Video Article

# Regioselective *O*-Glycosylation of Nucleosides *via* the Temporary 2',3'-Diol Protection by a Boronic Ester for the Synthesis of Disaccharide Nucleosides

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## Abstract

Disaccharide nucleosides, which consist of disaccharide and nucleobase moieties, have been known as a valuable group of natural products having multifarious bioactivities. Although chemical *O*-glycosylation is a commonly beneficial strategy to synthesize disaccharide nucleosides, the preparation of substrates such as glycosyl donors and acceptors requires tedious protecting group manipulations and a purification at each synthetic step. Meanwhile, several research groups have reported that boronic and borinic esters serve as a protecting or activating group of carbohydrate derivatives to achieve the regio- and/or stereoselective acylation, alkylation, silylation, and glycosylation. In this article, we demonstrate the procedure for the regioselective *O*-glycosylation of unprotected ribonucleosides utilizing boronic acid. The esterification of 2',3'-diol of ribonucleosides with boronic acid makes the temporary protection of diol, and, following *O*-glycosylation with a glycosyl donor in the presence of *p*-toluenesulfenyl chloride and silver triflate, permits the regioselective reaction of the 5'-hydroxyl group to afford the disaccharide nucleosides. This method could be applied to various nucleosides, such as guanosine, adenosine, cytidine, uridine, 5-metyluridine, and 5-fluorouridine. This article and the accompanying video represent useful (visual) information for the *O*-glycosylation of unprotected nucleosides and their analogs for the synthesis of not only disaccharide nucleosides, but also a variety of biologically relevant derivatives.

## Video Link

The video component of this article can be found at https://www.jove.com/video/57897/

## Introduction

Disaccharide nucleosides, which are conjugates of a nucleoside and a carbohydrate moiety linked *via* an *O*-glycosidic bond, constitute a valuable class of naturally-occurring carbohydrate derivatives[1,2,3,4,5,6,7]. For instance, they are incorporated in biological macromolecules such as tRNA (transfer ribonucleic acid) and poly(ADP-ribose) (ADP = adenosine diphosphate), as well as in some antibacterial agents and other biologically-active substances (*e.g.,* adenophostins, amicetins, ezomycin)[5,6,8,9,10,11,12,13,14,15,16,17,18,19]. Hence, disaccharide nucleosides and their derivatives are expected to be lead compounds for drug discovery research. The methodologies for the synthesis of disaccharide nucleosides are classified into three categories; enzymatic *O*-glycosylation[20,21], chemical *N*-glycosylation[5,9,16,22,23,24], and chemical *O*-glycosylation[7,9,14,16,18,19,24,25,26,27,28,29,30,31,32,33,34,35,36,37]. In particular, chemical *O*-glycosylation would be an efficient method for the stereoselective synthesis and large-scale synthesis of disaccharide nucleosides. Previous research has shown that the *O*-glycosylation of 2'-deoxyribonucleoside **2** with the thioglycosyl donor **1**, using the combination of *p*-toluenesulfenyl chloride and silver triflate, affords the desired disaccharide nucleoside **3** (**Figure 1A**; Ar = aryl and PG = protecting group)[38].

Following these results, we decided to develop the *O*-glycosylation of ribonucleosides applying the *p*-toluenesulfenyl chloride/silver triflate promoter system. While several examples of the *O*-glycosylation of partially protected ribonucleosides have been demonstrated[7,9,14,16,18,19,24,32,33,34,35,36,37], the use of unprotected or temporarily-protected ribonucleosides as a glycosyl acceptor for *O*-glycosylation has been negligibly reported. Therefore, the development of regioselective *O*-glycosylation of unprotected or temporarily-protected ribonucleosides would provide a more beneficial synthetic method without protecting group manipulations of ribonucleosides. In order to achieve the regioselective *O*-glycosylation of ribonucleosides, we focused on the boron compounds, because several examples of regio- and/or stereoselective acylation, alkylation, silylation, and glycosylation of carbohydrate derivatives assisted by boronic or borinic acid have been reported[39,40,41,42,43,44,45,46,47,48,49,50]. In this article, we demonstrate the procedure for the synthesis of disaccharide nucleosides utilizing regioselective *O*-glycosylation at the 5'-hydroxyl group of ribonucleosides *via* a boronic ester intermediate. In the strategy presented here, boronic ester intermediate **6** would be afforded by the esterification of the ribonucleoside **4** with the boronic acid **5**, which allows the regioselective *O*-glycosylation at the 5'-hydroxyl group with thioglycosyl donor **7** to give the disaccharide nucleoside **8** (**Figure 1B**)[51]. We also studied the interaction of a ribonucleoside and boronic acid by nuclear magnetic resonance (NMR) spectroscopy, to observe the formation of a boronic ester. Esterification to make a boronic ester and a glycosylation reaction require anhydrous conditions to prevent the hydrolysis of the

boronic ester and the glycosyl donor. In this article, we demonstrate the typical procedures to obtain the anhydrous conditions for successful glycosylation reactions for researchers and students not only in chemistry but also in other research fields.

## Protocol

NOTE: All experimental data [NMR, infrared spectroscopies (IR), mass spectroscopies (MS), optical rotations, and elemental analyses data] of the synthesized compounds were reported in a previous paper[51].

# 1. Procedure for *O*-Glycosylation Reactions

1. **Synthesis of compound α/β-12 (Entry 12 in Table 1)**
   NOTE: Entries 1 - 13 in **Table 1** were carried out using a similar procedure.
   1. **Temporary protection of 2',3'-diol of ribonucleoside**[40]
      1. In a 10 mL pear-shaped flask (flask 1), dissolve mannosyl donor α-**9** (28.4 mg, 0.0486 mmol)[52], uridine **10** (7.9 mg, 0.0324 mmol) and 4-(trifluoromethyl)phenylboronic acid **11c** (9.3 mg, 0.0490 mmol) in anhydrous pyridine (0.40 mL).
         NOTE: The use of a 10 mL pear-shaped flask is recommended because, in step 1.1.3.1, the reaction mixture will be transferred to flask 2 (a 10 mL two-neck round-bottom flask with a septum attached to it) containing molecular sieves powder.
      2. Co-evaporate the reaction mixture (obtained in step 1.1.1.1.) with anhydrous pyridine (0.40 mL, 3x) and anhydrous 1,4-dioxane (0.40 mL, 3x) at room temperature to *ca.* 40 °C to remove any water.
      3. Dissolve the residue (obtained in step 1.1.1.2.) in anhydrous 1,4-dioxane (0.32 mL) and stir the reaction mixture at its reflux temperature for 1 h to form a boronic ester (the temporary protection).
      4. Remove the solvent using a rotary evaporator followed by a vacuum pump.

   2. **Activation of molecular sieves**
      1. In a 10 mL two-neck round-bottom flask with a septum attached to it (flask 2), add 4 Å molecular sieves powder (64 mg).
         NOTE: Appropriate molecular sieves should be selected according to the solvent used for the glycosylation (3 Å for acetonitrile and 4 Å for 1,4-dioxane, dichloromethane, and propionitrile).
      2. Heat the molecular sieves in a microwave under atmospheric pressure and cool them under reduced pressure evacuated by a vacuum pump (3x), and then dry them with a heat gun under reduced pressure while replacing the air with argon gas several times.

   3. **Glycosylation**
      1. Dissolve the residue of step 1.1.1.4. in flask 1 in propionitrile (0.64 mL) or other solvents and transfer this solution to flask 2.
         NOTE: Acetonitrile, 1,4-dioxane, dichloromethane, and propionitrile were used for Entries 1 - 7 and 9, Entry 10, Entry 11, and Entries 8, 12, and 13, respectively.
      2. Stir the reaction mixture in flask 2 at room temperature for 0.5 h followed by cooling it to -40 °C.
         NOTE: The temperature was changed according to the solvent used for the glycosylation (-40 °C for dichloromethane and propionitrile, room temperature for 1,4-dioxane, and -20 °C for acetonitrile).
      3. Add silver triflate (49.9 mg, 0.194 mmol) and *p*-toluenesulfenyl chloride (12.8 µL, 0.0968 mmol) to the reaction mixture at the same temperature as used in step 1.1.3.2.
      4. Stir the reaction mixture at the same temperature for 1.5 h.
      5. Check the reaction by thin-layer chromatography (TLC) with hexane/ethyl acetate [3/1 (v/v)] to check the glycosyl donors [the retention factor ($R_f$) (donor α-**9**) = 0.63] and with chloroform/methanol [10/1 (v/v))] to check the glycosyl acceptors and products [$R_f$ (acceptor **10**) = 0.03, $R_f$ (desired product) = 0.50].
      6. Quench the reaction mixture with saturated aqueous sodium bicarbonate (1.0 mL), dilute it with chloroform (2.0 mL), remove the insoluble materials with *Celite*, and carefully wash the Celite with chloroform (20 mL).
      7. Wash the filtrate (organic layer) with saturated aqueous sodium bicarbonate (20 mL, 3x) and brine (20 mL) using a 100 mL separatory funnel.
      8. Dry the resulting organic layer with sodium sulfate, filter the insoluble materials, and concentrate the filtrate using a rotary evaporator.
      9. Roughly purify the remaining residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 50/1 (v/v)] to afford crude 5'-*O*-(6"-*O*-acetyl-2",3",4"-tri-*O*-benzyl-α/β-D-mannopyranosyl)uridine containing a small quantity of byproducts (15.2 mg, colorless syrup).

   4. **Acetylation**
      1. In a 5 mL vial, dissolve the resulting crude compound prepared in step 1.1.3.9 in anhydrous pyridine (0.20 mL).
      2. Add *N*,*N*-dimethyl-4-aminopyridine (a catalytic amount) and acetic anhydride (20.4 µL, 0.0216 mmol: 10 equivalents based on the crude compound) to the solution at 0 °C.
      3. Stir the reaction mixture at the same temperature for 0.5 h followed by a warming to room temperature.
      4. After stirring overnight, check the reaction by TLC with chloroform/methanol [30/1 (v/v)] [$R_f$ (α/β-**12**) = 0.45].
      5. Dilute the reaction mixture with chloroform (20 mL).
      6. Wash the organic layer with 1 M hydrochloric acid (20 mL, 3x), saturated aqueous sodium bicarbonate (20 mL, 3x), and brine (20 mL) using a 100 mL separatory funnel.
      7. Dry the resulting organic layer with sodium sulfate, filter the insoluble materials, and concentrate the filtrate using a rotary evaporator.
      8. Purify the remaining residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 90/1 (v/v)] to give α/β-**12** (15.8 mg, 61%, α/β = 1.6/1, colorless amorphous solid).

2. **Synthesis of compounds β-22 to β-30 (Table 2) and β-33 (Table 3)**
NOTE: The synthesis of β-**22** - β-**30** and β-**33** was carried out using a similar procedure.
    1. **Synthesis of compound β-22 (Entry 1 in Table 2)**
        1. **Temporary protection of 2', 3'-diol of ribonucleoside**
            1. In a 10 mL pear-shaped flask (flask 3), dissolve adenosine **13** (20.4 mg, 0.0763 mmol), galactosyl donor β-**21** (80.4 mg, 0.114 mmol)[53], and 4-(trifluoromethyl)phenylboronic acid **11c** (21.7 mg, 0.114 mmol) in anhydrous pyridine (0.76 mL).
NOTE: The use of a 10 mL pear-shaped flask is recommended because the reaction mixture will be transferred to flask 4 (a 10 mL two-neck round-bottom flask with a septum attached to it) containing molecular sieves powder in the step 1.2.1.3.1.
            2. Co-evaporate the reaction mixture (obtained in step 1.2.1.1.1.) with anhydrous pyridine (0.76 mL, 3x) and anhydrous 1,4-dioxane (0.76 mL, 3x) at room temperature to *ca.* 40 °C to remove any water.
            3. Dissolve the residue (obtained in step 1.2.1.1.2.) in anhydrous 1,4-dioxane (0.76 mL) and stir the reaction mixture at its reflux temperature for 1 h to form a boronic ester (a temporary protection).
            4. Remove the solvent using a rotary evaporator followed by a vacuum pump.

        2. **Activation of molecular sieves**
            1. In a 10 mL two-neck round-bottom flask with a septum attached to it (flask 4), add 4 Å molecular sieves powder (150 mg).
            2. Heat the molecular sieves in a microwave under atmospheric pressure and cool them under reduced pressure evacuated by a vacuum pump (3x), and then dry them with a heat gun under reduced pressure while replacing the air with argon gas several times.

        3. **Glycosylation**
            1. Dissolve the residue of step 1.2.1.1.4. in flask 3 in propionitrile (1.50 mL) and transfer this solution to flask 4.
            2. Stir the reaction mixture at room temperature for 0.5 h, followed by cooling it to -40 °C.
            3. Add silver triflate (117.6 mg, 0.458 mmol) and *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol) to the reaction mixture at the same temperature as mentioned in step 1.2.1.3.2.
            4. Stir the reaction mixture, at the same temperature for 1.5 h.
            5. Check the reaction by TLC with hexane/ethyl acetate [2/1 (v/v)] to check the glycosyl donors [$R_f$ (donor β-**21**) = 0.62] and with chloroform/methanol [10/1 (v/v)] to check the glycosyl acceptors and products [$R_f$ (acceptor **13**) = 0.05, $R_f$ (desired product) = 0.30].
            6. Quench the reaction mixture with saturated aqueous sodium bicarbonate (2.0 mL), dilute it with chloroform (3.0 mL), remove the insoluble materials through *Celite*, and carefully wash the *Celite* with chloroform (30 mL).
            7. Wash the filtrate (organic layer) with saturated aqueous sodium bicarbonate (30 mL, 3x) and brine (30 mL) using a 100 mL separatory funnel.
            8. Dry the resulting organic layer with sodium sulfate, filter the insoluble materials, and concentrate the filtrate using a rotary evaporator.
            9. Purify the remaining residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 30/1 (v/v)] to afford β-**22** (27.4 mg, 42%, colorless solid).

    2. **Synthesis of compound β-23 (Entry 2 in Table 2)**
        1. Conduct the reaction using **14** (28.4 mg, 0.0765 mmol)[54], β-**21** (80.5 mg, 0.115 mmol), **11c** (21.8 mg, 0.115 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.8 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 50/1 (v/v)] to give β-**23** (21.9 mg, 30%, colorless solid). TLC: $R_f$ (β-**23**) = 0.37 [chloroform/methanol = 10/1 (v/v)].

    3. **Synthesis of compound β-24 (Entry 3 in Table 2)**
        1. Conduct the reaction using **15** (21.6 mg, 0.0763 mmol), β-**21** (80.5 mg, 0.115 mmol), **11c** (21.8 mg, 0.115 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 8/1 (v/v)] to give β-**24** (8.1 mg, 12%, colorless solid). TLC: $R_f$ (β-**24**) = 0.20 [chloroform/methanol = 10/1 (v/v)].

    4. **Synthesis of compound β-25 (Entry 4 in Table 2)**
        1. Conduct the reaction using **16** (27.0 mg, 0.0764 mmol)[55], β-**21** (80.5 mg, 0.115 mmol), **11c** (21.8 mg, 0.115 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.8 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 20/1 (v/v)] to give β-**25** (31.4 mg, 44%, colorless solid). TLC: $R_f$ (β-**25**) = 0.27 [chloroform/methanol = 10/1 (v/v)].

    5. **Synthesis of compound β-26 (Entry 5 in Table 2)**
        1. Conduct the reaction using **10** (18.6 mg, 0.0762 mmol), β-**21** (80.4 mg, 0.114 mmol), **11c** (21.7 mg, 0.114 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 40/1 (v/v)] to give β-**26** (26.1 mg, 42%, colorless solid). TLC: $R_f$ (β-**26**) = 0.45 [chloroform/methanol = 10/1 (v/v)].

    6. **Synthesis of compound β-27 (Entry 6 in Table 2)**
        1. Conduct the reaction using **17** (19.7 mg, 0.0763 mmol), β-**21** (80.5 mg, 0.115 mmol), **11c** (21.8 mg, 0.115 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL),

anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 40/1 (v/v)] to give β-**27** (33.8 mg, 53%, colorless solid). TLC: $R_f$ (β-**27**) = 0.50 [chloroform/methanol = 10/1 (v/v)].

7. **Synthesis of compound β-28 (Entry 7 in Table 2)**
    1. Conduct the reaction using **18** (20.0 mg, 0.0763 mmol), β-**21** (80.4 mg, 0.114 mmol), **11c** (21.7 mg, 0.114 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform then ethyl acetate/chloroform = 1/1 (v/v)] to give β-**28** (38.8 mg, 61%, colorless solid). TLC: $R_f$ (β-**28**) = 0.33 [chloroform/methanol = 10/1 (v/v)].

8. **Synthesis of compound β-29 (Entry 8 in Table 2)**
    1. Conduct the reaction using **19** (18.5 mg, 0.0761 mmol), β-**21** (80.4 mg, 0.114 mmol), **11c** (21.7 mg, 0.114 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 10/1 (v/v)] to give β-**29** (34.1 mg, 55%, colorless solid). TLC: $R_f$ (β-**29**) = 0.25 [chloroform/methanol = 10/1 (v/v)].

9. **Synthesis of compound β-30 (Entry 9 in Table 2)**
    1. Conduct the reaction using **20** (26.6 mg, 0.0766 mmol)[56], β-**21** (80.6 mg, 0.115 mmol), **11c** (21.8 mg, 0.115 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.8 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 50/1 (v/v)] to give β-**30** (28.0 mg, 40%, colorless solid). TLC: $R_f$ (β-**30**) = 0.48 [chloroform/methanol = 10/1 (v/v)].

10. **Synthesis of compound β-33 (Entry 1 in Table 3)**
    1. Conduct the reaction using **18** (20.0 mg, 0.0762 mmol), β-**31** (80.4 mg, 0.114 mmol)[57], **11c** (21.7 mg, 0.114 mmol), *p*-toluenesulfenyl chloride (30.3 µL, 0.229 mmol), silver triflate (117.6 mg, 0.458 mmol), anhydrous 1,4-dioxane (0.76 mL), anhydrous propionitrile (1.50 mL), and 4 Å molecular sieves (150 mg). Purify the resulting residue by column chromatography [silica gel, chloroform/methanol = 1/0 - 30/1 (v/v)] to give β-**33** (34.5 mg, 54%, colorless solid). TLC: $R_f$ (β-**33**) = 0.33 [chloroform/methanol = 10/1 (v/v)].

## 2. Deprotection of β-28 (Figure 2)

1. In a 5 mL vial, add β-**28** (25.2 mg, 0.0300 mmol) and 10 M methylamine in methanol (2.0 mL)[58].
2. Stir the reaction mixture at 0 °C for 2 h followed by warming it to room temperature.
3. After stirring the mixture for 13 h, check the reaction by TLC with chloroform/methanol [10/1 (v/v)] [$R_f$ (β-**35**) = 0.20].
4. Concentrate the reaction mixture using a rotary evaporator.
5. Dissolve the resulting residue in water (15 mL) and wash the aqueous layer with dichloromethane (15 mL, 3x) using a 50 mL separatory funnel.
6. Concentrate the aqueous layer using a rotary evaporator.
7. Purify the remaining residue by preparative high-performance liquid chromatography (HPLC) [column: ODS (octadecylsilane) column (20$\Phi$ x 250 mm), eluent: water (contains 0.1% [v/v] trifluoroacetic acid), flow rate: 8.0 mL/min, detection: 266 nm, temperature: 25 °C, retention time: 20 min] to give β-**35** (7.9 mg, 62%, colorless amorphous solid)[59].

## 3. NMR Studies of Cyclic Boronic Ester (Figure 3 and 4)

1. **Preparation and measurement of 36**
    1. In 10 mL pear-shaped flask, dissolve uridine **10** (34.3 mg, 0.140 mmol) and 4-(trifluoromethyl)phenylboronic acid **11c** (40.0 mg, 0.211 mmol) in anhydrous pyridine (1.00 mL).
    2. Co-evaporate the reaction mixture with anhydrous pyridine (1.00 mL, 3x) and anhydrous 1,4-dioxane (1.00 mL, 3x) at room temperature to *ca.* 40 °C to remove any water.
    3. Dissolve the residue in anhydrous 1,4-dioxane (1.40 mL) and stir the reaction mixture at its reflux temperature for 1 h to form a boronic ester (a temporary protection).
    4. Dispense the reaction mixture (0.14 mL) to a 5 mL vial.
    5. Remove the solvent from the 5 mL vial using a rotary evaporator followed by a vacuum pump.
    6. Dissolve the resulting residue **36** in acetonitrile-$d_3$ (0.64 mL).
    7. Measure $^{1}$H, $^{11}$B and $^{19}$F NMR spectroscopies using a quartz NMR tube at 25 °C.

2. **Preparation and measurement of 38**
    1. Prepare the reaction mixture **38** from **11c** (40.0 mg, 0.211 mmol) using the similar procedure as that of the step 3.1.

## Representative Results

The results of the *O*-glycosylation of uridine **10** with thiomannoside α-**9** are summarized in **Table 1**[60,61]. In Entry 1, the *O*-glycosylation of **10** with α-**9** in the absence of boronic acid derivatives resulted in the formation of a complicated mixture. In Entry 2, **10** and phenylboronic acid **11a** were mixed and co-evaporated with pyridine and 1,4-dioxane and, then, stirred in 1,4-dioxane at its reflux temperature to form the temporary protection of 2',3'-*cis*-diol followed by an addition of α-**9** to conduct glycosylation.

In Entries 3 - 13, the *O*-glycosylations were carried out according to the protocol described here (step 1.1). The effect of substituents on the arylboronic acid was investigated in Entries 4 - 9. Electron-deficient arylboronic acids such as 4-(trifluoromethyl)phenylboronic acid **11c** and 2,4-difluorophenylboronic acid **11d** resulted in higher chemical yields of α/β-**12** than that of 4-methoxyphenylboronic acid **11b**, possibly due to the higher stability of boronic ester intermediate prepared from electron-deficient arylboronic acid[62]. However, the use of 4-nitrophenylboronic acid **11e**, which has also an electron-withdrawing group, resulted in a low chemical yield of α/β-**12** on account of the low solubility of boronic ester intermediate in acetonitrile. In Entry 8, the *O*-glycosylation using 4-hexylphenylboronic acid **11f** in propionitrile (to enhance the solubility of the boronic ester intermediate) did not improve the chemical yield. In Entry 9, alkylboronic acid (cyclopentylboronic acid **11g**) was used instead of arylboronic acid, which resulted in a lower chemical yield of α/β-**12** than in that of arylboronic acids.

The solvent effect for the chemical yield and stereoselectivity of the glycosylation product was studied in Entries 10 - 12. In Entry 10, the use of 1,4-dioxane as a solvent permitted a more α-stereoselective *O*-glycosylation than the use of acetonitrile did[63,64], while the yield of α/β-**12** was insufficient. In Entry 11, the *O*-glycosylation in dichloromethane gave a negligible amount of α/β-**12** because of the low solubility of the intermediate. In Entry 12, using propionitrile as the solvent resulted in a higher chemical yield of α/β-**12** than when using other solvents (Entries 5, 10, and 11) with nearly the same stereoselectivity compared with the use of acetonitrile (Entry 5). In Entry 13, the equivalents of *p*-toluenesulfenyl chloride and silver triflate were reduced to 1.8 and 3.6 against **10**, respectively (in Entries 1 - 12, 3.0 and 6.0 equivalents of *p*-toluenesulfenyl chloride and silver triflate were used against **10**, respectively) to afford α/β-**12** in the similar result.

In **Table 2**, the *O*-glycosylations of **10** and **13** - **20** with the thiogalactoside β-**21** were carried out under the optimized reaction conditions established in **Table 1** (Entry 12) (In this paper, adenine, guanine, cytosine, uracil, thymine, and 5-fluorouracil are abbreviated as Ade, Gua, Cyt, Ura, Thy, and 5-FUra, respectively, not as A, G, C, U, T, and 5-FU, which are their general abbriviations to avoid misunderstanding [for example, C-nucleoside generally means C(carbon)-glycosidic bonds]). In the case of adenosine, unprotected **13** afforded the corresponding disaccharide nucleoside in a higher yield than *N*-protected **14** could, possibly due to the depurination of **14** and/or β-**23** similar to our previous report (Entries 1 and 2)[38]. The *O*-glycosylation of *N*-protected guanosine **16** supplied β-**25** in a better yield compared with the glycosylation of unprotected **15** because of the higher solubility of the intermediate prepared from **16** than that from **15** (Entries 3 and 4). In Entries 5 - 7, the *O*-glycosylations of uridine **10** and analogs such as 5-metyluridine **17** and 5-fluorouridine **18** were examined. The use of **10** afforded the β-**26** (42% yield) with a side reaction to give a by-product in which the 5-position of the uracil moiety was substituted with a *p*-tolylthio group (Entry 5)[65]. On the other hand, **17** and **18**, in which the 5-position of the uracil moiety is a methyl or fluoro group, gave the corresponding disaccharide nucleosides β-**27** and β-**28** in moderate yields, respectively (Entries 6 and 7). Furthermore, a large-scale reaction using 250 mg of **18** (0.95 mmol) and 1.01 g of β-**21** (1.43 mmol) afforded β-**28** in a 58% yield (461.0 mg), which is almost the same yield as that of a small-scale reaction (61% in Entry 7 of **Table 2**). In the case of cytidine, the *O*-glycosylation of unprotected **19** gave β-**29** in a slightly better yield than the use of *N*-protected **20** resulting in β-**30** did.

Several glycosyl donors, such as glucosyl donor β-**31**, galactosyl donor β-**21**, and mannosyl donor α-**32,** were used in the *O*-glycosylation of 5-fluorouridine **18** (**Table 3**)[66]. The result of Entry 2 is the same as that of Entry 7 of **Table 2** in this manuscript. From these results, the use of galactosyl donor β-**21** afforded the corresponding product β-**28** in a high yield compared with the use of β-**31** and α-**32**. In Entry 3, the reaction using α-**32** gave a mixture of α-**34** with an unidentified byproduct, which possibly has a similar molecular weight as that of **34** (it is assumed that it could be a regio- or stereoisomer of **34**), because these compounds could not be separated by gel permeation chromatography (GPC), which separates the compounds having different molecular weights. Moreover, the mixture showed similar chemical shifts in the $^{19}$F NMR spectrum (164.0 and 165.2 ppm). The deprotection of the glycosylation product β-**28** using methylamine gave β-**35** (62%) (**Figure 2**).

The reaction mixture **36** prepared from **10** and **11c** according to step 3 of the protocol (**Figure 3**) was observed by $^{1}$H, $^{11}$B, and $^{19}$F NMR spectroscopy to investigate the formation of boronic ester intermediate **37** (**Figure 4**). The reaction mixture **38** was also prepared from **11c** for comparison. The results of the $^{1}$H NMR spectra indicated that the signal of 2'- and 3'-hydroxyl protons disappeared, and that of 2' and 3' protons dramatically shifted upfield in the presence of **11c** (**Figures 4A** and **4B**). In the $^{11}$B NMR spectra, we assumed that the peaks of boronic ester **37**, **11c** and/or boroxine **40** (which is a cyclic trimer generated by the dehydration condensation of three boronic acids), and boroxine pyridine complex **39** (which is a proposed structure based on the reported spectra data of boroxine pyridine complexes) were observed at 32 ppm, 28 ppm, and 21 ppm, respectively (**Figures 4C** - **4E**)[67,68,69]. In $^{19}$F NMR spectra, we hypothesized that the peaks of **37**, **11c** and/or **40**, and **39** correspond to -63.3 ppm, -63.2 ppm, and -62.8 ppm, respectively (**Figures 4F** - **4H**).

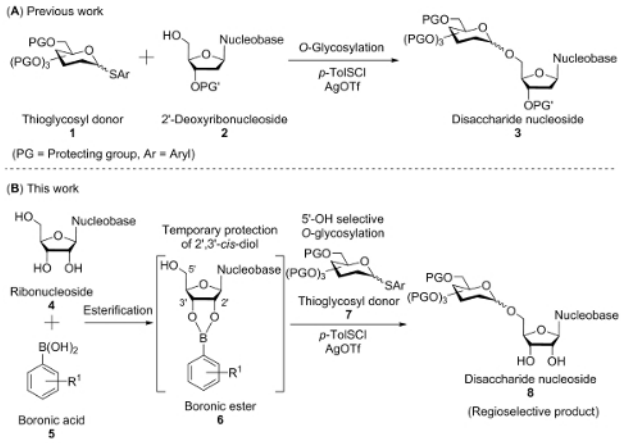



**Figure 1: Previous work and this work.** (**A**) This panel shows the *O*-glycosylation of 2′-deoxyribonucleoside with a thioglycoside promoted by *p*-toluenesulfenyl chloride (*p*-TolSCl) and silver triflate (AgOTf). (**B**) This panel shows the regioselective *O*-glycosylation of an unprotected ribonucleoside utilizing a cyclic boronic ester as a temporary protecting group. Please click here to view a larger version of this figure.

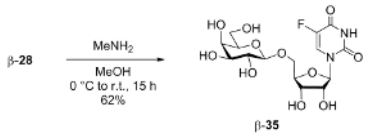



**Figure 2: Deprotection of β-28.** The cleavage of benzoyl groups was conducted with methylamine ($MeNH_2$) to afford β-**35**. Please click here to view a larger version of this figure.

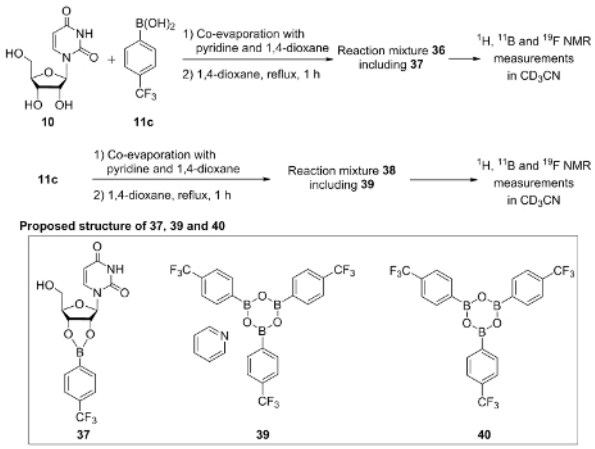



**Figure 3: Preparation of the reaction mixtures 36 and 38.** Mixtures **36** and **38** were prepared from uridine **10** and 4-(trifluoromethyl)phenylboronic acid **11c** and from **11c**, respectively. Please click here to view a larger version of this figure.

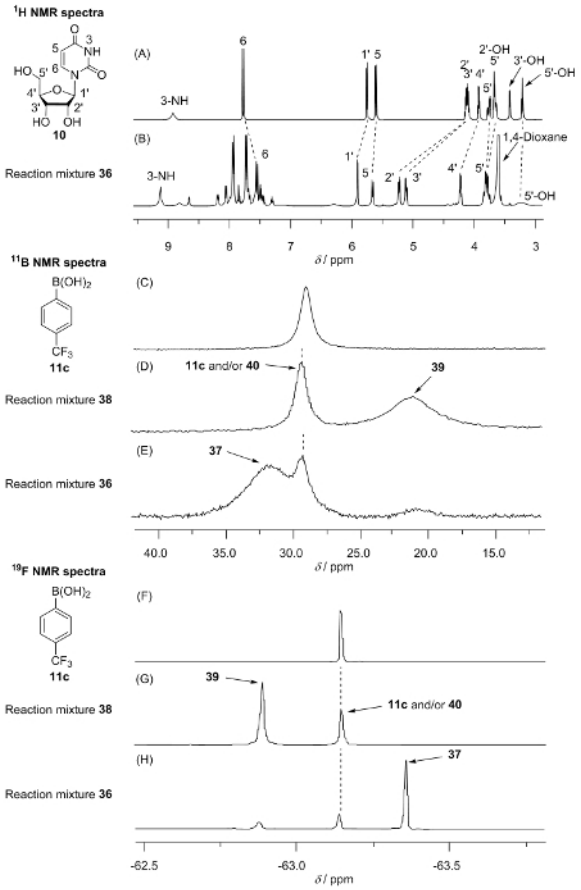
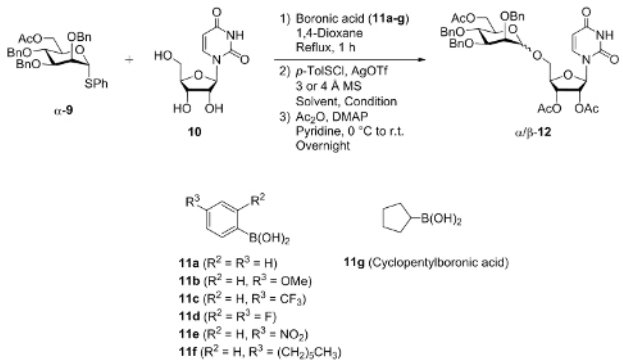
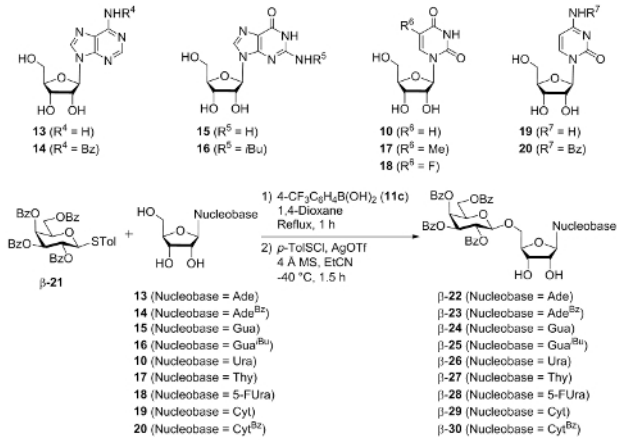



**Figure 4**: **NMR study of the cyclic boronic ester intermediate 37 prepared from uridine 10 and 4-(trifluoromethyl)phenylboronic acid 11c by $^1$H, $^{11}$B, and $^{19}$F NMR measurements in acetonitrile-$d_3$ at 25 °C**. The **37**, **39** and **40** were proposed structures, see Figure 3. (**A**) This panel shows **10** observed by $^1$H NMR. (**B**) This panel shows mixture **36** observed by $^1$H NMR. (**C**) This panel shows **11c** observed by $^{11}$B NMR. (**D**) This panel shows mixture **38** observed by $^{11}$B NMR. (**E**) This panel shows mixture **36** observed by $^{11}$B NMR. (**F**) This panel shows **11c** observed by $^{19}$F NMR. (**G**) This panel shows mixture **38** observed by $^{19}$F NMR. (**H**) This panel shows mixture **36** observed by $^{19}$F NMR. Please click here to view a larger version of this figure.



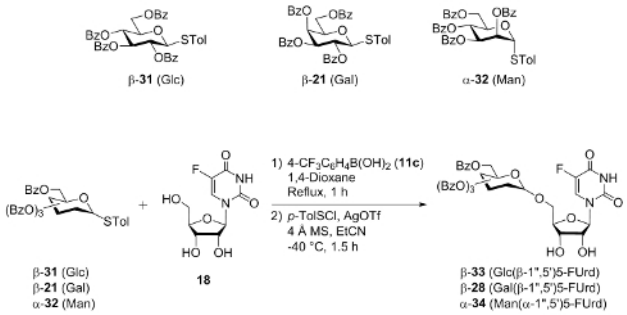
| Entry | Boronic Acid [b] | Solvent | Condition | Yield (for 3 Steps) [c] |
|---|---|---|---|---|
| 1 [a] | - | MeCN | −20 °C, 1.5 h | <16% (complex mixture) |
| 2 [a,d] | $PhB(OH)_2$ (**11a**) | MeCN | −20 °C, 1.5 h | 41% (α/β = 1.6/1) |
| 3 [a,e] | **11a** | MeCN | −20 °C, 1.5 h | 45% (α/β = 1.6/1) |
| 4 [a,e] | $4\text{-MeOC}_6H_4B(OH)_2$ (**11b**) | MeCN | −20 °C, 1.5 h | 39% (α/β = 1.8/1) |
| 5 [a,e] | $4\text{-CF}_3C_6H_4B(OH)_2$ (**11c**) | MeCN | −20 °C, 1.5 h | 51% (α/β = 1.8/1) |
| 6 [a,e] | $2,4\text{-F}_2C_6H_4B(OH)_2$ (**11d**) | MeCN | −20 °C, 1.5 h | 46% (α/β = 1.8/1) |
| 7 [a,e] | $4\text{-NO}_2C_6H_4B(OH)_2$ (**11e**) | MeCN | −20 °C, 1.5 h | 24% (α/β = 1.6/1) |
| 8 [a,e] | $4\text{-CH}_3(CH_2)_5C_6H_4B(OH)_2$ (**11f**) | EtCN | −40 °C, 1.5 h | 30% (α/β = 1.6/1) |
| 9 [a,e] | Cyclopentylboronic acid (**11g**) | MeCN | −20 °C, 1.5 h | 8% (α/β = 1.7/1) |
| 10 [a,e] | **11c** | 1,4-Dioxane | r.t., 1.5 h | 27% (α/β = 3.3/1) |
| 11 [a,e] | **11c** | $CH_2Cl_2$ | −40 °C, 1.5 h | trace |
| 12 [a,e] | **11c** | EtCN | −40 °C, 1.5 h | 61% (α/β = 1.6/1) |
| 13 [e,f] | **11c** | EtCN | −40 °C, 1.5 h | 57% (α/β = 1.5/1) |

**Table 1. Reaction conditions for regioselective *O*-glycosylation of uridine 10 with thiomannoside α-9.**[a] Glycosylations were conducted using 1.5 equivalents of α-**9**, 3.0 equivalents of *p*-toluenesulfenyl chloride, and 6.0 equivalents of silver triflate against **10**. The resulting products were acetylated with *ca.* 10 equivalents of acetic anhydride ($Ac_2O$) in the presence of a catalytic amount of *N*,*N*-dimethyl-4-aminopyridine (DMAP). [b] Boronic acid **11** was 1.5 equivalents against **10**. [c] The α/β ratio of α/β-**12** was checked by $^1$H NMR. [d] A mixture of **10** and **11a** was co-evaporated with pyridine and 1,4-dioxane and then stirred in 1,4-dioxane at its reflux temperature, followed by the addition of a solution of α-**9** in acetonitrile to conduct the glycosylation. [e] A mixture of α-**9**, **10**, and **11** was co-evaporated with pyridine and 1,4-dioxane and then stirred in 1,4-dioxane at its reflux temperature followed by a treatment with *p*-toluenesulfenyl chloride and silver triflate. [f] A glycosylation reaction was conducted using 1.5 equivalents of α-**9**, 1.8 equivalents of *p*-toluenesulfenyl chloride, and 3.6 equivalents of silver triflate against **10**. The resulting products were acetylated with *ca.* 10 equivalents of acetic anhydride in the presence of a catalytic amount of *N*,*N*-dimethyl-4-aminopyridine. Ac = acetyl, Bn = benzyl, Ph = phenyl.



| Entry [a] | Acceptor | Product | Yield (for 2 Steps) |
|---|---|---|---|
| 1 | **13** (Nucleobase = Ade) | β-**22** | 42% |
| 2 | **14** (Nucleobase = Ade$^{Bz}$) | β-**23** | 30% |
| 3 | **15** (Nucleobase = Gua) | β-**24** | 12% |
| 4 | **16** (Nucleobase = Gua$^{iBu}$) | β-**25** | 44% |
| 5 | **10** (Nucleobase = Ura) | β-**26** | 42% (*ca*. 15%: Nucleobase = 5-STol-Ura) |
| 6 | **17** (Nucleobase = Thy) | β-**27** | 53% |
| 7 | **18** (Nucleobase = 5-FUra) | β-**28** | 61% |
| 8 | **19** (Nucleobase = Cyt) | β-**29** | 55% |
| 9 | **20** (Nucleobase = Cyt$^{Bz}$) | β-**30** | 40% |

**Table 2. *O*-Glycosylations of nucleosides 10 and 13 - 20 with the thiogalactoside β-21 for the synthesis of disaccharide nucleosides β-22 - β-30**. [a] Glycosylations were conducted using 1.5 equivalents of β-**21**, 1.5 equivalents of 4-(trifluoromethyl)phenylboronic acid **11c**, 3.0 equivalents of *p*-toluenesulfenyl chloride, and 6.0 equivalents of silver triflate against the acceptor (**10** and **13** - **20**). A mixture of β-**21**, the acceptor (**10** and **13** - **20**), and **11c** was co-evaporated with pyridine and 1,4-dioxane and then stirred in 1,4-dioxane at its reflux temperature followed by a treatment with *p*-toluenesulfenyl chloride and silver triflate. Bz = benzoyl, *i*Bu = isobutyryl, Tol = tolyl, Ade = adenine, Gua = guanine, Ura = uracil, Thy = thymine, 5-FUra = 5-fluorouracil, Cyt = cytosine.




| Entry [a] | Donor | Product | Yield (for 2 Steps) |
|---|---|---|---|
| 1 | β-**31** (Glc) | β-**33** | 54% |
| 2 [b] | β-**21** (Gal) | β-**28** | 61% |
| 3 | α-**32** (Man) | α-**34** | <39% (mixture) |

**Table 3. *O*-Glycosylations of glycosyl donors β-21, β-31, and α-32 with 5-fluorouridine 18 for the synthesis of disaccharide nucleosides β-28, β-33, and α-34**. [a] Glycosylations were conducted using 1.5 equivalents of a donor (β-**21**, β-**31,** or α-**32**), 1.5 equivalents of 4-(trifluoromethyl)phenylboronic acid **11c**, 3.0 equivalents of *p*-toluenesulfenyl chloride, and 6.0 equivalents of silver triflate against **18**. A mixture of a donor (β-**21**, β-**31**, or α-**32**), **18**, and **11c** was co-evaporated with pyridine and 1,4-dioxane and then stirred in 1,4-dioxane at its reflux temperature followed by a treatment with *p*-toluenesulfenyl chloride and silver triflate. [b] This is the same result as Entry 7 of **Table 2**. Glc = glucoside, Gal = galactoside, Man = mannoside, 5-FUrd = 5-fluorouridine.

## Discussion

The purpose of this manuscript is to show a convenient synthetic method to prepare disaccharide nucleosides using unprotected ribonucleosides without tedious protecting group manipulations. We report herein on the regioselective *O*-glycosylations of nucleosides *via* the temporary 2',3'-diol protection by a cyclic boronic ester (**Figure 1B**)[51].

The preparation of the cyclic boronic ester intermediate is one of the important steps. Anhydrous solvents should be used for the co-evaporation of the reaction mixture (steps 1.1.1.2 and 1.2.1.1.2 of the protocol) and for the esterification step (steps 1.1.1.3 and 1.2.1.1.3) because the boronic esters prepared from nucleoside and boronic acid might be easily hydrolyzed. The *O*-glycosylation reactions also require anhydrous conditions to avoid the hydrolysis of the glycosyl donors. Therefore, the molecular sieves (steps 1.1.2 and 1.2.1.2), the two-neck round-bottom flask, and the anhydrous solvents (steps 1.1.3.1 and 1.2.1.3.1) should be sufficiently dried prior to their use for the *O*-glycosylation.

The *p*-toluenesulfenyl chloride-prepared according to our previous paper[38] - should be stored in the dark at -20 °C, to be used within 3 months. If the silver triflate is wet, it should be dried *in vacuo* prior to its use for the *O*-glycosylation.

This method could be applied to various nucleosides and glycosyl donors (**Table 1**, **2**, and **3**). The large-scale synthesis of β-**28** largely succeeded, except for some examples such as the combination of α-**32** and **18** (**Table 3**, Entry 3), in which the isolation of the desired

disaccharide nucleoside is not easy. In addition, this method is applied to the construction of a 1",5'-glicosidic linkage of disaccharide nucleosides (the construction of a 1",2'- and 1",3'-glicosidic linkage is yet to be studied).

The *O*-glycosylation utilizing unprotected nucleosides supplies disaccharide nucleosides in a shorter process than previous methods using protected nucleosides.

The *O*-glycosylation of unprotected nucleosides utilizing the temporary protection of a cyclic boronic ester could be applied to the preparation of various biologically-active disaccharide nucleosides and their analogs. Especially, β-**35** and its analogs are expected to be the new drug candidates since it has been known that 5-fluorouridine and 5-fluorouracil have anticancer, antivirus, and antibacterial activities[24,59,70,71,72,73,74,75,76]. We also believe that the application of a temporary protection of hydroxyl groups by a boronic ester will be useful for the synthesis of a variety of natural and artificial compounds, as well as disaccharide nucleosides.

## Disclosures

The authors have nothing to disclose.

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