ₃ CN aq. | AcONa | 1.5 | r. t. | 1.8 | 0.0 | 1.8 | 0.0 |
| 12 | 67% CH ₃ CN aq. | Na ₂ HPO ₄ buffer* | 1.5 | r. t. | 39.5 | 22.1 | 61.6 | 8.2 |

* pH=8, volume 5 ml.

The peak areas of the sample solutions and the standard solutions were given by HPLC injected a part of volume, and the weight of benzoates was calculated by the absolute calibration method from the peak areas, and converted to the yield percentages. See detail in reference 6.

Table 2. Proton NMR Spectrum (600 MHz) for **3** and the Mixture of the Di-*O*-benzoate **4a,b** in CDCl₃

| Compds | | H1' | H2' | H3' | H4' | H5'a | H5'b | 2', 3' or 5'OH |
|---------------------------------|-----|-----------|-----------|-----------|-----------|-----------------|-----------|----------------|
| 2',3'-Bz (3) | ppm | 6.36-6.39 | | 6.07 | 4.65 | 4.10-4.13 | | 5.29(5'OH) |
| | | m | | dt | m | m | | dd |
| <i>J</i> (Hz) | | | | 3.3, 1.6 | | 12.9, 10.7, 1.6 | | 10.7, 2.7 |
| 2',5'-Bz (30%) (4a) | ppm | 6.33 | 6.08 | 5.16-5.19 | 4.53-4.55 | 4.80 | 4.67 | 2.91(3'OH) |
| | | d | dd | m | m | q | dd | d |
| <i>J</i> (Hz) | | 3.6 | 5.5, 3.8 | | | 3.6 | 12.4, 4.1 | 5.2 |
| 3',5'-Bz (70%) (4b) | ppm | 6.14 | 5.27-5.30 | 5.84 | 4.78-4.80 | 4.86 | 4.61 | 3.95(2'OH) |
| | | d | m | dd | m | dd | dd | d |
| <i>J</i> (Hz) | | 5.8 | | 5.5, 3.3 | | 12.1, 3.6 | 12.1, 3.8 | 4.9 |

4a and its 3',5'-congener **4b** in the ratio 3:7 from ¹H-NMR spectrum as shown in Table 2. The discrepancy in the di-*O*-benzoate formation between that in aqueous solution and in dry pyridine is under investigation.

As a conclusion, we have developed a method to obtain the 3'-*O*-benzoate **2b** without stannum in aqueous solution. This method was expanded to a practical preparation of the 2',3'-di-*O*-benzoate **3**. We are looking forward to achieving regioselective acylation of various ribonucleosides for the synthesis of the 2',3'-di-*O*-benzoates, an important protected nucleoside for the synthesis of 5'-nucleotides or 5'-modified nucleosides.

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6. High-performance liquid chromatography: Apparatus of 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 the mono- and di-*O*-benzoate mixture; flow rate, 1 ml /min; column temperature, 50°C. Standard solutions were prepared as follows: 3'-*O*-benzoate 11.73 mg was dissolved in DMSO (50 ml) and 2',3'-di-*O*-benzoate (3.90 mg) was dissolved in DMSO (20 ml).
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9. Selected data for **3**: mp 129 - 131°C. (Found: C; 57.83, H; 3.81, N; 11.31. C₂₄H₁₉ClN₄O₆ requires C; 58.25, H; 3.87, N; 11.32%); MS *m/z* 464, 466 (M⁺-CH₂O), 372, 374 (M⁺-benzoic acid), 341 (M⁺-6-chloropurine), 154, 156 (6-chloropurine), λ_{max} (MeOH)/nm 264.
10. Selected data for **5**: MS *m/z* 445 (M⁺- 6-chloropurine), 154, 156 (6-chloropurine), λ_{max} (MeOH)/nm 264; ¹H-NMR (600 MHz, CDCl₃): δ 8.61, 8.28 (each 1H, s, 2-H, 8-H), 7.26-8.09 (ca 15H, m, 3×C₆H₃CO), 6.45 (1H, d, *J* 4.9, 1'-H), 6.42 (1H, dd, *J* 5.5, 4.9, 2'-H), 6.25 (1H, dd, *J* 5.5, 5.2, 3'-H), 4.94 (1H, dd, *J* 12.4, 3.3, 5'a-H), 4.85-4.87 (1H, m, 4'-H), 4.70 (1H, dd, *J* 12.4, 4.1, 5'b-H).