Synthesis of Protected 2′-Deoxy-2′-fluoro-β-D-arabinonucleosides

This unit describes in detail the preparation of protected 2′-deoxy-2′-fluoroarabinonucleosides. These building blocks are required for the synthesis of 2′-deoxy-2′-fluoroarabinonucleic acid (2′F-ANA), an oligonucleotide analog exhibiting very promising antisense properties (Damha et al., 1998; Wilds and Damha, 2000; Lok et al., 2002). The preparation of phosphoramidites from these building blocks and the synthesis of 2′F-ANA are described in *UNIT 4.15*.

SYNTHESIS AND CHARACTERIZATION OF N^2-ISOBUTYRYL-9-[2-DEOXY-2-FLUORO-5-*O*-(4-METHOXYTRITYL)-β-D-ARABINOFURANOSYL]GUANINE

BASIC PROTOCOL 1

Synthesis of araF-G (**S.6**; Figure 1.7.3) was accomplished via the condensation of 2,6-dichloropurine with either 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (**S.2**; Figure 1.7.1) or 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**) as a key chemical step (Figure 1.7.2). Starting from **S.3** gives a higher yield of **S.4** and simplifies its purification. The versatile intermediate N^9-β-glycoside (**S.4**) was transformed into araF-G in three steps (Figure 1.7.3). Treatment of **S.4** with NaN_3 in ethanol gave the 2,6-diazido derivative (Wower et al., 1994). This product was then subjected to reduction with $SnCl_2$ in a mixture of dichloromethane/methanol to give its 2,6-diamino derivative (Tennila et al., 2000). Standard debenzoylation and deamination gave araF-G. Transient protection of the 3′- and 5′-OH of araF-G (Kierzek, 1985), followed by acylation at N2 and 5′-*O*-tritylation, gave **S.7** (Figure 1.7.3) in acceptable yields.

Materials

Nitrogen gas source
1,3,5-Tri-*O*-benzoyl-α-D-ribofuranose (**S.1**; Pfanstiehl)
Dichloromethane, dry (see recipe)
[Bis(2-methoxyethyl)amino]sulfur trifluoride (MAST; Aldrich) or (diethylamino)sulfur trifluoride (DAST; Aldrich)
Saturated aqueous sodium bicarbonate
Sodium sulfate (Na_2SO_4), anhydrous
Silica gel (230 to 400 mesh)
Chloroform

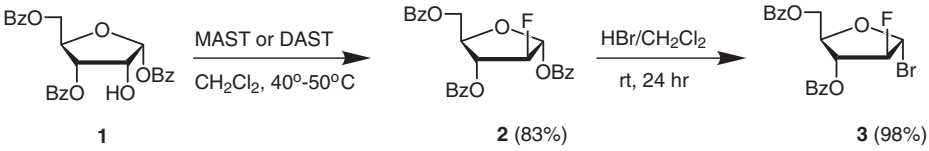

Figure 1.7.1 Synthesis of 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**). The expected yields are given in parentheses. Bz, benzoyl; DAST, diethylaminosulfur trifluoride; HBr, 30% (w/v) hydrogen bromide in acetic acid; MAST, [bis(2-methoxyethyl)amino]sulfur trifluoride.

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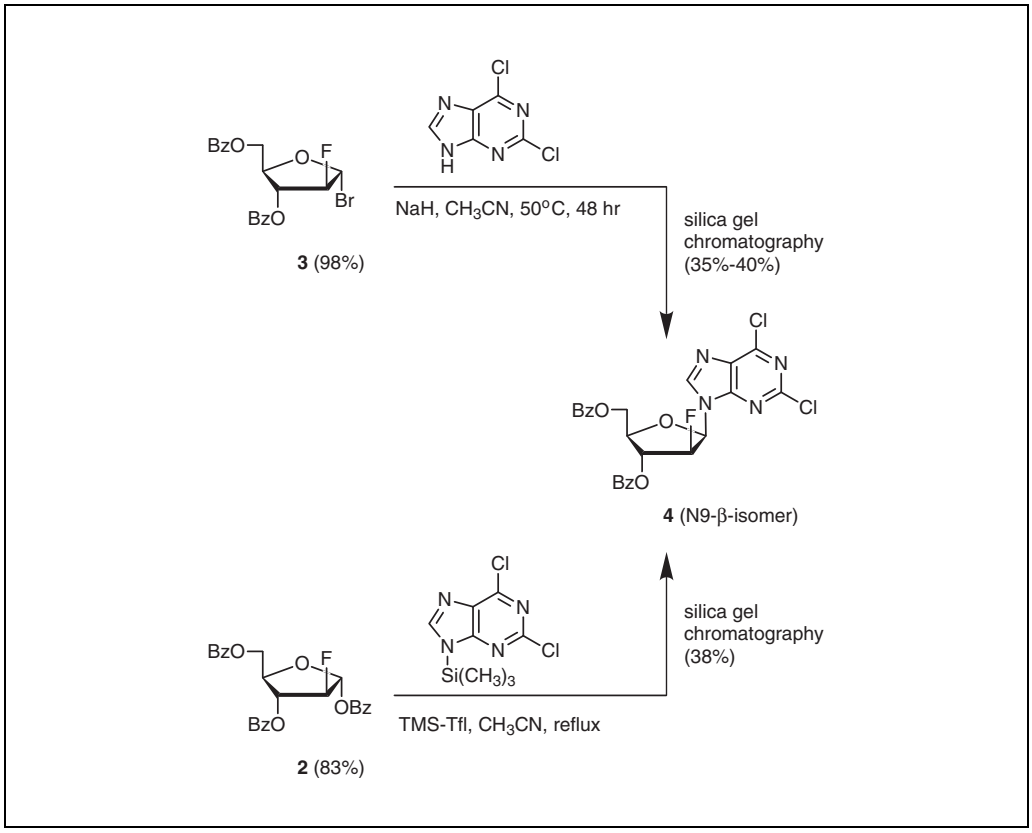

Figure 1.7.2 Synthesis of 2,6-dichloro-9-(3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)purine (**S.4**) from **S.3** or **S.2**. The expected yields are given in parentheses. Bz, benzoyl; NaH, 60% (w/v) sodium hydride in oil; TMS-Tfl, trimethylsilyl trifluoromethanesulfonate.

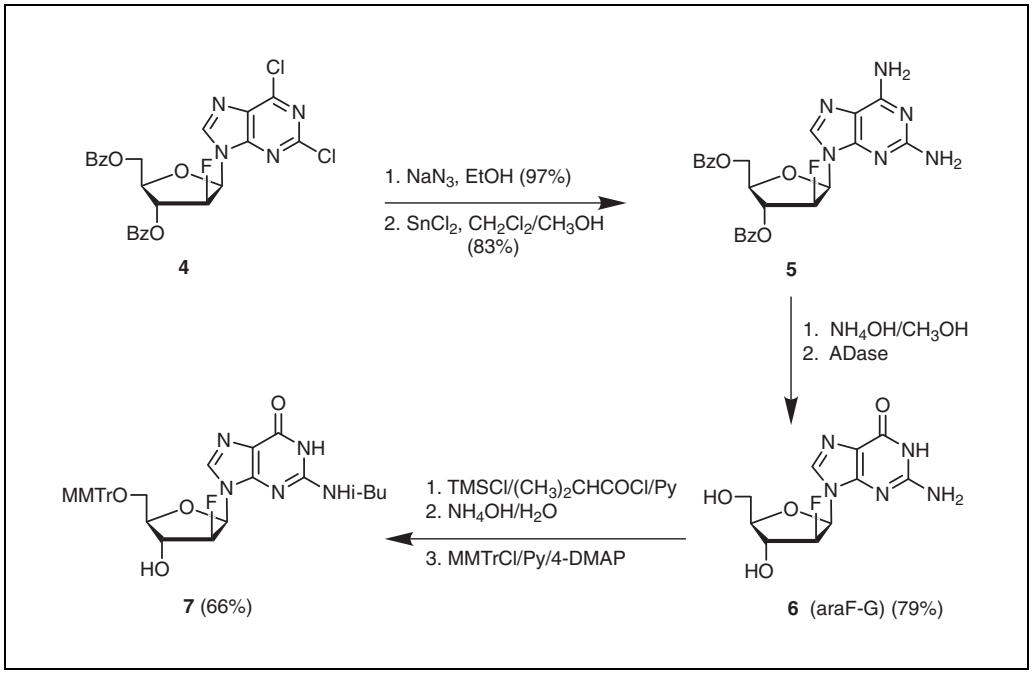

Figure 1.7.3 Synthesis of araF-G (**S.6**) in four steps from **S.4**, followed by introduction of isobutyryl (*i*-Bu) and monomethoxytrityl (MMTr) groups into the N2 and O5′ positions, respectively, to give **S.7**. The expected yields are given in parentheses. ADase, adenosine deaminase; 4-DMAP, 4-dimethylaminopyridine; EtOH, 95% (v/v) ethanol; NH_4OH, 29.7% (w/w) aqueous ammonia; MMTr·Cl, *p*-anisylchlorodiphenylmethane (or monomethoxytrityl chloride); Py, pyridine; TMSCl, chlorotrimethylsilane.

Sand
Merck thin-layer chromatography (TLC) silica plates (Kieselgel 60 F-254; 0.2 mm thick)
30% (w/v) HBr in acetic acid
Chlorotrimethylsilane (TMSCl)
2,6-Dichloropurine
Hexamethyldisilazane (HMDS)
Acetonitrile, dry (see recipe)
Trimethylsilyl trifluoromethanesulfonate (TMS-Tfl)
5:1 (v/v) toluene/ethyl acetate
Sodium hydride, as a 60% (w/v) solution in mineral oil (Aldrich)
Sodium azide (NaN_3)
95% (v/v) ethanol
Tin dichloride ($SnCl_2$)
Methanol
29.7% (w/w) aqueous ammonia (Fisher)
5% and 10% (v/v) methanol in dichloromethane
Adenosine deaminase solution in 50% glycerol (from calf intestine mucosa, specific activity 160 to 200 U/mg protein; Sigma)
Phosphorus pentoxide (P_2O_5)
Pyridine, dry (see recipe)
Isobutyryl chloride
2:1:1 (v/v/v) water/ethyl acetate/ether
4-Dimethylaminopyridine (4-DMAP)
p-Anisylchlorodiphenylmethane (monomethoxytrityl chloride or MMTr·Cl)
0% to 5% (v/v) gradient of methanol in dichloromethane

Oven-dried glassware, including:
25-, 100-, and 250-mL round-bottom flasks
1-L Erlenmeyer flasks
Reflux condenser
Oil bath, 40° to 50°C
1-L separatory funnels
Rotary evaporator equipped with a vacuum pump or water aspirator
Chromatography columns: 5×50 cm, 3×15 cm, and 3×20 cm

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

Fluorinate 1,3,5-tri-O-benzoyl-α-D-ribofuranose to give S.2

1. In an oven-dried 250-mL round-bottom flask equipped with a reflux condenser, a stir bar, and nitrogen gas source, dissolve 10 g **S.1** in 100 mL dry dichloromethane.

2. While stirring, add 8 mL (43.39 mmol) MAST or 9 mL (68.12 mmol) DAST dropwise.

   It is better to use MAST. It is less expensive, gives higher yields, and makes purification easier.

   CAUTION*: DAST is a flammable and corrosive liquid. MAST is a toxic and corrosive liquid. Both must be handled with gloves in a well-ventilated fume hood. In case of skin contact freely apply calcium gluconate gel (Pharmascience). Reapply and continue application for an additional 10 to 15 min while seeking medical assistance.*

3. Place the flask in an oil bath preheated to 40° to 50°C and stir 24 hr or until the reaction is complete as analyzed by TLC using Merck TLC (*APPENDIX 3D*) silica plates.

   *The starting material should be run alongside the reaction for comparison. The plates are developed using dichloromethane, and the bands are visualized by UV shadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. Typically, the R_f value of the desired compound (**S.2**) is 0.34 (dichloromethane).*

4. Dilute the reaction mixture with 200 mL dichloromethane and add it carefully to 300 mL saturated aqueous sodium bicarbonate solution.

5. Pour the mixture into a 1-L separatory funnel and allow the phases to separate. Pour the lower, yellow organic phase into a 1-L Erlenmeyer flask.

6. Dry the organic layer over anhydrous Na_2SO_4. Filter and evaporate the filtrate to dryness in a rotary evaporator equipped with a vacuum pump or water aspirator to give a yellow solid.

7. Prepare a slurry of 350 g silica gel in chloroform. Pour slurry into a 5 × 50–cm chromatography column and carefully layer 2 cm sand on top of slurry (see *APPENDIX 3E* for column chromatography).

8. Dissolve the crude product (step 6) in a minimal amount of chloroform and layer it carefully on top of the column.

9. Elute with chloroform and collect 200-mL fractions (typically a total of 2 to 4 L). Combine fractions that contain pure product, as determined by TLC. Evaporate to dryness in a rotary evaporator and dry overnight under high vacuum.

   *Typically, the R_f value of the desired product (**S.2**) is 0.34 (dichloromethane).*

10. Check purity of the product.

    *2-Deoxy-2-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose (**S.2**): 8.3 g (83%); TLC (dichloromethane) 0.34; ^{1}H NMR (400 MHz, acetone-d_6, tetramethylsilane as internal reference): 8.1 to 7.4 (15 H, m, Bz), 6.7 (1H, d, $J_{1,F}$ = 9.2 Hz, H-1), 5.6 and 5.7 (1H, dd, $J_{3,F}$ = 20 Hz, $J_{3,2}$ = 3.6 H_Z, H-3), 5.5 to 5.7 (1H, d, $J_{2,F}$ = 48 Hz, H-2), 4.9 (1H, m, H-4), 4.7 to 4.8 (2H, m, H-5 and H-5′); ^{19}F NMR (300 MHz, dimethyl sulfoxide [DMSO]-d_6, 99% trifluoracetic acid as external standard): −113 (ddd).*

    *To prepare **S.4** from **S.2**, proceed to step 17a. It is better, however, to brominate as in steps 11 to 16 and use **S.3** in the condensation reaction (go to step 17b). This gives a higher yield of **S.4**, and affords only the N7- and N9-β-isomers, making purification easier.*

Brominate to give S.3

11. In an oven-dried 100-mL round-bottom flask, dissolve 2.2 g (4.85 mmol) **S.2** in 30 mL dry dichloromethane and add 4 mL of 30% HBr in acetic acid.

12. Stir the reaction mixture 24 hr at room temperature.

13. Analyze the reaction by TLC.

    *The starting material should be run alongside the reaction for comparison. The plates are developed using dichloromethane, and the bands are visualized by UV shadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. Typically, the R_f value of the desired compound (**S.3**) is ~0.55 (dichloromethane).*

14. Dilute the reaction mixture with 100 mL dichloromethane and add it carefully to 200 mL saturated aqueous sodium bicarbonate.

15. Pour the mixture into a clean 1-L separatory funnel and allow the phases to separate. Pour the lower, brown organic phase into a 1-L Erlenmeyer flask. Dry the organic layer over anhydrous Na_2SO_4. Filter and evaporate the filtrate to dryness in the rotary evaporator to give a brown oil.

16. Check the purity of the product.

*2-Deoxy-2-fluoro-3,5-di-O-benzoyl-α-D-arabinofuranosyl bromide (**S.3**): 2.0 g (98%); TLC (dichloromethane) 0.55; ^{1}H NMR (500 MHz, CD_2Cl_2): 8.2 to 7.3 (10 H, m, Bz), 6.8 (1H, d, $J_{1,F}$ = 19 Hz, H-1), 5.6 (1H, d, $J_{2,F}$ = 50 Hz, H-2), 5.4 (1H, dd, H-3), 4.8 (3H, m, H-4, H-5, and H-5″).*

Condense 2,6-dichloropurine with S.2 or S.3

For S.2:

17a. In an oven-dried 250-mL round-bottom flask equipped with a reflux condenser, stir bar, and nitrogen gas source, add 4 mL TMSCl to a suspension of 2.84 g (15 mmol) 2,6-dichloropurine in 40 mL HMDS. Reflux 2 to 3 hr at 120°C, cool down, and evaporate to dryness in the rotary evaporator.

18a. Azeotrope (co-evaporate) the residue with 50 mL dry acetonitrile. To this residue, add 4 g (8.0 mmol) **S.2** in 100 mL dry acetonitrile followed by 4 mL TMS-Tfl. Reflux the resulting solution 45 min at 80°C.

19a. Analyze the reaction by TLC.

*The starting material should be run alongside the reaction for comparison. The plates are developed using 9:1 (v/v) toluene/ethyl acetate, and the bands are visualized by UV shadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. Typically, the R_f value of the desired product, the N9-β-isomer (**S.4**), is 0.28 (9:1 toluene/ethyl acetate).*

20a. Cool the mixture, dilute with 200 mL dichloromethane, and wash it carefully with 300 mL saturated sodium bicarbonate solution.

21a. Dry the dichloromethane layer over anhydrous Na_2SO_4, filter, and evaporate to dryness.

22a. Prepare a 5 × 50–cm silica chromatography column as described in step 7. Apply the residue (step 21a) to the column and elute with a mixture of 5:1 toluene/ethyl acetate. Collect 50-mL fractions and combine those that contain pure product (**S.4)** as determined by TLC.

23a. Evaporate combined fractions to dryness in the rotary evaporator and dry overnight under a high vacuum. Proceed to step 24.

For S.3:

17b. In a clean oven-dried 250-mL round-bottom flask equipped with reflux condenser, stir bar, and nitrogen gas source, dissolve 0.54 g (2.84 mmol) of 2,6-dichloropurine and 0.07 g (2.95 mmol) sodium hydride in 30 mL dry acetonitrile. Stir 45 min at room temperature.

18b. Dissolve 1.2 g (2.84 mmol) **S.3** in 20 mL dry acetonitrile and add it in portions to the flask. Place the flask in an oil bath and stir 48 hr at 50°C or until the reaction is complete as determined by TLC.

*The starting material should be run alongside the reaction for comparison. The plates are developed using 9:1 (v/v) toluene/ethyl acetate, and the bands are visualized by UV shadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. Typically, the R_f value of the desired N9-β-isomer (**S.4**) is 0.28 (9:1 toluene/ethyl acetate). Side products have R_f values in the range of 0.20 to 0.23 with the same solvent system.*

19b. Vacuum filter the resulting mixture and evaporate to dryness.

20b. Prepare a 5 × 50–cm silica chromatography column as described in step 7. Apply the residue (step 19b) to the column and elute with 5:1 toluene/ethyl acetate. Collect 50-mL fractions and combine those that contain the pure product (**S.4**) as determined by TLC.

21b. Evaporate combined fractions to dryness in the rotary evaporator and dry overnight under high vacuum. Proceed to step 24.

24. Check the purity of the product.

*9-(2-Deoxy-2-fluoro-3,5-di-O-benzoyl-β-D-arabinofuranosyl)-2,6-dichloropurine (**S.4**): average yield of N9-β-isomer is 38% from each condensation (i.e., 2,6-dichloropurine with **S.2** or **S.3**); TLC (9:1 [v/v] toluene/ethyl acetate) 0.28; ^{1}H NMR (400 MHz, $CDCl_3$): 8.1 to 7.1 (10 H, m, Bz), 8.4 (1H, d, $J_{8,F}$ = 3.2 Hz, H-8), 6.6 (1H, dd, $J_{1',2'}$ = 2.8 Hz, $J_{1',F}$ = 22 Hz, H-1′), 5.7 (1H, dd, $J_{3',F}$ = 17 Hz, $J_{3',4'}$ = 2.8 Hz, H-3′), 5.4 (1H, dd, $J_{2',F}$ = 50 Hz, $J_{2',3'}$ = 2.0 Hz, H-2′), 4.7 to 4.8 (2H, dd, H-5′ and H-5″), 4.61 (1H, m, H-4′); ^{19}F NMR (300 MHz, DMSO-d_6, 99% [v/v] trifluoroacetic acid as external reference): −113 (ddd). FAB-MS (fast atom bombardment mass spectrometry, NBA-matrix): 531 [M+H$^+$].*

Transform N^9-β-glycoside (S.4) into araF-G (S.6)

25. In a clean oven-dried 250-mL round-bottom flask equipped with reflux condenser and stir bar, dissolve 0.4 g (0.75 mmol) **S.4** and 0.25 g (3.7 mmol) NaN_3 in 50 mL of 95% ethanol.

Use sodium azide instead of lithium azide to avoid partial debenzoylation of the intermediary 2,6-diazido derivative.

26. Reflux the reaction mixture 2 hr in the 80°C oil bath. Cool mixture and evaporate to dryness.

Precipitation of sodium chloride is a good sign of a successful reaction.

The R_f values for the 2,6-diazido and 2,6-dichloro derivatives are 0.48 and 0.52, respectively, in 3:1 (v/v) toluene/ethyl acetate.

27. Dissolve the residue in dichloromethane, wash the organic layer with water, dry it over anhydrous Na_2SO_4, and evaporate to yield a syrupy product.

A yield of 0.4 g (97%) is expected. Silica column chromatography is not required after this step.

28. Treat 0.4 g (0.73 mmol) of 2,6-diazidopurine nucleoside (step 27) with 0.42 g (2.22 mmol) $SnCl_2$ in a mixture of 30 mL dichloromethane and 3 mL methanol for 40 min at room temperature.

*The R_f value for the 2,6-diamino derivative (**S.5**) is 0.42 in 9:1 (v/v) dichloromethane/methanol.*

29. Dilute the mixture with 100 mL dichloromethane, wash the organic layer with water, dry it over anhydrous Na_2SO_4, and evaporate to yield a white solid.

The expected yield is 0.3 g (83%). Silica column chromatography is not required after this step.

30. Treat 0.3 g (0.61 mmol) **S.5** with a mixture containing 3 mL of 29.7% aqueous ammonia and 12 mL methanol for 48 hr at room temperature.

The R_f value for the desired product is 0.06 in 9:1 (v/v) dichloromethane/methanol and 0.16 in 3:1 (v/v) chloroform/ethanol.

31. Evaporate the resulting solution to dryness. Dissolve the crude product in a minimal amount of 5% methanol in dichloromethane and place it carefully on the top of a 3 × 15–cm chromatography column containing 25 g silica gel.

32. Elute with 10% methanol in dichloromethane and collect 50-mL fractions. Combine fractions containing the desired product as determined by TLC and evaporate to dryness in the rotary evaporator to yield a white foam.

33. Check the product.

    *9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-2,6-diaminopurine (**S.5**): 0.14 g (83%); TLC (3:1 [v/v] chloroform/ethanol) 0.16, (9:1 [v/v] dichloromethane/methanol) 0.06; UV (H_2O) λ_{max}, 280 nm; FAB-MS (NBA-matrix): 285 [M+H$^+$], 307 [M+Na$^+$].*

34. Treat 0.14 g (0.49 mmol) of the product with 100 µL adenosine deaminase solution in 5 mL water for 24 hr at room temperature.

35. Collect the precipitated white material by filtration and wash it with 10 mL water and then with 10 mL methanol. Dry the white powder (**S.6**) over P_2O_5.

    This reaction has also been performed on a larger scale using 0.5 g substrate. A yield of 71% was obtained.

36. Check purity of the product.

    *9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (**S.6**): 0.11 g (79%); UV (H_2O) λ_{max}, 251 nm; ^{1}H NMR (400 MHz, DMSO-d_6): 10.6 (1H, s, N-H), 7.8 (1H, d, $J_{8,F}$ = 2.8 Hz, H-8), 6.5 (2H, br s, NH_2), 6.1 (1H, dd, $J_{1',2'}$ = 4.4 Hz, $J_{1',F}$ = 16 Hz, H-1′), 5.9 (1H, d, $J_{OH,3'}$ = 4.8 Hz, HO-C3′), 5.0 and 5.1 (1H, dt or ddd, $J_{2',F}$ = 33 Hz, $J_{2',3'}$ = 3.6 Hz, H-2′), 5.0 (1H, t, HO-C5′), 4.3 (1H, m, $J_{3',F}$ = 14 Hz, H-3′), 3.8 (1H, m, H-4′), 3.6 (2H, m, H-5′ and H-5″); ^{19}F NMR (300 MHz, DMSO-d_6, 99% [v/v] trifluoroacetic acid as external reference): −120 (ddd); FAB-MS (NBA-matrix): 286 [M+H$^+$]. See Figure 1.7.4 for ^{1}H NMR.*

Isobutyrylate and monomethoxytritylate to give S.7

37. In a clean oven-dried 25-mL round-bottom flask, dissolve 0.3 g (1.05 mmol) araF-G (**S.6**) in 5 mL dry pyridine, add 3 mL TMSCl, and stir mixture 45 min at room temperature.

38. Add 500 µL isobutyryl chloride and stir 2 hr at room temperature.

39. Immerse the reaction flask in an ice bath and add 1 mL water. After 10 min, add 1 mL of 29.7% aqueous ammonia and stir 20 min at room temperature.

    The R_f value for N^2-isobutyryl-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine is 0.45 in 3:1 (v/v) chloroform/ethanol.

40. Carefully evaporate the resulting mixture to near dryness and take up the residue in 120 mL 2:1:1 water/ethyl acetate/ether. Evaporate the aqueous layer to get the desired compound as a colorless powder.

41. Check purity of the product.

    N^2-Isobutyryl-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine: 0.35 g (94%); TLC (3:1 [v/v] chloroform/ethanol) 0.45; ^{1}H NMR (300 MHz, DMSO-d_6): 12.1 (1H, N-H), 11.7 (1H, N-H), 8.1 (1H, d, $J_{8,F}$ = 2.4 Hz, H-8), 6.17 (1H, dd, $J_{1',2'}$ = 5.6 Hz, $J_{1',F}$ = 14 Hz, H-1′), 6.0 (1H, d, $J_{OH,3'}$ = 6.0 Hz , HO-C3′), 5.1, 5.2 (1H, dt or ddd, $J_{2',F}$ = 70 Hz, H-2'), 4.3 (1H, ddd, H-3′), 3.8 (1H, dd, H-4′), 3.6 (2H, m, H-5′ and H-5″, 2.5(1H, m, H-C[CH_3]$_2$), 1.1 (6H, s,[CH_3]$_2$- CH).

42. Dissolve 0.3 g (0.85 mmol) of the product in 20 mL dry pyridine and add catalytic amount (~30 mg) 4-DMAP and 0.4 g (1.3 mmol) MMTr·Cl. Stir mixture 48 hr at room temperature.

    The R_f value for the desired tritylated product is 0.41 in 9:1 (v/v) chloroform/methanol.

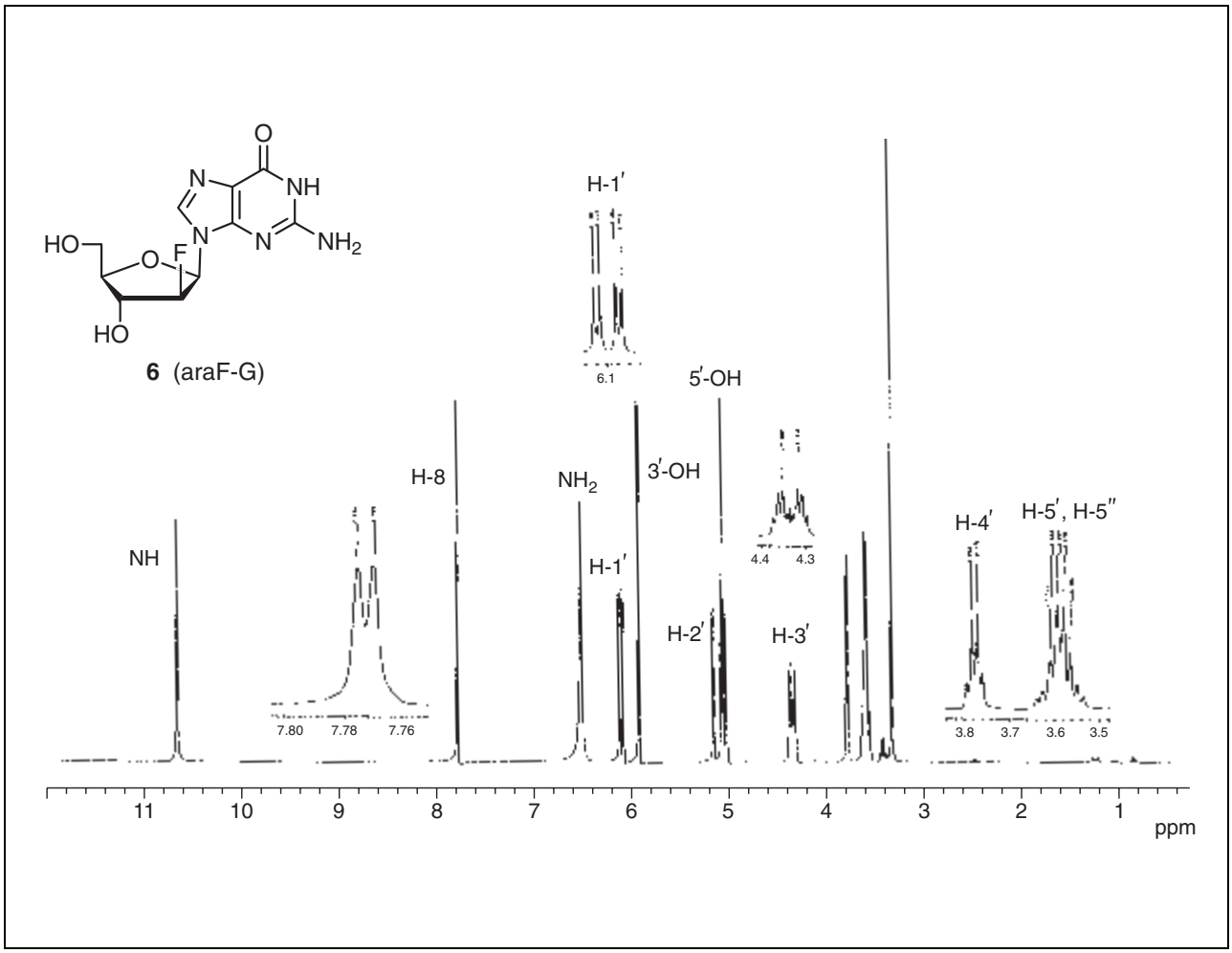

Figure 1.7.4 ^{1}H NMR spectrum (400 MHz) of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine, araF-G (**S.6**), in DMSO-d_6.

43. Evaporate pyridine, take up the residue in dichloromethane, wash the organic layer with saturated aqueous sodium bicarbonate, dry organic layer over anhydrous Na_2SO_4, and evaporate to dryness.

44. Dissolve the crude product in a minimal amount of dichloromethane and place it carefully on the top of a 3 × 20–cm chromatography column containing 38 g silica gel.

45. Elute with 0% to 5% methanol in dichloromethane and collect 50-mL fractions. Combine those containing pure product as determined by TLC.

46. Evaporate combined fractions to dryness in the rotary evaporator to get the desired product as a yellow foam.

47. Check purity of the final product.

*N^2-Isobutyryl-9-[2-deoxy-2-fluoro-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl]guanine (**S.7**): 0.35 g (66%); TLC (9:1 [v/v] chloroform/methanol); ^{1}H NMR (400 MHz, acetone-d_6): 7.8 (1H, d, $J_{8,F}$ = 3.2 Hz, H-8), 7.6 to 6.8 (14H, m, trityl), 6.2 (1H, dd, $J_{1',2'}$ = 6 Hz, $J_{1',F}$ = 16 Hz, H1′), 5.1 (1H, dt or ddd, $J_{2',F}$ = 54 Hz, H-2′), 4.6 (1H, m, H-3′), 4.2 (1H, m, H-4′), 3.6 (3H, s, CH_3O), 3.5 and 3.2 (2H , 2dd, H-5′ and H-5″), 2.1 (1H, m, H-C[CH_3]$_2$), 1.2 and 1.3 (6H, s, [CH_3]$_2$-CH).*

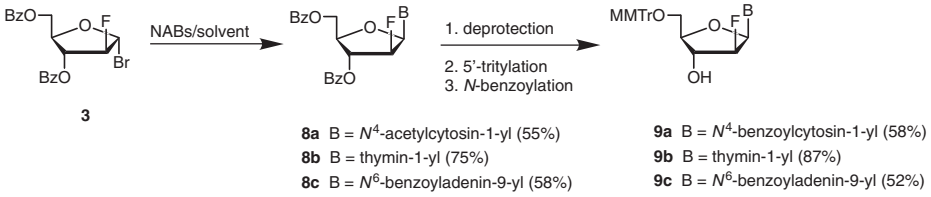

Figure 1.7.5 Synthesis of 2′-deoxy-2′-fluoro-β-D-arabinonucleosides (**S.9a-c**). The expected yields are given in parentheses. Bz, benzoyl; MMTr, *p*-anisyldiphenylmethyl; NABs, nucleic acid bases (silylated-*N*-acetylcytosine or silylated-thymine or *N*-benzoylated-adenine); solvent, dichloromethane or carbon tetrachloride.

SYNTHESIS AND CHARACTERIZATION OF N^4-BENZOYL-1-[2-DEOXY-2-FLUORO-5-*O*-(4-METHOXYTRITYL)-β-D-ARABINOFURANOSYL]CYTOSINE

BASIC PROTOCOL 2

Synthesis of araF-C was accomplished via the condensation of N^4-acetylcytosine with 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**) as a key chemical step (Figure 1.7.5). This was followed by deprotection, benzoylation, and tritylation to give **S.9a**. Cytosine, N^4-benzoylcytosine, or N^4-acetylcytosine can be used for the condensation step. Better yields are observed with N^4-acetylcytosine.

Materials

Nitrogen gas source
Chlorotrimethylsilane (TMSCl)
N^4-Acetylcytosine
Hexamethyldisilazane (HMDS)
2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**; see Basic Protocol 1)
Carbon tetrachloride (CCl_4), anhydrous (Aldrich)
Merck thin-layer chromatography (TLC) silica plates (Kieselgel 60 F-254; 0.2 mm thick)
Dichloromethane, dry (see recipe)
Saturated aqueous sodium bicarbonate
Sodium sulfate (Na_2SO_4), anhydrous
29.7% (w/w) aqueous ammonia (Fisher)
Methanol
5% (v/v) ethanol in chloroform
Silica gel (230 to 400 mesh)
Chloroform
3:1 (v/v) chloroform/ethanol
Benzoic anhydride
N,N-Dimethylformamide (DMF), anhydrous (Aldrich)
Pyridine, dry (see recipe)
p-Anisylchlorodiphenylmethane (monomethoxytrityl chloride or MMTr·Cl)
4-Dimethylaminopyridine (4-DMAP)
0% to 1% (v/v) gradient of methanol in chloroform

250-mL round-bottom flasks, oven dried
Reflux condenser
Oil bath, 120°C and 77°C
Rotary evaporator equipped with a vacuum pump or water aspirator
3 × 20–cm chromatography column

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

Condense N^4-acetylcytosine with S.3

1. In an oven-dried 250-mL round-bottom flask equipped with a reflux condenser, stir bar, and nitrogen gas source, add 15 mL TMSCl to a suspension of 11 g (153.14 mmol) N^4-acetylcytosine in 80 mL HMDS.
2. Reflux 24 hr in an oil bath at 120°C, cool mixture, and evaporate to dryness under reduced pressure using a rotary evaporator equipped with a vacuum pump or water aspirator.
3. Add 10 g (23.64 mmol) **S.3** in 40 mL anhydrous CCl_4 and reflux the resulting solution 4 days at 77°C.
4. Analyze the reaction by TLC (*APPENDIX 3D*) using Merck TLC silica plates.

   *The starting material should be run alongside the reaction for comparison. The plates are developed using 9:1 (v/v) chloroform/methanol, and the bands are visualized by UV shadowing and dipping the plate in 10% (v/v) sulfuric acid in methanol followed by heating. Typically, the R_f value of the desired product N1·β-isomer is 0.6 (9:1 chloroform/methanol). The starting material, **S.3**, has an R_f value of 0.9 under these conditions.*
5. Dilute reaction with 400 mL dry dichloromethane and wash it carefully with 500 mL saturated aqueous sodium bicarbonate solution.
6. Dry the dichloromethane layer over anhydrous Na_2SO_4, filter, and evaporate to dryness.

   No purification is needed at this step.

Deprotect 3′,5′-O-dibenzoyl araF-C^{Ac}

7. Treat 8.5 g crude compound with a mixture of 120 mL of 29.7% aqueous ammonia and 150 mL methanol for 48 hr at room temperature.

   This step removes O-benzoyl and N-acetyl groups.

   The R_f value for the desired product is 0.09 in 9:1 (v/v) chloroform/methanol and 0.17 in 3:1 (v/v) chloroform/ethanol.
8. Evaporate the resulting solution to dryness. Dissolve the crude product in a minimal amount of 5% ethanol in chloroform and place it carefully on top of a 3 × 20–cm silica gel chromatography column (see *APPENDIX 3E* for column chromatography).
9. Elute with 3:1 chloroform/ethanol and collect 50-mL fractions. Combine fractions containing pure product as determined by TLC.
10. Evaporate combined fractions to dryness in the rotary evaporator to yield the desired product as a white foam.

    *A yield of 3.1 g (54% from **S.3**) is expected.*

N^4-Benzoylate araF-C

11. Dissolve 3.1 g (12.65 mmol) product and 3.43 g (15.16 mmol) benzoic anhydride in 20 mL anhydrous DMF and stir 35 hr to get araF-C^{Bz}.

    The R_f value of the desired product is 0.63 in 3:1 (v/v) chloroform/ethanol and 0.67 in 3:1 (v/v) chloroform/methanol.

5′-Tritylate araF-C^{Bz}

12. Remove DMF under reduced pressure and co-evaporate the residue with dry pyridine. Add the following and stir 24 hr at room temperature:

    20 mL pyridine
    4.0 g (12.95 mmol) MMTr·Cl
    30 mg 4-DMAP (catalytic amount).

    *The R_f value for the desired product (**S.9a**) is 0.50 in 9:1 (v/v) chloroform/methanol and 0.20 in 20:1 (v/v) chloroform/methanol.*

13. Quench with 10 mL methanol, evaporate the resulting mixture, take up the residue in 150 mL chloroform, wash the organic layer with 150 mL saturated aqueous sodium bicarbonate, dry organic layer over anhydrous Na_2SO_4, and evaporate to dryness.

14. Dissolve the crude product in a minimal amount of chloroform and place it carefully on the top of a 3 × 20–cm silica gel chromatography column.

15. Elute with 0% to 1% methanol in chloroform and collect 50-mL fractions. Combine the fractions containing pure product as determined by TLC.

16. Evaporate combined fractions to dryness in the rotary evaporator to get the desired product as a yellow foam.

17. Check purity of the final product.

    *N^4-Benzoyl-1-[2-deoxy-2-fluoro-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl]cytosine (**S.9a**): 4.6 g (58% from araF-C); TLC (20:1 [v/v] chloroform/methanol); ^{1}H NMR (400 MHz, acetone-d_6, tetramethylsilane as internal reference): 8.0 (1H, d, H-6), 7.6 to 7.2 (19H, m, trityl, Bz), 6.9 (1H, d, H-5), 6.3 (1H, dd, $J_{1',2'}$ = 3.6 Hz, $J_{1',F}$ = 14 Hz, H-1′), 5.2 (1H, m, $J_{2',F}$ = 49 Hz, H-2′), 4.5 (1H, m, H-3′), 4.3 (1H, m, H-4′), 3.8 (3H, s, CH_3O), 3.5 (2H, m, H-5′ and H-5″).*

SYNTHESIS AND CHARACTERIZATION OF N^6-BENZOYL-9-[2-DEOXY-2-FLUORO-5-*O*-(4-METHOXYTRITYL)-β-D-ARABINOFURANOSYL]ADENINE

BASIC PROTOCOL 3

N^6-Benzoyl-9-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)adenine was prepared by direct condensation of N^6-benzoyladenine with 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**; Figure 1.7.5; Watanabe et al., 1988). Flash chromatography and the removal of benzoyl groups with ammonia gave araF-A, which was successfully 5′-tritylated and N^6-benzoylated in a one-pot procedure to give the title compound, **S.9c**.

Materials

2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**; see Basic Protocol 1)
Dichloromethane, dry (see recipe)
N^6-Benzoyladenine, anhydrous
Activated molecular sieves (type 4A)
Pyridine, dry (see recipe)
Chloroform
Silica gel (230 to 400 mesh)
7:3 (v/v) chloroform/dichloromethane
Merck thin-layer chromatography (TLC) silica plates (Kieselgel 60 F-254; 0.2 mm thick)
Ethanol
29.7% (w/w) aqueous ammonia (Fisher)
p-Anisylchlorodiphenylmethane (monomethoxytrityl chloride or MMTr·Cl)

Trimethylsilyl chloride (TMSCl)
Benzoyl chloride
Brine (saturated aqueous NaCl)
Magnesium sulfate, anhydrous
33:1 (v/v) dichloromethane/methanol

250- and 500-mL round-bottom flasks, oven dried
Reflux condenser
Oil bath, 40° to 50°C
Rotary evaporator equipped with vacuum pump
7 × 15–cm chromatography column

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

Condense N^6-benzoyladenine with S.3

1. In a 250-mL round bottom flask equipped with a reflux condenser, dissolve 7.5 g (17.7 mmol) **S.3** in 150 mL dry dichloromethane.
2. Add 10.5 g (44 mmol) anhydrous N^6-benzoyladenine and 21 g activated molecular sieves (type 4A).
3. Place the flask in an oil bath preheated to 40° to 50°C and reflux for 4 days.
4. Cool reaction mixture to room temperature, add 50 mL dry pyridine, and filter. Rinse molecular sieves with 50 mL pyridine and combine both fractions.
5. Evaporate filtrate to dryness in a rotary evaporator to yield a brown oil.
6. Dissolve the crude product in a minimal amount of chloroform.
7. Apply the resulting solution to a 7 × 15–cm chromatography column packed with 100g silica gel in chloroform.

   Column chromatography is performed by using a small amount of air pressure at a rate of ~1 inch of solvent per minute (Still et al., 1978; APPENDIX 3E).

8. Elute the product with chloroform and then continue with 7:3 chloroform/dichloromethane. Collect 100-mL fractions and combine those that contain pure product as determined by TLC (*APPENDIX 3D*) using Merck TLC silica plates.

   The R_f value for the desired product is 0.75 in 9:1 (v/v) dichloromethane/methanol.

9. Evaporate combined fractions and dry overnight under a high vacuum to get a white foam.
10. Check the purity of the product.

    *N^6-Benzoyl-9-(2-deoxy-2-fluoro-3,5-di-O-benzoyl-β-D-arabinofuranosyl)adenine (**S.8c**): 6 g as a white foam, (58% from **S.3**); TLC (9:1 [v/v] dichloromethane/methanol); ^{1}H NMR (270 MHz, DMSO-d_6): 8.8 (s, H-2), 8.5 (d, $J_{H8,F}$ = 2 Hz, H-8), 8.1 to 7.3 (m, Bz), 6.8 (dd, $J_{1',2'}$ = 4 Hz, $J_{1',F}$ = 18 Hz, H-1′), 6.0 (ddd, $J_{3',F}$ = 19 Hz, H-3′), 5.8 (ddd, $J_{1',2'}$ = 4 Hz, $J_{2',3'}$ = 2 Hz, $J_{2',F}$ = 52 Hz, H-2′), 4.8 (m, H-4′), 4.7 (m, H-5′ and H-5″); ^{19}F NMR (270 MHz, DMSO-d_6, no external reference was used) –197 (ddd); FAB-MS (NBA-matrix): 582 ($M+H^+$).*

    The purified product contains ~5% of α-isomer, which is removed in steps 24 to 26.

Deprotect N^6-benzoyl-9-(2-deoxy-2-fluoro-3,5-di-O-benzoyl-β-D-arabinofuranosyl) adenine

11. In a 500-mL round-bottom flask equipped with magnetic stirrer, suspend 6 g (10.33 mmol) **S.8c** in 150 mL ethanol.

12. Add 150 mL of 29.7% aqueous ammonia and stir 2 days. Use TLC to determine when deprotection is complete.

    The R_f value for araF-A is 0.20 in 17:3 (v/v) dichloromethane/methanol.

13. Evaporate the resulting solution to dryness.

    No purification is needed after this step.

Tritylate and benzoylate 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine

14. Co-evaporate 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (step 13) twice with 50 mL each dry pyridine.

15. Dissolve the residue in 50 mL dry pyridine.

16. Add 3.7 g (12 mmol) MMTr·Cl and stir the reaction mixture overnight at room temperature. Use TLC to ensure that the tritylation is complete.

    The R_f value for the desired product is 0.40 in 9:1 (v/v) dichloromethane/methanol.

    An additional portion of MMTr·Cl can be added if tritylation is not complete.

17. Add 6.4 mL (50 mmol) TMSCl and stir the resulting solution 30 min at room temperature.

18. Add 5.8 mL (50 mmol) benzoyl chloride and stir 2 hr.

19. Place the reaction flask into an ice bath to cool and add 10 mL water. Stir 5 min.

20. Add 20 mL of 29.7% aqueous ammonia and stir 30 min.

21. Carefully evaporate the reaction mixture to dryness. Avoid formation of foam, which makes evaporation difficult.

22. Redissolve the mixture in 200 mL chloroform, wash with 200 mL brine, dry over anhydrous magnesium sulfate, filter, and evaporate.

23. Dissolve the crude product in a minimal amount of dichloromethane.

24. Place it carefully on the top of a 7 × 15–cm silica gel chromatography column and elute with dichloromethane and then with 33:1 dichloromethane/methanol. Collect 100-mL fractions and combine those that contain pure product as determined by TLC.

    The R_f value for the desired product is 0.38 in 9:1 (v/v) chloroform/ethanol.

25. Combine fractions that contain pure product and evaporate and dry overnight at high vacuum to get a white foam.

26. Check the purity of the product.

    *N^6-Benzoyl-9-[2-deoxy-2-fluoro-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl] adenine (**S.9c**): 3.8 g (as a white foam, 52% yield from **S.8c**); TLC (9:1 [v/v] chloroform/ethanol) 0.38; ^{1}H NMR (500 MHz, acetone-d_6) 10.0 (br s, N-H), 8.6 (s,H-2), 8.3 (d, $J_{H8\text{-}F}$ = 3 Hz, H-8), 8.1 to 6.9 (m, MMTr, Bz), 6.7 (dd, $J_{1',F}$ = 18 Hz, $J_{1',2'}$ = 4 Hz, H-1′), 5.3 (ddd, $J_{1',H2'}$ = 4 Hz, $J_{2',F}$ = 52 Hz, $J_{2',3'}$ = 4 Hz, H-2′), 4.7 (ddd, $J_{2',3'}$ = 4 Hz, $J_{3',F}$ = 19 Hz, $J_{3',4'}$ = 3 Hz, H-3′), 4.2 (dd, $J_{4',5',5''}$ = 4 Hz, $J_{3',4'}$ = 3 Hz, H-4′), 3.8 (s, CH_3O-), 3.5 to 3.4 (m, H-5′ and H-5″); FAB-MS (NBA-matrix): 646 ($M+H^+$).*

BASIC PROTOCOL 4

SYNTHESIS AND CHARACTERIZATION OF 1-[2-DEOXY-2-FLUORO-5-*O*-(4-METHOXYTRITYL)-β-D-ARABINOFURANOSYL]THYMINE

1-(Deoxy-2-fluoro-3,5-di-*O*-benzoyl-2-β-D-arabinofuranosyl)thymine was synthesized by direct coupling of silylated thymine and 2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**). Crystallization of the crude product gave pure 1-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)thymine, which gave **S.9b** after deprotection and tritylation using standard procedures.

Materials

Thymine
Ammonium sulfate
Phosphorous pentoxide (P_2O_5)
Acetonitrile, dry (see recipe)
Hexamethyldisilazane (HMDS)
2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (**S.3**; see Basic Protocol 1)
Magnesium sulfate, anhydrous
Carbon tetrachloride (CCl_4), anhydrous (Aldrich)
Dichloromethane, dry (see recipe)
Ethanol
Concentrated aqueous ammonia
Merck thin-layer chromatography (TLC) silica plates (Kieselgel 60 F-254; 0.2 mm thick)
Pyridine, dry (see recipe)
p-Anisylchlorodiphenylmethane (monomethoxytrityl chloride or MMTr·Cl)
Chloroform
Brine (aqueous saturated NaCl)
Silica gel (230 to 400 mesh)
19:1 (v/v) dichloromethane/methanol

250- and 500-mL round-bottom flasks, oven dried
Oil bath, 100°
Rotary evaporator attached to a vacuum pump
7 × 15–cm chromatography column chromatography (*APPENDIX 3E*)

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

Silylate thymine

1. Dry 5.4 g (43 mmol) thymine and 540 mg ammonium sulfate in an oven-dried 250-mL round-bottom flask in a vacuum desiccator over P_2O_5 overnight.
2. Dissolve dry mixture in 160 mL dry acetonitrile and add 9 mL HMDS.
3. Put flask in a 100°C oil bath and allow reaction mixture to reflux for 4 hr.

   Once the reaction mixture becomes clear, the reaction is considered to be complete.
4. Let reaction cool to room temperature and evaporate excess solvents under reduced pressure.

Condense 2,4-bis-O-(trimethylsilyl)thymine with S.3

5. Dissolve 7g (16.5 mmol) **S.3** in 70 mL dry CCl_4.
6. Carefully transfer solution with a syringe to the flask containing silylated thymine (step 4).

7. Put flask in an oil bath and leave it to reflux for 4 days.

*The R_f value for the desired product, **S.8b,** is 0.49 in 9:1 (v/v) chloroform/methanol.*

8. Cool down mixture to room temperature, dilute it with 500 mL dichloromethane, and wash with 1 L water.

A large volume of water is necessary to get rid of salts formed during the coupling step.

9. Dry organic layer with anhydrous magnesium sulfate and concentrate under reduced pressure to obtain the crude product as a slightly brown solid.

Usually 1-(2-deoxy-2-fluoro-3,5-di-O-benzoyl-2-β-D-arabinofuranosyl)thymine contains ~2% to 3% of 1-(2-deoxy-2-fluoro-3,5-di-O-benzoyl-2-α-D-arabinofuranosyl)thymine, which cannot be removed by silica gel chromatography at this step. Crystallization (step 10), however, gives the analytically pure compound.

10. Dissolve the crude product in 40 mL dichloromethane and crystallize from 200 mL ethanol.

11. Check purity of the product.

*1-(2-Deoxy-2-fluoro-3,5-di-O-benzoyl-2-β-D-arabinofuranosyl)thymine (**S.8b**): 5.8 g (75% yield from **S.3**); TLC (9:1 [v/v] chloroform/methanol) 0.49; ^{1}H NMR (500 MHz, DMSO-d_6): 11.5 (s, N-H), 8.0 to 7.4 (m, H-6 and Bz), 6.3 (dd, $J_{1',2'}$ = 4 Hz, $J_{1'\text{-}F}$ = 19 Hz, H-1′), 5.7 (ddd, $J_{2',3'}$ = 2 Hz, $J_{3',4'}$ = 4 Hz, $J_{3',F}$ = 19 Hz, H-3′), 5.5 (ddd, $J_{1'',2'}$ = 4 Hz, $J_{2',3'}$ = 2 Hz, $J_{2',F}$ = 53 Hz, H-2′), 4.8 to 4.7 (m, H-5′ and H-5″), 4.6 (m, H-4′), 1.6 (s, CH_3-C5); FAB-MS (NBA-matrix): 468 [M^+].*

Deprotect 1-(2-deoxy-2-fluoro-3,5-di-O-benzoyl -β-D-arabinofuranosyl)thymine

12. In a 500-mL round bottom flask equipped with magnetic stirrer, suspend 5.8 g (12.38 mmol) **S.8b** in 150 mL ethanol.

13. Add 150 mL concentrated aqueous ammonia and stir the resulting mixture for 3 days.

The R_f value for the desired product is 0.49 in 3:1 (v/v) chloroform/ethanol.

14. When deprotection is complete, as determined by TLC (*APPENDIX 3D*) using Merck TLC silica plates, evaporate solution to dryness.

Tritylate 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine

15. Co-evaporate 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (step 14) twice with 50 mL each dry pyridine and dissolve in 50 mL dry pyridine.

16. Add 4.4 g (14.23 mmol) MMTr·Cl and stir the reaction overnight.

*The R_f value for the desired product (**S.9b**) is 0.3 in 19:1 (v/v) chloroform/methanol.*

17. Dilute mixture with 200 mL chloroform, wash with 200 mL brine, dry the organic layer over magnesium sulfate, filter, and evaporate to obtain the crude product.

18. Dissolve the crude product in a minimal amount of dichloromethane.

19. Load it onto a 7 × 15–cm chromatography column packed with 100 g silica gel (*APPENDIX 3E*). Elute with dichloromethane and then with 19:1 (v/v) dichloromethane/methanol. Collect 100-mL fractions and combine those that contain pure product as determined by TLC.

The R_f value for the desired product is 0.3 in 19:1 (v/v) choloroform/methanol.

20. Combine fractions that contain pure product, evaporate, and dry overnight at high vacuum to obtain a white foam.

21. Check the purity of the product.

*1-[2-Deoxy-2-fluoro-5-O-(4-methoxytrityl)-β-D-arabinofuranosyl]thymine (**S.9b**): 5.7 g (87%); TLC (9:1 [v/v] chloroform/methanol) 0.3; ^{1}H NMR (500 MHz, DMSO-d_6) 11.5 (br s, NH), 7.4 to 6.9 (m, H-6 and MMTr), 6.1 (dd, $J_{1'-2'}$ = 4.5 Hz, $J_{1'-F}$ = 16 Hz, H-1′), 5.0 (ddd, $J_{1'-2'}$ = 4 Hz , $J_{2',3'}$ = 2.5 Hz, $J_{2'-F}$ = 53 Hz, H-2′), 4.3 (ddd, $J_{2'-3'}$ = 2.5 Hz, $J_{3'-4'}$ = 4.5 Hz $J_{H3'-F}$ = 19 Hz, H-3′), 3.9 (m, H-4′), 3.7 (s, CH_3O-), 3.3 (m, H-5′ and H-5′), 1.6 (s, CH_3-C5); FAB-MS (NBA-matrix): 532 [M^+].*

REAGENTS AND SOLUTIONS

*Use deionized, distilled water in all recipes and protocol steps. For work up of reaction mixtures and chromatography, use reagent grade solvents. For common stock solutions, see **APPENDIX 2A**; for suppliers, see **SUPPLIERS APPENDIX**.*

Acetonitrile

Dry acetonitrile by refluxing over calcium hydride (Aldrich) or phosphorus pentoxide (P_2O_5; Fisher) for 10 hr followed by distillation under inert atmosphere.

Dichloromethane

Dry dichloromethane by refluxing over calcium hydride (Aldrich) for 24 hr followed by distillation under inert atmosphere.

Pyridine

Dry pyridine by refluxing over calcium hydride (Aldrich) for 8 hr followed by distillation under inert atmosphere.

COMMENTARY

Background Information

The interests of medicinal and organic chemists towards the construction of compounds that contain fluorine is derived from the relative stability of the carbon-fluorine bond, both chemically and metabolically, and from the strong electronegative character of fluorine, which alters the electronic properties of the molecule. In fact, many fluorinated organic compounds exhibit interesting biophysical properties. This has been shown to be the case for anti-inflammatory steroids (Herdewijn et al., 1989), collagen helices (Holmgren et al., 1999), nucleosides (Ma et al., 1997; Pankiewicz, 2000), and nucleic acids (Damha et al., 1998). For example, collagen chains in which fluorine atoms replace the hydroxyl groups form triple helices with extraordinary stability (Eberhardt et al., 1996). A number of highly selective nucleoside antiviral and antileukemic agents based on 2-deoxy-2-fluoroarabinose have been prepared over the past 20 years (for reviews, see Burchenal et al., 1983; Pankiewicz, 2000). These compounds also serve as building blocks for the synthesis of 2′-deoxy-2′-fluoroarabinonucleic acids (2′F-ANAs). It has recently been shown that 2′F-ANA forms a duplex with RNA that is more stable than a DNA:RNA duplex of identical sequence (Damha et al., 1998; Wilds and Damha, 2000). The authors' laboratory has also shown that 2′F-ANA:RNA heteroduplexes, like the natural DNA:RNA heteroduplex, are substrates of RNase H, an enzyme implicated in the mechanism of action of antisense oligonucleotides (Damha et al., 1998).

This unit describes methods for the synthesis of protected 2′-deoxy-2′-fluoroarabinonucleosides. These methods, based on experience and facilities at hand, allow straightforward synthesis of suitable monomers that can be phosphitylated and used as building blocks for the synthesis of antisense oligonucleotides (Lok et al., 2002). The authors have optimized some of the key steps reported in the literature (Tann et al., 1985; Howell et al., 1988; Pankiewicz et al., 1992; Scharer and Verdine, 1995; Maruyama et al., 1999; Wilds and Damha, 2000), particularly those involved in the synthesis of araF-G (Tennila et al., 2000).

A critical step is the fluorination of the ribose sugars, which may lead to loss of water (dehydration) via an elimination reaction. Elimination is virtually avoided by using DAST (or MAST) as the fluorinating reagent instead of HF, SF_4, and HF-amine reagents (Middleton, 1975).

The most convenient method for the synthesis of araF-U, araF-T, and araF-C involves directly coupling 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide with silylated pyrimidines (e.g., Howell et al., 1988; Wilds and Damha, 2000). This produces primarily the desired β-anomer with <5% of the α-stereoisomer, which is removed by chromatography and/or crystallization. This is in contrast to the results obtained during the synthesis of 2′-deoxynucleosides (lacking the 2′-fluorine atom) to afford anomeric mixtures that generally favor the α-anomer. This may be accounted for by the electronegative nature of the 2′-F atom, which effectively prevents the ionization of the C1′-leaving group to produce an S_N1 oxonium ion intermediate. Therefore, the glycosylation reaction mainly proceeds via the energetically preferred S_N2 pathway (Howell et al., 1988; Figure 1.7.6).

Originally, the authors synthesized araF-A (Pankiewicz et al., 1992) and araF-G (Chu et al., 1989) starting from the ribonucleosides (rA and rG). Coupling the purines to the fluorinated arabinose sugar **S.3**, however, largely improves yields and minimizes the number of steps. A presilylation step for the synthesis of araF-A is not required. Synthesis of araF-G is accomplished with a masked base, namely 2,6-dichloropurine, followed by its transformation into guanine in subsequent steps (Ma et al., 1997; Tennila et al., 2000; Figure 1.7.3). All steps work well, except for the *N*-glycosylation reaction, which proceeds to give the desired N9-β-anomer in 35% to 40% yield (optimized). The authors are currently investigating coupling of **S.3** to various purine derivatives in order to simplify and increase the yield of araF-G.

Critical Parameters and Troubleshooting

During the synthesis of araF-G (**S.6**), two important considerations have to be taken into account. First, the refluxing time for the synthesis of **S.4** from **S.2** should not exceed 1 hour; otherwise the yield of the desired product (**S.4**) will be low. Second, purification of the 2,6-diaminopurine derivative is very important in order to get good yields of araF-G during the adenosine deaminase step. In the synthesis of araF-C and araF-T, both the coupling time and the equivalents of the heterocyclic bases used in the N-glycosylation reactions are very important factors for obtaining high yields of nucleosides. During synthesis of araF-A and

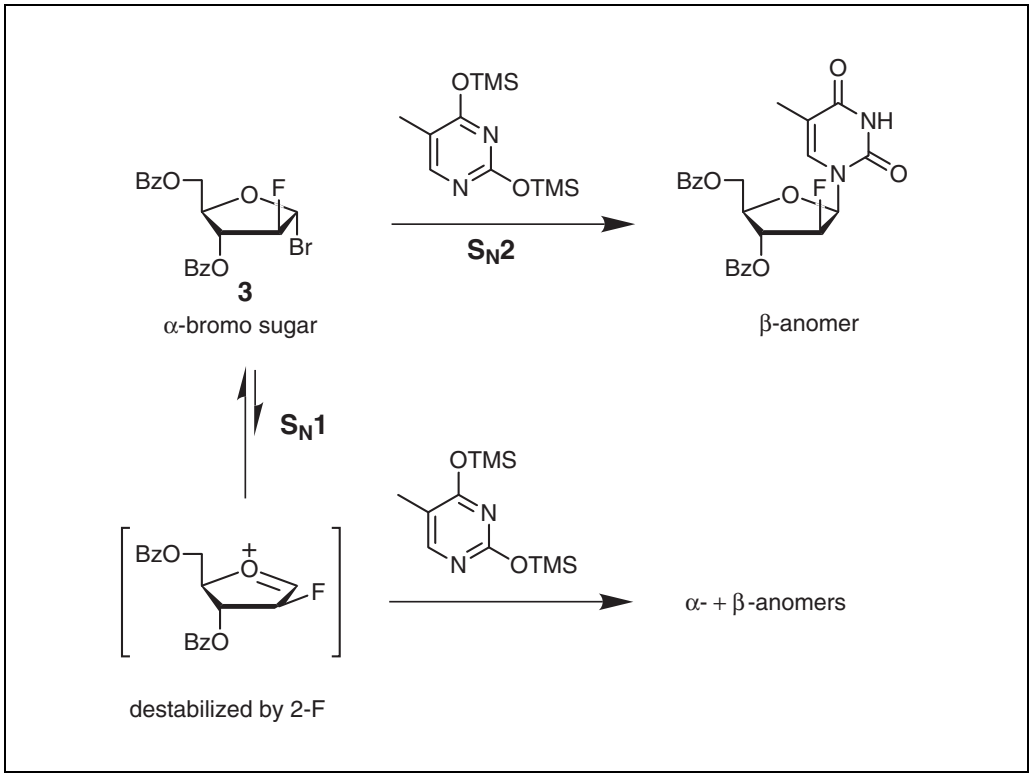

Figure 1.7.6 Coupling of 2,4-bis(trimethylsilyl)thymine with 2-fluoroarabinose sugar **S.3**. Under appropriate conditions (e.g., CCl_4 solvent, reflux), the reaction proceeds primarily via an S_N2 pathway, affording the β and α anomers in a ratio of 98:2. Bz, benzoyl; TMS, trimethylsilane.

araF-C, it is better to use N^6-benzoyladenine (without silylation) and N^4-acetylcytosine (with silylation), respectively, for coupling with **S.3**.

Anticipated Results

The black sheep of the family of nucleosides is guanosine, and araF-G is no exception. The synthesis of araF-G requires more steps than for other araF nucleosides. Nevertheless, these procedures yield araF-G in high purity and moderate yields.

Figure 1.7.4 shows the ^{1}H NMR spectrum of araF-G. The purity of the isolated nucleoside (and all others reported here) is usually >98%. The additional splitting of the sugar proton signals is indicative of the presence of fluorine (spin = ½). The β-configuration of the *N*-glycosidic bond for all nucleosides was established by two-dimensional nuclear Overhauser effect (2D-NOE) NMR experiments (Wilds and Damha, 2000). A characteristic feature of the araF-A and araF-G spectra (^{1}H NMR) is the doublet corresponding to the H-8 proton. The splitting of the H-8 signal is due to long-range ^{1}H-^{19}F coupling (likely through space via H-8...2′-F bonding) that arises only for the β-anomers (Wilds and Damha, 2000).

Time Considerations

Each of the araF nucleosides presented in this unit can be prepared in 6 to 7 days except for araF-G, for which close to 2 weeks are required.

Literature Cited

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This article reviews the application of DAST to the synthesis of fluorinated nucleosides.

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This article describes key connections and synthetic strategies of araF-nucleosides that form the basis of the procedures outlined in these protocols.

Pankiewicz, 2000. See above.

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This article also describes synthetic strategies of araF-nucleosides.

Wilds and Damha, 2000. See above.

This article provides procedures for the synthesis of araF-nucleosides and 2′F-ANA.

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