Regioselective 2′-Silylation of Purine Ribonucleosides for Phosphoramidite RNA Synthesis

UNIT 2.8

This unit describes high-yield procedures for protection of purine ribonucleosides based on a reaction that allows highly regioselective 2′-silylation. The *H*-phosphonate monoester group produced in the silylation reaction is then cleaved, without silyl migration (Song et al., 1999; Zhang et al., 1997), to give intermediates ready for phosphitylation to yield the phosphoramidites. This method gives overall yields that are three times the best yields available by conventional procedures for adenosine (see Basic Protocol 1) and guanosine (see Basic Protocol 2), but offers no advantage for cytidine or uridine.

BASIC PROTOCOL 1

SYNTHESIS OF 5′-*O*-(4,4′-DIMETHOXYTRITYL)-2′-*O*-*tert*-BUTYLDIMETHYLSILYL-6-*N*-ACYLADENOSINE

This protocol makes use of transient protection of the 2′,3′-diol moiety of a ribonucleoside (**S.2** in Fig. 2.8.1) by reaction with *N,N*-dimethylformamide dimethylacetal (Zemlicka, 1963) to prevent the small, but potentially troublesome, tritylation of the 2′-hydroxyl that otherwise accompanies tritylation of the 5′-hydroxyl (Zhang et al., 1997). The 2′,3′-*O*-dimethylaminomethylene group is cleaved by any protic solvent. The *N*-dimethylaminomethylene group is cleaved by treatment with either aqueous ammonia or methylamine. The phenoxyacetylation reaction is carried out using the hydroxybenzotriazole active ester of phenoxyacetic acid after transient hydroxyl protection with trimethylchlorosilane.

The regioselective silylation of the *N*- and 5′-*O*-protected adenosine and guanosine derivatives (**S.4** and **S.13**, respectively) presumably occurs by a reaction sequence in which the phenyl-*H*-phosphonate reacts first with *tert*-butyldimethylchlorosilane to generate the corresponding diester, which then undergoes a transesterification with **S.4/S.13** to generate a mixture of isomers (**S.5/S.14**). Subsequent transfer of the *tert*-butyldimethylsilyl (TBDMS) group predominantly to the more acidic 2′-hydroxyl gives **S.6a/S15a** along with 10% to 15% of the 3′-*O*-TBDMS isomers.

The *H*-phosphonate moiety is removed by reaction of the isomers of **S.6a,b/S15a,b** with ethylene glycol or glycerol. The extraordinarily facile transesterification of *H*-phosphonate diesters in the presence of a vicinal hydroxyl group effects the conversion to **S.8a,b/S.17a,b** quantitatively within minutes, presumably via the intermediate **S.7a,b/S.16a,b**.

After careful purification, **S8a/S.17a** are converted to the phosphoramidites **S.9/S.18** by reaction with 2-cyanoethyl tetraisopropylphosphorodiamidite using diisopropylammonium tetrazolide as a catalyst. A short silica gel column removes the excess reagent.

For *H*-phosphonate synthesis, monomers like **S.6a/S.15a** but without amino protection can be prepared by a similar route (Zhang et al., 1997). The labile phenoxyacetyl group used here does not survive the polar conditions required for purification of the charged *H*-phosphonates.

Materials

- Adenosine
- Pyridine (reagent grade or better)
- Dimethylformamide dimethyl acetal
- Nitrogen source

Contributed by Barbara L. Gaffney and Roger A. Jones
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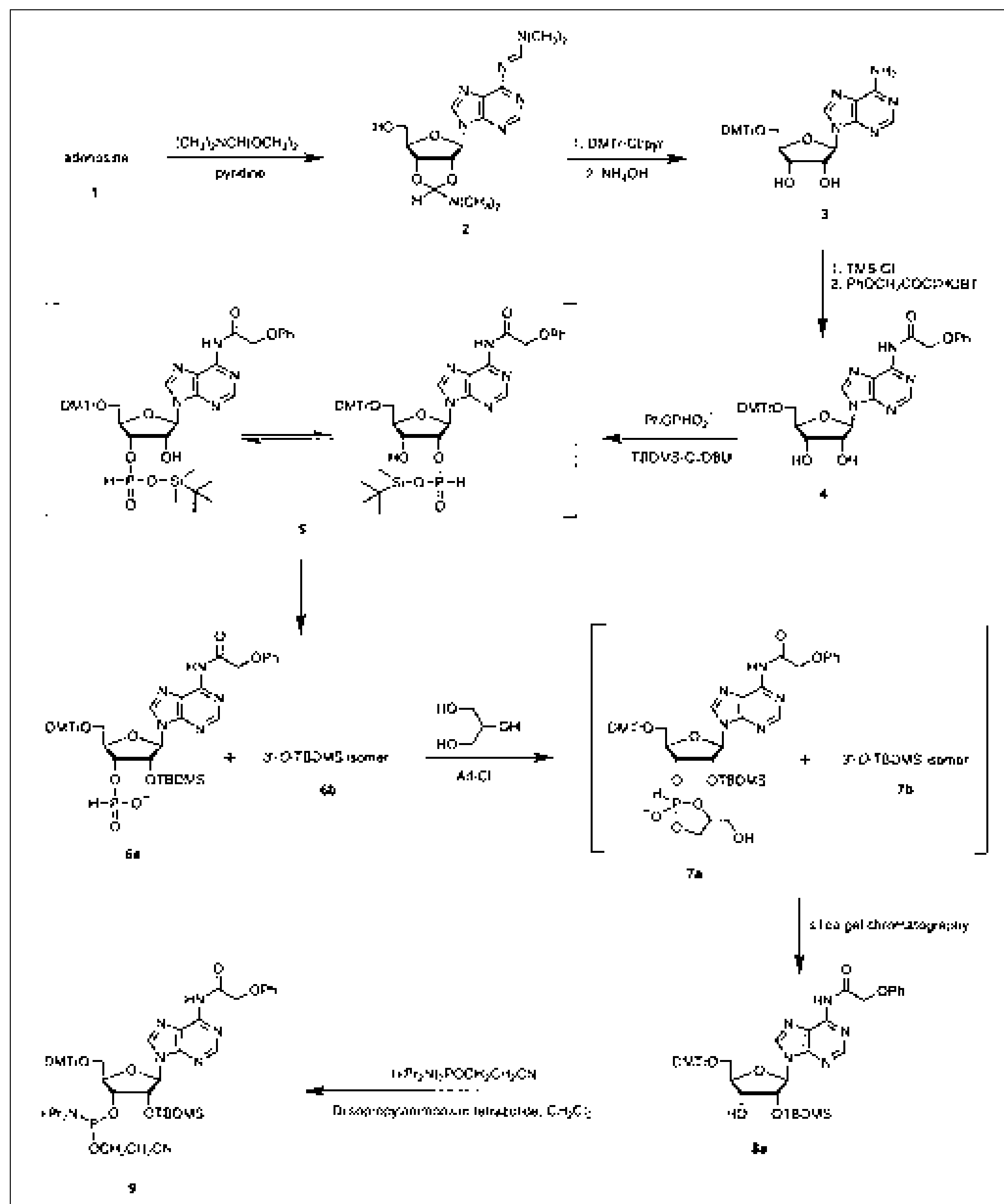

Figure 2.8.1 Dimethoxytritylation *N*-phenoxyacetylation, silylation, dephosphonylation, and phosphitylation of adenosine. Abbreviations: Ad, adamantanecarbonyl; DBU, 1,8-diazabycyclo[5.4.0]undex-7-ene; DMTr, 4,4′-dimethoxytrityl; HOBT, 1-hydroxybenzotriazole; Ph, phenyl; *i*-Pr, isopropyl; TMS, trimethylsilyl; TBDMS, *tert*-butyldimethylsilyl.

Acetonitrile (anhydrous, dried over 3Å molecular sieves)
0.1 M triethylammonium acetate (TEAA)
4,4′-Dimethoxytrityl chloride (DMTr-Cl)
5% (v/v) methanol in dichloromethane
Dichloromethane
Concentrated aqueous ammonium hydroxide
N-Methylmorpholine
Trimethylchlorosilane
Adenosine phenoxyacetylating reagent (see recipe)
Sodium bicarbonate
Ethyl acetate

Petroleum ether
Ammonium phenyl-*H*-phosphonate (see recipe)
1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU)
tert-Butyldimethylsilyl chloride (TBDMS·Cl)
0.5 M potassium phosphate buffer, pH 7.0 (*APPENDIX 2A*)
Glycerol
1-Adamantanecarbonyl chloride
Diisopropylammonium tetrazolide
Argon source
2-Cyanoethyl tetraisopropylphosphorodiamidite
Methylene chloride (anhydrous)
Triethylamine (anhydrous)

250-mL and 25-mL round-bottom flask
Rotary evaporator
Silica gel 60F TLC plates (Merck)
Waters XTerra 2.5-µm C18 chromatography column
Vacuum pump
Septum
Vent needle
Desiccator with P_2O_5

Additional reagents and equipment for TLC (*APPENDIX 3D*), column chromatography (*APPENDIX 3E*), and HPLC (*UNIT 10.5*)

Tritylate adenosine

1. Suspend 1.34 g (5 mmol) adenosine in 10 mL pyridine in a 250-mL round-bottom flask with a magnetic stir bar and concentrate to dryness using a rotary evaporator and vacuum pump. Repeat this azeotropic drying process two times with 10-mL portions of pyridine.

2. Suspend the dry adenosine in 60 mL pyridine, reduce the volume to ~50 mL, and add 2.6 mL (20 mmol) dimethylformamide dimethyl acetal.

3. Seal the flask with a septum and displace the air with nitrogen through a vent needle in the septum. After a few minutes, remove the nitrogen line and vent needle. Allow to sit for 1 hr.

4. Concentrate to an oil, dissolve the oil in 60 mL pyridine, and concentrate to ~50 mL.

5. Add 2.03 g (6 mmol) 4,4′-dimethoxytrityl chloride and stir 2 to 3 hr.

6. Check the reaction by HPLC using a gradient of 2:98 to 80:20 acetonitrile: 0.1 M TEAA, pH 6.8 on a C18 column or by TLC (*APPENDIX 3D*) on silica gel plates using 60F methanol in dichloromethane (Table 2.8.1). Examine the plate under UV light, and then hold it over an open container of fresh aqueous HCl to observe trityl-containing spots.

   When the TLC plate is held over an open container of HCl, the fumes will cause any trityl-containing spots to turn a bright orange.

   If the reaction is not complete after 1 hr, add 0.5 mmol more of 4,4′-dimethoxytrityl chloride and wait an additional 1 hr.

   HPLC and TLC mobility of compounds in the reaction are listed in Table 2.8.1.

Table 2.8.1 HPLC and TLC Mobility of Compounds Described in Figures 2.8.1 and 2.8.2

Compound (structure number in figures)	TLC R_f (methanol:dichloromethane) (5:95)	TLC R_f (methanol:dichloromethane) (10:90)	HPLC[a] Retention time, min
Adenosine			
3	0.09	0.27	11.3
4	0.08	0.37	12.5
6a,6b	0.01 minor 0.03 major	0.08 minor 0.13 major	12.7 major 13.4 minor
8a	0.55	0.83	15.7
8b	0.48	0.75	16.2
9	0.80, 0.84	0.73, 0.90	—[b]
Guanosine			
11	0.00	0.10	—
13	0.05	0.44	11.0
15a,b	—	—	11.1 major 12.2 minor
17a	0.41	0.58	13.9
17b	0.32	0.53	14.5
18	0.38, 0.43	0.58, 0.83	—[b]

[a]Gradient of 2:98 to 80:20 acetonitrile:0.1 M TEAA (pH 6.8) over 10 min, remaining at 80:20 for 5 min, then back down to 2:98 over 2 min, at 1 mL/min on a Waters XTerra 2.5 µm C18 column.
[b]Too hydrophobic to analyze conveniently on HPLC.

7. Add 10 mL methanol to quench the excess reagent, wait 5 min, and then pour the solution into 100 mL water containing 1 g (12 mmol) sodium bicarbonate.

8. Extract the solution two times with 80-mL portions of dichloromethane, concentrate the combined organic layers, dissolve the residue in 25 mL pyridine, and add 25 mL concentrated aqueous ammonium hydroxide. Seal the flask tightly and heat 5 hr at 60°C.

   Use caution in heating this closed system. Use a shield and carefully inspect the flask for cracks or defects that might weaken it.

9. Cool to room temperature, open the flask carefully, and check the mixture by HPLC or TLC to have a record of the retention time or R_f value of **S.3** (Table 2.8.1).

10. Concentrate the mixture with frequent additions of pyridine so that the water is removed azeotropically to give **S.3** as an oil. Dry by evaporation of pyridine as in step 1, leaving dry **S.3** in ~50 mL pyridine.

Phenoxyacetylate

11. To the solution of **S.3** in 50 mL dry pyridine, add 5.5 mL (50 mmol) *N*-methylmorpholine. Seal the flask with a septum and displace the air with nitrogen through a vent needle in the septum. Keep the nitrogen flowing slowly.

12. Cool this mixture in an ice-bath and add 3.2 mL (25 mmol) trimethylchlorosilane over 3 to 5 min.

    Trimethylchlorosilane is highly moisture sensitive. Use of special bottles such as the Aldrich SURE-SEAL system will help to prevent deterioration of the reagent.

13. Remove the flask from the ice-bath and maintain for 1 hr at room temperature.

14. Add all of the freshly prepared adenosine phenoxyacetylating reagent using a syringe. Remove the nitrogen line and vent needle and stir 12 to 18 hr.

15. Check the reaction by HPLC or TLC.

    If the reaction is not complete, add 0.5 mmol more of the phenoxyacetylating reagent and wait an additional 2 hr.

16. Pour the mixture into 100 mL water containing 2.5 g (30 mmol) sodium bicarbonate, extract two times with 100-mL portions of dichloromethane, and concentrate the combined organic layers to dryness.

17. Dissolve the residue in 50 mL pyridine, add 25 mL water, and stir 12 to 18 hr.

18. Concentrate the solution and check the mixture by HPLC or TLC.

19. Purify the residue by column chromatography (*APPENDIX 3E*) on silica gel using 0:100 to 15:85 (v/v) methanol/dichloromethane to give pure **S.4** in yields of up to ~90%.

Silylate

20. To 2.63 g (15 mmol) of ammonium phenyl-*H*-phosphonate, add 2.3 mL (15 mmol) DBU and co-evaporate with 50 mL pyridine.

21. Dissolve the residue in 120 mL pyridine, concentrate to ~100 mL, add 2.26 g (15 mmol) TBDMS-Cl and mix. Place 3.52 g (5 mmol) **S.4** in a dry 250-mL round-bottom flask and dry by evaporation of pyridine as in step 1. Using a syringe, add the mixture prepared in step 21 to **S.4**, followed by 3.8 mL (25 mmol) DBU.

    The silylation reaction can be performed using other N- and 5′-O-protected adenosine derivatives.

22. Stir 5 to 8 hr and check the reaction by HPLC or TLC.

    If the reaction is not complete, add 1 mmol more of DBU and wait another 2 hr.

23. Pour the mixture into 100 mL of 0.5 M aqueous potassium phosphate buffer, pH 7.0, and extract two times with 100-mL portions of dichloromethane. Concentrate the combined organic layers to dryness.

Dephosphonylate

24. Add 1.38 g (15 mmol) glycerol to the residue and dry the mixture by co-evaporation with 50 mL pyridine.

25. Dissolve the residue in 60 mL pyridine, concentrate to ~50 mL, and add 2.98 g (15 mmol) of 1-adamantanecarbonyl chloride. Stir 10 min.

26. Pour the solution into 100 mL of 0.5 M aqueous potassium phosphate buffer and extract two times with 100-mL portions of dichloromethane. Concentrate the combined organic layers to dryness.

27. Check the mixture by HPLC or TLC and purify the residue by column chromatography in silica gel using 60:40 to 100:0 (v/v) ethyl acetate petroleum ether to give **S.8a** in yields of ~65% from **S.4**.

    The isomers can be distinguished because the 2′-O-silyl isomer runs faster than the 3′-O-silyl isomer both on silica gel and C18 reversed-phase chromatography. See Table 2.8.2 for NMR data. If desired, the identity can be verified by two-dimensional COSY NMR: for the 2′-O-silyl isomer, the hydroxyl resonance only shows a cross-peak to the 3′-H resonance, while for the 3′-O-silyl isomer, the hydroxy resonance only shows a cross-peak to the 2′-H resonance.

Phosphitylate

28. In an oven-dried 25-mL round-bottom flask, place 3.05 g (3.0 mmol) of pure **S.8a** and 0.28 g (1.5 mmol) of diisopropylammonium tetrazolide. Dry the flask and a rubber septum (not inserted) in an evacuated desiccator over P_2O_5 overnight.

    It is critical that the reaction be absolutely anhydrous. Further, the starting material must be pure, since the product can only tolerate a very fast chromatographic purification to remove the excess reagent.

29. Open the desiccator under argon and immediately insert the septum. Displace any air with argon through a vent needle in the septum. Add 15 mL of dry dichloromethane through the septum and swirl 5 to 10 min to dissolve the solids completely.

30. Cool the flask in an ice bath at 0° and add 1.00 mL (3.0 mmol) of 2-cyanoethyl tetraisopropylphosphorodiamidite. Keep the mixture at 0° for one hour and swirl it every 15 min or so.

31. Remove a small sample carefully with a syringe with an oven-dried needle to check by HPLC or TLC. Normally, the reaction will be about 75% to 85% done.

32. Remove the flask from the ice bath and keep it at room temperature. Add another 0.5 mL (1.5 mmol) of the phosphitylating reagent and allow the reaction to proceed for several more hours. Check by HPLC or TLC no more than once per hr, each time using a dry syringe needle.

    Note that the product exists as a pair of diastereomers, since the phosphorus atom is chiral. Any inadvertent hydrolysis of the product results in a pair of hydrogen phosphonate diesters that are much more polar than the product. Some hydrolysis will occur during HPLC and TLC analysis.

33. Prepare a small glass column containing about 10 cm of silica gel packed in 98:2 dry methylene chloride:triethylamine.

34. Place the reaction mixture directly onto this column and load it using nitrogen pressure. Wash the column using nitrogen pressure with about 30 mL of 98:2 dry methylene chloride:triethylamine, followed by 49:1:50 dry methylene chloride:triethylamine:dry acetonitrile.

    The product normally elutes after 20 to 50 mL, sometimes just after a yellow impurity.

    It is very important to work as quickly as possible so as not to leave the product in solution any longer than necessary, since it will start to degrade immediately. A quick way to check the fractions for product is to spot them on a grid marked on a TLC plate. The plate does not have to be developed, just checked for UV-active material. Then the first and last fractions can be checked by HPLC or on a developed TLC plate.

36. Combine the fractions containing pure product and evaporate to a foam. Dry the product in a desiccator over P_2O_5.

37. Check the product for purity by ^{31}P NMR (Table 2.8.2).

BASIC PROTOCOL 2

SYNTHESIS OF 5′-*O*-(4,4′-DIMETHOXYTRITYL)-2′-*O*-*tert*-BUTYLDIMETHYSILYL-2-*N*-ACYLGUANOSINE

In this protocol, *N*- and 5′-*O*-protected guanosine (**S.13** in Fig. 2.8.2) is generated in a similar fashion as **S.4** in Basic Protocol 1, except that guanosine is first amino protected (whereas adenosine is first tritylated) because the *N*-phenoxyacetyl derivative is crystalline and therefore easier to isolate. Silylation, dephosphorylation, and phosphitylation are performed following the procedures for adenosine.

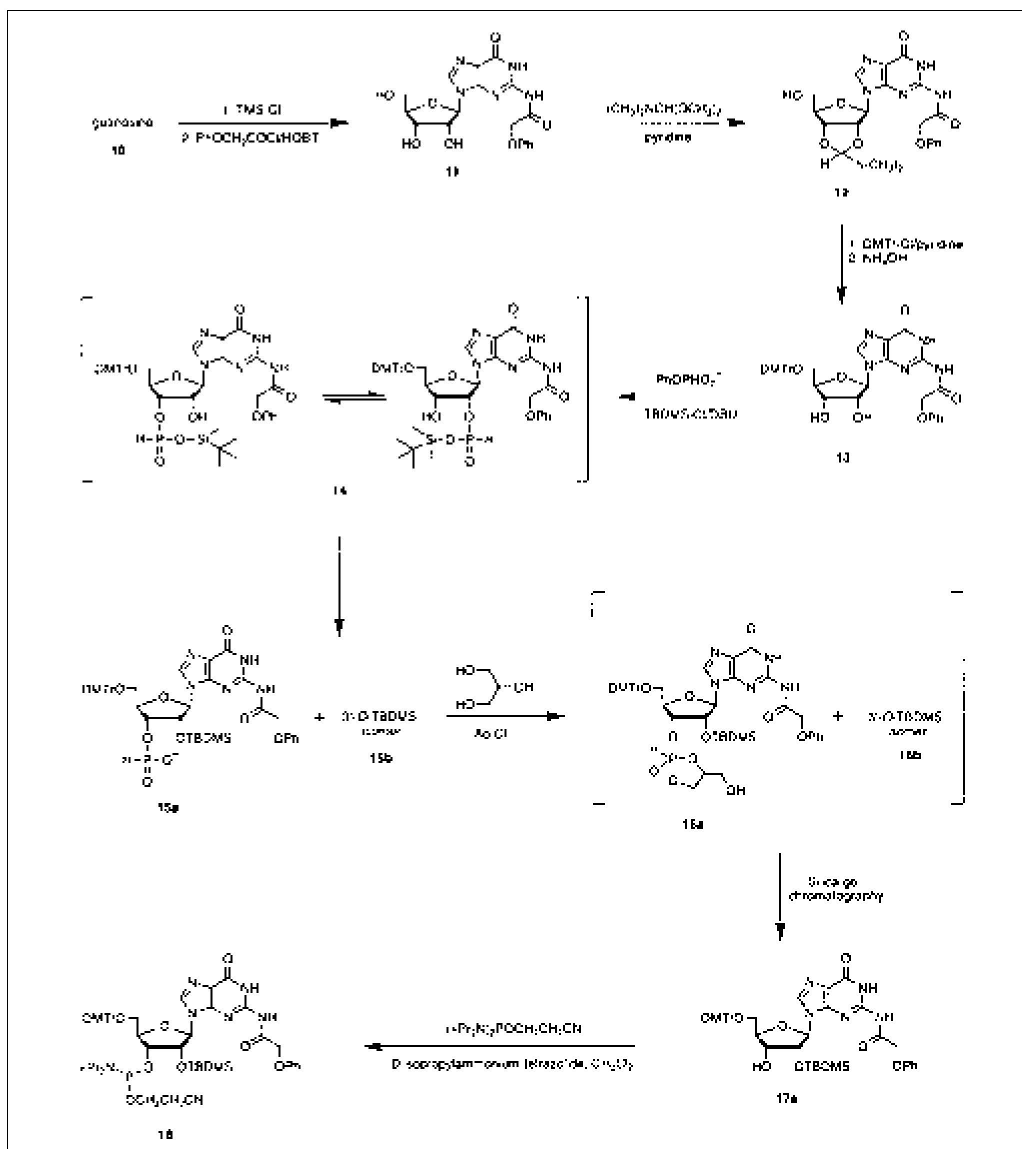

Figure 2.8.2 *N*-Phenoxylacetylation, dimethoxytritylation, silylation, dephosphonylation, and phosphitylation of guanosine. Abbreviations: Ad, adamantanecarbonyl; DBU, 1,8-diazabycly-clo[5.4.0]undec-7-ene; DMTr, 4,4′-dimethoxytrityl; HOBT, 1-hydroxybenzotriazole; Ph, phenyl; *i*-Pr, isopropyl; TMS, trimethylsilyl; TBDMS, *tert*-butyldimethylsilyl.

Materials

Guanosine
Pyridine
Nitrogen source
Trimethylchlorosilane
Guanosine phenoxyacetylating reagent (see recipe)
2-Propanol
Methanol
Dichloromethane
Acetonitrile (anhydrous, dried over 3Å molecular sieves)
0.1 M triethylammonium acetate (TEAA)
Dimethylformamide dimethyl acetal
4,4′-Dimethoxytrityl chloride (DMTr-Cl)
Methanol

Sodium bicarbonate
Dichloromethane
Ammonium phenyl-*H*-phosphonate (see recipe)
1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU)
tert-Butyldimethylsilyl chloride (TBDMS-Cl)
0.5 M potassium phosphate buffer, pH 7.0 (*APPENDIX 2A*)
Glycerol
1-Adamantanecarbonyl chloride
5:95 to 15:85 (v/v) acetone/dichloromethane (optional; for phosphoramidite synthesis)

250-mL round-bottom flasks
Magnetic stir bar
Rotary evaporator
Silica gel 60F TLC plates (Merck)
Waters XTerra 2.5-µm C18 chromatography column
Vacuum pump
Septum
Vent needle
Desiccator with P_2O_5

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

Phenoxyacetylate guanosine

1. Suspend 1.42 g (5 mmol) guanosine in 10 mL pyridine in a 250-mL round-bottom flask with a magnetic stir bar and concentrate to dryness using a rotary evaporator and vacuum pump. Repeat this azeotropic drying process two times with 10-mL portions of pyridine.

2. Suspend the dry guanosine in 60 mL pyridine and concentrate to ~50 mL.

3. Seal the flask with a septum and displace the air with nitrogen through a vent needle in the septum. Keep the nitrogen flowing slowly.

4. Cool this mixture in an ice-bath and add 3.8 mL (30 mmol) trimethylchlorosilane over 3 to 5 min.

   Trimethylchlorosilane is highly moisture sensitive. Use of special bottles such as the Aldrich SURE-SEAL system will help to prevent deterioration of the reagent.

5. Remove the flask from the ice bath and maintain 1 hr at room temperature.

6. Add all the freshly prepared guanosine phenoxyacetylating reagent using a syringe. Remove the nitrogen line and vent needle and stir 36 hr.

7. Add 30 mL water, concentrate the solution, and co-evaporate two times with 30-mL portions of water to a final volume of ~15 mL.

8. Filter the slurry.

   *The solid contains both the product (**S.11**) and some 1-hydroxybenzotriazole from the guanosine phenoxyacetylating reagent.*

9. To remove the 1-hydroxybenzotriazole, shake the solid thoroughly with a 50-mL portion of water and filter. Repeat with 20 mL water followed by three 20-mL portions of 2-propanol to give **S.11** as a colorless solid in yields of up to 95%.

10. Check the product by HPLC using a gradient of 2:98 to 80:20 acetonitrile: 0.1 M TEAA pH 6.8 on a C18 column or TLC (*APPENDIX 3D*) on silica gel 60F plates using the appropriate concentration of methanol in dichloromethane (Table 2.8.1).

11. Dry the product in a desiccator over P_2O_5 at least overnight and check that the yield is <100%.

    A yield >100% means that the product is contaminated with hydroxybenzotriazole and one should repeat the thorough shaking with 2-propanol.

Tritylate

12. Dissolve the dry **S.11** in 60 mL pyridine, concentrate to ~50 mL, and add 0.8 mL (6 mmol) dimethylformamide dimethyl acetal.

13. Seal the flask with a septum and displace the air with nitrogen through a vent needle in the septum. After a few minutes, remove the nitrogen line and vent needle. Allow to sit for 1 hr.

    The dimethylformamide dimethyl acetal will slowly replace the phenoxyacetyl group so the time and the amount of this reagent used is kept to a minimum.

14. Concentrate to an oil, dissolve the oil in 60 mL pyridine, and concentrate to ~50 mL.

15. Add 2.03 g (6 mmol) 4,4′-dimethoxytrityl chloride and stir 2 to 3 hr.

16. Check the reaction by HPLC or TLC. Examine the plate under UV light, and then hold it over an open container of fresh aqueous HCl to observe trityl-containing spots.

    When the TLC plate is held over an open container of HCl, the fumes will cause any trityl-containing spots to turn a bright orange.

    If the reaction is not complete after 1 hr, add an additional 0.5 mmol of 4,4′-dimethoxytrityl chloride and wait another 1 hr.

17. Add 10 mL methanol to quench the excess reagent, wait 5 min, and then pour the solution into 100 mL water containing 1 g (12 mmol) sodium bicarbonate.

18. Extract the solution two times with 80-mL portions of dichloromethane and concentrate the combined organic layers to dryness.

19. Check the mixture by HPLC or TLC. Purify the residue by column chromatography (*APPENDIX 3E*) on silica gel using 0:100 to 10:90 (v/v) methanol/dichloromethane to give pure **S.13** in yields of up to ~90%.

Silylate

20. To 2.63 g (15 mmol) ammonium phenyl-*H*-phosphonate, add 2.3 mL (15 mmol) DBU and co-evaporate with 50 mL pyridine.

21. Dissolve the residue in 120 mL pyridine, concentrate to ~100 mL, add 2.26 g (15 mmol) TBDMS-Cl and mix. Place 3.52 g (5 mmol) **S.4** in a dry 250-mL round-bottom flask and dry by evaporation of pyridine as in step 1. Using a syringe, add the mixture prepared in step 21 to **S.4**, followed by 3.8 mL (25 mmol) DBU.

    The silylation reaction can be performed using other N- and 5′-O-protected guanosine derivatives.

22. Stir 5 to 8 hr and check the reaction by HPLC or TLC.

    If the reaction is not complete, add 1 mmol more of DBU and wait another 2 hr.

23. Pour the mixture into 100 mL of 0.5 M aqueous potassium phosphate buffer, pH 7.0, and extract two times with 100-mL portions of dichloromethane. Concentrate the combined organic layers to dryness.

Dephosphonylate

24. Add 1.38 g (15 mmol) glycerol to the residue and dry the mixture by co-evaporation with 50 mL pyridine.

25. Dissolve the residue in 60 mL pyridine, concentrate to about ~50 mL, and add 2.98 g (15 mmol) of 1-adamantanecarbonyl chloride. Stir 10 min.

26. Pour the solution into 100 mL of 0.5 M potassium phosphate buffer, pH 7.0, and extract two times with 100-mL portions of dichloromethane. Concentrate the combined organic layers to dryness.

27. Check the mixture by HPLC or TLC and purify the residue by column chromatography on silica gel using 5:95 to 15:85 acetone/dichloromethane to give **S.17a** in yields of ~65% from **S.13**.

    The isomers can be distinguished because the 2′-O-silyl isomer runs faster than the 3′-O-silyl isomer both on silica gel and C18 reversed-phase chromatography. See Table 2.8.2 for NMR data. If desired, the identity can be verified by two-dimensional COSY NMR: for the 2′-O-silyl isomer, the hydroxyl resonance only shows a cross-peak to the 3′-H resonance, while for the 3′-O-silyl isomer, the hydroxyl resonance only shows a cross-peak to the 2′-H resonance.

Phosphitylate

28. In an oven-dried 25-mL round-bottom flask, place 3.05 g (3.0 mmol) of pure **S.17a** and 0.28 g (1.5 mmol) of diisopropylammonium tetrazolide. Dry the flask and a rubber septum (not inserted) in an evacuated desiccator over P_2O_5 overnight.

    It is critical that the reaction be absolutely anhydrous. Further, the starting material must be pure, since the product can only tolerate a very fast chromatographic purification to remove the excess reagent.

29. Open the desiccator under argon and immediately insert the septum. Displace any air with argon through a vent needle in the septum. Add 15 mL of dry dichloromethane through the septum and swirl 5 to 10 min to dissolve the solids completely.

30. Cool the flask in an ice bath at 0° and add 1.00 mL (3.0 mmol) of 2-cyanoethyl tetraisopropylphosphorodiamidite. Keep the mixture at 0° for 1 hr and swirl it every 15 min or so.

31. Remove a small sample carefully with a syringe with an oven-dried needle to check by HPLC or TLC. Normally, the reaction will be about 75% to 85% done.

32. Remove the flask from the ice bath and keep it at room temperature. Add another 0.5 mL (1.5 mmol) of the phosphitylating reagent and allow the reaction to proceed for several more hours. Check by HPLC or TLC no more than once per hr, each time using a dry syringe needle.

    Note that the product exists as a pair of diastereomers, since the phosphorus atom is chiral. Any inadvertent hydrolysis of the product results in a pair of hydrogen phosphonate diesters that are much more polar than the product. Some hydrolysis will occur during HPLC and TLC analysis.

33. Prepare a small glass column containing about 10 cm of silica gel packed in 98:2 dry methylene chloride:triethylamine.

Table 2.8.2 NMR Chemical Shifts (ppm)[a]

Compound	N1-H and N2-H	N6-H	H-8	H-2	aromatic	1′-H	2′-OH	3′-OH	CH_2	2′-H	3′-H	4′-H	MeO	5′,5″-H	tBuSi	MeSi
Adenosine ^1H NMR (DMSO-d_6)																
4	—	10.97 s	8.61 s	8.57 s	6.7-7.4	6.04 d	5.64 d	5.29 d	5.03 s	4.76 m	4.34 m	4.10 m	3.70 s	3.23 m	—	—
8a	—	10.97 s	8.62 s	8.59 s	6.7-7.4	6.05 d	—	5.22 d	5.02 s	4.87 m	4.28 m	4.13 m	3.71 s	3.28 m	0.74 s	–0.05 –0.15
8b	—	10.95 s	8.67 s	8.59 s	6.7-7.4	6.00 d	5.48 s	—	5.02 s	4.89 m	4.50 m	4.06 m	3.7 s	3.15 m	0.83 s	0.08 0.05
Guanosine ^1H NMR (DMSO-d_6)																
13	11.84 s 11.79 s	—	8.16 s	—	6.8-7.4	5.88 s	5.65 d	5.23 d	4.87 s	4.55 m	4.23 m	4.16 m	3.7 s	3.22 m	—	—
17a	11.80 br s	—	8.14 s	—	6.8-7.4	5.88 d	—	5.11 d	4.85 s	4.62 m	4.15 m	4.07 m	3.71 s	3.24 m	0.76	–0.01 –0.11
17b	11.85 s 11.79 s	—	8.18 s	—	6.8-7.4	5.80 d	5.52 d	—	4.85 s	4.58 m	4.21 m	3.94 m	3.71 s	3.17 m	0.81 s	0.05 0.00

[a]For ^{31}P NMR, the chemical shift for compound 9 is 147, 149 and that for compound 18 is 147, 151.

34. Place the reaction mixture directly onto this column and load it using nitrogen pressure. Wash the column using nitrogen pressure with about 30 mL of 98:2 dry methylene chloride:triethylamine, followed by 49:1:50 dry methylene chloride:triethylamine:dry acetonitrile.

The product normally elutes after 20 to 50 mL, sometimes just after a yellow impurity.

It is very important to work as quickly as possible so as not to leave the product in solution any longer than necessary, since it will start to degrade immediately. A quick way to check the fractions for product is to spot them on a grid marked on a TLC plate. The plate does not have to be developed, just checked for UV-active material. Then the first and last fractions can be checked by HPLC or on a developed TLC plate.

36. Combine the fractions containing pure product and evaporate to a foam. Dry the product in a desiccator over P_2O_5.

37. Check the product for purity by ^{31}P NMR (Table 2.8.2).

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****.*

Adenosine phenoxyacetylating reagent

Co-evaporate 2.03 g (15 mmol) of 1-hydroxybenzotriazole and 2.2 mL (20 mmol) *N*-methylmorpholine two times with 20-mL portions of acetonitrile. Dissolve the residue in 50 mL dichloromethane. Add 2.1 mL (15 mmol) phenoxyacetyl chloride and shake for 10 min. Prepare fresh.

Ammonium phenyl-H-phosphonate

Add 38.3 mL (0.20 mol) diphenyl phosphite over 10 min to 400 mL of 7.4 M aqueous ammonia. Stir for 1 hr. Concentrate to dryness and co-evaporate the residue two times with 100-mL portions of absolute ethanol. Stir the residue with 400 mL ethyl ether for 30 min to give up to 85% of the colorless crystalline product. Store up to 1 month at –20°C.

Diisopropylammonium tetrazolide

Place 2.00 g (28.5 mmol) of solid tetrazole in a dry 250-mL round-bottom flask containing a dry stir bar and add 130 mL of dry acetonitrile. Stir until dissolved, then add 9.0 mL (63.9 mmol) of freshly distilled diisopropylamine. After 2 min of stirring, collect the white precipitate by filtration in a dry glass funnel and wash it four times with 10-mL portions of dry acetonitrile. Dry the solid in a desiccator over P_2O_5. Store up to 3 months at –20°C.

Guanosine phenoxyacetylating reagent

Co-evaporate 1.35 g (10 mmol) of 1-hydroxybenzotriazole and 1.1 mL (10 mmol) *N*-methylmorpholine two times with 20-mL portions of acetonitrile. Dissolve the residue in 25 mL dichloromethane. Add 1.39 mL (10 mmol) phenoxyacetyl chloride and shake for 10 min. Prepare fresh.

COMMENTARY

Background Information

The procedures described here use adenosine and guanosine that are 5′-protected with the 4,4′-dimethoxytriyl (DMTr) group and amino-protected with the labile phenoxyacetyl group (Wu et al., 1988; Chaix et al., 1989; Singh and Nahar, 1995; Sinha et al., 1995). They should also be applicable to nucleosides containing most other amino-protecting groups. Amino protection of adenosine with the benzoyl group and guanosine with the isobutyryl group can be carried out by standard literature

procedures, and these derivatives are more easily handled than are the more-labile phenoxyacetylated compounds. A general discussion of amino-protecting groups and literature references is given in *UNIT 2.1*.

The procedures developed by Ogilvie for 2′-silylation proceed with only modest regioselectivity (Ogilvie, 1978, 1979). Although use of silver nitrate improves the selectivity, the results are variable and do not approach the selectivity of the above protocols (Hakimelahi, 1982). A general discussion of 2′-protecting groups, including the 2′-TBDMS group, is given in *UNIT 2.2*.

Critical Parameters

The most difficult of the above protocols to carry out are the tritylation and phenoxyacetylation steps used for preparation of **S.4** and **S.13**. Skill and practice are required to achieve high yields on these protection reactions, largely because of the phenoxyacetyl group. In contrast, the regioselective silylation and dephosphonylation reactions work well even in the hands of unskilled researchers.

Anticipated Results

For the reasons discussed above, some experience is required to achieve high yields for the phenoxyacetylation reactions, and initial efforts are likely be give more modest yields (e.g., 50%). With experience, yields of ~90% can be expected. The regioselectivity of the silylation reaction is invariably 85% to 90% regardless of experience.

Time Considerations

The total time for conversion of adenosine or guanosine to the fully protected derivatives **S.8a** and **S.17a**, respectively, is ~1 week.

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