Synthesis of N2-Substituted Deoxyguanosine Nucleosides from 2-Fluoro-6-*O*-(Trimethylsilylethyl)-2′-Deoxyinosine

This unit describes the synthesis of 2-fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine and gives examples of its use for the preparation of N2-substituted deoxyguanosine nucleosides. Such nucleoside derivatives are used for a variety of purposes including chemotherapy, enzyme mechanism studies, nuclear magnetic resonance (NMR) studies (when isotopically labeled), and as synthetic standards for identification of adducts formed by the reaction of DNA with xenobiotics. In addition, the O6-protected 2-fluoro-2′-deoxyinosine compounds can be converted to phosphoramidites and used in the synthesis of oligonucleotides, thus allowing substitution reactions to be carried out after oligonucleotide assembly.

2-Halopurine derivatives have been used for many years for the preparation of N2-substituted guanosine derivatives, with the 2-fluoro substituent being the most easily displaced by nucleophiles (Montgomery and Hewson, 1960; Gerster and Robins, 1965, 1966). The 2-fluoro group is introduced by aqueous diazotization of guanosine in the presence of potassium fluoride or fluoroboric acid. However, these conditions are too harsh for 2′-deoxyguanosine and lead to depurination; hence, different synthetic methodology is needed for the deoxynucleoside. The fluorine atom can be introduced successfully by diazotization under anhydrous conditions with *t*-butyl nitrite as the diazotizing agent and HF in pyridine as the fluoride source (Robins and Uznanski, 1981; Lee et al., 1990; Harris et al., 1991). Success in the fluoridation step requires protection of the C6 oxygen group, which is done by Mitsunobu alkylation (Mitsunobu, 1981) with trimethylsilylethanol or other alcohols. The O6-protecting group also facilitates displacement of the halogen by nucleophiles.

Basic Protocol 1 in this unit describes the synthesis of 2-fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine and comprises three separate procedures: (1) protection of the 2-NH_2, 3′-OH, and 5′-OH groups of 2′-deoxyguanosine to make a triacetyl derivative, (2) protection of the O6 group by Mitsunobu alkylation with trimethylsilylethanol, and (3) introduction of the 2-fluoro group. Alternate Protocol 1 describes the preparation and use of 3′,5′-*O*-diacetyl-2′-deoxyguanosine and its use in the Mitsunobu reaction described in Basic Protocol 1. Alternate Protocol 1 gives lower yields than Basic Protocol 1, but is quicker and is better for the preparation of 6-*O*-(*p*-nitrophenethyl)-2′-deoxyguanosine, another commonly used O6-protected derivative.

Basic Protocol 2 describes a general procedure for the synthesis of N2-substituted 2′-deoxyguanosines. Two specific examples are then given in Alternate Protocols 2 and 3, which give detailed directions for synthesis using an unhindered diamine to give a derivative with an alkylamine sidechain, and for using an amino alcohol to yield an N2 hydroxyalkenyl derivative. A Support Protocol outlines the procedure for carrying out reactions in an inert atmosphere.

CAUTION: Several of the steps in these protocols involve the use of toxic, corrosive, and flammable chemicals. It is highly recommended that all operations be carried out in a fume hood. Good laboratory safety practices should be observed at all times, including the use of safety goggles, a laboratory coat, and disposable gloves. It is recommended that this synthesis be done only by personnel experienced in the handling of reactive and toxic chemicals.

Contributed by Thomas M. Harris and Constance M. Harris
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NOTE: A variety of methods is used in these procedures to remove volatile solvents and reagents. The choice of method depends upon the boiling points of the volatiles, the stability of the products (e.g., sometimes heat cannot be used to hasten evaporation), and the volume to be removed. For additional details, see Critical Parameters and Troubleshooting.

BASIC PROTOCOL 1

SYNTHESIS OF 2-FLUORO-6-*O*-(TRIMETHYLSILYLETHYL)-*2′*-DEOXYINOSINE USING *2-N*-3′,5′-*O*-TRIACETYL-2′-DEOXYGUANOSINE

This protocol describes the synthesis of the 2-fluoro derivative of 6-*O*-(trimethylsilylethyl)-2′-deoxyinosine (Fig. 1.3.1). It is divided into three basic procedures. (1) Protection of the 2-NH_2, 3′-OH, and 5′-OH groups of 2′-deoxyguanosine (**S.1**) is performed by acetylation (**S.2**). (2) Protection of the O6 group is carried out via Mitsunobu alkylation with trimethylsilylethanol, diethyl azodicarboxylate, and triphenylphosphine. Sodium methoxide and methanol are then added to the reaction to remove the acetyl protecting groups, yielding the 6-*O*-trimethylsilylethyl (TMSE) derivative (**S.3**). (3) The 2-NH_2 group is converted to the 2-fluoro substituent by performing nonaqueous diazotization and fluoridation at low temperature with *t*-butyl nitrite and HF/pyridine (Robins and Uznanski, 1981). An Alternate Protocol utilizing 3′,5′-*O*-diacetyl-2′-deoxyguanosine is also described (see Alternate Protocol 1).

NOTE: Anhydrous solvents are required for several steps in these procedures. They can be purchased (e.g., from Aldrich in Sure/Seal bottles) or dried by distillation from appropriate desiccants and stored under nitrogen.

Materials

2′-Deoxyguanosine (dG) monohydrate
Pyridine, anhydrous (Aldrich; packed under nitrogen in a Sure/Seal bottle)
Acetic anhydride, freshly distilled
Triethylamine (*d* 0.726), distilled from calcium hydride
4-Dimethylaminopyridine (DMAP)

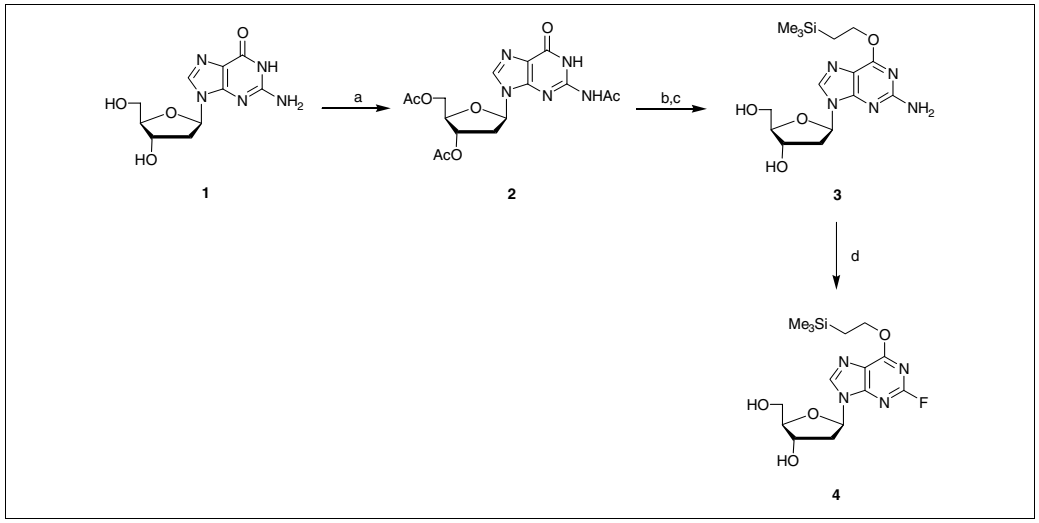

Figure 1.3.1 Synthesis of 2-fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine (**S.4**) using 2-*N*-3′,5′-*O*-triacetyl-2′-deoxyguanosine (**S.2**; see Basic Protocol 1). Reagents: (a) acetic anhydride, pyridine, 4-dimethylaminopyridine (steps 1 to 13): (b) triphenylphosphine, diethyl azodicarboxylate, 2-trimethylsilylethanol (steps 14 to 19); (c) sodium methoxide, methanol (steps 20 to 33); (d) HF/pyridine, *t*-butyl nitrite (steps 34 to 46).

Dry nitrogen (N_2) or argon (Ar)
Methanol, anhydrous
Methylene chloride (CH_2Cl_2), anhydrous
Anisaldehyde/sulfuric acid spray (see recipe)
Acetonitrile
Dioxane, anhydrous, distilled from sodium metal before use
Triphenylphosphine
2-Trimethylsilylethanol
Diethyl azodicarboxylate (DEAD; from a fresh, unopened bottle)
0.35 M sodium methoxide in methanol (see recipe)
Aqueous acetic acid: 6.2 mL glacial acetic acid in 30 mL water
Sodium sulfate (Na_2SO_4), anhydrous
63- to 200-mesh silica gel
Sand
Dry ice/acetonitrile cooling bath (−35° to −40°C)
70% HF/pyridine solution (Aldrich)
t-Butyl nitrite
Potassium carbonate (K_2CO_3)
Ethyl acetate

2-liter round-bottom flask
Rotary evaporator equipped with a condenser cooled with chilled water or a dry ice condenser
Reflux condenser with 24/40 joint and gas inlet adapter
Temperature-controlled oil bath (up to ~115°C)
0.25-mm silica gel $60_{F\text{-}254}$ glass thin-layer chromatography (TLC) plates
UV light source
Vacuum system (oil pump) capable of creating <1 mmHg pressure, with manifold and cold trap
Filter paper (Whatman no. 1, 7-cm diameter)
Buchner funnel
500-mL and l-liter Erlenmeyer flasks
1-liter, three-neck flask with 24/40 joints (oven dried) and rubber septa
10-mL glass syringes (oven dried)
1-liter, single-neck flask with 24/40 joint
Water aspirator
500-mL separatory funnels
Heavy-walled glass column (5-cm i.d. × 40-cm length)
Abderhalden apparatus (drying pistol; 78°C)
50-mL polypropylene conical tubes with rubber septa
23-G syringe needles
20-mL plastic syringes with 3-in. (7.6-cm) 20-G needles
1-mL glass syringe (oven dried)

Additional reagents and equipment for performing reactions under nitrogen (see Support Protocol)

Protection of 2-NH_2, 3′-OH, and 5′-OH Groups

Remove water from dG

1. Place 8.54 g dG (**S.1**) in a 2-liter round-bottom flask.

Table 1.3.1 Synthesis of 2-*N*-3′,5′-*O*-Triacetyl-2′-Deoxyguanosine (**S.2**)

Reagent	Amount	MW (g/mol)	Millimoles	Equivalents
2′-Deoxyguanosine·H_2O	8.54 g	285	30.0	1.0
Pyridine (for coevaporation)	450 mL	79	5600	187
Pyridine (for reaction)	1200 mL	79	14900	497
Acetic anhydride	28 mL	102	299	10
Triethylamine	46 mL	101	330	11
4-Dimethylaminopyridine (DMAP)	0.36 g	122	2.99	0.1
Methanol	350 mL	32	8640	288

*Table 1.3.1 lists quantities of reagents for the synthesis of N^2,$O^{3'}$,$O^{5'}$-triacetyl-2′-deoxyguanosine (**S.2**).*

2. Add 150 mL anhydrous pyridine and then remove the pyridine under vacuum using a rotary evaporator equipped with a dry ice condenser and connected to an oil pump.

   The same oil pump used in step 8, capable of creating <1 mmHg pressure, can be used here; however, it is not necessary. For this step, 1 mmHg pressure would be sufficient.

3. Repeat the addition of pyridine and evaporation twice more.

   Alternatively, the dG can be dried under vacuum in an Abderhalden drying apparatus at 78°C (refluxing ethanol) for 16 hr.

Acetylate dG

4. Add the following reagents (see Table 1.3.1):

   1200 mL anhydrous pyridine

   28 mL acetic anhydride

   46 mL triethylamine

   0.36 g DMAP.

5. Place a large magnetic stir bar in the flask, fit the flask with a reflux condenser, and place the flask in a 55°C temperature-controlled oil bath on top of a magnetic stir plate. Stir the reaction at 55°C for 72 hr under nitrogen (see Support Protocol).

6. Monitor the reaction by occasionally removing a small sample (1 to 2 µL) and analyzing it by TLC. Perform TLC on 0.25-mm silica gel $60_{F\text{-}254}$ glass plates. Elute with 1:9 (v/v) methanol/methylene chloride. Detect product under UV light and also with anisaldehyde/sulfuric acid spray. Spray (or dip) the plate and heat on a hot plate for several seconds.

   Anisaldehyde/sulfuric acid spray is used for the detection of sugars. Spots should appear in various shades of blue and purple. The R_f of the product is 0.31 and that of the starting material is <0.1.

   CAUTION: *Anisaldehyde/sulfuric acid spray should be used in a well-ventilated fume hood. It is corrosive and the vapors are highly irritating.*

Work up and purify 2-N-3′,5′-O-triacetyl-2′-deoxyguanosine

7. When TLC analysis indicates that the reaction is complete, cool in an ice bath and add 350 mL methanol. Stir for 5 min and remove solvents under reduced pressure using a rotary evaporator.

   A red oily residue should be present at this point.

8. Connect the flask to a vacuum system capable of obtaining a vacuum of <1 mmHg and allow to dry overnight.

   It is important to remove the solvents as thoroughly as possible before recrystallization.

9. Add 100 mL acetonitrile and incubate at −20°C overnight.

10. While the reaction mixture is still cold, collect the resulting crystals by vacuum filtration using Whatman no. 1 filter paper and a Buchner funnel.

11. Rinse the precipitate with cold acetonitrile (4°C) and allow the crystals to air dry.

12. Recrystallize from a minimum amount of hot methanol. Place the solid in a 500-mL Erlenmeyer flask and add a few milliliters of methanol. Heat with gentle shaking on a hot plate or in a steam bath until boiling. Gradually add methanol until all the solid is dissolved. Remove from heat and allow to cool.

    Recrystallization can easily be carried out on a larger scale.

13. Cool at −20°C overnight, filter as in step 10, and air dry.

    *The yield of compound **S.2** should be 80% to 90% (9.5 to 10.5 g). The product can be stored indefinitely at room temperature.*

Protection of the O6 Group by Mitsunobu Alkylation

Perform Mitsunobu reaction

14. Equip a 1-liter, 24/40 three-neck flask with rubber septa on the two side necks, a 24/40 reflux condenser in the center neck, and a magnetic stir bar. Place a gas inlet adapter on top of the condenser and connect to a nitrogen source (see Support Protocol).

15. Remove one rubber septum, add the following reagents (see Table 1.3.2), and replace the septum.

    430 mL dioxane

    5.10 g triacetyl deoxyguanosine (**S.2**)

    6.82 g triphenylphosphine.

    *Table 1.3.2 lists quantities of reagents for the synthesis of 6-O-(trimethylsilylethyl)-2′-deoxyguanosine (**S.3**).*

Table 1.3.2 Synthesis of 6-*O*-TMSE-2′-Deoxyguanosine (**S.3**) from **S.2**[a]

Reagent	Amount	MW (g/mol)	Millimoles	Equivalents
Dioxane	430 mL	88	5052	389
2-*N*-3′,5′-*O*-Triacetyl-dG (**S.2**)	5.10 g	393	13	1.00
Triphenylphosphine	6.82 g	262	26	2
2-Trimethylsilylethanol	3.75 mL	118	26	2
Diethyl azodicarboxylate (DEAD)	4.10 mL	174	26	2
Methanol	120 mL	32	2940	227
Sodium methoxide solution (0.35 M)	200 mL	54	70	5
Glacial acetic acid[b]	6.2 mL	60	109	8

[a]For synthesis from the diacetyl derivative, use 4.56 g **S.7** (MW 351) in place of **S.2**, and substitute 60 mL concentrated NH_4OH for the 200 mL sodium methoxide solution.
[b]Diluted in 30 mL water.

16. Transfer the reaction flask to a temperature-controlled oil bath placed on a magnetic stir plate. Start water circulating through the condenser and raise the temperature of the oil bath to ~115°C.

    The solvent should begin gently refluxing.

17. Simultaneously add 3.75 mL of 2-trimethylsilylethanol with a 10-mL glass syringe through one rubber septum and 4.10 mL DEAD through the other.

    Best results are obtained when using DEAD from a fresh unopened bottle. The cloudy yellow suspension should become translucent and darker yellow in color.

18. Continue stirring the reaction mixture for 15 min at 100°C, and then cool to room temperature over ~2 hr.

19. Transfer the solution to a 1-liter single-neck flask. Remove the solvents under reduced pressure using a rotary evaporator with a dry ice condenser and a water aspirator.

Remove acetyl protecting groups

20. Redissolve the viscous red residue in 120 mL anhydrous methanol and slowly add 200 mL of 0.35 M sodium methoxide in methanol. Stir at room temperature for 7 hr.

21. Neutralize the reaction by adding 36.2 mL aqueous acetic acid. Stir for 1 hr at room temperature.

22. Check the pH of the solution using pH paper, and adjust to pH 7.0, if necessary.

Work up and purify 6-O-(trimethylsilylethyl)-2′-deoxyguanosine

23. Evaporate the solution under reduced pressure as in step 19. Suspend the residue in 100 mL methylene chloride and transfer to a 500-mL separatory funnel.

24. Extract with 40 mL water. Back extract the aqueous layer with five 100-mL aliquots of methylene chloride. Combine the organic layers.

25. Add sufficient anhydrous Na_2SO_4 to the extract to cover the bottom of the flask. Swirl gently and allow the salt to settle. If the solution looks cloudy, add more Na_2SO_4. Allow the solution to stand for 30 to 60 min.

26. Filter by gravity through fluted filter paper into a round-bottom flask to remove the drying agent. Evaporate under reduced pressure (see step 19).

27. While the organic extract is drying, pack a 5 × 20–cm silica gel column using 250 g of 63- to 200-mesh silica gel in methylene chloride containing 0.2% triethylamine in a heavy-walled glass column.

    Column chromatography is carried out by flash chromatography techniques employing heavy-walled glass columns that can be connected to a gas inlet via a ball joint. Air pressure can be applied to the top of the column via the inlet to increase the flow rate.

28. Dissolve the crude product in 10 mL methylene chloride.

29. Load the solution on the column and allow the solution to sink to the level of the column bed.

30. Rinse the flask with two 1-mL aliquots of methylene chloride, add each rinse to the bed, and allow them to sink to the level of the bed.

31. Carefully add a 1-cm layer of sand to the top of the column and begin elution with a gradient of CH_2Cl_2/methanol/triethylamine ranging from 97.8:2.0:0.2 to 94.8:5.0:0.2 (v/v/v).

32. Collect 10-mL fractions and analyze by TLC (step 6; $R_f = 0.41$).

33. Combine fractions containing pure product in a round-bottom flask and evaporate to dryness with a rotary evaporator and a dry ice condensor, first under reduced pressure using a water evaporator and then under high vacuum using an oil pump.

Impure fractions can be combined, dried, and reanalyzed under the same TLC conditions to yield additional pure product.

*The product is a pale yellow powder. Approximately 4.2 g (>85% yield) of purified 6-O-TMSE-2′-dG (**S.3**) should be obtained. It can be stored indefinitely at 4° or –20°C.*

The Mitsunobu reaction is readily scaled up as much as five fold.

Introduction of the 2-Fluoro Group

Fluoridate 6-O-TMSE-2′-dG

34. Dry 0.53 g of 6-*O*-TMSE-2′-dG (**S.3**) overnight in an Abderhalden apparatus under vacuum at 78°C.

*The **S.3** product should be quite dry after step 33. The weight should change very little after drying in the Abderhalden apparatus.*

*Table 1.3.3 lists quantities of reagents for the synthesis of 2-fluoro-6-O-TMSE-2′-dI (**S.4**).*

35. Transfer 6-*O*-TMSE-2′-dG to a 50-mL polypropylene conical tube with a stir bar and a rubber septum. Maintain the reaction under nitrogen using a 23-G syringe needle inserted through the septum.

36. Using a 10-mL glass syringe, place 5.4 mL anhydrous pyridine in a second conical tube, similarly equipped.

37. Place both conical tubes in a dry ice/acetonitrile cooling bath (−35° to −40°C).

38. Using a 20-mL plastic syringe with a 3-in. 20-G needle, add 9.6 mL of 70% HF/pyridine solution to the tube containing the anhydrous pyridine over a period of 3 min (final 45% HF). Stir for 15 min at −35° to −40°C.

CAUTION: *HF is highly corrosive and should be handled with care.*

The quality of the 70% HF/pyridine solution is critical to the success of this reaction. The color should be no darker than pale amber. Using a solution that is a dark red or brown leads to increased decomposition products.

Table 1.3.3 Synthesis of 2-Fluoro-6-*O*-TMSE-2′-Deoxyinosine (**S.4**) from **S.3**

Reagent	Amount	MW (g/mol)	Millimoles	Equivalents
6-*O*-TMSE-2′-dG (**S.3**)	0.53 g	368	1.44	1.0
Pyridine	5.4 mL	79	66	46
70% HF/pyridine	9.6 mL	20	~336	~233
t-Butyl nitrite	0.43 mL	103	3.60	2.5
K_2CO_3	23 g	138	166	115[a]

[a]230 neutralizing eq.

If the solution appears to be freezing, 2 to 4 mL anhydrous toluene can be added to keep the solution homogeneous.

39. Using another 20-mL plastic syringe and 3-in. 20-G needle, slowly transfer the HF/pyridine solution into the tube containing the dried 6-*O*-TMSE-2′-dG (**S.3**). Stir for 5 min at −35° to −40°C.

40. Using a 1-mL glass syringe, add 0.43 mL *t*-butyl nitrite to the reaction over 5 min while maintaining the bath temperature at −35° to −40°C. Stir the red reaction mixture for 25 min.

Work up and purify 2-fluoro-6-O-TMSE-2′-dI

41. Dissolve 23 g K_2CO_3 in 34 mL water in a 1-liter Erlenmeyer flask. Begin stirring vigorously at 0°C (ice bath).

42. Quench the reaction by slowly pouring the reaction mixture (step 40) into the cold, stirring K_2CO_3 solution. Rinse the reaction tube with ethyl acetate and add the wash to the neutralized solution.

43. Transfer the solution to a 500-mL separatory funnel and extract five times with 40 mL ethyl acetate. Combine the ethyl acetate extracts.

44. Dry over anhydrous Na_2SO_4, filter to remove the drying agent, and evaporate to a red oil (steps 25 and 26). Place under high vacuum overnight (see step 8).

45. Purify the crude product by flash chromatography (steps 27 to 31) using an ~2.5 × 15–cm silica gel column (50 g silica gel). Elute with a gradient of CH_2Cl_2/methanol/triethylamine ranging from 95:4:1 to 90:9:1 (v/v/v).

46. Analyze by TLC and evaporate appropriate fractions to dryness (steps 32 and 33; R_f = 0.36).

*Approximately 0.50 g (>90%) of 2-fluoro-6-O-(trimethylsilylethyl)-2′-deoxyinosine (**S.4**) should be obtained. The product can be stored indefinitely at –20°C under anhydrous conditions.*

NOTE: *This reaction can be carried out starting with 6.7 g of **S.3** using a 500-mL round-bottomed flask for the reaction. A mixture of 100 mL anhydrous pyridine and 50 mL toluene is used to dissolve the nucleoside before the addition of the HF/pyridine, which is not diluted before addition at –40°C. The yield of purified **S.4** should be ~70%.*

ALTERNATE PROTOCOL 1

SYNTHESIS OF 2-FLUORO-6-*O*-(TRIMETHYLSILYLETHYL)-2′-DEOXYINOSINE USING 3′,5′-*O*-DIACETYL-2′-DEOXYGUANOSINE

In this procedure, the Mitsunobu reaction can be carried out starting with 3′,5′-*O*-diacetyl-2′-deoxyguanosine (Fig. 1.3.2; Zajc et al., 1992), which is synthesized by the procedure of Matsuda et al. (1986). Although the yield of the Mitsunobu product (**S.3**) is not as high when the diacetyl derivative **S.7** is used, the overall synthesis is much quicker because the synthesis of the diacetyl derivative takes only ~1 hr compared to the 72 hr required for the synthesis of 2-*N*-3′,5′-*O*-triacetyl-2′-deoxyguanosine (**S.2**). This procedure is preferred when 6-*O*-(*p*-nitrophenethyl)-2′-deoxyinosine is desired instead of compound **S.3**, because the *p*-nitrophenethyl group is not stable in the presence of the sodium methoxide used for deacylation of the triacetyl derivative.

***Additional Materials** (also see Basic Protocol 1)*

Concentrated ammonium hydroxide (NH_4OH)
1-liter, three-neck, round-bottom flask with 24/40 joints

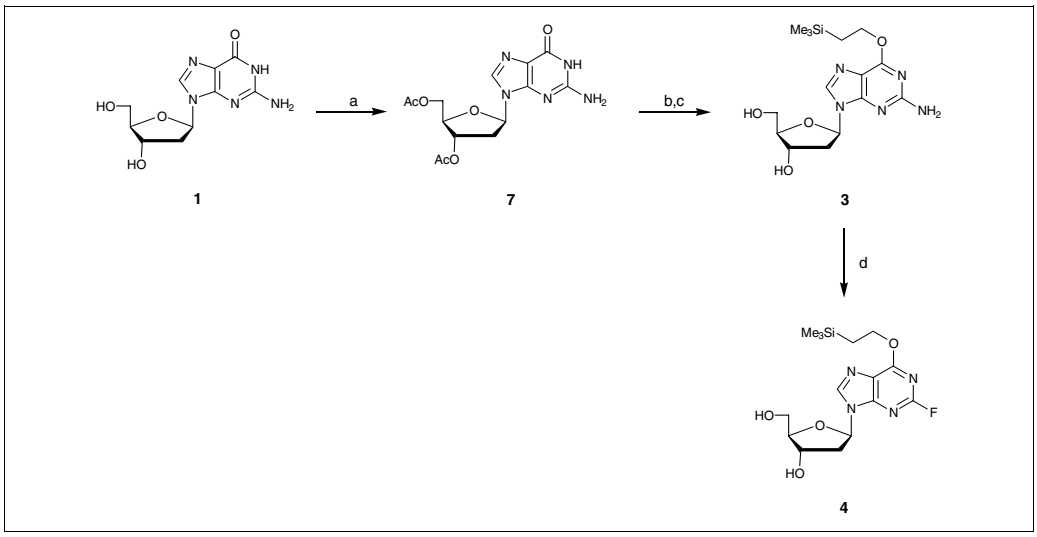

Figure 1.3.2 Synthesis of 2-fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine (**S.4**) using 3′,5′-*O*-diacetyl-2′-deoxyguanosine (**S.7**; see Alternate Protocol 1). Reagents: (a) acetic anhydride, 4-dimethylaminopyridine, triethylamine (steps 1 to 5); (b) triphenylphosphine, diethyl azodicarboxylate, 2-trimethylsilylethanol (step 6); (c) NH_4OH, methanol (steps 7 and 8); (d) HF/pyridine, *t*-butyl nitrite (step 9).

Protection of 3′- and 5′-OH Groups

Remove water from dG

1. Dry 8.56 g dG in an Abderhalden apparatus (drying pistol) under vacuum at 78°C.

   *Table 1.3.4 lists quantities of reagents used for the synthesis of 3′,5′-O-diacetyl-2′-deoxyguanosine (**S.7**).*

   Water can also be removed by evaporation with pyridine, and this method is appropriate if the reaction is to be carried out in pyridine. Drying with an Abderhalden apparatus has the advantage of not involving solvent; however, there is a limit to the amount of material that can be effectively dried in a drying pistol.

Acetylate dG

2. Place dried dG in a 1-liter, three-neck, round-bottom flask equipped with a large magnetic stir bar, glass stoppers in two side arms, and a gas inlet tube in the center neck. Attach a nitrogen source to the gas inlet tube as described (see Support Protocol).

3. Add the following reagents (see Table 1.3.4) to produce a yellowish suspension:

   0.36 g DMAP

   325 mL acetonitrile

   85 mL triethylamine

   82 mL acetic anhydride.

4. Stir for 30 min to 1 hr at room temperature.

   During this time the suspension will become whiter.

Table 1.3.4 Synthesis of 3′,5′-*O*-Diacetyl-2′-Deoxyguanosine (**S.7**)

Reagent	Amount	MW (g/mol)	Millimoles	Equivalents
2′-Deoxyguanosine·H_2O	8.56 g	285	32.0	1.0
4-Dimethylaminopyridine (DMAP)	0.36 g	122	3.2	0.1
Acetonitrile	325 mL	41	623	195
Triethylamine	85 mL	101	610	19
Acetic anhydride	82 mL	102	869	27

Work up and purify 3′,5′-O-diacetyl-2′-deoxyguanosine

5. Filter the suspension using Whatman no. 1 filter paper, a Buchner funnel, and a water aspirator. Wash the precipitate with acetonitrile and dry overnight in the Abderhalden apparatus (78°C).

   *This should yield 7.5 to 8.0 g (70%) of the **S.7** product. If desired, the compound can be recrystallized from water, but this is not necessary. The compound can be stored indefinitely at room temperature.*

Protection of the O6 Group by Mitsunobu Alkylation

6. Perform Mitsunobu reaction as described (see Basic Protocol 1, steps 14 to 19), starting with 4.56 g of the 3′,5′-*O*-diacetyl compound (**S.7**; Table 1.3.2).

7. Remove acetyl protecting groups as described (see Basic Protocol 1, steps 20 to 22), but use 60 mL concentrated NH_4OH instead of 200 mL sodium methoxide (Table 1.3.2).

   The yield of the Mitsunobu reaction is not as high (~50%) when starting with the diacetyl compound, but the overall reaction time is shortened considerably.

8. Work up and purify 6-*O*-TMSE-2′-dG as described (see Basic Protocol 1, steps 23 to 33).

   The Mitsunobu reaction is readily scaled up as much as five fold.

Introduction of the 2-Fluoro Group

9. Introduce the 2-fluoro group as described (see Basic Protocol 1, steps 34 to 46).

BASIC PROTOCOL 2

GENERAL GUIDELINES FOR SYNTHESIS OF N2-SUBSTITUTED NUCLEOSIDES

This protocol outlines a general procedure for the preparation of N2-substituted deoxyguanosines (Fig. 1.3.3A) from the 2-fluoro derivative **S.4** synthesized using Basic Protocol 1 or Alternate Protocol 1. Alternate Protocols 2 and 3 give detailed directions for preparing specific aminoalkyl and hydroxyalkanyl derivatives. As can be seen in the Alternate Protocols, considerable latitude can be exercised in the relative amounts of diisopropylethylamine (DIEA) and dimethyl sulfoxide (DMSO) that are used; a greater excess of amine can also be employed without harm.

Materials

2-Fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine (**S.4**; see Basic Protocol 1 or Alternate Protocol 1)
Amine of choice
N,*N*-Diisopropylethylamine (DIEA), anhydrous
Dimethylsulfoxide (DMSO), anhydrous, vacuum distilled from calcium hydride

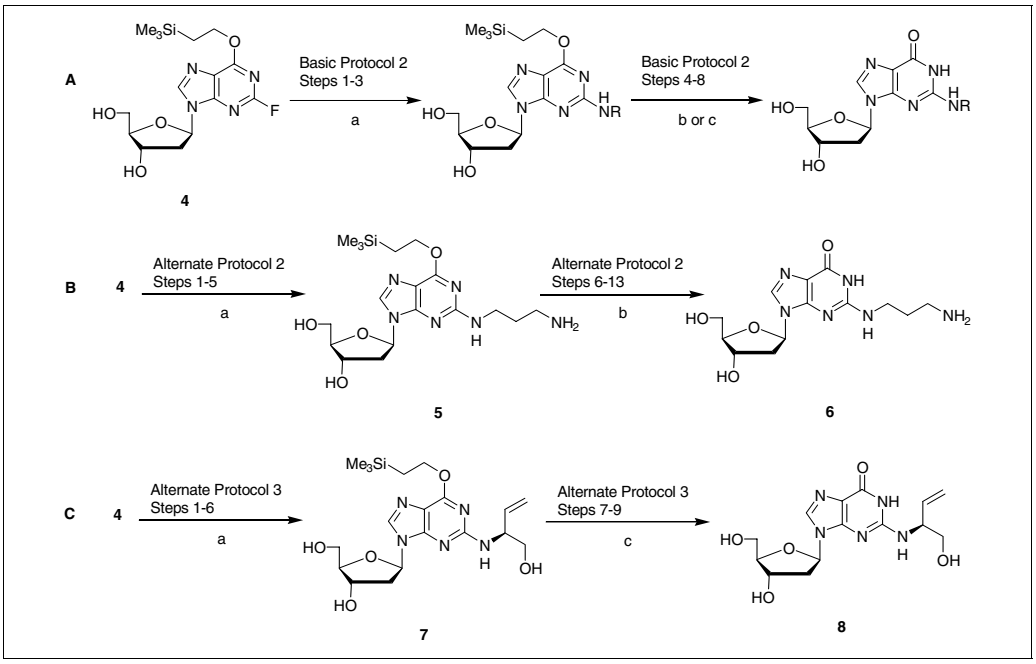

Figure 1.3.3 (**A**) General guidelines for synthesis of N2-substituted nucleosides (see Basic Protocol 2). (**B**) Synthesis of 2-*N*-(3-aminopropyl)-2′-deoxyguanosine (**S.6**; see Alternate Protocol 2). (**C**) Synthesis of 2-*N*-[2(*S*)-1-hydroxybut-3-en-2-yl]-2′-deoxyguanosine (**S.8**; see Alternate Protocol 3). Reagents: (a) amine, diisopropylethylamine, dimethyl sulfoxide; (b) 0.1 M tetrabutylammonium fluoride; (c) 0.1 M acetic acid.

0.1 M aqueous acetic acid *or* 1 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF)

Methanol, anhydrous

0.1 M sodium bicarbonate ($NaHCO_3$)

Vial or test tube with secure cap

Temperature-controlled oil bath or heating block at 45° to 60°C

Rotary evaporator with water aspirator and oil pump

Additional reagents and equipment for thin-layer chromatography (TLC; see Basic Protocol 1), or high-performance liquid chromatography (HPLC)

1. Combine the following in a vial or a test tube that can be securely capped.

   1 equivalent 2-fluoro-6-*O*-(trimethylsilylethyl)-2′-deoxyinosine

   1.5 to 2.0 equivalents amine

   2 equivalents DIEA

   20 μL DMSO per mg of nucleoside.

2. Heat in a temperature-controlled oil bath or heating block at 45° to 60°C. If the reaction is not homogeneous after warming up, add additional anhydrous DMSO.

3. Continue heating for 1 to 2 days, until the fluoronucleoside (**S.4**) has been completely eliminated. Check the reaction periodically by TLC (see Basic Protocol 1, step 6; **S.4** R_f ~0.36).

4. Evaporate the solvent under reduced pressure using a rotary evaporator, first with a water aspirator and then under high vacuum with an oil pump.

   A centrifugal vacuum evaporator (e.g., Speedvac) can also be used and is often more convenient for small-scale syntheses such as those described in Alternate Protocols 2 and 3.

5. Add 20 µL of 0.1 M aqueous acetic acid (or 20 µL of 1 M TBAF in THF) per mg nucleoside and incubate at room temperature for 2 hr to remove the trimethylsilylethyl protecting group. If necessary, add an organic solvent such as methanol to improve solubility. Monitor by TLC until deprotection is complete.

   Some of the TMSE protecting group will probably have fallen off the product by the end of the reaction. The deprotected product will have a lower R_f than the 6-O-TMSE compound.

6. Neutralize the reaction with 0.1 M $NaHCO_3$ (20 µL if 0.1 M acetic acid was used) and check the pH (6.5 to 7.5) with pH paper.

7. Evaporate solvents under reduced pressure (step 4).

8. Purify the product by flash chromatography on silica gel, by preparative TLC, or by HPLC.

   The choice of purification technique will depend primarily on the scale of the reaction. The choice of solvent system will depend on the product characteristics. The alkylated nucleosides can be stored indefinitely at 4° or –20°C.

ALTERNATE PROTOCOL 2

SYNTHESIS OF 2-*N*-(3-AMINOPROPYL)-2′-DEOXYGUANOSINE

This protocol describes the preparation of a 2-*N*-aminopropyl derivative of 2′-deoxyguanosine (**S.6**) using tetrabutylammonium fluoride (TBAF) to remove the 6-*O*-trimethylsilylethyl group (see Fig. 1.3.3B).

Additional Materials *(also see Basic Protocol 2)*

1,3-Diaminopropane
Acetonitrile
Concentrated NH_4OH
Silica gel
Sand
Tetrahydrofuran (THF), anhydrous
0.1 M ammonium formate in water, pH 6.4

12 × 75–mm glass test tube or conical vial with magnetic stir bar
2.5 × 20–cm column
100-mL round-bottom flask
10 × 250–mm C18 reversed-phase HPLC column (e.g., YMC-ODS-AQ column; YMC)

1. Combine the following in a 12 × 75–mm glass test tube or conical vial with a small magnetic stir bar.

   50 mg (0.13 mmol) 2-fluoro-6-*O*-TMSE-2′-dI (**S.4**)

   16.8 mg (0.13 mmol) DIEA

   200 µL DMSO.

2. Add 23 mg (0.31 mmol) of 1,3-diaminopropane, cap securely, and heat while stirring in a 45°C oil bath or heating block.

Additional amine (well in excess of the 1 eq that is theoretically required) is used to ensure that only one of the amino groups in the diaminopropane reacts with the fluoronucleoside. It is possible for the diamine to react at both ends to form a bis(nucleoside) cross-linked with a propyl chain. If this is the desired product, the diamine can be used as the limiting reagent (e.g., 1 mmol of diamine to 3 mmol fluoronucleoside).

3. Continue heating until the fluoronucleoside (**S.4**) has been completely eliminated. Check the reaction periodically by TLC (see Basic Protocol 1, step 6), eluting with 85:8:7 (v/v/v) acetonitrile/H_2O/concentrated NH_4OH.

   *The reaction should be finished after ~20 hr. The R_f of 1-(6-O-trimethylsilylethyl)-N^2-(3-aminopropyl)-2′-deoxyguanosine (**S.5**) is ~0.25, and that of **S.4** is ~0.8.*

4. Evaporate the solvent under reduced pressure (see Basic Protocol 2, step 4).

5. Prepare a column for flash chromatography by packing a 2.5 × 20–cm column with 20 g silica gel using the TLC solvent system (step 3).

6. Place the sample in a 100-mL round-bottom flask and add a few milliliters of methanol to dissolve it. Add ~1 g dry silica gel and evaporate the methanol using a rotary evaporator with a water aspirator.

7. Carefully add the powdery sample/gel mixture as evenly as possible to the top of the column. Add a layer of sand and elute using the TLC solvent mixture.

8. Collect 5- to 7-mL fractions and analyze by TLC (step 3).

9. Combine fractions containing pure product and evaporate to dryness using a rotary evaporator, first with a water aspirator and then under high vacuum with an oil pump.

   *Yields of 70% to 75% (35 to 40 mg) of **S.5** should be obtained.*

10. Dissolve **S.5** in 500 µL anhydrous THF. Add 200 µL of 1 M TBAF in THF and stir the reaction for 12 hr at room temperature.

11. Evaporate the solvent under reduced pressure (see Basic Protocol 2, step 4).

12. Monitor the deprotection reaction by TLC (step 3).

    *The R_f of **S.5** is 0.25. The deprotected product will have a much lower R_f.*

13. Add 200 µL of 1:1 (v/v) DMSO/methanol and purify by HPLC using the following conditions:

    Column: 10 × 250–mm YMC-ODS-AQ

    Solvents: (A) 0.1 M ammonium formate, pH 6.4; (B) acetonitrile

    Flow rate: 5 mL/min

    Gradient: 99% to 90% A over 15 min, 90% to 80% A over 5 min, 80% to 0% A over 10 min, return to 99% A over 5 min.

*2-N-(3-Aminopropyl)-2′-deoxyguanosine (**S.6**) elutes at ~12 min. Yields of ~50% should be expected for the deprotection and HPLC purification steps. The product can be stored indefinitely at 4° or –20°C.*

ALTERNATE PROTOCOL 3

SYNTHESIS OF 2-*N*-[2(*S*)-1-HYDROXYBUT-3-EN-2-YL]-2′-DEOXYGUANOSINE

This protocol describes the use of 2-amino-3-butenol to prepare an 2-*N*-hydroxyalkene-substituted 2′-deoxyguanosine (**S.8**) using acetic acid to remove the 6-*O*-trimethylsilylethyl group (Fig. 1.3.3C).

Additional Materials *(also see Basic Protocol 2)*

2(*S*)-Amino-3-butenol
0.1 M ammonium formate in water, pH 6.4

12 × 75–mm glass test tube or conical vial with a magnetic stir bar
Lyophilizer
10 × 250–mm C18 reversed-phase HPLC column (e.g., YMC-ODS-AQ column; YMC)

1. Dry 10 mg of 2-fluoro-6-*O*-TMSE-2′-dI (**S.4**; 0.027 mmol) overnight in a 12 × 75–mm glass test tube under vacuum at room temperature using a rotary evaporator and an oil pump.
2. Add a small magnetic stir bar and the following reagents:

   35 mg (0.041 mmol) 2(*S*)-amino-3-butenol

   10 µL (0.057 mmol) DIEA

   50 µL anhydrous DMSO.
3. Cap the tube securely and heat with stirring in a temperature-controlled oil bath or heating block at 60°C for 20 hr.
4. Monitor the reaction by TLC (see Alternate Protocol 2, step 3).

   *The reaction mixture may contain both product with TMSE (**S.7**) and desilylated product (**S.8**). Starting material **S.4** has an R_f of 0.8, and **S.8** has an R_f of 0.22. **S.7** will have an R_f between those of **S.4** and **S.8**.*
5. Remove the solvent under reduced pressure (see Basic Protocol 2, step 4).
6. Suspend the residue in 0.5 mL water and lyophilize.
7. Remove remaining TMSE protecting group by treating the product mixture with 200 µL of 0.1 M aqueous acetic acid at room temperature for 2 hr.
8. Neutralize with an equal volume of 0.1 M $NaHCO_3$ (final pH 6.5 to 7.5) and lyophilize.
9. Purify by HPLC using the following conditions:

   Column: 10 × 250-mm YMC-ODS-AQ

   Solvents: (A) 0.1 M ammonium formate, pH 6.4; (B) methanol

   Flow rate: 5 mL/min

   Gradient: 90% to 10% A over 25 min.

   *The deprotected product (**S.8**) elutes at ~12 min; protected product (**S.7**) elutes at ~26 min. The yield from this reaction is ~80%. The product can be stored indefinitely at 4° or –20°C.*

SETTING UP A NITROGEN ATMOSPHERE

Many of the steps are carried out under a slight positive pressure of inert gas (usually nitrogen, although argon can also be used) to keep moisture and oxygen out of reactions. When a procedure is described as being carried out under nitrogen, this is accomplished by attaching a source of dry nitrogen gas via tubing to a Y- or T-shaped glass connector. One arm of the connector is attached to the inlet of a bubbler that is partially filled with a high-boiling inert liquid such as mineral oil; the outlet of the bubbler is open to the atmosphere. The third arm of the connector is connected via tubing to the reaction flask equipped with either a gas inlet adapter or a syringe needle inserted through a rubber septum in one of the joints of the flask. To ensure a slight positive pressure of inert gas in the system, the flow of nitrogen is adjusted so that a slow stream of bubbles is created in the bubbler. This arrangement is preferable to having nitrogen pass through the flask, which causes evaporation of solvents. A useful guide to handling air-sensitive reagents and working in an inert atmosphere can be found in Technical Bulletin AL-134 (Aldrich Chemical, 1983).

REAGENTS AND SOLUTIONS

*Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2A**; for suppliers, see **SUPPLIERS APPENDIX**.*

Anisaldehyde/sulfuric acid spray

Dissolve 5 mL of *p*-anisaldehyde in 90 mL of 95% ethanol. Carefully add 5 mL concentrated H_2SO_4 followed by three drops of glacial acetic acid. Store up to several months at 4° to 6°C or several days at room temperature.

Reagent can be applied to TLC plates with a spray bottle or by dipping.

CAUTION: *Prepare and use this reagent only in a well-ventilated fume hood. It is corrosive and the vapors are highly irritating.*

Sodium methoxide in methanol, 0.35 M

Prepare a 0.35 M solution by adding 18.9 g solid sodium methoxide (Aldrich) to 1 liter anhydrous methanol. Prepare fresh before use or store for several days at room temperature under an inert atmosphere.

Prepared solutions of sodium methoxide in methanol can be purchased from Aldrich.

COMMENTARY

Background Information

Syntheses of N2-substituted guanine nucleosides have been studied for many years, primarily with ribosides. The primary route to these compounds has been via nucleophilic displacement of a halogen at the 2 position of an inosine bearing a substituent (such as *O*-benzyl or thione) at the 6 position (Montgomery and Hewson, 1960; Gerster and Robins, 1965, 1966). The halogen (bromo, chloro, or fluoro) is introduced by aqueous diazotization of the 2-amino group of a guanosine derivative in the presence of a halide source (Gerster and Robins, 1965), and the O6 substituent is converted by hydrogenation (for the benzyl substituent) or oxidation (for thione) to the 2-fluoro guanine derivative.

This strategy, while successful for ribosides, requires acidic conditions that are too vigorous for the acid-labile deoxyribosides. The current strategy takes advantage of methodology developed by Robins and Uznanski (1981) for low-temperature, nonaqueous diazotization of ribosides in organic solvents using *t*-butyl nitrite as the diazotizing agent and HF/pyridine as the fluoride source for the preparation of fluoro derivatives. Other fluoridating agents have also been explored (Acedo et al., 1994; Adib et al., 1997). The fluoro derivatives have proved to be the most useful because they are more reactive toward nucleophilic displacement than the other halogen derivatives (Gerster and Robins, 1965). Lee et al. (1990) reported that the nonaqueous diazotization strat-

egy could be used for the preparation of 2-fluoro-6-*O*-benzyl-2′-deoxyinosine. Harris et al. (1991) then reported similar experiments that had been extended to the synthesis of 2-fluoro-2′-deoxyinosine itself, and its incorporation into oligonucleotides via phosphoramidite chemistry. However, the 2-fluoro derivative without O6-protection did not react as well with nucleophiles, and its conversion to the corresponding phosphoramidite did not proceed in very high yield.

Although the benzyl protecting group is easily introduced, its removal requires catalytic hydrogenation, which can cause problems if a polycyclic aromatic hydrocarbon (PAH) substituent has been introduced at N2 (Lee et al., 1990). Hence, it became apparent that a different O6-protecting group was required. The 4-nitrophenethyl (NPE) group, which had been explored by Gaffney and Jones (1982) and Himmelsbach et al. (1984) for the protection of deoxyguanosine during oligonucleotide synthesis, has found wide use for preparation of the 2-fluoroinosine derivative (Zajc et al., 1992; Erlanson et al., 1993; Eritja et al., 1995; Lee et al., 1995; Schmid and Behr, 1995; Diaz et al., 1997). 2-Fluoro-6-*O*-(4-nitrophenethyl)-2′-deoxyinosine can be prepared by the series of reactions described in this unit, starting with either triacetyl (Lee et al., 1995; see Basic Protocol 1) or diacetyl (Zajc et al., 1992; see Alternate Protocol 1) deoxyguanosine. Use of the 3′,5′-*O*-diacetyl derivative greatly shortens the synthesis, because several days of ammonia/methanol treatment are required for removal of the N2-acetyl group. There is risk of losing the NPE group by β-elimination if sodium methoxide is used for this step. The trimethylsilylethyl group is also very useful for O6-protection (Tsarouhtsis et al., 1995; DeCorte et al., 1996). Both groups can be used for preparation of O6-protected 2-fluoro-2′-deoxyinosine phosphoramidites and for oligonucleotide synthesis. The NPE group is usually removed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); it may be slowly lost if the substitution reaction requires prolonged heating in the presence of excess amine. The TMSE group, while quite stable in the fluoro derivative under anhydrous neutral or alkaline conditions, is often lost spontaneously once there is an amine substituent at C2, and generally requires only mild acid treatment to achieve complete deprotection. Allerson et al. (1997) have recently reported a synthesis of the 2-fluoro-6-*O*-(4-nitrophenethyl)-*riboside* derivative, in which the hydroxyls and N2 were protected as triethylsilyl (TES) derivatives; the TES groups were removed in the course of the fluoridation reaction.

Quite a number of substitution reactions have now been reported using a 2-fluoro-2′-deoxyinosine derivative, either at the nucleoside stage or in oligonucleotides. Amine nucleophiles have been used to attach a wide variety of substituents including amino alcohols (Zajc et al., 1992), polyamines (Schmid and Behr, 1995; Diaz et al., 1997), disulfide-containing diamines (Wolfe and Verdine, 1993), pyrrolizidine amines (Woo et al., 1993; Tsarouhtsis et al., 1995), heterocyclic amines (Wang and Bergstrom, 1993; Ramasamy et al., 1994), benzylic amines bearing large polycyclic substituents (Lee et al., 1990, 1995; Sangaiah et al., 1992; Zajc et al., 1992), and metal-binding ligands (Bergstrom and Gerry, 1994). In addition, ^{15}N is easily introduced by use of $^{15}NH_3$ at either the nucleoside or oligonucleotide stage (Acedo et al., 1994).

An acylated derivative of 2-fluoro-6-*O*-NPE-2′-deoxyinosine has been used in a reaction with the severely hindered triol amines derived from opening of the diol epoxide of benzo[*a*]pyrene with ammonia (Zajc et al., 1992); however, a high temperature was required for the reaction. The 2-triflate derivative of 2′-deoxyinosine (Steinbrecher et al., 1993; Edwards et al., 1997) offers promise for syntheses requiring very bulky, highly sterically hindered nucleophiles.

Compound Characterization

2-N-3′,5′-O-Triacetyl-2′-deoxyguanosine (***S.2***): mp 193° to 194°C (soften), 225°C (decompose); TLC R_f 0.31 (9:1 CH_2Cl_2/MeOH); ^1H NMR (DMSO-d_6) δ 12.20 to 11.40 (br, 2H, 2×NH), 8.23 (s, 1H, H8), 6.22 (dd 1H, J = 7.2 Hz, H1′), 5.33 (d, 1H, J = 5.7 Hz, H3′), 4.22 to 4.20 (m, 3H, H4′, H5′, H5″), 2.95 (m, 1H, H2′), 2.52 (m, 1H, H2″), 2.16 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.00 (s, 3H, CH_3).

3′,5′-O-Diacetyl-2′-deoxyguanosine (***S.7***): mp 300°C (decompose); ^1H NMR (DMSO-d_6) δ 7.80 (s, 1H, H8), 6.40 (2H, bs, NH_2), 6.03 (s, 1H, H1′), 5.19 (1H, m, H3′), 4.15 (1H, m, H5″), 4.11 (2H, m, H5″H4′), 2.81 (m, 1H, H2′), 2.33 (m, 1H, H2″, 1.97 (s, 3H, CH_3), 1.94 (s, 3H, CH_3).

*6-O-(Trimethylsilylethyl)-2′-deoxyguano*sine (***S.3***): mp 66° to 68°C; TLC R_f 0.41 (9:1 CH_2Cl_2/MeOH); ^1H NMR (MeOH-d_4) δ 8.00 (s, 1H, H8), 6.30 (dd, 1H, J = 6.1 Hz, J = 2.1 Hz, H1′), 4.61 to 4.53 (m, 3H, OCH_2CH_2Si, H3′), 4.03 (q, 1H, J = 2.7 Hz, H4′), 3.83 (dd,

1H, J = 3.3, 12.2, H5′), 3.73 (dd, 1H, J = 12.2, 2.9, H5″), 2.76 (m, 1H, H2′), 2.34 (m, 1H, H2″), 1.21 (m, 2H, CH_2Si), 0.08 (s, 9H, $(CH_3)_3Si$); ^{1}H NMR ($CDCl_3$) δ 7.64 (s, 1H, H8), 6.16 (dd, 1H, J = 8.8 Hz, J = 5.7 Hz, H1′), 5.04 (s, 2H, NH_2), 4.56 (d, 1H, J = 4.8 Hz, H3′), 4.39 (t, 2H, J = 8.6 Hz, OCH_2CH_2Si), 4.07 (br, 1H, H4′), 3.70 (m, 2H, H5′, H5″), 2.73 (m, 1H, H2′), 2.21 (m, 1H, H2″), 1.02 (t, 2H, J = 8.6 Hz, CH_2Si), -0.10 (s, 9H, $(CH_3)_3Si$).

2-Fluoro-6-O-(trimethylsilylethyl)-2′-deoxyinosine (***S.4***): mp 120° to 122°C; TLC R_f 0.36 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (MeOH-d_4) δ 8.46 (s, 1H, H8), 6.41 (t, 1H, J = 6.7 Hz, H1′), 4.67 to 4.72 (m, 2H, OCH_2CH_2Si), 4.56 (m, 1H, H3′), 4.02 (q, 1H, J = 3.6 Hz, H4′), 3.81 (dd, 1H, J = 3.6, H5″), 3.73 (dd, 1H, J = 2.9, H5′), 2.77 (m, 1H, H2″), 2.44 (m, 1H, H2′), 1.26 (m, 2H, CH_2Si), 0.1 (s, 9H, $(CH_3)_3Si$); ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H, H8), 6.31 (t, 1H, J = 6.7 Hz, H1′), 5.35 (d, 1H, J = 4.2 Hz, 3′-OH), 4.94 (t, 1H, J = 5.5 Hz, 5′-OH), 4.64 (t, 2H, J = 8.3 Hz, OCH_2CH_2Si), 4.40 (m, 1H, H3′), 3.87 (q, 1H, J = 4.0 Hz, H4′), 3.55 (m, 2H, H5′ and H5″), 2.68 (m, 1H, H2′), 2.32 (m, 1H, H2″), 1.20 (t, 2H, J = 8.3 Hz, CH_2Si), 0.08 (s, 9H, $(CH_3)_3Si$); ^{19}F NMR (MeOH-d_4) δ -51.55; ^{19}F NMR ($CDCl_3$) δ –50.40.

Critical Parameters and Troubleshooting

Overall, it is important that starting nucleosides for each step in the synthesis be thoroughly dried either by coevaporation with anhydrous pyridine or in an Abderhalden drying apparatus (overnight under vacuum at 78°C).

Anhydrous solvents are also important. The solvents should either be freshly distilled and stored under nitrogen or argon, or should be taken from a freshly opened bottle of commercially prepared anhydrous solvent. It is best to purchase anhydrous solvents in the smallest size bottle that will fit the requirements of the synthesis.

Acylation. Dry reagents are important for acylation. The main effect of moisture is to decrease the yield of product. Serious problems are seldom encountered in this step.

Mitsunobu alkylation. This reaction is most sensitive to the quality of the DEAD reagent. The best yields are obtained when using reagent from a freshly opened bottle.

Fluoridation. This reaction requires careful attention to three factors: (1) dryness of the O6-protected nucleoside, (2) quality of the HF/pyridine reagent, which should be pale amber or lighter in color, and (3) maintenance of low temperature during the course of the reaction. Lack of attention to any of these factors can lead to depurination and failure to obtain any product.

Substitution reactions. The nature of the nucleophile is the most critical parameter in this reaction. Sterically hindered amines require longer time and/or higher temperature for reaction. The rate of reaction is also determined by the concentrations of nucleoside and amine. Temperatures as high as 80° to 85°C have been used in certain cases. If the substituent is a bulky hydrocarbon, removal of the TMSE group may require more vigorous deprotection conditions (e.g., 5 mM HCl in methanol at 50°C for several hours).

Evaporation of solvents. This is usually accomplished using rotary evaporation under vacuum. It is very helpful if the rotary evaporator is equipped with a dry ice condenser or with a condenser that is cooled with chilled water. The vacuum is normally applied with a water aspirator (25 to 30 mmHg), which is quite sufficient for materials with boiling points <100°C (at 760 mm). However, several of the reagents and solvents used in these procedures have boiling points >100°C. For these steps, it is better to use an oil pump to produce a vacuum of ≤1 mmHg. This allows higher-boiling compounds to be removed with the application of little or no heat. The general procedure is to remove the more volatile materials first using the water aspirator, and then remove the higher-boiling compounds using the oil pump. The oil pump should not be used to remove the lower-boiling materials, particularly if there are chlorinated solvents present.

If the only volatile material is water, it can also be removed by lyophilizaton or vacuum centrifugal evaporation (e.g., with a Speedvac evaporator). Small volumes of volatile organics can also be removed by this method if the instrument is equipped with a chemically resistant cold trap. This is, for example, an effective way to removed small volumes of DMSO.

Anticipated Results

An overall yield of 45% to 55% of 2-fluoro-6-*O*-TMSE-2′-deoxyinosine from 2′-deoxyguanosine can be obtained in this series of reactions. The yields of substitution reactions are generally very high, although the recovered yield of purified product may be considerably

lower, depending upon the scale and method of purification.

Time Considerations

An approximate time scale for the sequence of reactions described in this unit would be 3 to 5 days for acylation, depending upon whether the tri- or diacetyl derivative is chosen as the starting material; 3 days for the Mitsunobu reaction; 2 days for fluoridation; and 2 to 4 days for substitution reactions, depending upon the nucleophile and the mode of purification (e.g., HPLC generally takes longer than preparatory TLC). The reaction can be greatly accelerated by using a large excess of amine.

Considerable variation in these times is to be expected and is based upon the time allowed for drying and/or distillation of reagents, recrystallization, chromatographic separation, and analysis, as well as on the experimenter's level of synthetic chemistry experience.

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Key References

Gerster and Robins, 1965, 1966. See above.

These papers describe the synthesis and some of the reactions of 2-fluoro-6-O-benzyl-inosine.

Tsarouhtsis et al., 1995; DeCorte et al., 1996. See above.

Synthesis of 2-fluoro-6-O-(trimethylsilylethyl)-2′-deoxyinosine and the related phosphoramidite; oligonucleotide synthesis and substitution reactions.

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