Synthesis of 2′-*O*-β-D-Ribofuranosylnucleosides

This unit describes a three-step procedure for the preparation of 2′-*O*-β-D-ribofuranosylnucleosides (Mikhailov et al., 1997a; Markiewicz et al., 1998). First, the complete synthesis of 2′-*O*-β-D-ribofuranosyladenosine is described (see Basic Protocol). As shown in Figure 1.14.1, the procedure involves (1) condensation of a small excess of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose activated with tin tetrachloride with an *N*-protected 3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl-ribonucleoside in 1,2-dichloroethane, (2) removal of silyl protecting group with tetrabutylammonium fluoride, and (3) deacylation with ammonia in methanol. Using these procedures, 2′-*O*-β-D-ribofuranosyladenosine is prepared in a 61% overall yield. The 2′-*O*-ribosylation proceeds stereospecifically with the formation of a β-glycosidic bond. The presence of a participating 2-*O*-benzoyl group leads exclusively to 1,2-*trans*-ribofuranoside. This reaction is carried out under mild conditions (0°C, 1,2-dichloroethane, 2 hr for pyrimidine nucleosides, 7 to 16 hr for purine derivatives) and yields of the target compounds are 72% to 80% (Mikhailov et al., 1997a). At room temperature, the reaction occurs faster (30 min), but the yields in the coupling steps are lower (40% to 77%; Markiewicz et al., 1998). These variations are presented as slightly modified procedures for the preparation of four other 2′-*O*-β-D-ribofuranosylnucleosides (see Alternate Protocols 1 through 4; Fig. 1.14.3).

CAUTION: Carry out all operations involving organic solvents and reagents in a well-ventilated fume hood, and wear gloves and protective glasses.

PREPARATION OF 2′-*O*-β-D-RIBOFURANOSYLADENOSINE

BASIC PROTOCOL

The first step of preparation of 2′-*O*-β-D-ribofuranosyladenosine involves the condensation of N^6-benzoyl-3′,5′-(1,1,3,3-*O*-tetraisopropyldisiloxane-1,3-diyl)adenosine (**S.1**; Markiewicz and Wiewiorowski, 1986; *UNIT 2.4*) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) in the presence of tin tetrachloride in 1,2-dichloroethane at 0°C (Fig. 1.14.1). To obtain the product **S.3** in high yield and to simplify its isolation, it is recommended to run the reaction under nitrogen and to perform preactivation of the sugar with tin tetrachloride; usually the reaction is complete in 7 to 8 hr. During workup of the reaction mixture with saturated sodium bicarbonate solution, a suspension is formed, which should be filtered through a 2- to 3-cm layer of Hyflo Super Cel before the separation of organic and aqueous layers. The second step involves removal of the silyl protection from the 3′- and 5′-hydroxyl groups with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) for 15 to 20 min at room temperature followed by silica gel column chromatography. In the last step, all benzoyl groups from **S.4** are removed with 5 M ammonia (half-saturated at 0°C) in methanol for 2 to 3 days at room temperature followed by recrystallization from methanol to give 2′-*O*-β-D-ribofuranosyladenosine (**S.5**) in an overall yield of 61%.

Materials

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**)
N^6-Benzoyl-3′,5′-(1,1,3,3-*O*-tetraisopropyldisiloxane-1,3-diyl)adenosine (**S.1**; *UNIT 2.4*; Fig. 2.4.5)
Phosphorus pentoxide (P_2O_5)
Balloon of nitrogen or argon
1,2-Dichloroethane, anhydrous
Tin tetrachloride ($SnCl_4$)
Methanol (MeOH), analytical grade

Contributed by Sergey N. Mikhailov, Ekaterina V. Efimtseva, Andrei A. Rodionov, Georgii V. Bobkov, Irina V. Kulikova, and Piet Herdewijn
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Methylene chloride (CH_2Cl_2), reagent grade
Saturated sodium bicarbonate solution (sat. $NaHCO_3$)
Hyflo Super Cel (Fluka)
Sodium sulfate, anhydrous (Na_2SO_4)
Silica gel (e.g., Kieselgel 60, 0.06 to 0.20 mm; Merck)
Tetrabutylammonium fluoride trihydrate (TBAF)
Tetrahydrofuran (THF), reagent grade
Chloroform ($CHCl_3$), reagent grade
5 M ammonia in methanol (half-saturated at 0°C)
Diethyl ether, reagent grade

50- and 250-mL round-bottom flasks
Vacuum desiccator
Vacuum oil pump
TLC plate: silica-coated aluminum plate with fluorescent indicator (Merck silica gel 60 F_{254})
254-nm UV lamp
Long disposable capillaries
100-mL funnels with sintered glass disc filters (porosity 3)
100- and 250-mL separatory funnel
Rotary evaporator equipped with a water aspirator
3 × 20–cm and 3 × 15–cm sintered glass chromatography columns, porosity 3
Stainless steel spatula

Additional reagents and equipment for TLC (*APPENDIX 3D*) and column chromatography (*APPENDIX 3E*)

Prepare S.3

1. Weigh 1.26 g (2.5 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) into a 250-mL round-bottom flask containing a stir bar and weigh 1.23 g (2 mmol) of nucleoside **S.1** into a separate flask.
2. Put both flasks in a vacuum desiccator with phosphorus pentoxide and evacuate using a vacuum oil pump for 10 to 15 min. Close the stopcock of the desiccator and leave overnight at room temperature.
3. Connect the desiccator with a balloon of nitrogen or argon and open the stopcock of the desiccator. Open the desiccator and quickly close the flasks.
4. Dissolve 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) in 25 mL of 1,2-dichloroethane, close with stopcock and balloon of nitrogen or argon, and put the flask in the ice-water bath (Fig. 1.14.2A).
5. Add 0.35 mL (3 mmol) of tin tetrachloride in one portion under stirring and keep the reaction mixture for 10 min at 0°C.
6. Add 1.23 g (2 mmol) of nucleoside **S.1** in one portion and stir the reaction mixture for 7 hr at 0°C.
7. Monitor reaction by TLC (*APPENDIX 3D*) using 2% (v/v) MeOH in CH_2Cl_2. To protect the reaction mixture from atmospheric moisture, remove the sample using a long capillary as shown in Figure 1.14.2B.

   *The starting compound **S.1** ($R_f = 0.21$) usually disappears after 6 to 7 hr at 0°C. The product **S.3** ($R_f = 0.31$) moves faster in the same solvent.*

8. Add 10 mL saturated sodium bicarbonate solution and stir the suspension for 20 min at 0°C.
9. Lay a 2- to 3-cm layer of Hyflo Super Cel on a 100-mL funnel with sintered disc (porosity 3) and wash it with 20 mL CH_2Cl_2. Filter the suspension using a vacuum

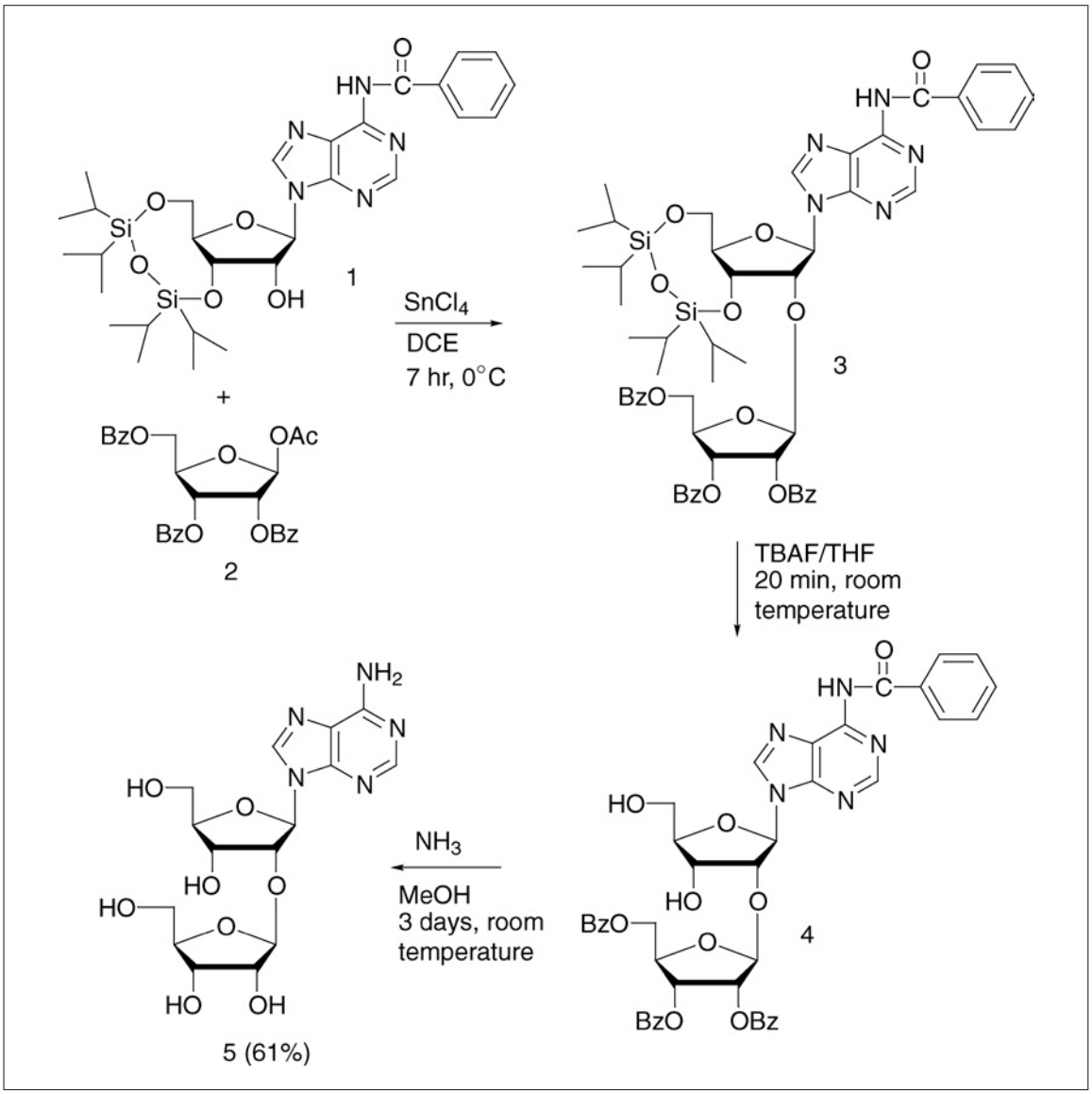

Figure 1.14.1 Synthesis of 2′-*O*-β-D-ribofuranosyladenosine (**S.5**). The expected overall yield of **S.5** from **S.1** is given in parentheses. DCE, 1,2-dichloroethane; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran.

pump, and wash the layer with 20 mL CH_2Cl_2 and 20 mL of 5% (v/v) MeOH in CH_2Cl_2.

10. Separate the organic layer using a 250-mL separatory funnel and wash the organic layer with 20 mL water.
11. Dry the organic layer over ~10 g Na_2SO_4, filter off Na_2SO_4 by gravity filtration, wash the precipitate with 20 mL CH_2Cl_2, and evaporate the combined filtrates using a rotary evaporator connected to vacuum system.
12. Prepare a slurry of 50 g silica gel in CH_2Cl_2 and pour into a 3 × 20–cm chromatography column (*APPENDIX 3E*).
13. Dissolve the residue in a minimal amount of CH_2Cl_2 and layer it carefully on top of the silica gel.
14. Wash the column with 300 mL CH_2Cl_2 and 300 mL of 0.5% (v/v) MeOH in CH_2Cl_2. Elute with 1% (v/v) MeOH in CH_2Cl_2. Collect 25-mL fractions.

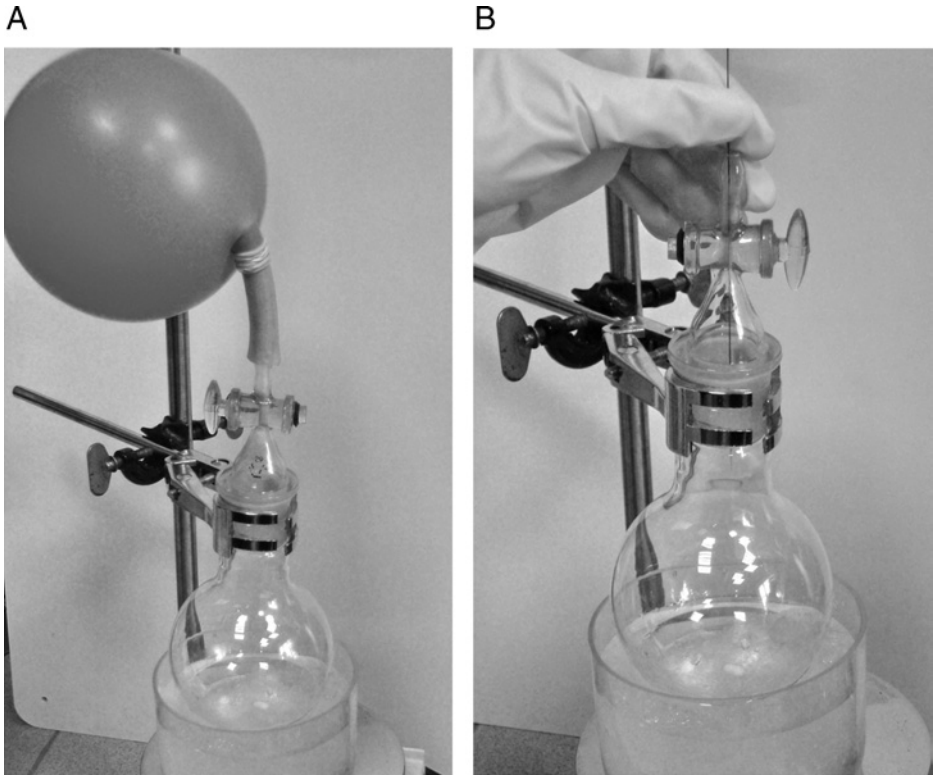

Figure 1.14.2 Protection from atmospheric moisture for synthesis of 2′-*O*-β-D-ribofuranosylnucleosides. (**A**) A 250-mL round-bottom flask with adaptor and stopcock connected with a balloon of nitrogen or argon in an ice-water bath. (**B**) Checking the reaction mixture with a long capillary (diameter ~1 mm). The diameter of the hole in the stopcock is ~3 mm.

15. Evaluate fractions by TLC using 2% (v/v) MeOH in CH_2Cl_2 and combine the fractions that contain only **S.3**. Evaporate the volatile materials from the combined fractions using a rotary evaporator connected to vacuum system, and dry the residual foam for 2 to 3 hr using a vacuum oil pump.

16. Grind the foam with a stainless steel spatula and dry the resulting powder 12 to 16 hr using a vacuum oil pump.

17. Characterize the product by TLC, ^{1}H NMR, and ^{13}C NMR.

The compound is stable for at least 12 months at ambient temperature.

*N^6-Benzoyl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]adenine (**S.3**). Yield of white amorphous solid 1.57 g (74%). TLC: R_f 0.31 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 9.02 brs (1H, NH), 8.72 s (1H, H8), 8.15 s (1H, H2), 8.03-7.87 m (8H, Bz), 7.61-7.28 m (12H, Bz), 6.09 d (1H, $J_{1',2'}$ = 1.0 Hz, H1′, Ado), 5.96 dd (1H, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 6.2 Hz, H3′, Rib), 5.88 d (1H, H2′, Rib), 5.82 s (1H, H1′, Rib), 4.96 dd (1H, $J_{3',2'}$ = 4.7 Hz, $J_{3',4'}$ = 9.0 Hz, H3′, Ado), 4.90 dd (1H, H2′, Ado), 4.81-4.65 m (3H, H4′, Ado; H4′,5′a, Rib), 4.18 d (1H, $J_{5'a,5'b}$ = −13.4 Hz, H5′a, Ado), 4.13 dd (1H, $J_{5'b,4'}$ = 1.0 Hz, $J_{5'b,5'a}$ = −9.5 Hz, H5′b, Rib), 4.03 dd (1H, $J_{5'b,4'}$ = 1.5 Hz, H5′b, Ado), 1.08-1.03 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 166.01, 165.37, 164.97 and 164.43 (C=O), 152.70 (C2), 150.76 (C6), 149.35 (C4), 141.80 (C8), 133.41, 133.16, 132.69, 129.71, 129.64, 129.10, 128.83, 128.34 and 127.78 (Bz), 123.40 (C5), 105.65 (C1′, Rib), 88.81 (C1′, Ado), 81.34 (C4′, Ado), 79.55 (C4′, Rib), 78.47 (C2′, Ado), 75.51 (C2′, Rib), 72.53 (C3′, Rib), 69.84 (C3′, Ado), 65.27 (C5′, Rib), 59.70 (C5′, Ado), 17.26, 17.04, 16.87, 16.77, 13.31, 12.89, 12.74 and 12.59 (iPr).*

Prepare S.4

18. Weigh 1.06 g (1 mmol) **S.3** into a 50-mL round-bottom flask, add 5 mL of 0.5 M TBAF in THF, stopper the flask, and keep the solution for 20 to 30 min at room temperature. Monitor deprotection by TLC using 5% (v/v) MeOH in CH_2Cl_2.

    *The starting compound **S.3** ($R_f = 0.93$) usually disappears after 20 to 30 min at 20°C. The product **S.4** ($R_f = 0.22$) moves slower in the same solvent.*

19. When the reaction is complete, evaporate all volatile material to dryness in vacuo using a rotary evaporator. Add 10 mL chloroform to the residue and evaporate to dryness.

20. Dissolve the residue in a minimal amount (3 to 4 mL) of chloroform and apply on a 3 × 15–cm column containing 30 g silica gel. Wash column with 300 mL CH_2Cl_2 and 200 mL of 1% (v/v) MeOH in CH_2Cl_2. Elute with 2% (v/v) MeOH in CH_2Cl_2 and collect 25-mL fractions.

21. Evaluate fractions by TLC using 5% (v/v) MeOH in CH_2Cl_2 and combine the fractions that contain only **S.4**. Evaporate the combined fractions using a rotary evaporator connected to a vacuum system, and dry the residual foam for 2 to 3 hr using a vacuum oil pump.

22. Grind the foam with a stainless steel spatula and dry the resulting powder 12 to 16 hr using a vacuum oil pump.

23. Characterize the compound by TLC, ^{1}H NMR, and ^{13}C NMR.

    The compound is stable for at least 12 months at 0°C.

    *N^6-Benzoyl-9-[2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-adenine (**S.4**). Yield of white amorphous solid 0.74 g (91%). TLC: R_f 0.22 (95:5 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 9.52 brs (1H, NH), 8.78 s (1H, H8), 8.18 s (1H, H2), 8.06-7.88 m (8H, Bz), 7.53-7.34 m (12H, Bz), 6.05 d (1H, $J_{1',2'} = 7.2$ Hz, H1′, Ado), 5.72 dd (1H, $J_{3',2'} = 5.3$ Hz, $J_{3',4'} = 5.0$ Hz, H3′, Rib), 5.64 dd (1H, $J_{2',1'} = 2.2$ Hz, H2′, Rib), 5.21 d (1H, H1′, Rib), 5.20 dd (1H, $J_{2',3'} = 4.7$ Hz, H2′, Ado), 4.59 brd (1H, H3′, Ado), 4.54 dd (1H, $J_{5'a,4'} = 4.1$ Hz, $J_{5'a,5'b} = -11.8$ Hz, H5′a, Rib), 4.48 ddd (1H, $J_{4',5'b} = 4.2$ Hz, H4′, Rib), 4.30 brs (1H, H4′, Ado), 4.11 dd (1H, H5′b, Rib), 3.96 brd (1H, $J_{5'a,5'b} = -12.7$ Hz, H5′a, Ado), 3.75 brd (1H, H5′b, Ado). ^{13}C NMR ($CDCl_3$): 165.71, 165.21 and 164.66 (C=O), 151.96 (C2), 150.26 (C6), 150.11 (C4), 143.85 (C8), 133.47, 133.36, 133.16, 132.54, 129.51, 129.41, 128.94, 128.54 and 127.82 (Bz), 123.80 (C5), 106.19 (C1′, Rib), 88.86 (C1′, Ado), 86.99 (C4′, Ado), 80.48 (C4′, Rib), 79.53 (C2′, Ado), 75.61 (C2′, Rib), 72.18 (C3′, Rib), 71.06 (C3′, Ado), 64.12 (C5′, Rib), 62.73 (C5′, Ado).*

Prepare S.5

24. Dissolve 408 mg (0.5 mmol) **S.4** in 15 mL of 5 M ammonia in methanol (half-saturated at 0°C) in a 50-mL round-bottom flask, stopper the flask, and keep the solution for 2 to 3 days at room temperature.

25. Evaporate all volatile material under reduced pressure using a rotary evaporator.

26. Partition the residue between 10 mL CH_2Cl_2 and 20 mL water using a 100-mL separatory funnel. Separate the aqueous layer and wash it two additional times with 10 mL CH_2Cl_2.

27. Concentrate the aqueous layer to a volume of ∼1 mL, add 7 mL MeOH, and keep the mixture 16 hr at 0°C.

28. Collect the precipitate by vacuum filtration on a glass filter (porosity 3), wash with 2 to 3 mL MeOH followed by 5 mL diethyl ether, and dry in a vacuum desiccator with phosphorus pentoxide for 24 hr at room temperature.

29. Characterize the compound by TLC, UV spectroscopy, ^{1}H NMR, and ^{13}C NMR.

The compound is stable for at least 12 months at 0°C.

*9-(2-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)adenine (**S.5**): Yield of white crystals 190 mg (91%). According to elemental analysis, **S.5** is obtained as a monohydrate. m.p. 212°-214°C (softening at 162°-164°C). TLC: R_f 0.12 (8:2 v/v CH_2Cl_2/MeOH). $[\alpha]^{20}_D$ −97° (c 0.76, DMSO). UV (pH 7-13): λ_{max} 261 nm (ε 14200); (pH 1): λ_{max}261 nm (ε 13700). LSIMS ($C_{15}H_{21}N_5O_8$ + H): calcd. 400.1468, found 400.1465. ^{1}H NMR (400 MHz, D_2O): 8.32 s (1H, H8, Ade), 8.19 s (1H, H2, Ade), 6.12 d (1H, $J_{1',2'}$ = 6.4 Hz, H1′, Ado), 5.07 s (1H, H1′, Rib), 4.80 dd (1H, $J_{2',3'}$ = 5.0 Hz, H2′, Ado), 4.55 dd (1H, $J_{3',4'}$ = 3.3 Hz, H3′, Ado), 4.29 ddd (1H, $J_{4',5'a}$ = 2.6 Hz, $J_{4',5'b}$ = 3.6 Hz, H4′ Ado), 4.13 d (1H, $J_{2',3'}$ = 4.5 Hz, H2′, Rib), 3.99 dd (1H, $J_{3',4'}$ = 7.4 Hz, H3′, Rib), 3.92 dd (1H, $J_{5'a,5'b}$ = −13.0 Hz, H5′a, Ado), 3.83 dd (1H, H5′b, Ado), 3.82 ddd (1H, $J_{4',5'a}$ = 3.7 Hz, $J_{4',5'b}$ = 6.8 Hz, H4′ Rib), 3.32 dd (1H, $J_{5'a,5'b}$ = −12.0 Hz, H5′a, Rib), 2.75 dd (1H, H5′b, Rib). ^{13}C NMR (D_2O): 156.14 (C6), 153.06 (C2), 149.74 (C4), 141.08 (C8), 119.42 (C5), 106.42 (C1′, Rib), 87.44 (C1′, Ado), 86.84 (C4′, Ado), 83.11 (C4′, Rib), 78.64 (C2′, Ado), 74.75 (C2′, Rib), 71.31 (C3′, Rib), 69.43 (C3′, Ado), 63.16 (C5′, Rib), 61.88 (C5′, Ado).*

ALTERNATE PROTOCOL 1

PREPARATION OF 2′-*O*-β-D-RIBOFURANOSYLURIDINE

3′,5′-(1,1,3,3-*O*-Tetraisopropyldisiloxane-1,3-diyl)uridine (**S.6a**; Markiewicz and Wiewiorowski, 1986; *UNIT 2.10*; Fig. 2.10.2) is converted to 2′-*O*-β-D-ribofuranosyluridine (**S.9a**) with an overall yield of 56% (Mikhailov et al., 1997a) using the steps outlined in the Basic Protocol (Fig. 1.14.3). The reaction uses the same molar equivalent of starting nucleoside (i.e., 2 mmol **S.6a**) and the same amounts of other reagents as in the Basic Protocol. The condensation reaction of pyrimidine nucleoside **S.6a** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) is carried out in 1,2-dichloroethane for 2 hr at 0°C. 2′-*O*-β-D-Ribofuranosyluridine (**S.9a**) is isolated by crystallization from a minimal amount of water.

*1-[3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]uracil (**S.7a**). Yield of white amorphous solid 76%. TLC: R_f 0.30 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 8.35 brs (1H, NH), 8.05-7.85 m (6H, Bz), 7.80 d (1H, $J_{6,5}$ = 8.0 Hz, H6), 7.56-7.26 m (9H, Bz), 5.88 dd (1H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 6.5 Hz, H3′, Rib), 5.82 d (1H, H2′, Rib), 5.81 s (1H, H1′, Urd), 5.80 s (1H, H1′, Rib), 5.65 d (1H, H5), 4.80-4.75 m (3H, H3′,4′, Urd; H4′, Rib), 4.41 d (1H, $J_{2',3'}$ = 4.8 Hz, H2′, Urd), 4.30 dd (1H, $J_{5'a,4'}$ = 4.0 Hz, $J_{5'a,5'b}$ = −9.6 Hz, H5′a, Rib), 4.23 d (1H, $J_{5'a,5'b}$ = −13.4 Hz, H5′a, Urd), 4.08 dd (1H, $J_{5'b,4'}$ = 1.0 Hz, H5′b, Rib), 3.95 dd (1H, $J_{5',4'}$ = 1.5 Hz, H5′b, Urd), 1.09-0.96 m (28H, iPr). ^{13}C NMR (400 MHz, $CDCl_3$): 166.01, 165.22 and 164.95 (C=O), 163.51 (C4), 149.68 (C2), 139.41 (C6), 133.31, 133.21, 133.00, 132.83, 129.64, 129.10, 128.87, 128.32 and 128.21 (Bz), 105.44 (C1′, Rib), 101.45 (C5), 89.25 (C1′, Urd), 81.52 (C4′, Urd), 79.33 (C4′, Rib), 78.47 (C2′, Urd), 75.47 (C2′, Rib), 72.96 (C3′, Rib), 68.71 (C3′, Urd), 65.60 (C5′, Rib), 59.22 (C5′, Urd), 17.22, 17.12, 17.01, 16.77, 16.67, 13.26, 12.91, 12.75 and 12.42 (iPr).*

*1-[2-O-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]uracil (**S.8a**). Yield of white amorphous solid 92%. TLC: R_f 0.20 (95:5 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 8.47 brs (1H, NH), 8.08-7.91 m (6H, Bz), 7.54-7.36 m (10H, H6, Bz), 5.82 dd (1H, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 5.9 Hz, H3′, Rib), 5.74 dd (1H, $J_{2',1'}$ = 1.9 Hz, H2′, Rib), 5.70 d (1H, $J_{1',2'}$ = 4.7 Hz, H1′, Urd), 5.65 d (1H, $J_{5,6}$ = 8.0 Hz, H5), 5.48 d (1H, H1′, Rib), 4.78-4.69 m (3H, H2′, Urd; H4′,5′a, Rib), 4.49-4.42 m (2H, H3′, Urd; H5′b, Rib), 3.93-3.87 m (2H, H4′,5′a, Urd), 3.75 dd (1H, $J_{5'b,4'}$ = 2.3 Hz, $J_{5'b,5'a}$ = −12.4 Hz, H5′b, Urd). ^{13}C NMR (400 MHz, $CDCl_3$): 166.12 and 165.46 (C=O), 163.78 (C4), 150.41 (C2), 142.54 (C6), 133.51, 133.47, 133.28, 129.69, 129.34, 128.68 and 128.42 (Bz), 106.82 (C1′, Rib), 102.08 (C5), 91.10 (C1′, Urd), 84.51 (C4′, Urd), 80.65 (C4′, Rib), 79.75 (C2′, Urd), 75.77 (C2′, Rib), 72.40 (C3′, Rib), 69.08 (C3′, Urd), 64.65 (C5′, Rib), 61.14 (C5′, Urd).*

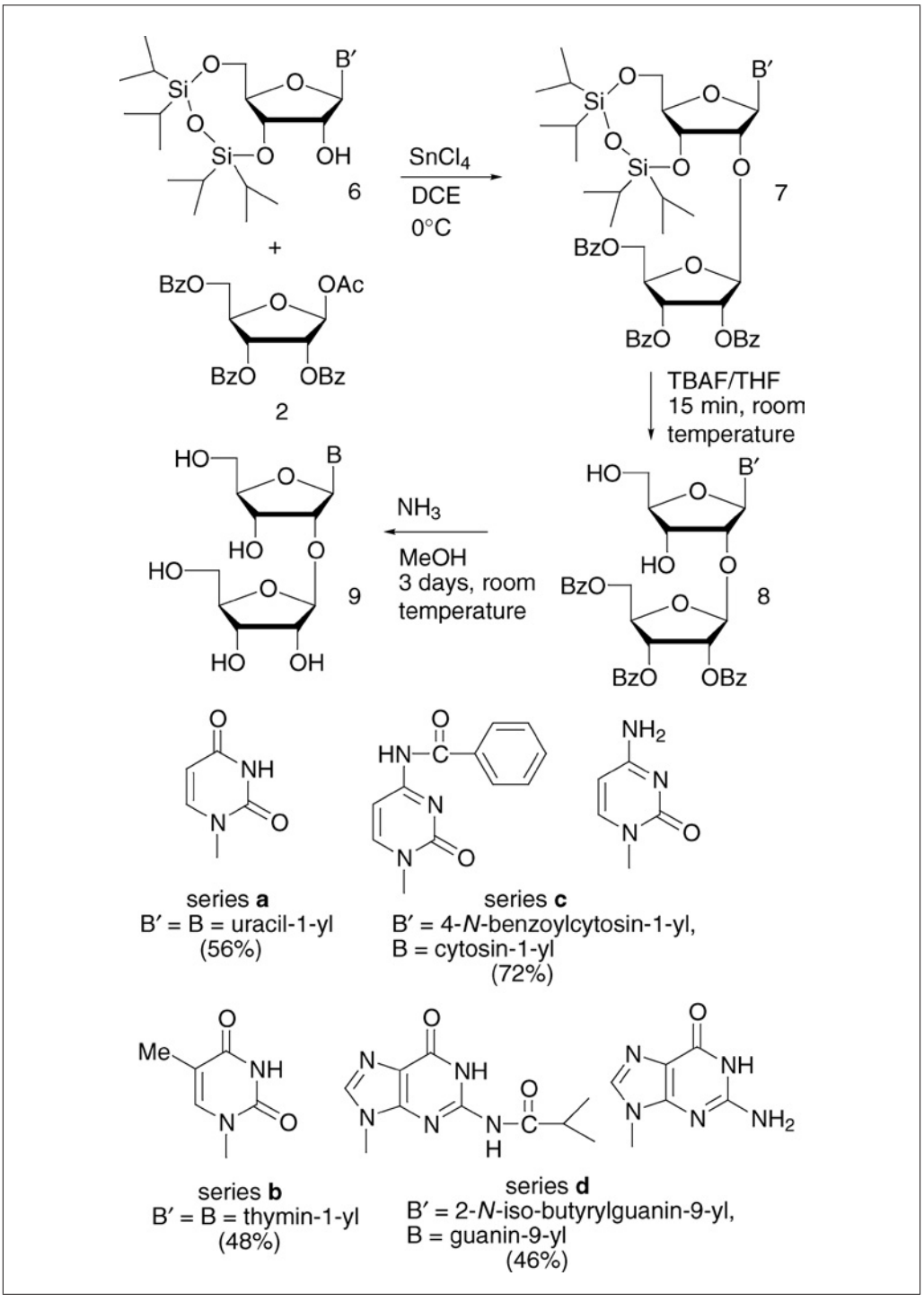

Figure 1.14.3 Synthesis of 2′-*O*-β-D-ribofuranosyluridine (**S.9a**), 2′-*O*-β-D-ribofuranosylthymidine (**S.9b**), 2′-*O*-β-D-ribofuranosylcytidine (**S.9c**), and 2′-*O*-β-D-ribofuranosylguanosine (**S.9d**). The expected overall yields are given in parentheses. DCE, 1,2-dichloroethane; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran.

*1-(2-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)uracil (**S.9a**): Yield of white crystals 80%. m.p. 224°-225°C (water). TLC: R_f 0.15 (8:2 v/v CH_2Cl_2/MeOH). $[\alpha]^{20}_D$ −36° (c 0.68, DMSO). UV (pH 1-7): λ_{max} 262 nm (ε 9400); (pH 13): λ_{max} 262 nm (ε 6900). LSIMS ($C_{14}H_{20}N_2O_{10}$+ H): calcd. 377.1196, found 377.2801. ^{1}H NMR (400 MHz, D_2O): 7.87 d (1H, $J_{5,6}$ = 8.1 Hz, H6, Ura), 6.04 d (1H, $J_{1',2'}$ = 5.0 Hz, H1′, Urd), 5,92 d (1H, H5, Ura), 5.15 s (1H, H1′, Rib), 4.44 dd (1H, $J_{2',3'}$ = 5.4 Hz, H2′, Urd), 4.36 dd (1H, $J_{3',4'}$ = 5.1 Hz, H3′, Urd), 4.19 dd (1H, $J_{3',2'}$ = 4.8 Hz, $J_{3',4'}$ = 6.9 Hz, H3′, Rib), 4.16 d (1H, H2′, Rib), 4.11 ddd (1H, $J_{4',5'a}$ = 2.9 Hz, $J_{4',5'b}$ = 4.5 Hz, H4′, Urd), 3.99 ddd (1H, $J_{4',5'a}$ = 3.4 Hz, $J_{4',5'b}$ = 6.6 Hz, H4′, Rib), 3.88 dd (1H, $J_{5'a,5'b}$ = −12.7 Hz, H5′a, Urd), 3.79 dd (1H, H5′b, Urd), 3.75 dd (1H, $J_{5'a,5'b}$ = −12.1 Hz, H5′a, Rib), 3.48 dd (1H, H5′b, Rib). ^{13}C NMR (D_2O): 166.66 (C4), 152.17 (C2), 142.55 (C6), 107.28 (C1′, Rib), 103.06 (C5), 88.47 (C1′, Urd), 85.11 (C4′, Urd), 83.35 (C4′, Rib), 78.93 (C2′, Urd), 74.85 (C2′, Rib), 71.14 (C3′, Rib), 68.87 (C3′, Urd), 63.34 (C5′, Rib), 61.17 (C5′, Urd).*

ALTERNATE PROTOCOL 2

PREPARATION OF 2′-*O*-β-D-RIBOFURANOSYLTHYMIDINE

3′,5′-(1,1,3,3-*O*-Tetraisopropyldisiloxane-1,3-diyl)thymidine (**S.6b**; Markiewicz and Wiewiorowski, 1986; see Support Protocol) is converted to 2′-*O*-β-D-ribofuranosylthymidine (**S.9b**) with an overall yield of 48% (Mikhailov et al., 1997a) using the steps outlined in the Basic Protocol (Fig. 1.14.3). The reaction uses the same molar equivalent of starting nucleoside (i.e., 2 mmol **S.6b**) and the same amounts of other reagents as in the Basic Protocol. The condensation reaction of pyrimidine nucleoside **S.6b** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) is carried out in 1,2-dichloroethane for 2 hr at 0°C. 2′-*O*-β-D-Ribofuranosylthymidine (**S.9b**) is isolated by crystallization from a minimal amount of 9:1 (v/v) ethanol/water.

*1-[3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]thymine (**S.7b**). Yield of white amorphous solid 74%. TLC: R_f 0.30 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 8.21 brs (1H, NH), 8.00-7.83 m (6H, Bz), 7.55-7.25 m (10H, Bz, H6), 5.85 dd (1H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 6.5 Hz, H3′, Rib), 5.78 d (1H, H2′, Rib), 5.76 s (1H, H1′, Thd), 5.75 s (1H, H1′, Rib), 4.77-4.69 m (3H, H3′,4′, Thd; H4′, Rib), 4.39 d (1H, $J_{2',3'}$ = 4.3 Hz, H2′, Thd), 4.30 dd (1H, $J_{5'a,4'}$ = 4.4 Hz, $J_{5'a,5'b}$ = −9.5 Hz, H5′a, Rib), 4.18 d (1H, $J_{5'a,5'b}$ = −13.4 Hz, H5′a, Thd), 4.04 dd (1H, $J_{5'b,4'}$ = 2.0 Hz, H5′b, Rib), 3.92 dd (1H, $J_{5'b,4'}$ = 2.6 Hz, H5′b, Thd), 1.86 d (3H, $J_{5,6}$ = 1.2 Hz, Me5), 1.07-0.92 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 166.11 and 165.32 (C=O), 165.01 (C4), 149.55 (C2), 135.27 (C6), 133.39, 133.30, 132.96, 129.72, 129.18, 128.94, 128.41, 128.29 and 128.25 (Bz), 110.09 (C5), 105.51 (C1′, Rib), 89.64 (C1′, Thd), 81.48 (C4′, Thd), 79.38 (C4′, Rib), 78.52 (C2′, Thd), 75.53 (C2′, Rib), 73.05 (C3′, Rib), 69.02 (C3′, Thd), 65.77 (C5′, Rib), 59.25 (C5′, Thd), 17.41, 17.33, 17.22, 17.13, 17.01, 16.88, 16.77, 13.42, 12.86 and 12.68 (iPr), 12.57 (Me5).*

*1-[2-O-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]thymine (**S.8b**). Yield of white amorphous solid 87%. TLC: R_f 0.20 (95:5 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD): 7.98-7.81 m (6H, Bz), 7.42 q (1H, $J_{6,5}$ = 1.2 Hz, H6), 7.49-7.25 m (9H, Bz), 5.76 d (1H, $J_{1',2'}$ = 4.2 Hz, H1′, Thd), 5.74 dd (1H, $J_{3',2'}$ = 5.1 Hz, $J_{3',4'}$ = 6.2 Hz, H3′, Rib), 5.69 dd (1H, $J_{2',1'}$ = 1.2 Hz, H2′, Rib), 5.44 d (1H, H1′, Rib), 4.53 ddd (1H, $J_{4',5'a}$ = 4.8 Hz, $J_{4',5'b}$ = 5.9 Hz, H4′, Rib), 4.41 dd (1H, $J_{5'a,5'b}$ = −11.8 Hz, H5′a, Rib), 4.30 dd (1H, H5′b, Rib), 4.28 dd (1H, $J_{2',3'}$ = 5.3 Hz, H2′, Thd), 4.13 dd (1H, $J_{3',4'}$ = 5.6 Hz, H3′, Thd), 3.77 ddd (1H, $J_{4',5'a}$ = 2.3 Hz, $J_{4',5'b}$ = 2.5 Hz, H4′, Thd), 3.66 dd (1H, $J_{5'a,5'b}$ = −12.4 Hz, H5′a, Thd), 3.52 dd (1H, H5′b, Thd), 1.76 d (3H, Me5). ^{13}C NMR ($CDCl_3$-CD_3OD): 166.15 and 165.48 (C=O), 164.33 (C4), 150.55 (C2), 137.74 (C6), 133.62, 133.47, 133.21, 129.62, 129.54, 129.18, 128.55, 128.48, 128.38, 128.31 and 128.28 (Bz), 110.48 (C5), 106.31 (C1′, Rib), 89.92 (C1′, Thd), 84.36 (C4′, Thd), 79.31 (C4′, Rib), 77.32 (C2′, Thd), 75.57 (C2′, Rib), 72.36 (C3′, Rib), 68.89 (C3′, Thd), 64.69 (C5′, Rib), 60.79 (C5′, Thd), 11.96 (Me5).*

*1-(2-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)thymine (**S.9b**). Yield of white crystals 75%. m.p. 236°-237°C (aq. EtOH). TLC: R_f 0.16 (8:2 v/v CH_2Cl_2/MeOH). $[\alpha]^{20}_D$ −52° (c 0.82, water). UV (pH 1-7): λ_{max}269 nm (ε9400); (pH 13): λ_{max} 262 nm (ε7100). LSIMS ($C_{15}H_{22}N_2O_{10}$+ H): calcd. 391.1352, found 391.1362. ^{1}H NMR (400 MHz, D_2O): 7.66 q (1H, $J_{6,CH3}$ = 1.2 Hz, H6, Thy), 6.03 d (1H, $J_{1',2'}$ = 5.5 Hz, H1′, Thd), 5.12 s (1H, H1′, Rib), 4.44 dd (1H, $J_{2',3'}$ = 5.5 Hz, H2′, Thd), 4.38 dd (1H, $J_{3',4'}$ = 4.7 Hz, H3′, Thd), 4.14 dd (1H, $J_{2',3'}$ = 4.7 Hz, $J_{3',4'}$ = 6.7 Hz, H3′, Rib), 4.13 dd (1H, H2′, Rib), 4.11 ddd (1H, $J_{4',5'a}$ = 3.1 Hz, $J_{4',5'b}$ = 4.5 Hz, H4′, Urd), 3.99 ddd (1H, $J_{4',5'a}$ = 3.6 Hz, $J_{4',5'b}$ = 6.7 Hz, H4′, Rib), 3.88 dd (1H, $J_{5'a,5'b}$ = −12.7 Hz, H5′a, Thd), 3.81 dd (1H, H5′b, Urd), 3.71 dd (1H, $J_{5'a,5'b}$ = −12.1 Hz, H5′a, Rib), 3.44 dd (1H, H5′b, Rib), 1.91 d (1H, Me5). ^{13}C NMR (D_2O): 167.28 (C4), 152.71 (C2), 138.49 (C6), 112.82 (C5), 107.71 (C1′, Rib), 88.50 (C1′, Thd), 85.60 (C4′, Thd), 83.88 (C4′, Rib), 79.17 (C2′, Thd), 75.32 (C2′, Rib), 71.74 (C3′, Rib), 69.32 (C3′, Thd), 64.03 (C5′, Rib), 61.71 (C5′, Thd), 12.47 (Me5).*

PREPARATION OF 2′-*O*-β-D-RIBOFURANOSYLCYTIDINE

ALTERNATE PROTOCOL 3

N^4-Benzoyl-3′,5′-(1,1,3,3-*O*-tetraisopropyldisiloxane-1,3-diyl)cytidine (**S.6c**; Markiewicz and Wiewiorowski, 1986; *UNIT 2.4*; Fig. 2.4.4) is converted to 2′-*O*-β-D-ribofuranosylcytidine (**S.9c**) with an overall yield of 72% (Mikhailov et al., 1997a) using the steps outlined in the Basic Protocol (Fig. 1.14.3). The reaction uses the same molar equivalent of starting nucleoside (i.e., 2 mmol **S.6c**) and the same amounts of other reagents as in the Basic Protocol. The condensation reaction of pyrimidine nucleoside **S.6c** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) is carried out in 1,2-dichloroethane for 2 hr at 0°C. After step 26, the aqueous layer containing product **S.9c** is evaporated to dryness in vacuo, the residue is dissolved in 5 mL methanol, and is evaporated to dryness again. The residual foam is dried for 2 to 3 hr using a vacuum oil pump, and is finally dried in a vacuum desiccator with phosphorus pentoxide.

*N^4-Benzoyl-1-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]cytosine (**S.7c**). Yield of white amorphous solid 80%. TLC: R_f 0.32 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 8.77 brs (1H, NH), 8.30 d (1H, $J_{6,5}$ = 7.6 Hz, H6), 8.02-7.82 m (8H, Bz), 7.55-7.24 m (13H, H5, Bz), 5.96 s (1H, H1′, Cyd), 5.92 dd (1H, $J_{3',2'}$ = 5.0 Hz, $J_{3',4'}$ = 6.3 Hz, H3′, Rib), 5.89 s (1H, H1′, Rib), 5.81 d (1H, H2′, Rib), 4.83-4.78 m (3H, H3′,4′, Cyd; H4′, Rib), 4.49 d (1H, $J_{2',3'}$ = 4.1 Hz, H2′, Cyd), 4.28 dd (1H, $J_{5'a,4'}$ = 3.8 Hz, $J_{5'a,5'b}$ = −9.4 Hz, H5′a, Rib), 4.24 d (1H, $J_{5'a,5'b}$ = −13.4 Hz, H5′a, Cyd), 4.17 dd (1H, $J_{5'b,4'}$ = 1.0 Hz, H5′b, Rib), 3.98 dd (1H, $J_{5',4'}$ = 1.5 Hz, H5′b, Cyd), 1.10-0.96 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 166.09, 165.28 and 165.05 (C=O) 162.40 (C4), 155.02 (C2), 144.36 (C6), 133.31, 133.18, 132.80, 129.74, 129.23, 128.98, 128.37, 128.21 and 127.53 (Bz), 105.48 (C1′, Rib), 96.02 (C5), 90.14 (C1′, Cyd), 81.81 (C4′, Cyd), 79.07 (C4′, Rib), 78.51 (C2′, Cyd), 75.63 (C2′, Rib), 73.10 (C3′, Rib), 68.71 (C3′, Cyd), 65.51 and 59.32 (C5′), 17.41, 17.28, 17.06, 16.91, 16.77, 13.31, 13.02, 12.85 and 12.50 (iPr).*

*N^4-Benzoyl-1-[2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-cytosine (**S.8c**). Yield of white amorphous solid 95%. TLC: R_f 0.25 (95:5 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 9.18 brs (1H, NH), 8.05-7.89 m (9H, H6, Bz), 7.58-7.33 m (13H, H5, Bz), 5.84 dd (1H, $J_{3',2'}$ = 5.2 Hz, $J_{3',4'}$ = 5.3 Hz, H3′, Rib), 5.77 d (1H, $J_{1',2'}$ = 3.8 Hz, H1′, Cyd), 5.74 dd (1H, $J_{2',1'}$ = 1.6 Hz, H2′, Rib), 5.60 d (1H, H1′, Rib), 4.92 dd (1H, $J_{2',3'}$ = 5.0 Hz, H2′, Cyd), 4.76 dd (1H, $J_{5'a,4'}$ = 4.0 Hz, $J_{5'a,5'b}$ = −11.9 Hz, H5′a, Rib), 4.69 ddd (1H, $J_{4',3'}$ = 5.3 Hz, $J_{4',5'a}$ = 4.3 Hz, H4′, Rib), 4.49-4.45 m (2H, H3′, Cyd; H5′b, Rib), 4.03 brd (1H, $J_{4',3'}$ = 5.3 Hz, H4′, Cyd), 3.94 brd (1H, $J_{5'a,5'b}$ = −12.8 Hz, H5′a, Cyd), 3.77 brd (1H, H5′b, Cyd). ^{13}C NMR ($CDCl_3$): 166.66, 166.05 and 165.37 (C=O), 162.77 (C4), 155.31 (C2), 147.17 (C6), 133.46, 133.35, 133.14, 132.95, 129.67, 129.42, 128.76, 128.35 and 127.71 (Bz), 106.66 (C1′, Rib), 96.87 (C5), 92.38 (C1′, Cyd), 84.87 (C4′, Cyd), 80.67 (C4′, Rib), 79.51 (C2′, Cyd), 75.87 (C2′, Rib), 72.57 (C3′, Rib), 68.34 (C3′, Cyd), 64.82 (C5′, Rib), 60.56 (C5′, Cyd).*

*1-(2-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)cytosine (**S.9c**): Yield of white amorphous solid 95%. TLC: R_f 0.04 (8:2 v/v CH_2Cl_2/MeOH). $[\alpha]^{20}{}_D$ −29° (c 0.53, water). UV (pH 7-13): λ_{max}271 nm (ε8200); (pH 1): λ_{max} 279 nm (ε 12200). LSIMS ($C_{14}H_{21}N_3O_9 + H$): calcd. 376.1355, found 376.1338. ^{1}H NMR (400 MHz, D_2O): 7.81 d (1H, $J_{5,6} = 7.6$ Hz, H6, Cyt), 6.06 d (1H, H5, Cyt), 6.04 d (1H, $J_{1',2'} = 5.1$ Hz, H1′, Cyd), 5.12 s (1H, H1′, Rib), 4.37 dd (1H, $J_{2',3'} = 5.5$ Hz, H2′, Cyd), 4.33 dd (1H, $J_{3',4'} = 4.7$ Hz, H3′, Cyd), 4.17 dd (1H, $J_{3',2'} = 4.6$, $J_{3',4'} = 6.8$, H3′, Rib), 4.16 d (1H, H2′, Rib), 4.11 ddd (1H, $J_{4',5'a} = 3.0$ Hz, $J_{4',5'b} = 4.4$ Hz, H4′, Cyd), 3.97 ddd (1H, $J_{4',5'a} = 3.4$ Hz, $J_{4',5'b} = 6.7$ Hz, H4′, Rib), 3.88 (1H, $J_{5'a,5'b} = -12.7$ Hz, H5′a, Cyd), 3.79 dd (1H, H5′b, Cyd), 3.71 dd (1H, $J_{5'a,5'b} = -12.2$ Hz, H5′a, Rib), 3.37 dd (1H, H5′b, Rib). ^{13}C NMR (D_2O): 166.57 (C4), 158.03 (C2), 142.28 (C6), 106.96 (C1′, Rib), 97.17 (C5), 88.70 (C1′, Cyd), 85.08 (C4′, Cyd), 83.30 (C4′, Rib), 78.92 (C2′, Cyd), 74.84 (C2′, Rib), 71.23 (C3′, Rib), 68.90 (C3′, Cyd), 63.51 (C5′, Rib), 61.35 (C5′, Cyd).*

ALTERNATE PROTOCOL 4

PREPARATION OF 2′-*O*-β-D-RIBOFURANOSYLGUANOSINE

N^2-Isobutyryl-3′,5′-(1,1,3,3-*O*-tetraisopropyldisiloxane-1,3-diyl)guanosine (**S.6d**; Markiewicz and Wiewiorowski, 1986; *UNIT 2.4*; Fig. 2.4.6) is converted to 2′-*O*-β-D-ribofuranosylguanosine (**S.9d**) with an overall yield of 46% (Mikhailov et al., 1997a) using the steps outlined in the Basic Protocol (Fig. 1.14.3) with some modifications described here. The reaction uses the same molar equivalent of starting nucleoside (i.e., 2 mmol **S.6d**) and the same amounts of other reagents as in the Basic Protocol. The condensation reaction of guanosine nucleoside **S.6d** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) is carried out in 1,2-dichloroethane for 16 hr at 0°C. The guanosine derivatives form stable complexes with tin tetrachloride. After the condensation reaction (step 6), the reaction mixture is taken up with a long capillary (Fig. 1.14.2B) and is added to a microcentrifuge tube containing 0.2 mL saturated sodium bicarbonate solution and 0.2 mL ethyl acetate. The tube is shaken and kept for 5 to 10 min at 20°C to destroy the tin tetrachloride complexes. A sample from the upper organic layer is then checked by TLC using 2% (v/v) MeOH in CH_2Cl_2 (step 7). The starting compound **S.6d** ($R_f = 0.14$) usually disappears after 16 hr at 0°C, and the product **S.7d** ($R_f = 0.25$) moves faster in the same solvent. After TLC, the purification of **S.7d** resumes with step 8. After step 26, the aqueous layer containing 2′-*O*-β-D-ribofuranosylguanosine (**S.9d**) is evaporated to dryness in vacuo. The residue is dissolved in 10 mL ethanol and the mixture is kept for 16 hr at 0°C. The resulting precipitate is collected by vacuum filtration on a glass filter (porosity 3), washed with 5 ml diethyl ether, and dried for 24 hr in a vacuum desiccator with phosphorus pentoxide.

*N^2-Isobutyryl-9-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]guanine (**S.7d**). Yield of white amorphous solid 72%. TLC: R_f 0.25 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 12.19 brs (1H, NH), 9.81 brs (1H, NH), 8.08 s (1H, H8), 8.00-7.82 m (6H, Bz), 7.55-7.26 m (9H, Bz), 6.10 dd (1H, $J_{3',2'} = 5.1$ Hz, $J_{3',4'} = 5.8$ Hz, H3′, Rib), 5.86 dd (1H, $J_{2',1'} = 0.9$ Hz, H2′, Rib), 5.79 d (1H, H1′, Rib), 5.72 s (1H, H1′, Guo), 4.87-4.53 m (5H, H2′,3′,4′, Guo; H4′,5′a, Rib), 4.23 d (1H, $J_{5'a,5'b} = -13.5$ Hz, H5′a, Guo), 4.15 dd (1H, $J_{5'b,4'} = 2.2$ Hz, $J_{5'b,5'a} = -9.4$ Hz, H5′b, Rib), 3.96 dd (1H, $J_{5',4'} = 2.5$ Hz, H5′b, Guo), 2.86 sep (1H, J = 6.8 Hz, CH, iBu), 1.32 d (3H, Me, iBu), 1.22 d (3H, Me, iBu), 1.07-0.92 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 179.42, 167.87, 165.34 and 165.00 (C=O), 155.42 (C6), 148.27 (C2), 147.09 (C4), 135.91 (C8), 134.01, 133.57, 129.71, 128.95, 128.83, 128.66 and 128.45 (Bz), 121.60 (C5), 105.46 (C1′, Rib), 87.32 (C1′, Guo), 81.17 (C4′, Guo), 79.38 (C4′, Rib), 78.75 (C2′, Guo), 75.81 (C2′, Rib), 73.29 (C3′, Rib), 69.31 (C3′, Guo), 65.65 (C5′, Rib), 59.41 (C5′, Guo), 36.10 (CH, iBu), 19.21 (Me, iBu), 18.92 (Me, iBu), 17.48, 17.31, 17.13, 16.96, 16.80, 16.71, 13.32, 13.06, 12.82 and 12.53 (iPr).*

*N^2-Isobutyryl-9-[2-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-β-D-ribofuranosyl]-guanine (**S.8d**). Yield of white amorphous solid 84%. TLC: R_f 0.15 (95:5 v/v CH_2Cl_2/MeOH). ^1H NMR (400 MHz, $CDCl_3$): 12.27 brs (1H, NH), 9.67 brs (1H, NH), 8.62 s (1H, H8), 7.99-7.88 m (6H, Bz), 7.55-7.32 m (9H, Bz), 6.07 dd (1H, $J_{3',2'} = 5.0$ Hz, $J_{3',4'} = 5.9$ Hz, H3′, Rib), 5.88 d (1H, $J_{1',2'} = 1.9$ Hz, H1′, Guo), 5.85 d (1H, H2′, Rib), 5.80 s (1H, H1′, Rib), 4.76 dd (1H, $J_{5'a,4'} = 5.9$ Hz, $J_{5'a,5'b} = -11.3$ Hz, H5′a, Rib), 4.73-4.66 m (2H, H3′, Guo; H4′, Rib), 4.53 dd (1H, $J_{5'b,4'} = 3.7$ Hz, H5′b, Rib), 4.40 dd (1H, $J_{2',3'} = 4.4$ Hz, H2′, Guo), 4.17 brd (1H, $J_{4',3'} = 8.2$ Hz, H4′, Guo), 4.06brs (2H, H5′a,5′b, Guo), 2.82 sep (1H, J = 6.8 Hz, CH, iBu), 1.31 d (3H, Me, iBu), 1.24 d (3H, Me, iBu). ^{13}C NMR ($CDCl_3$): 179.73, 167.77 and 165.27 (C=O), 155.95 (C6), 148.03 (C2), 147.48 (C4), 138.98 (C8), 133.93, 133.48, 129.87, 129.73, 129.69, 128.99, 128.83, 128.64, 128.45 and 128.39 (Bz), 120.50 (C5), 105.48 (C1′, Rib), 87.97 (C1′, Guo), 83.67 (C4′, Guo), 79.25 (C4′, Rib), 77.32 (C2′, Guo), 75.82 (C2′, Rib), 73.07 (C3′, Rib), 68.79 (C3′, Guo), 65.57 (C5′, Rib), 59.83 (C5′, Guo), 36.05 (CH, iBu), 19.13 (Me, iBu), 18.85 (Me, iBu).*

*9-(2-O-β-D-Ribofuranosyl-β-D-ribofuranosyl)guanine (**S.9d**): Yield of white crystals 76%. m.p. 215°-216°C (EtOH). TLC: R_f 0.09 (8:2 v/v CH_2Cl_2/MeOH). $[\alpha]^{20}_D$ −75° (c 0.92, water). UV (pH 1): λ_{max} 257 nm (ε 10800); (pH 7): λ_{max} 254 nm (ε 12000); (pH 13): λ_{max} 263 nm (ε 10000). LSIMS ($C_{15}H_{21}N_5O_9$ + H): calcd. 416.1417, found 416.1413. ^1H NMR (400 MHz, D_2O): 8.03 s (1H, H6, Guo), 6.01 d (1H, $J_{1',2'} = 6.3$ Hz, H1′, Guo), 5,12 d (1H, $J_{1',2'} = 0.8$ Hz, H1′, Rib), 4.81 dd (1H, $J_{2',3'} = 5.3$ Hz, H2′, Guo), 4.56 dd (1H, $J_{3',4'} = 3.3$ Hz, H3′, Guo), 4.26 ddd (1H, $J_{4',5'a} = 3.1$ Hz, $J_{4',5'b} = 4.1$ Hz, H4′, Guo), 4.17 dd (1H, $J_{2',3'} = 4.6$ Hz, H2′, Rib), 4.08 dd (1H, $J_{3',4'} = 7.3$ Hz, H3′, Rib), 3.92 ddd (1H, $J_{4',5'a} = 3.8$ Hz, $J_{4',5'b} = 6.8$ Hz, H4′, Rib), 3.91 dd (1H, $J_{5'a,5'b} = -12.8$ Hz, H5′a, Guo), 3.85 dd (1H, H5′b, Guo), 3.47 dd (1H, $J_{5'a,5'b} = -11.9$ Hz, H5′a, Rib), 3.01 dd (1H, H5′b, Rib). ^{13}C NMR (D_2O): 159.89 (C6), 154.86 (C2), 150.85 (C4), 139.28 (C8), 117.67 (C5), 107.18 (C1′, Rib), 87.52 (C1′, Guo), 87.03 (C4′, Guo), 83.80 (C4′, Rib), 78.98 (C2′, Guo), 75.37 (C2′, Rib), 72.06 (C3′, Rib), 69.96 (C3′, Guo), 64.02 (C5′, Rib), 62.41 (C5′, Guo).*

PREPARATION OF THE 3′,5′-PROTECTED RIBOTHYMIDINE

SUPPORT PROTOCOL

1-[3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]thymine (**S.6b**) is prepared starting from thymine and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (Fig. 1.14.4). A procedure proposed by Vorbrüggen and Ruh-Pohlenz (2000) has completely replaced the previously employed methods for the synthesis of nucleosides and their analogs. The method involves glycosylation of trimethylsilyl derivatives of heterocyclic bases with peracylated sugars in aprotic solvents in the presence of Lewis acids such as $SnCl_4$ or trimethylsilyl trifluoromethanesulfonate (TMSOTf; *UNIT 1.13*). The use of this approach has significantly simplified the reaction procedure and increased the yields of the target products. The presence of a 2-*O*-acyl group in a carbohydrate residue is believed to be crucial for the stereochemistry of this reaction, since it stabilizes C1 carbonium ion generated via an intermediate 1,2-acyloxonim ion and yields 1,2-*trans* derivatives.

In the first step of this procedure, crystalline 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-thymine (**S.11**) is prepared from thymine derivative **S.10** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) in a yield of 82%. In the second step, the cleavage of the benzoyl protecting groups is achieved using sodium methoxide in methanol under mild conditions, and 1-(β-D-ribofuranosyl)thymine (**S.12**) is purified by crystallization from ethanol. In the third step, nucleoside **S.12** is simultaneously protected on its 3′- and 5′-hydroxy groups by the bifunctional protecting reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane developed by Markiewicz and Wiewiorowski (1986). The derivative **S.6b** is formed in high yield (86% from **S.12**) due to the higher reactivity of the primary 5′-hydroxy group and the subsequently favorable cyclization to the 3′-hydroxy group. Chromatography is required only in the last step of the sequence. Using this three-step procedure, **S.6b** is prepared in an overall yield of 55%.

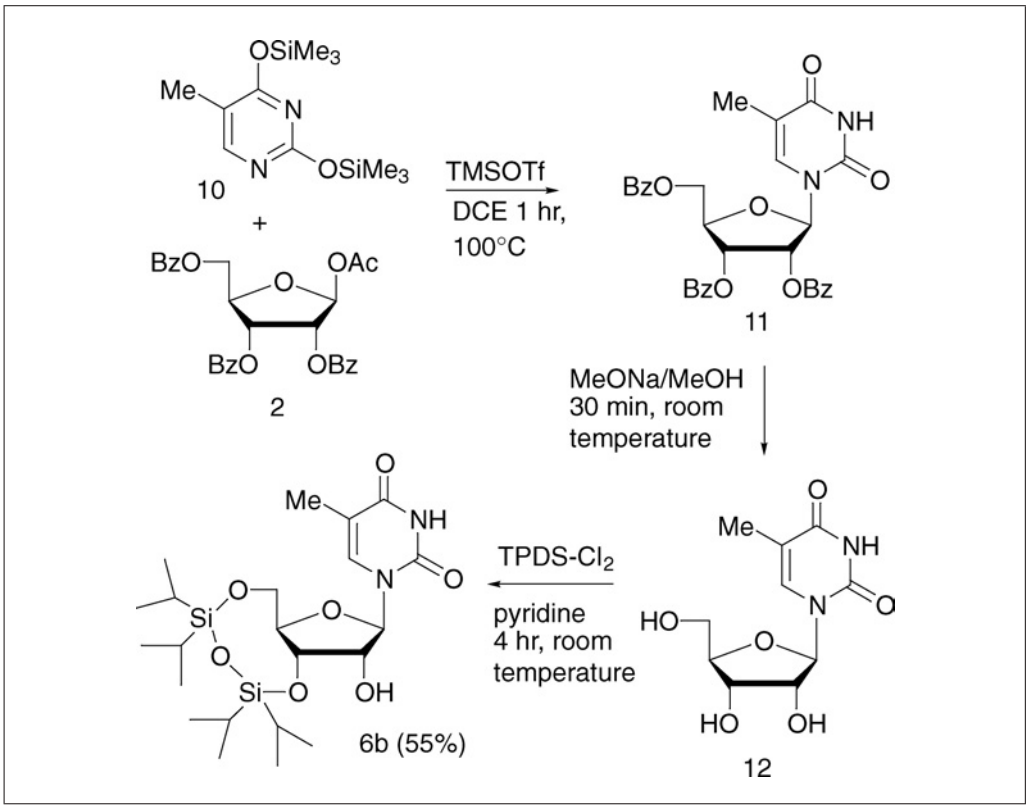

Figure 1.14.4 Synthesis of 1-[3,5-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]-thymine (**S.6b**) The expected overall yield is given in parentheses. DCE, 1,2-dichloroethane; TMSOTf, trimethylsilyl trifluoromethanesulfonate; $TPDSCl_2$, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane.

Additional Materials *(also see Basic Protocol)*

Thymine
Ammonium sulfate [$(NH_4)_2SO_4$]
1,1,1,3,3,3-Hexamethyldisilazane, reagent grade
Calcium chloride ($CaCl_2$), anhydrous
Trimethylsilyl trifluoromethanesulfonate (TMSOTf)
0.2 N sodium methoxide (NaOMe), freshly prepared from sodium and dry methanol
Dowex 50 × 4 (100 to 200 mesh) in H+ form
Pyridine, anhydrous
Markiewicz reagent: 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, 96% pure (Wacker)
Toluene, reagent grade

100-, 250-, and 500-mL round-bottom flasks
Reflux condensers
$CaCl_2$ protection tubes
Oil bath with temperature control
Adapters with stopcocks and vacuum pump (Fig. 1.14.5)
250- and 500-mL separatory funnels
Glass filters (porosity 3)
3 × 20–cm chromatography columns

Prepare S.11

1. Weigh 3.78 g (30 mmol) of thymine and 20 mg of $(NH_4)_2SO_4$ into a 250-mL round-bottom flask, add 50 mL of 1,1,1,3,3,3-hexamethyldisilazane (b.p. 125°C), and attach a condenser equipped with $CaCl_2$ protection tube.

2. Reflux the mixture in an oil bath set at 130°C until complete dissolution of thymine (10 to 12 hr).

3. Cool the flask, remove the condenser, and attach an adaptor with a stopcock (Fig. 1.14.5A). Concentrate the solution to a viscous oil using a rotary evaporator connected to a vacuum system (bath temperature ~30° to 35°C).

   *The resulting bis-O-trimethylsilylthymine **S.10** is very hygroscopic.*

4. Close the stopcock of adaptor and attach a second adaptor with stopcock to the flask. Connect the system to a vacuum pump (Fig. 1.14.5B), evacuate the system, and close the upper stopcock. Connect the system with a balloon of nitrogen or argon and open the upper stopcock to fill the adaptor with nitrogen. Repeat this procedure (evacuation and flash with nitrogen) and then open both stopcocks to fill the entire system with nitrogen (Fig. 1.14.5C).

5. Dissolve the residue in 30 mL of anhydrous 1,2-dichloroethane and concentrate the solution to a viscous oil using a rotary evaporator with a bath temperature ~30° to 35°C.

6. Repeat step 4.

7. Dissolve the residue in 120 mL of anhydrous 1,2-dichloroethane. Add 12.6 g (25 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**S.2**) in one portion and 5.79 mL (32 mmol) of trimethylsilyl trifluoromethanesulfonate (TMSOTf) with gentle hand stirring.

8. Attach a condenser equipped with $CaCl_2$ protection tube and heat the clear solution for 1 hr in a 100°C oil bath. Monitor reaction by TLC in 98:2 (v/v) CH_2Cl_2/MeOH.

   *The starting **S.2** ($R_f = 0.91$) usually disappears after 1 hr at 100°C. The product **S.11** ($R_f = 0.45$) moves slower in the same solvent.*

9. Cool the flask to room temperature. Remove the condenser, add 50 mL of saturated sodium bicarbonate solution, and stir the suspension for 20 min at 20°C.

10. Put a 2- to 3-cm layer of Hyflo Super Cel on a 100-mL funnel with a sintered disc (porosity 3) and wash with 20 mL CH_2Cl_2. Filter the suspension using vacuum pump and wash the layer with 50 mL CH_2Cl_2.

11. Separate the organic layer using a 250-mL separatory funnel and wash the organic layer with 50 mL of water.

12. Dry the organic layer over ~20 g Na_2SO_4, filter off Na_2SO_4 by gravity filtration, and wash the precipitate with 40 mL CH_2Cl_2.

13. Concentrate the combined filtrates to a solid using a rotary evaporator connected to vacuum system.

14. Add 100 mL of ethanol, attach a condenser, and reflux (79°C) until the solid is completely dissolved. Cool the flask to room temperature and keep the mixture for 16 hr at 0°C.

15. Collect the precipitate by vacuum filtration on a glass filter (porosity 3), wash the precipitate with 10 mL ethanol, and dry **S.11** over phosphorus pentoxide in a vacuum desiccator overnight.

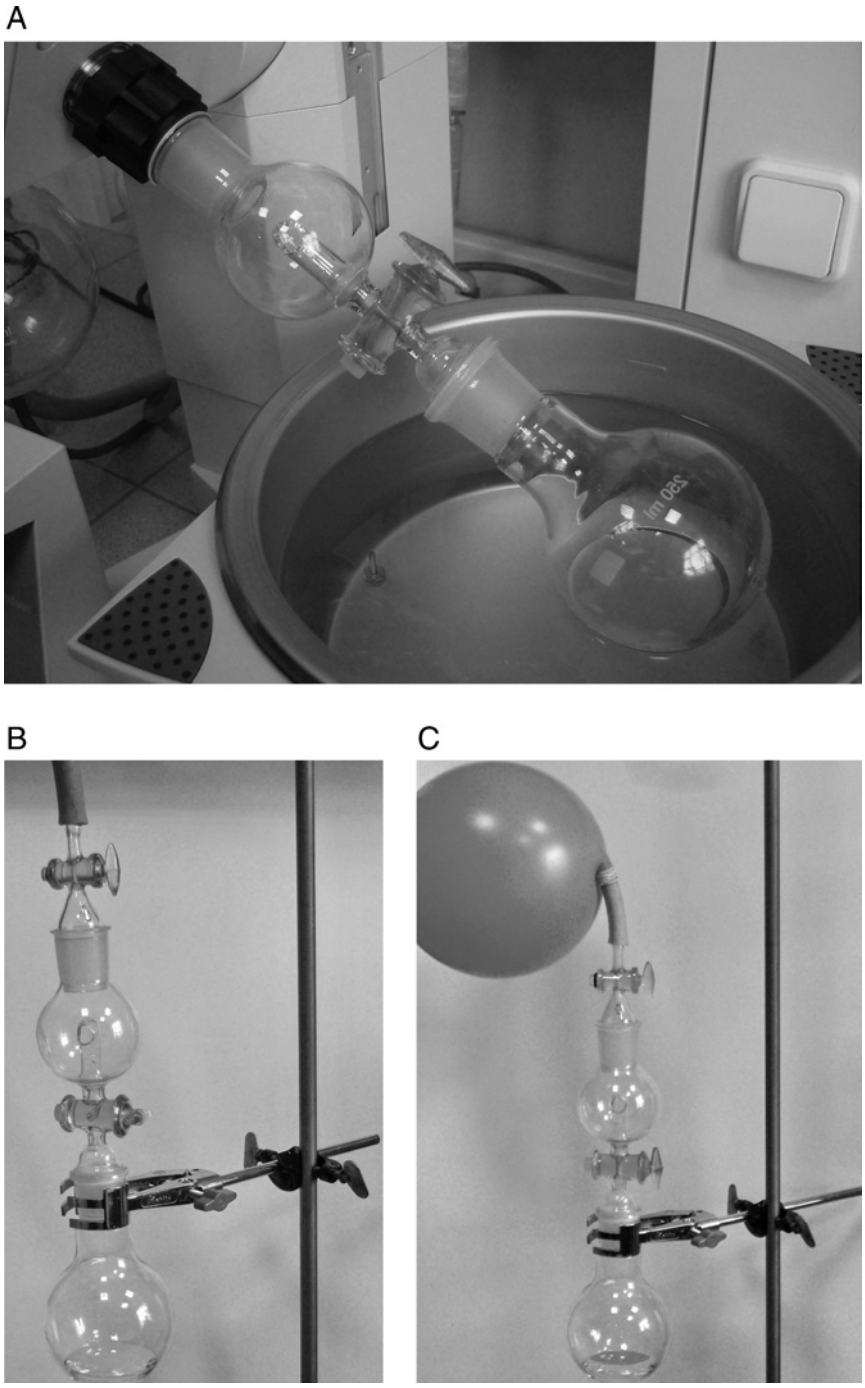

Figure 1.14.5 Protection from atmospheric moisture for synthesis of the 3′,5′-protected starting thymidine nucleoside. (**A**) Evaporation of volatile solvents from a 250-mL round-bottom flask with adaptor and stopcock using a rotary evaporator connected to a vacuum system. (**B**) A 250-mL round-bottom flask with two adaptors and stopcocks connected to a vacuum system. The upper stopcock is opened. (**C**) Flashing the whole reaction system with nitrogen or argon using a 250-mL round-bottom flask with two adaptors connected to a balloon of nitrogen or argon with both stopcocks opened.

16. Characterize the compound by TLC and ^{1}H NMR.

The compound is stable for at least 12 months at 20°C.

*1-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)thymine (**S.11**): Yield of white crystals 11.7 g (82%). m.p. 158°-159°C (EtOH). TLC: R_f 0.45 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 8.32 brs (1H, NH), 8.15-7.32 m (15H, Ph), 7.13 q (1H, $J_{6,Me} = 1.2$, H6), 6.39 d (1H, $J_{1',2'} = 6.4$, H1′), 5.87 dd (1H, $J_{3',2'} = 6.0$, $J_{3',4'} = 3.7$, H3′), 5.74 dd (1H, H2′), 4.83 dd (1H, $J_{5'a,4'} = 2.6$, $J_{5'a,5'b} = -12.2$, H5′a), 4.63 ddd (1H, $J_{4',5'b} = 3.4$, H4′), 4.61 dd (1H, H5′b), 1.55 d (3H, Me).*

Prepare S.12

17. Weigh 11.4 g (20 mmol) **S.11** into a 500-mL round-bottom flask. Add 200 mL of 0.2 N NaOMe, stopper the flask, and stir the reaction for 30 min at room temperature.

18. Monitor reaction by TLC in 98:2 (v/v) CH_2Cl_2/MeOH.

*The starting **S.11** ($R_f = 0.45$) usually disappears after 30 min at 20°C. The product **S.12** ($R_f = 0.01$) moves slower in the same solvent.*

19. Remove MeOH under reduced pressure using a rotary evaporator in vacuo and dissolve the residue in 100 mL water and 100 mL chloroform.

20. Separate the aqueous layer using a 500-mL separatory funnel and wash with 50 mL chloroform.

21. Apply aqueous solution to a 3 × 20–cm chromatography column containing 50 mL of Dowex 50 × 4 (100 to 200 mesh) in H+ form packed in water.

22. Elute with water and collect 50-mL UV-containing fractions until elution is complete (elution volume will vary from 200 to 400 mL). Monitor fractions by TLC using 9:1 (v/v) CH_2Cl_2/MeOH ($R_f = 0.11$.).

23. Combine the fractions that contain **S.12** in a 500-mL round-bottom flask and evaporate to dryness using a rotary evaporator connected to a vacuum system.

24. Add 30 mL EtOH, stopper the flask, and keep the mixture for 16 hr at 0°C.

25. Collect the precipitate by vacuum filtration on a glass filter (porosity 3), wash the precipitate with 10 mL EtOH followed by 10 mL diethyl ether, and dry **S.12** over phosphorus pentoxide in a vacuum desiccator overnight.

26. Characterize the compound by TLC, UV, and ^{1}H NMR.

The compound is stable for at least 12 months at 20°C.

*1-(β-D-Ribofuranosyl)thymine (**S.12**): Yield of white crystals 4.0 g (78%). m.p. 180°-181°C (EtOH). TLC: R_f 0.11 (9:1 v/v CH_2Cl_2/MeOH). UV (pH 1-7): λ_{max} 269 nm (ε9200); (pH 13): λ_{max} 264 nm (ε7300). ^{1}H NMR (400 MHz, D_2O): 7.62 s (1H, H6), 5.85 d (1H, $J_{1',2'} = 4.9$ Hz, H1′), 4.29 dd (1H, $J_{2',3'} = 5.2$ Hz, H2′), 4.19 dd (1H, $J_{3',4'} = 5.4$ Hz, H3′), 4.08 ddd (1H, $J_{4',5'a} = 3.0$ Hz, $J_{4',5'b} = 4.3$ Hz, H4′), 3.87 dd (1H, $J_{5'a,5'b} = -12.8$ Hz, H5′a), 3.78 dd (1H, H5′b), 1.85 s (3H, Me).*

Prepare S.6b

27. Weigh 2.6 g (10 mmol) of dry **S.12** in a 100-mL round-bottom flask and add 20 mL anhydrous pyridine. Evaporate using a rotary evaporator equipped with a water aspirator and then apply a dry nitrogen atmosphere (steps 3 to 4, Fig. 1.14.5).

28. Dissolve the residue in 40 mL pyridine with magnetic stirring. Rapidly add 3.14 mL (10 mmol) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (Markiewicz reagent) in one portion, stopper the flask, and keep 4 hr at 20°C.

29. Monitor reaction by TLC using 98:2 (v/v) CH_2Cl_2/MeOH.

*The starting **S.12** ($R_f = 0.01$) usually disappears after 4 to 6 hr at 20°C. The product **S.6b** ($R_f = 0.23$) moves faster in the same solvent.*

30. Remove nearly all the pyridine using a rotary evaporator connected to a vacuum system and dissolve the residue in 50 mL water and 100 mL CH_2Cl_2.

31. Separate the organic layer using a 250-mL separatory funnel and wash with 20 mL saturated sodium bicarbonate solution and then with 20 mL water.

32. Dry the organic layer over ~10 g Na_2SO_4, filter off Na_2SO_4 by gravity filtration, and wash the precipitate with 20 mL CH_2Cl_2.

33. Evaporate the combined filtrates using a rotary evaporator connected to a vacuum system. Remove traces of pyridine by co-evaporating two times with 20 mL toluene.

34. Prepare a slurry of 50 g of silica gel in CH_2Cl_2 and pour it into a 3 × 20–cm chromatography column.

35. Dissolve the obtained residue in a minimal amount CH_2Cl_2 and layer it carefully on top of the silica gel.

36. Wash column with 300 mL CH_2Cl_2 and 300 mL of 0.5% (v/v) MeOH in CH_2Cl_2. Elute with 1% (v/v) MeOH in CH_2Cl_2. Collect 25-mL fractions.

37. Evaluate fractions by TLC and combine the fractions that contain only **S.6b.** Evaporate the volatile materials from the combined fractions using a rotary evaporator connected to a vacuum system, and dry the residual foam for 2 to 3 hr using a vacuum oil pump.

38. Grind the foam with a stainless steel spatula and dry the resulting powder 12 to 16 hr using a vacuum oil pump.

39. Characterize the product by TLC and ^{1}H NMR.

The compound is stable for at least 12 months at ambient temperature.

*1-[3,5-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]thymine (**S.6b**): Yield of white amorphous solid 4.30 g (86%). TLC: R_f 0.23 (98:2 v/v CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): 9.18 brs (1H, NH), 7.44 s (1H, H6), 5.70 s (1H, H1′), 4.38 dd (1H, $J_{3',2'} = 5.1$, Hz $J_{3',4'} = 8.6$ Hz, H3′), 4.18 dd (1H, $J_{5'a,4'} = 2.5$ Hz, $J_{5'a,5'b} = -13.2$ Hz, H5′a), 4.17 d (1H, H2′), 3.95 ddd (1H, $J_{4',5'b} = 2.9$ Hz, H4′), 4.01 dd (1H, H5′b), 1.89 s (3H, Me), 1.09-1.02 m (28H, iPr). ^{13}C NMR ($CDCl_3$): 163.93 (C4), 150.21 (C2), 135.99 (C6), 110.74 (C5), 91.42 (C1′), 82.14 (C4′), 75.15 (C2′), 69.57 (C3′), 60.81 (C5′), 17.57, 17.50, 17.41, 17.38, 17.22, 17.14, 17.10, 17.02, 13.59, 13.15, 12.91 and 12.76 (iPr), 12.63 (Me).*

COMMENTARY

Background Information

Disaccharide nucleosides belong to an important group of natural compounds that form poly(ADP-ribose) and are found in tRNA, antibiotics, and other physiologically active compounds. To date, ~100 disaccharide nucleosides and related derivatives have been isolated from a variety of natural sources. These compounds contain an extra carbohydrate residue linked to one of the nucleoside hydroxyl groups via an *O*-glycosidic bond. The disaccharide residue and heterocyclic base make their properties similar to those of carbohydrates and nucleosides. These compounds manifest a broad spectrum of biological activities, including antibacterial, fungicidal, herbicidal, antitumoral, and antiviral (Lerner, 1991; Efimtseva and Mikhailov, 2004; Nauwelaerts et al., 2004).

An effective and simple synthesis of 2′-*O*-β-D-ribofuranosylnucleosides, minor tRNA components, has been recently elaborated (Mikhailov et al., 1997a; Markiewicz et al., 1998). The method consists of the condensation of a small excess of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose activated with

tin tetrachloride with *N*-protected 3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl-ribonucleosides in 1,2-dichloroethane. O-Glycosylation proceeds stereospecifically with the formation of a β-glycosidic bond. Yields of disaccharide nucleosides reach 70% to 80% if the reaction is performed at 0°C (Mikhailov et al., 1997a). O-Glycosylation proceeds stereospecifically with formation of a β-glycosidic bond.

After complete deprotection, 2′-*O*-β-D-ribofuranosylnucleoside disaccharide nucleosides are obtained in good overall yields (Mikhailov et al., 1997a; Markiewicz et al., 1998). This method has been used for the preparation of pyrimidine 3′-*O*-β-D-ribofuranosyl-2′-deoxyribonucleosides and 3′-*O*-β-D-ribopyranosyl-2′-deoxyribonucleosides (Mikhailov et al., 1996), and for 5′-*O*-β-D-ribofuranosyl-2′-deoxyribonucleosides and 5′-*O*-β-D-ribofuranosylnucleosides (Mikhailov et al., 1997b, 1998). To study the broad applicability of the method, some other sugars such as fully acylated D- and L-arabinofuranose, D-ribopyranose, and D-erythrofuranose have been used in the O-glycosylation reaction, and the corresponding disaccharide nucleosides have been incorporated into oligodeoxyribonucleotides and oligoribonucleotides (Efimtseva et al., 2001). Melting point determinations demonstrated that modified oligonucleotides containing disaccharide nucleosides form stable duplexes with complementary RNA ($\Delta T_m = 0°C$; Efimtseva et al., 2001). The duplex RNA maintains an A-type helical geometry with the extra 2′-*O*-ribose moiety located in the minor groove. This implies that the voluminous extra sugar moiety has almost no effect on the stability of the RNA duplex (Luyten et al., 2000).

Oligonucleotides containing 2′-*O*-ribofuranosylnucleosides have been used as modified primers in RNA-templated DNA synthesis catalyzed by HIV reverse transcriptase. It was shown that an additional 2′-ribofuranose residue in a specific position (-3-4) of the primer prevents elongation (Andreeva et al., 2002). An important feature of these oligonucleotides is the presence of an additional cis-diol group, which may be readily oxidized with sodium periodate to give oligonucleotide derivatives with aldehyde groups placed anywhere in the sequence (Efimtseva and Mikhailov, 2002). Such oligonucleotides were used for affinity labelling of different enzymes (Tunitskaya et al., 1999; Gritsenko et al., 2002). The incorporation of disaccharide nucleosides into oligonucleotides opens new possibilities for the functionalization of nucleic acids. The extra sugar ring attached to a nucleoside can serve several purposes. For the synthetic work, it is important that acyl blocking groups used in disaccharide synthesis are compatible with the chemistry of automated oligonucleotide synthesis.

Compound Characterization

The structure of the compound is supported by NMR spectroscopy and mass spectrometry. The ^{1}H NMR spectra of the obtained compounds are rather complicated due to the presence of two ribofuranose residues. In spite of this, most of the chemical shifts and coupling constants may be calculated directly from the NMR spectra. In some cases, comparison with the published spectra of disaccharide nucleosides and ^{1}H-^{13}C correlation and COSY spectra were used for assignment. The chemical shifts were assigned using double resonance techniques and COSY experiments.

Several conclusions were drawn from the ^{1}H NMR spectral analysis. In nucleosides **S.1** and **S.6** and disaccharide nucleosides **S.3** and **S.7** with a 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl group, the coupling constants $J_{1',2'}$ of nucleoside moieties are <0.5 Hz (Robins et al., 1983). The O-glycosylation reaction proceeded stereospecifically with the formation of 1″,2″-*trans*-substituted disaccharide nucleosides **S.3** and **S.7**. The coupling constants ($J_{1'',2''}$) in the additional ribose moiety are <0.5 Hz. As in the case of disaccharide nucleosides (Mikhailov et al., 1997a) and 2′-*O*-methylnucleosides (Robins et al., 1981), incorporation of the 2′-*O*-substitutent results in a low field shift (+2+3 ppm) of C2′ of the nucleoside moiety in ^{13}C NMR spectra.

Critical Parameters and Troubleshooting

The synthesis of 2′-*O*-β-D-ribofuranosylnucleosides is short, straightforward, and fairly efficient. However, careful attention to details of basic organic synthesis procedures is required. Preparation of the various compounds requires prior experience with routine chemical laboratory techniques such as solvent evaporation, extraction, TLC, and column chromatography. Characterization of the products demands knowledge of ^{1}H and ^{13}C NMR, UV, and mass spectroscopy. General laboratory safety is also of primary concern when hazardous materials are involved. Strict adherence to the outlined methods is therefore highly recommended.

All solvents should be distilled before use. Anhydrous solvents are very important. They should be either freshly distilled and stored over molecular sieves, or be taken from a freshly opened bottle of commercially prepared anhydrous solvent. It is important for each step of the syntheses that the starting compounds be thoroughly dried either by coevaporation with anhydrous pyridine or in a desiccator over P_2O_5. Protection of reaction mixtures from atmospheric moisture (as shown in Figs. 1.14.2 and 1.14.5) will enhance yields, especially in the case of ribosylation and silylation reactions.

For all compounds, a sample of 50 to 100 mg should be kept as a reference. In all cases, the reaction progress is followed by TLC. A baseline is marked and the spots (~1 optical unit) of the reaction mixture and starting material are placed at equal distances. For the preparation of **S.3** and **S.7**, where the R_f values of the starting compounds and products are very similar, a mixed sample containing both starting compound and reaction mixture may be placed on the TLC plate. After developing in the appropriate solvent, the end-line of the TLC is marked, and spots are identified under a UV lamp (254 nm).

Before each column purification, TLC is performed on the reaction mixture to evaluate the completeness of the reaction and to choose the optimal elution system for column chromatography. The silica gel for column chromatography (0.06 to 0.20 mm) is suspended in methylene chloride and loaded into the column, which is tightly packed by gentle tapping until there is a constant volume. The crude product is dissolved in a minimal volume of solvent and is carefully applied using a glass Pasteur pipet. After sample application, the flask and column walls are washed with a minimal volume of solvent. For protection, the silica layer is topped by a 1-cm layer of sand.

Anticipated Results

The method presented here generally produces high yields even for inexperienced workers. Good overall yields (46% to 72%) of the final 2′-*O*-ribofuranosylnucleosides (**S.5** and **S.9**) from the Markiewicz-protected nucleosides (**S.1** and **S.6**) are expected following these procedures. 2′-*O*-Ribosylation proceeds stereospecifically with formation of a β-glycosidic bond. The first ribosylation reaction is carried out under mild conditions (0°C, 1,2-dichloroethane, 2 hr for pyrimidine nucleosides, 7 to 16 hr for purine derivatives) and the yields of the target compounds are 72% to 80% (Mikhailov et al., 1997a). At room temperature, the reaction occurs faster (30 min), but the yields in the coupling steps are lower (40% to 77%; Markiewicz et al., 1998).

Time Considerations

The three-step preparation of 2′-*O*-β-D-ribofuranosylnucleosides starting from Markiewicz-protected nucleosides can be accomplished in 1 week. Each column chromatography step requires 3 to 5 hr. Normally the first two steps (ribosylation and silylation) may be performed in 3 to 4 working days, including purification and spectroscopic analysis. The final deacylation reaction normally proceeds in 2 to 3 days at room temperature and may be run over a weekend. The three-step preparation of the protected thymine starting nucleoside (**S.6b**) may be accomplished in 1 week.

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