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Synthesis of 5'-O-DMT-2'-O-TBS mononucleosides using an organic catalyst

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Abstract

This unit describes a highly effective method to produce 5'-O-DMT-2'-O-TBS mononucleosides selectively using a small organic catalyst. This methodology avoids the tedious protection/deprotection strategy necessary to differentiate the 2'- and 3'-hydroxyl groups in a ribonucleoside. The catalyst was synthesized in two steps starting from the condensation of valinol and cyclopentyl aldehyde, followed by anionic addition of *N*-methylimidazole. Ring closure of the amino alcohol with *N,N*-dimethylformamide dimethyl acetal in methanol furnishes the catalyst. All four 2'-O-TBS protected mono-nucleosides, U, A^{Bz}, G^{Ib}, and C^{Ac}, were produced in a single step using 10 to 20 mol% of the catalyst at room temperature with excellent yields and selectivity. Further transformation to phosphoramidite demonstrates the utility of this protocol toward the preparation of monomers useful for automated synthesis of RNA.

Keywords

2'-O-TBS ribonucleosides; protection by site-selective functionalization; organocatalyst; phosphoramidation

INTRODUCTION

The manipulation of RNA is currently of high importance due to its critical role as riboswitches (Serganov and Nudler 2013) and RNAi (Kurreck 2009). In order to diversify the types of synthetic RNA, many have tried to develop various protected monoribonucleosides. Differentiated from deoxyribonucleotide synthesis, selective protection of 2'-hydroxyl group is an essential requirement for ribonucleoside monomers. Among the various protecting groups, the *tert*-butyldimethylsilyl (TBS) group is the most common protecting group for RNA monomers in automated synthesis because of its chemical orthogonality (Unit 3.6).

Historically, 2'-O-TBS protected monoribonucleosides can be acquired by chromatographic separation of a mixture of 2'- and 3'-O-TBS monoribonucleosides (Ogilvie et al., 1978) or a multistep protection/deprotection strategy, which involves an exocyclic silicon tether

between 3'- and 5'-hydroxyl groups (Markiewicz and Wiewiórowski 1978). To overcome these inefficient methods, we developed a site selective organocatalyst protocol. The catalyst was synthesized from commercial material in two steps and produced all four 2'-*O*-TBS protected mononucleosides in good yield and excellent selectivity (Blaisdell et al., 2013). The efficiency of this method was confirmed by transferring the silylated product to the phosphoramidite using *N*-methylimidazole as an activator.

CAUTION: Carry out all operations involving organic solvents and reagents in a well-ventilated fume hood. Wear appropriate protective clothing and glasses.

NOTE: All glassware should be oven or flame dried, and all reactions should be performed under anhydrous conditions.

BASIC PROTOCOL 1: TWO-STEP PREPARATION OF ORGANOCATALYST

The organocatalyst described in this report is derived from inexpensive and commercial starting materials. The first step involves a rhodium-catalyzed hydroformylation of cyclopentene, directly followed by condensation of the subsequent aldehyde with (*S*)-valinol. The addition of mono-lithiated *N*-methylimidazole to this imine forms the amino alcohol intermediate after purification. The second step is the ring closure of this intermediate with *N,N*-dimethylformamide dimethyl acetal in methanol (Figure 1). This closure requires the removal of solvent and addition of extra methanol to convert small amounts of the dimethylamine bound catalyst to the methanol bound catalyst. Once isolated, the solid organocatalyst is stored in a glovebox.

Materials

Cyclopentene
Tris(2,4-di-*tert*-butylphenyl)phosphite
Rh(acac)(CO)₂
Anhydrous benzene
1:1 H₂/CO ("syn" gas)
(*S*)-Valinol
Anhydrous tetrahydrofuran
Anhydrous Magnesium sulfate
N-methylimidazole
n-Butyllithium (10 M in hexanes)
Saturated aqueous ammonium chloride
Diethyl ether
Anhydrous methanol
N,N-dimethylformamide dimethyl acetal

Anhydrous pentane

Silica gel (EMD Silica Gel 60, 230–400 mesh)

Hexane

Ethyl acetate

Pressurized bomb reactor

Glovebox with balance, –40 °C freezer and vacuum ports

500 mL round bottom flask

PTFE syringe driven filter

Cannula

500 mL separatory funnel

Additional reagents and equipment for column chromatography (APPENDIX 3E) and thin-layer chromatography (TLC; APPENDIX 3D)

Prepare amino alcohol intermediate 3

- 1 Dissolve cyclopentene (22.1 mL, 250 mmol) and tris(2,4-di-*tert*-butylphenyl)phosphite (2.10 g, 3.25 mmol) in bomb reactor (purged with N₂).
- 2 In the glovebox, dissolve Rh(acac)(CO)₂ (226 mg, 0.875 mmol) in anhydrous benzene (50 mL). Add this solution to bomb reactor and purge the system three times with 1:1 H₂/CO. Then, pressurize the reactor to 150 psi with 1:1 H₂/CO and heat to 80 °C. Stir at these conditions for 6 hr.
- 3 Use crude reaction mixture for the following reaction with no purification or solvent evaporation.
- 4 Dissolve (*S*)-valinol (88 mmol) in anhydrous tetrahydrofuran (180 mL) in a 500 mL round-bottomed flask. Add the crude cyclopentanecarboxaldehyde (88 mmol) in benzene (18 mL). Add magnesium sulfate (18 grams) and stir the reaction mixture at room temperature for 3 hr.
- 5 Cannula filter the oxazolidine (the formed imine undergoes a cyclization) into a 500 mL round-bottomed flask.
- 6 Dissolve *N*-methylimidazole (310 mmol) in anhydrous tetrahydrofuran (180 mL) under a nitrogen atmosphere in a 500 mL round-bottomed flask. Cool the flask to –78 °C in a dry ice/acetone bath. Add *n*-butyllithium in hexanes (31 mL, 310 mmol) dropwise to the cooled solution. Stir the reaction mixture at –78 °C for 30 min.
- 7 Transfer via cannula the lithiated *N*-methylimidazole dropwise into the flask containing the oxazolidine at –78 °C. Stir the reaction overnight while gradually warming to room temperature.
- 8 Slowly add saturated aqueous ammonium chloride (50 mL) to the reaction flask.

- 9 Transfer the quenched reaction solution to a 500 mL separatory funnel. Separate the organic layer and extract the aqueous layer three times with diethyl ether (3 × 200 mL). Combine the organic layers and dry with magnesium sulfate. Filter the solution and wash the magnesium sulfate with diethyl ether (25 mL). Concentrate the organic layer using a rotary evaporator.
- 10 Dissolve the crude product in a minimal amount of dichloromethane and carefully layer on top of a silica gel column. Elute with a mixture of dichloromethane : methanol (30:1 v/v).
- 11 Monitor column fractions by TLC and combine fractions containing pure product. Evaporate fractions to dryness using a rotary evaporator to obtain the product as colorless oil.

(*S*)-2-(((*R*)-Cyclopentyl(1-methyl-1*H*-imidazol-2-yl)methyl)amino)-3-methylbutan-1-ol: 12.0 g (63 %, d.r. = 94:6) ¹H NMR (CDCl₃, 500 MHz) δ 0.77 (d, 0.18H, *J* = 6.8), 0.82 (d, 0.18H, *J* = 6.8), 0.87 (d, 2.82H, *J* = 6.8), 0.91 (d, 2.82H, *J* = 6.8), 1.20–1.32 (m, 0.54H), 1.33–1.74 (m, 8.46H), 2.13–2.22 (m, 1.88H), 2.24–2.27 (m, 0.12H), 3.26 (d, 0.06H, *J* = 7.1), 3.28 (d, 0.94H, *J* = 5.9), 3.30 (d, 0.94H, *J* = 2.9), 3.32 (d, 0.06H, *J* = 4.4), 3.61 (s, 2.82H), 3.62 (s, 0.18H), 3.72 (d, 1H, *J* = 7.6), 6.72 (d, 0.06H, *J* = 1.2), 6.76 (d, 0.94H, *J* = 1.2), 6.93 (d, 0.94H, *J* = 1.2); ¹³C NMR (CDCl₃, 125 MHz) δ 38.1, 38.3, 38.4, 38.6, 44.4, 44.5, 44.6, 48.6, 48.7, 48.9, 49.8, 50.1, 52.1, 65.0, 77.7, 78.0, 79.3, 81.6, 82.0, 82.7, 139.7, 140.2, 146.1, 146.2, 169.8, 170.6; HRMS (ESI +) calcd. for C₁₅H₂₈N₃O [M+H]⁺: 266.2227, found: 266.2247. [α]_D²⁰ = +46.7 (*c* = 1.0, CH₂Cl₂, *l* = 50 mm).

Note: The mixture of diastereomers can be taken forward because only the major isomer precipitates during the purification in the next step.

Ring closure reaction

- 12 Dissolve the amino alcohol intermediate (28 mmol) prepared above in anhydrous methanol (56 mL) under nitrogen atmosphere in a 500 mL round-bottomed flask. Add *N,N*-dimethylformamide dimethyl acetal (140 mmol) to the flask. Heat the reaction to 50 °C and stir overnight.
- 13 Cool reaction to room temperature. Evaporate the solvent using high vacuum to dryness.
- 14 Redissolve the residue in anhydrous methanol (56 mL) and heat to 50 °C for two hr.
- 15 After two hr, cool reaction to room temperature and evaporate the solvent using high vacuum to dryness. Bring the residue into the glovebox.
- 16 In the glovebox, dissolve the residue in anhydrous pentane (250 mL). Place the solution in a –40 °C freezer overnight. A dark yellow oil will form at the bottom of the flask. Decant off the organic layer into a round-bottomed flask.

Concentrate the organic layer to approximately 100 mL using high vacuum. Place this solution in the $-40\text{ }^{\circ}\text{C}$ freezer overnight.

- 17** Collect the white solid that has formed by filtration. Wash the solid with a small portion of cold pentane (10 mL). Collect the solid and store within the glovebox.

(2*R*,4*S*)-3-((*R*)-Cyclopentyl(1-methyl-1*H*-imidazol-2-yl)methyl)-4-isopropyl-2-methoxyoxazolidine: 3.9 g (46%). ¹**H NMR** (CDCl₃, 500 MHz) δ 0.69 (d, 3H, *J* = 7.1), 0.73–0.80 (m, 1H), 0.87 (d, 3H, *J* = 6.9), 1.46–1.78 (m, 7H), 2.33–2.40 (m, 1H), 2.75 (ddd, 1H, *J* = 8.8, 6.9, 5.1), 2.92 (s, 3H), 2.97–3.06 (m, 1H), 3.34 (s, 3H), 3.48 (d, 1H, *J* = 11.0), 3.59 (t, 1H, *J* = 7.8), 3.75 (t, 1H, *J* = 8.3), 6.27 (d, 1H, *J* = 1.2), 6.72 (s, 1H), 7.16 (d, 1H, *J* = 1.2); ¹³**C NMR** (CDCl₃, 125 MHz) δ 16.8, 20.1, 25.6, 26.0, 29.5, 31.8, 32.2, 32.3, 45.1, 52.3, 58.8, 65.4, 66.0, 112.6, 120.2, 128.5, 149.1; **Elemental Analysis**: C₁₇H₂₉N₃O₂ requires: C = 66.42%, H = 9.51%, N = 13.67%, found: C = 66.51%, H = 9.28%, N = 13.82%. [α]_{D20} = -37.3 (*c* = 1.0, CH₂Cl₂, *l* = 50 mm).

NOTE 1: Steps 16 and 17 are done entirely in a glovebox, free from any moisture.

BASIC PROTOCOL 2: TBS Protection of 2'-hydroxyl group in presence of free 3'-hydroxyl group

Selective protection of the 2'-hydroxyl group was performed with the organic catalyst. The 2'-hydroxyl group in dimethoxytrityl-protected ribonucleosides, A^{bz}, C^{Ac}, G^{lb} and U, was differentiated with the organocatalyst in presence of DIPEA, DIPEA-HCl, and TBSCl (Figure 2). The 2'-O-TBS protected compounds are obtained in excellent yield and selectivity after column chromatography on silica gel. And further modification with 2-cyanoethyl-diisopropylchlorophosphordiamidite afforded the amidite (Figure 3) building-block for commercial RNA synthesizer.

Materials

5'-O-DMTr-Uridine

N⁶-Bz-5'-O-DMTr-Adenosine

N⁴-Ac-5'-ODMTr-Cytidine

N²-5'-O-DMTr-Guanosine

Catalyst **4**

N,N-diisopropylethylamine hydrochloride

N,N-diisopropylethylamine (distilled from CaH₂)

Tert-butyldimethylsilyl chloride

Tetrahydrofuran (anhydrous)

Methanol

Silica gel (EMD Silica Gel 60, 230–400 mesh)

Hexane

Ethyl acetate

Glove box equipped with balance

Round bottom flask (10 mL)

Rubber septa for 14/20 glass joint

Magnetic stirring bar

3.0 mL disposable Syringe (Norm-Ject)

Rotary evaporator

Additional reagents and equipment for column chromatography (APPENDIX 3E) and thin-layer chromatography (TLC; APPENDIX 3D)

For Uridine Derivative

- 1 Place oven dried 10 mL round bottom flask with magnetic stirring bar into glove box.
- 2 Place 5'-*O*-DMTr-Uridine (1.639 g, 3.0 mmol), catalyst (92 mg, 0.3 mmol) and *N,N*-diisopropylethylamine hydrochloride (14.9 mg, 0.03 mmol) in 10 mL round bottom flask.
- 3 Add anhydrous THF (2.0 mL) by syringe and seal the flask with rubber septa.
- 4 Take out round bottom flask from glove box.
- 5 Add *N,N*-diisopropylethylamine (784 μ L, 4.5 mmol) and solution of *tert*-butyldimethylsilyl chloride (904 mg, 6.0 mmol) in anhydrous THF (1.0 mL).
- 6 Allow reaction to stir for 24 h at room temperature. Observe the formation of *N,N*-diisopropylethylamine hydrogen chloride salt.
- 7 To quench the reaction mixture, add *N,N*-diisopropylethylamine (522 μ L, 3.0 mmol) and methanol (243 μ L, 6.0 mmol) to the reaction. Allow the reaction to stir for 1 min at room temperature.
- 8 Remove salt by passing the reaction mixture through 5.0 g of silica gel using 80 mL of ethyl acetate.
- 9 Concentrate the filtrate using a rotary evaporator connected to a vacuum pump.
- 10 Add CH₂Cl₂ with pipet to dissolve crude material.
- 11 Load product mixture onto the top of a silica gel column.
- 12 Flush with 30% ethyl acetate in hexane to remove *tert*-butyldimethylsilanol, then 1–5% MeOH in CH₂Cl₂. Collect fractions monitored by TLC.
- 13 Remove the liquid with rotatory evaporator followed by high vacuum pump to afford the product.

2'-O-TBS-5'-O-DMTr-uridine (**2a**) 1.90 g, 2.88 mmol, 96.0 %, selectivity 2':3' = 98.7:1.3 (HPLC condition: C18(150 × 4.6mm, 5μm, 1.0 mL/min 20% NH₄HCO₃(aq)/80% acetonitrile), rt = 5.2 min (2'-OTBS), 6.1 min (3'-OTBS)), TLC (95:5 [v/v] CH₂Cl₂/MeOH): R_f = 0.33. ¹H NMR (CDCl₃, 500 MHz) δ 9.28 (br s, 1H), 7.94 (d, 1H, J = 8.5), 7.38 (d, 1H, J = 7.0), 7.32-7.23 (m, 7H), 6.85 (d, 1H, J = 9.0), 5.96 (d, 1H, J = 3.0), 5.30 (d, 1H, J = 8.5), 4.37-4.34 (m, 2H), 4.11-4.10 (m, 1H), 3.80 (s, 6H), 3.54-3.48 (m, 2H), 2.59 (d, 1H, J = 6.0), 0.93 (s, 9H), 0.19 (s, 3H), 0.16 (s, 3H). ¹³C NMR (CDCl₃, 125 MHz) δ 163.5, 159.0, 158.9, 150.5, 144.5, 140.4, 135.4, 135.2, 130.4, 130.3, 128.3, 128.2, 127.4, 113.5, 113.5, 102.5, 88.9, 87.4, 83.7, 76.5, 70.6, 62.5, 55.4, 25.9, 18.2, -4.4, -5.0 HRMS (DART-ESI+) calcd. for C₃₆H₄₄N₂O₈Si: [M+H]⁺: 661.29452, found: 661.29258.

Perform phosphoramidation

- 14 Place 2'-O-TBS-5'-O-DMTr-uridine (132 mg, 0.2 mmol) into 10 mL round bottom flask with a magnetic stirring bar.
- 15 Add anhydrous CH₂Cl₂ (8.0 mL), *N,N*-diisopropylethylamine (522 uL, 3.0 mmol), 2-Cyanoethyl *N,N*-diisopropylchlorophosphordiamidite (133 uL, 0.6 mmol) and *N*-methylimidazole (15 uL, 0.2 mmol) at room temperature.
- 16 Stir the mixture for 1 hr and add methanol (1.0 mL) to quench the reaction. Stir for 5 min more and concentrate under rotatory evaporator.
- 17 Load the reaction mixture to silica gel column.
- 18 Elute with 50/50/1(v/v/v)=Ethylacetate/Hexane/TEA and collect the fraction monitoring with TLC. Pool the fractions containing product (R_f= 0.20, 0.16) and concentrate using rotatory evaporator connected to vacuum pump to afford foamy product as a mixture of diastereomers.

2'-O-TBS-5'-(2-cyanoethyl *N,N*-diisopropylphosphoamidyl)-5'-O-DMTr-uridine (**7**) yield 161 mg, 0.187 mmol, 92.0 %, selectivity 2':3' = 98.1:1.9 (HPLC condition: C18(150 × 4.6mm, 5μm, 1.0 mL/min 5% NH₄HCO₃/95% acetonitrile), rt=5.3, 5.4 min (2'-OTBS), 4.8, 6.4 min (3'-OTBS)), TLC (50:50:1 [v/v/v] Ethylacetate:Hexane:TEA): R_f = 0.20, 0.16. ¹H NMR (500 MHz, CDCl₃) δ 8.48 (s, br, 1H), 7.99 (d, 0.55H, J = 8.3), 7.91 (d, 0.45H, J=8.3), 7.25 – 7.42 (m, 9H), 6.82 – 6.85 (m, 4H), 5.99 (d, 0.45H, J=4.9), 5.90 (d, 0.55H, J = 3.9), 5.31 (d, 0.55H, J = 8.3), 5.27 (d, 0.45H, J = 8.3), 4.44 (t, 0.45H, J = 4.6), 4.30 – 4.60 (m, 2.10H), 4.20 – 4.23 (m, 0.45H), 3.91 – 3.98 (m, 0.45H), 3.80, 3.79 (2s, 6H), 3.68 – 3.72 (m, 0.55H), 3.53 – 3.62 (m, 5H), 3.41 (dd, 0.55H, J = 11.0, 1.7), 3.38 (dd, 0.45, J = 10.8, 2.4), 2.63 – 2.66 (m, 0.90H), 2.40 (t, 1.1H, J = 6.4), 1.15 – 1.17 (m, 12H), 0.91, 0.89 (2s, 9H), 0.14, 0.14, 0.13, 0.12 (4s, 6H) ³¹P NMR (121.5 MHz, CDCl₃) 150.0, 149.6 HRMS (DART-ESI+) calcd. for C₄₅H₆₂N₄O₉PSi: [M+H]⁺: 861.40182, found: 861.40308.

For Adenosine Derivative

- 1a** Perform selective TBS protection as described (see Basic Protocol 2, step 1–13) with following modification to afford **6b** as a foamy solid.

Step 2: Use protected Adenosine **5b** (2.02 g, 3.0 mmol), catalyst **4** (84.4 mg, 0.3 mmol).

Step 3: Use 3.0 mL THF.

Step 6: Stir for 48 hr.

*N*⁶-Benzoyl-2'-O-TBS-5'-O-DMTr-adenosine (**5b**) yield 2.18 g, 2.77 mmol, 92.2 %, selectivity 2':3' = 96.6:3.4 (HPLC condition: C18(150 × 4.6mm, 5μm, 1.0 min 20% NH₄HCO₃/80% acetonitrile), rt = 6.9 min (2'-OTBS), 8.3 min (3'-OTBS)), TLC (95:5 [v/v] CH₂Cl₂/MeOH): R_f = 0.53. ¹H NMR (400 MHz, CDCl₃) δ 9.10 (s, 1H), 8.73 (s, 1H), 8.23 (s, 1H), 8.03 (d, 2H, J = 7.8), 7.63 – 7.59 (m, 1H), 7.54 – 7.50 (m, 2H), 7.45 (d, 2H, J = 7.8), 7.33 (d, 4H, J = 8.8), 7.31 – 7.26 (m, 2H), 7.24 – 7.19 (m, 1H), 6.81 (d, 4H, J = 8.8), 6.11 (d, 1H, 5.4), 5.02 (t, 1H, J = 5.4), 4.38 – 4.35 (m, 1H), 4.30 – 4.28 (m, 1H), 3.78 (s, 6H), 3.54 (dd, 1H, J = 10.8, 2.9), 3.40 (dd, 1H, J = 10.8, 3.9), 2.72 (d, 1H, J = 3.9), 0.84 (s, 9H), –0.01 (s, 3H), –0.14 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ 164.7, 158.8, 153.1, 151.9, 149.8, 144.7, 141.9, 135.8, 133.9, 133.0, 130.3, 129.1, 128.3, 128.1, 128.0, 127.2, 123.4, 113.4, 88.6, 86.9, 84.5, 75.9, 71.7, 63.5, 55.4, 25.8, 18.1, –4.7, –5.0. HRMS (DART-ESI+) calcd. for C₄₄H₅₀N₅O₇Si: [M+H]⁺: 788.34740, found: 788.34780.

For Cytidine Derivative

- 1b** Perform selective TBS protection as described (see Basic Protocol 2, step 1–13) with following modification to afford **6c** as a foamy solid.

Step 2: Use protected cytidine **5c** (1.76 g, 3.0 mmol), catalyst **4** (168.8 mg, 0.6 mmol).

Step 3: Use 5.0 mL THF.

Step 6: Stir for 48 hr.

*N*⁴-Acetyl-2'-O-TBS-5'-O-DMTr-cytidine (**6c**) yield 2.01 g, 2.86 mmol, 95.5%, selectivity 2':3' = 98.8:1.2 (HPLC condition: C18(150 × 4.6mm, 5μm, 1.0 mL/min 15% TBAH(aq)/85% acetonitrile), rt = 4.4 min (2'-OTBS), 4.7 min (3'-OTBS)), TLC (95:5 [v/v] CH₂Cl₂/MeOH): R_f = 0.40. ¹H NMR (500 MHz, CDCl₃) δ 9.20 (br s, 1H), 8.45 (d, 1H, J = 7.8), 7.41 (d, 2H, J = 6.8), 7.29 – 7.35 (m, 6H), 7.25 – 7.29 (m, 1H), 7.07 (d, 1H, J = 7.3 Hz), 6.84 – 6.89 (m, 4H), 5.90 (d, 1H, J = 1.5 Hz), 4.32 – 4.38 (m, 1H), 4.27 (dd, 1H, J = 4.4, 1.0), 4.08 (dt, 1H, J = 7.8, 2.4 Hz), 3.82

(s, 6H), 3.58 (dd, 1H, $J=11.2, 2.0$), 3.52 (dd, 1H, $J=11.2, 2.9$), 2.36 (d, 1H, $J=9.3$), 2.24 (s, 3H), 0.93 (s, 9H), 0.30 (s, 3H), 0.18 (s, 3H). $^{13}\text{C NMR}$ (acetone- d_6 , 125 MHz) δ 170.3, 162.6, 162.2, 158.8(2C), 154.7, 144.7, 144.6, 135.8, 135.6, 130.1, 130.0, 128.2, 127.9, 126.9, 113.2, 95.4, 91.4, 86.7, 82.2, 68.8, 54.7, 25.4, 23.9, 17.9, -5.2, -5.5. **HRMS** (DART-ESI+) calcd. for $\text{C}_{38}\text{H}_{48}\text{N}_3\text{O}_8\text{Si}$: $[M+H]^+$: 702.32052, found: 702.32190.

For Guanosine Derivative

- 1c** Perform selective TBS protection as described (see Basic Protocol 2, step 1–13) with following modification to afford **6d** as a foamy solid.

Step 2: Use protected Guanosine **5d** (1.50 g, 2.29 mmol), catalyst **4** (138.0 mg, 0.458 mmol), *N,N*-diisopropylethylamine hydrochloride (11.2 mg, 0.0687 mmol).

Step 3: Use 3.5 mL THF.

Step 5: Use *N,N*-diisopropylethylamine (1.17 mL, 6.87 mmol) and *tert*-butyldimethylsilyl chloride (1.35 g, 9.16 mmol).

Step 6: Stir for 24 hr.

Step 7: Use *N,N*-diisopropylethylamine (1.17 mL, 6.87 mmol) and methanol (586 μL , 12.0 mmol).

*N*²-Isobutyl-2'-*O*-TBS-5'-*O*-DMTr-guanosine (**6d**) yield 1.71 g, 2.22 mmol, 96.9%, selectivity 2':3' = 95.9:4.1 (HPLC condition: C18(150 $\times$ 4.6mm, 5 μm , 1.0 mL/min 20% TBAH(aq)/80% acetonitrile), *rt* = 4.7 min (2'-OTBS), 6.0 min (3'-OTBS)), TLC (95:5 [v/v] $\text{CH}_2\text{Cl}_2/\text{MeOH}$): R_f = 0.41. $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 11.92 (br s, 1H), 7.78 (s, 1H), 7.61 (d, 2H, $J=8.2$), 7.45 (d, 2H, $J=8.8$), 7.43 (d, 2H, $J=8.8$), 7.30–7.22 (m, 3H), 7.18 (s, 1H), 6.81 (d, 2H, $J=8.8$), 6.78 (d, 2H, $J=8.8$), 5.71 (d, 1H, $J=7.3$), 5.32 (dd, 1H, $J=7.3, 5.4$), 4.34 (d, 1H, $J=5.4$), 4.23 (s, 1H), 3.77 (s, 3H), 3.75 (s, 3H), 3.60 (dd, 1H, $J=10.8, 1.5$), 3.02 (dd, 1H, $J=10.7, 2.4$), 2.78 (s, 1H), 1.21–1.14 (m, 1H), 0.85 (s, 9H), 0.78 (d, 3H, $J=6.8$), 0.49 (d, 3H, $J=6.8$), 0.04 (s, 3H), -0.18 (s, 3H). $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz) δ 178.3, 159.1, 159.1, 148.3, 147.2, 145.5, 139.6, 136.5, 135.9, 135.9, 130.2, 130.2, 128.3, 128.2, 127.5, 123.1, 113.6, 113.6, 88.6, 86.3, 84.7, 74.0, 71.3, 63.9, 55.5, 36.2, 25.8, 18.5, 18.1, -4.8, -4.9. **HRMS** (DART-ESI+) calcd. for $\text{C}_{41}\text{H}_{52}\text{N}_5\text{O}_8\text{Si}$: $[M+H]^+$: 770.35797, found: 770.35740.

REAGENTS AND SOLUTIONS

Diisopropylethylamine hydrogen chloride

Charge 100 mL round bottom flask with 30 mL diethyl ether at ambient temperature. Add diisopropylethylamine (0.870 mL, 5.0 mmol) and hydrogen chloride (5.0 mL, 5.0 mmol),

1.0M in diethyl ether) sequentially. Filter the white solid with glass filter and washed with 20 mL diethyl ether. Collect white solid and dry under vacuum for 24 hr.

Ammonium bicarbonate 10 mM aqueous solution

Dissolve ammonium bicarbonate (790 mg, 10 mmol) into deionized water (1L).

COMMENTARY

Background Information

2'-O-TBS ribonucleosides—The development of synthetic RNA as a potential drug has resulted in tremendous attention towards artificial RNA synthesis. The role of RNA has not been restricted to classical transcription, but expanded to riboswitches and RNAi. Despite the importance of synthetic RNA, industrial application has been limited due to the lack of efficient synthetic methods. Among the various synthetic attempts, the 2'-O-TBS based monomers are regarded as the most classical and the most useful building blocks for a commercial RNA synthesizer. Despite some drawbacks, such as long coupling reaction time with a phosphoramidite and migration under deprotection conditions, the TBS group has satisfied the basic requirement for the system. It is stable enough under weak acidic condition for the deprotection of 5'-O-trityl group, and stays bounded to the 2'-hydroxyl group when the base is liberated by an alkaline reagent. Additionally, the deprotection of TBS group can be achieved by the use of a fluoride anion reagent, which is an orthogonal method to other protecting groups.

Unlike deoxynucleoside monomers, chemical similarity of 2'- and 3'-hydroxyl group is a major obstacle in the selective protection of the 2'-hydroxy group in naturally occurring monomers. The classical way is a chromatographic separation of mixture of the two silylated monomers, which produces large amount of unwanted side products (Ogilvie et al., 1978). Another method requires the installation of a silicon tether, protecting both the 2'- and 5'-hydroxyl groups (Markiewicz and Wiewiórowski 1978). The method requires an excessive time and reagents caused by the three-step sequence. To circumvent such inefficiency, the organic catalyst, which can recognize a specific site of a molecule, was successively applied to synthesize 2'-O-TBS protected ribonucleosides. This unit describes the utility of a new catalyzed method resulting in excellent selectivity along with high yields.

Site selective functionalization—Site-selective functionalization is a long-standing goal for synthetic organic chemists (Mahatthanachai et al., 2012). Site selectivity can be achieved using inherent chemical selectivity among active functional groups or directed by a catalyst (Lee and Taylor, 2012). Enzymes are considered the most efficient catalyst for this purpose due to their ability to recognize multiple sites and modulate one specific group. However, the necessity to have one specific enzyme for each substrate diminishes its utility. To this end, synthetic chemists have tried to differentiate multiple functional groups and have succeeded in the case of selective modulation of polyhydroxyl molecules. A chiral DMAP derivative (Kawabata et al., 2007, 2009), peptide-based organocatalyst (Griswold and Miller, 2003), and amino-alcohol based boron reagent (Gouliaras et al., 2011, Chan et

al., 2011, Lee et al., 2011, 2012) were employed for selective functionalization of carbohydrates. Another peptide-based organocatalyst was developed to perform a selective silylation of 1,2- and 1,3-diols (Zhao et al., 2006, 2007, Rodrigo et al., 2011, Mannville et al, 2013).

We have focused on site-selective functionalization using reversible covalent bonding, which accelerates the reaction through proximity effects similar to an enzyme (Sun et al., 2011, 2013, Worthy et al., 2012, Giustra and Tan 2012). This catalyst accompanies a covalent substrate-binding site resulting in a strong specific recognition of 1,2-diol in a complex molecule (Figure 1). An imidazole-containing catalytic site delivers electrophiles within the spatially well-defined substrate-catalyst intermediate. As a consequence, one of the hydroxyl groups is successively transformed into an acyl, silyl or mesylated groups in a single step with excellent yields and selectivity.

Critical Parameters and Troubleshooting

All the reactions need to be carried out under nitrogen atmosphere and all the reagents and materials should be dried. Any remaining water content will contaminate the catalyst resulting in low conversion as well as low selectivity. In order to completely exclude 3'-O-TBS residue from mixture, a different eluent system (Ethyl acetate/Hexane) is suggested for chromatography. The pure 2'-O-TBS compounds come out first but have tailing characteristics during chromatography.

Anticipated Results

The scope of this protocol can be extended to any *cis*-1,2-diol compounds. Additionally, the use of other electrophiles, such as acid and sulfonyl chlorides, will be applicable to this system with high yields and selectivity. Purity of the resulting mixture can be determined by either NMR or HPLC methods.

Time Considerations

Preparation of catalyst requires 5 days, including ~8 hr for hydroformylation, ~22 hr for aminoalcohol intermediate, ~17 hr for ring closure, 28 hr for recrystallization. The site selective protection was normally carried out between 24 and 48 h for the reaction and ~4 hr for purification. Phosphoramidation took 3 hr for the reaction and 2 hr for column chromatography.

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	base	cat loading	time	yield	selectivity (2'-/3'-OTBS)
	U	10 mol%	24	96.0	98.7/1.3
	A ^{Bz}	10 mol%	48	92.2	96.6/3.4
	C ^{Ac}	20 mol%	48	95.5	98.8/1.2
	G ^{lb}	20 mol%	24	96.9	95.9/4.1

Figure 2.
Selective 2'-O-TBS protection

Figure 3.
Preparation of 3'-O-phosphoramidite