Two-Step, One-Pot Synthesis of Inosine, Guanosine, and 2′-Deoxyguanosine O^6-Ethers via Intermediate O^6-(Benzotriazol-1-yl) Derivatives

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ABSTRACT

A simple method for the etherification at the O^6-position of silyl-protected inosine, guanosine, and 2′-deoxyguanosine is described. Typically, a THF solution of the silylated nucleoside is treated with 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and Cs_2CO_3 under a nitrogen atmosphere. Conversion to the O^6-(benzotriazol-1-yl) ethers occurs within about 10 min for inosine, and within about 60 min for guanosine and 2′-deoxyguanosine. Then, for reaction with alcohols, the reaction mixture is evaporated and the O^6-(benzotriazol-1-yl) ether is treated with Cs_2CO_3 and an appropriate alcohol, at room temperature. On the other hand, for reaction with phenols, Cs_2CO_3 and the appropriate phenol are added to the reaction mixture without evaporation, and the reaction is carried out at 70°C. Subsequently, workup, isolation, and purification lead to the requisite O^6-alkyl or O^6-aryl ethers in good to excellent yields. *Curr. Protoc. Nucleic Acid Chem.* 49:1.26.1-1.26.16. © 2012 by John Wiley & Sons, Inc.

Keywords: inosine • guanosine • 2′-deoxyguanosine • BOP • reactive nucleosides • ethers • benzotriazolyl

BASIC PROTOCOL

The ubiquitous presence of nucleosides in living systems makes them excellent scaffolds for chemical modification, and the heterocycle as well as the sugar moiety are targets for this purpose. The ensuing modified nucleoside analogs are highly important for a variety of purposes. To name a few, these include treatment of diseases, nucleic acid labeling, study of DNA damage, and use as probes for base-pair recognition and for other biological processes.

In this unit, a two-step, one-pot operational procedure is described for the modification of the purine bases of nucleosides. Specifically, an efficient protocol for etherification of the C-6 amide carbonyl in silyl-protected inosine, guanosine, and 2′-deoxyguanosine is the focus. The silylated nucleoside precursors are readily accessible from commercially available inosine, guanosine, and 2′-deoxyguanosine. Reactions of these with 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and Cs_2CO_3 result in efficient conversion of each to a reactive intermediate, the corresponding O^6-(benzotriazol-1-yl) derivative. These O^6-(benzotriazol-1-yl) derivatives then undergo reaction with an alcohol or a phenol, also in the presence of Cs_2CO_3, to produce the purine nucleoside O^6-ether derivatives. In the broader context, this approach provides a relatively simple procedure for the synthesis of various ether derivatives from purine nucleosides, and this Basic Protocol describes the synthesis of alkyl and aryl ethers from three purine nucleosides.

This protocol is related to one previously published (*UNIT 1.22*), wherein we explained the synthesis of O^6-(benzotriazol-1-yl)inosine and 2′-deoxyinosine derivatives as

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discrete isolated reactive entities. In a subsequent step, these isolated O^6-(benzotriazol-1-yl) nucleoside derivatives were converted to C-6 modified purine nucleosides via reactions with amine, thiol, and phenol nucleophiles. The original work on hypoxanthine nucleosides (Bae and Lakshman, 2007) was then extended to guanine nucleosides (Lakshman and Frank, 2009). Related to these reports, one-pot conversions of inosine and 2′-deoxyinosine to adenosine derivatives has been accomplished via the use of BOP (Wan et al., 2005). What has been less easy to achieve is a one-pot etherification of purine nucleosides, which eliminates isolation of the O^6-(benzotriazol-1-yl) intermediates. This is the subject of the present unit.

Synthesis of silyl-protected inosine, guanosine, and 2′-deoxyguanosine. As required, inosine (**S.1**), guanosine (**S.5**), or 2′-deoxyguanosine (**S.9**) is dried by addition and evaporation of anhydrous pyridine. Dry *N*,*N*-dimethylformamide (DMF) is added, followed by addition of imidazole and *tert*-butyldimethylsilyl chloride (TBDMS-Cl). After allowing the reactions to proceed for an appropriate length of time, the silylated product **S.2, S.6**, or **S.10** is isolated by precipitation into water, the solids are redissolved in dichloromethane and dried, and the solvent is evaporated. The crude material is purified by column chromatography on silica gel, using an appropriate eluting solvent.

*Synthesis of the O^6-alkyl and -aryl ethers of inosine, guanosine and 2′-deoxyguanosine from the silylated precursors **S.2**, **S.6**, and **S.10**.* The substrates and nucleophiles used in this protocol, as well as the yields of the O^6-ethers that were synthesized, are shown in Figure 1.26.1. (1) For synthesis of O^6-alkyl ethers (**S.4a-e, S.8a**, and **S.12a**), reaction of silyl-protected nucleosides **S.2, S.6**, or **S.10** and BOP is conducted at room temperature, in anhydrous THF, with Cs_2CO_3 as base. Typically, formation of the intermediate O^6-(benzotriazol-1-yl)inosine is complete within 10 min, whereas formation of O^6-(benzotriazol-1-yl)guanosine and O^6-(benzotriazol-1-yl)-2′-deoxyguanosine is complete within 1 hr. The reaction mixture is evaporated to dryness on a rotary evaporator. Cs_2CO_3 and the appropriate alcohol are added, and the reaction mixture is stirred at room temperature for an appropriate length of time (**S.4a** = 10 min, **S.4b** = 20 min, **S.4c** = 10 min, **S.4d** = 7 hr, **S.4e** = 2 hr, **S.8a** = 2 hr, and **S.12a** = 3.5 hr). (2) For synthesis of O^6-aryl ethers (**S.4f**, **S.8b**, **S.12b**), reaction of silyl-protected nucleosides **S.2**, **S.6**, or **S.10** and BOP is conducted at room temperature, in anhydrous THF, with Cs_2CO_3 as base. Typically, formation of the intermediate O^6-(benzotriazol-1-yl)inosine is complete within 10 min at room temperature, whereas formation of O^6-(benzotriazol-1-yl)guanosine and O^6-(benzotriazol-1-yl)-2′-deoxyguanosine is complete within 1 hr. The reaction mixture is not evaporated, but Cs_2CO_3 and the appropriate phenol are added to the reaction mixture. The mixture is stirred at 70°C for an appropriate length of time (**S.4f** = 4 hr, **S.8b** = 1.5 hr, **S.12b** = 1.5 hr).

For either (1) or (2) above, workup of the reaction mixture, to isolate the crude material, followed by chromatographic purification on silica gel, then affords the desired products.

CAUTION: All operations involving organic solvents and reagents should be conducted in a well-ventilated hood. Use of protective equipment such as gloves, safety glasses, and laboratory coats is strongly recommended.

CAUTION: Reactions with BOP produce hexamethylphosphoramide (HMPA), a suspected carcinogen. Appropriate precautions should be taken when using this compound.

Materials

Inosine (**S.1**), 99% (Acros)
Anhydrous pyridine, distilled from KOH (stored over KOH)
N,N-Dimethylformamide (DMF), anhydrous (Aldrich)

Imidazole, 99% pure (Sigma)
tert-Butyldimethylsilyl chloride (TBDMS-Cl), 98% pure (Acros)
Hexanes, ACS grade (Fisher Scientific)
Dichloromethane (CH_2Cl_2), ACS grade (Fisher Scientific)
Sodium sulfate (Na_2SO_4), anhydrous, 99% pure (Spectrum)
200- to 300-mesh silica gel (Natland, *http://www.natland.com/*)
Ethyl acetate (EtOAc), HPLC grade (Fisher Scientific)
Guanosine (**S.5**), 98% (Lancaster Synthesis)
2′-Deoxyguanosine (**S.9**), 98% (Transgenomic, *http://www/transgenomic.com*)
1*H*-Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), ≥98% pure (Chem-Impex, *http://www.chemimpex.com*)
Tetrahydrofuran (THF): distilled over lithium aluminum hydride ($LiAlH_4$) and then distilled over sodium just prior to use
Cesium carbonate (Cs_2CO_3), 99% pure (Aldrich)
Nitrogen gas and balloons
Alcohols and phenols for reaction with **S.2**, **S.6**, and **S.10**:
Methanol (for **S.4a**, **S.8a**, and **S.12a**)
Allyl alcohol (for **S.4b**)
Propargyl alcohol (for **S.4c**)
Isopropyl alcohol (for **S.4d**)
Ethylene glycol (for **S.4e**)
p-Nitrophenol (for **S.4f)**
p-Methoxyphenol (for **S.8b**)
Phenol (for **S.12b**)

50- and 100-mL round-bottom flasks
Rotary evaporator equipped with a water aspirator
Magnetic stirrer and stir bars
Büchner funnel and appropriate filter flask
Water aspirator
Glass funnel, plugged with cotton
Oil pump for vacuum drying
TLC plates: 200-μm aluminum foil-backed silica gel plates with fluorescent indicator (for TLC analysis; Analtech)
Dual-wavelength UV lamp (254 and 365 nm; for TLC analysis)
70°C temperature-controlled sand bath
4-mL clear glass vials with Teflon/rubber-lined, closed-top, screw caps (Wheaton) for conducting etherification reactions
60- and 125-mL separatory funnels
Fraction collector

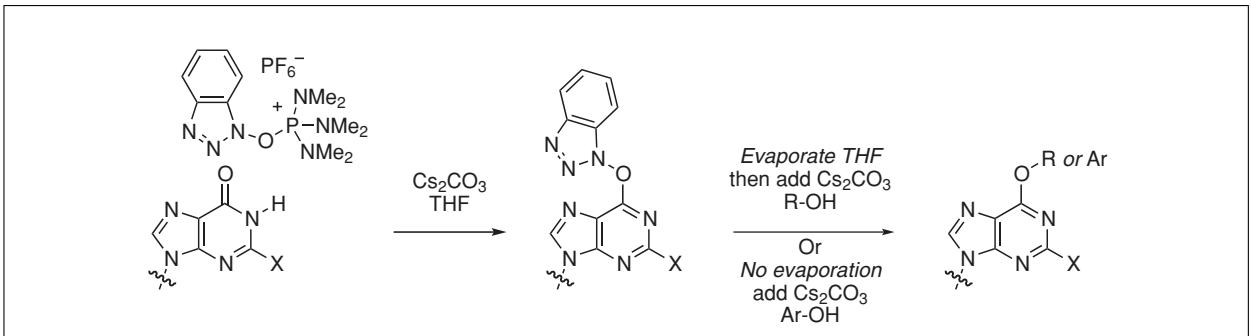
Additional reagents and equipment for thin-layer chromatography (TLC; *APPENDIX 3D*) and silica gel column chromatography (*APPENDIX 3E*)

NOTE: Except where indicated above, all reagents were obtained from commercial sources and used without further purification.

Silylate hydroxyl groups

Inosine

1. Using a round-bottom flask, dry inosine (**S.1**) two times by addition and evaporation of anhydrous pyridine (add an appropriate volume of pyridine to completely wet the nucleoside), using a rotary evaporator. Dissolve the residue in anhydrous DMF, and then add imidazole and TBDMS-Cl with magnetic stirring. Stir the reaction mixture at room temperature as indicated below.



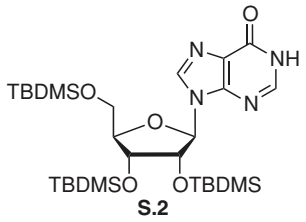
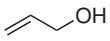
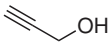
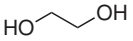
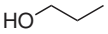
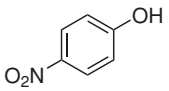
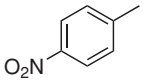
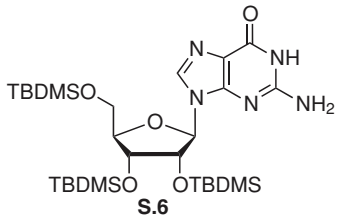
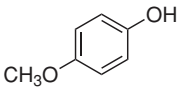
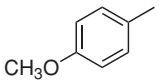
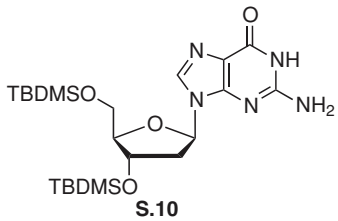
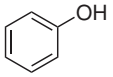
Nucleoside substrate	R-OH or Ar-OH	Product	R or Ar =	% Yield
 **S.2**	CH_3–OH	S.4a	CH_3—	94
		S.4b		90
		S.4c		86
		S.4d		75
		S.4e		63 (+ 15% dimer)
		S.4f		85
 **S.6**	CH_3–OH	S.8a	CH_3—	73
		S.8b		75
 **S.10**	CH_3–OH	S.12a	CH_3—	75
		S.12b		73

Figure 1.26.1 Substrates and alcohols used for the two-step, one-pot etherification, product numbers, and corresponding yields.

*Reagent quantities for **S.2**: 1.0 g (3.72 mmol) inosine **S.1**, 2.03 g (29.76 mmol, 8 eq.), imidazole, 2.24 g (14.8 mmol, 4 eq.) TBDMS-Cl, and 5 mL DMF. Reaction time: 16 hr.*

Volumes for some reagents (e.g., pyridine in this step, water and hexanes in the next step) are not provided for methods such as co-evaporation, precipitation, and washing because they are considered standard operating procedure.

2. Add water to the mixture and stir for 10 min. Filter the white precipitate in a Büchner funnel, using a water aspirator, and then wash the precipitate with water followed by hexanes.

3. Dissolve the resulting white precipitate in CH_2Cl_2. Dry over anhydrous Na_2SO_4 and filter out the drying reagent using a glass funnel plugged with cotton. Evaporate the filtrate to dryness, using a rotary evaporator, and then dry the product overnight under oil pump vacuum.

   *Compound **S.2** may need purification to remove partially protected products. Purify by silica gel column chromatography, using 3% (v/v) methanol in CH_2Cl_2 as the solvent system.*

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

   *2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)inosine (**S.2**). Yield of white powder 2.1 g (94%). R_f: 0.41 (9:1 v/v CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.74 (br s, 1H, NH), 8.23 (s, 1H, purinyl-H), 8.08 (s, 1H, purinyl-H), 6.01 (d, 1H, H-1′, J = 4.9 Hz), 4.51 (t, 1H, H-2′, J = 4.4 Hz), 4.30 (t, 1H, H-3′, J = 3.9 Hz), 4.13 (m, 1H, H-4′ Hz), 3.99 (dd, 1H, H-5′, J = 3.9, 11.2 Hz), 3.80 (dd, 1H, H-5′, J = 2.6, 11.2 Hz), 0.96, 0.93, and 0.81 (3s, 27H, tert-Bu), 0.15, 0.14, 0.10, 0.09, –0.02, and –0.18 (6s, 18H, $SiCH_3$).*

Guanosine

5. Using a round-bottom flask, dry guanosine (**S.5**) two times by addition and evaporation of anhydrous pyridine (add an appropriate volume of pyridine to completely wet the nucleoside), using a rotary evaporator. Dissolve the residue in anhydrous DMF, and then add imidazole and TBDMS-Cl with magnetic stirring. Stir the reaction mixture at room temperature as indicated below.

   *Reagent quantities for **S.6**: 1.0 g (3.53 mmol) guanosine **S.5**, 1.92 g (28.2 mmol, 8 eq.), imidazole, 2.12 g (14.1 mmol, 4 eq.) TBDMS-Cl, and 5 mL DMF. Reaction time: 16 hr.*

   Volumes for some reagents (e.g., pyridine in this step, water and hexanes in the next step) are not provided for methods such as co-evaporation, precipitation, and washing because they are considered standard operating procedure.

6. Add water to the mixture and stir for 10 min. Filter the white precipitate in a Büchner funnel, using a water aspirator, and then wash the precipitate with water followed by hexanes.

7. Dissolve the resulting white precipitate in CH_2Cl_2. Dry over anhydrous Na_2SO_4 and filter out the drying reagent using a glass funnel plugged with cotton. Evaporate the filtrate to dryness, using a rotary evaporator, and then dry the product overnight under oil pump vacuum.

   *Compound **S.6** may need purification to remove partially protected products. Purify by silica gel column chromatography, using 3% (v/v) EtOAc in hexanes as the solvent system.*

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

   *2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)guanosine (**S.6**). Yield of white powder 2.15 g (97%). R_f: 0.50 (9:1 v/v CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.01 (br s, 1H, NH), 7.90 (s, 1H, purinyl-H), 6.30 (br s, 2H, NH_2), 5.81 (d, 1H, H-1′, J = 2.9 Hz), 4.42 (m, 1H, H-2′), 4.27 (t, 1H, H-3′, J = 3.9 Hz), 4.09 (m, 1H, H-4′), 3.98 (dd, 1H, H-5′, J = 2.4, 11.2 Hz), 3.79 (dd, 1H, H-5′, J = 2.4, 11.2 Hz), 0.95, 0.91, and 0.86 (3s, 27H, tert-Bu), 0.13, 0.12, 0.09, 0.08, 0.01, and –0.04 (6s, 18H, $SiCH_3$).*

2′-Deoxyguanosine

9. Using a round-bottom flask dry 2′-deoxyguanosine (**S.9**) two times by addition and evaporation of anhydrous pyridine (add an appropriate volume of pyridine to completely wet the nucleoside), using a rotary evaporator. Dissolve the residue in anhydrous DMF, and then add imidazole and TBDMS-Cl with magnetic stirring. Stir the reaction mixture at room temperature as indicated below.

   *Reagent quantities for **S.10**: 1.0 g (3.7 mmol) 2′-deoxyguanosine **S.9**, 1.5 g (22.2 mmol, 6 eq.), imidazole, 1.6 g (11.1 mmol, 3 eq.) TBDMS-Cl, and 5 mL DMF. Reaction time: 16 hr.*

Volumes for some reagents (e.g., pyridine in this step, water and hexanes in the next step) are not provided for methods such as co-evaporation, precipitation, and washing because they are considered standard operating procedure.

10. Add water to the mixture and stir for 10 min. Filter the white precipitate in a Büchner funnel, using a water aspirator, and then wash the precipitate with water followed by hexanes.

11. Dissolve the resulting white precipitate in CH_2Cl_2. Dry over anhydrous Na_2SO_4 and filter out the drying reagent using a glass funnel plugged with cotton. Evaporate the filtrate to dryness, using a rotary evaporator, and then dry the product overnight under oil pump vacuum.

    *Compound **S.10** may need purification to remove partially protected products. Purify by silica gel column chromatography, using 10% (v/v) MeOH in CH_2Cl_2 solvent system.*

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

    *2′,3′-Di-O-(tert-butyldimethylsilyl)-2′-deoxyguanosine (**S.10**). Yield of white powder 1.75 g (95%). R_f: 0.42 (9:1 v/v CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.61 (br s, 1H, NH), 7.87 (s, 1H, purinyl-H), 6.47 (br s, 2H, NH_2), 6.10 (t, 1H, H-1′, J = 6.8 Hz), 4.48 (m, 1H, H-3′), 3.80 (m, 1H, H-4′), 3.69 (dd, 1H, H-5′, J = 6.3, 11.2 Hz), 3.64 (dd, 1H, H-5′, J = 4.3, 11.2 Hz), 2.64 (app quint, 1H, H-2′, J_{app} = 6.0 Hz), 2.23 (ddd, 1H, H-2′, J = 3.1, 6.0, 13.5 Hz), 0.88 and 0.86 (2s, 18H, tert-Bu), 0.10, 0.04, and 0.03 (3s, 12H, $SiCH_3$).*

Step one of the two-step etherification process: Activate S.2 or S.6 or S.10 with BOP and Cs_2CO_3

13. To a solution of **S.2, S.6**, or **S.10** and BOP in anhydrous THF, add Cs_2CO_3 under a nitrogen atmosphere (balloon filled with nitrogen gas). Stir the reaction mixture at room temperature for the period of time indicated below.

    *Reagent quantities for **S.4a-f**: 100 mg (0.163 mmol) silyl-protected inosine **S.2**, 144 mg (0.326 mmol) BOP, 106 mg (0.326 mmol) Cs_2CO_3, and 1.5 mL THF. Reaction time: 10 min.*

    *Reagent quantities for **S.8a,b**: 50 mg (0.079 mmol) silyl-protected guanosine **S.5**, 71 mg (0.159 mmol) BOP, 52 mg (0.159 mmol) Cs_2CO_3, and 1.5 mL THF. Reaction time: 1 hr.*

    *Reagent quantities for **S.12a,b**: 50 mg (0.100 mmol) silyl-protected 2′-deoxyguanosine **S.10**, 88.5 mg (0.200 mmol) BOP, 65 mg (0.200 mmol) Cs_2CO_3, and 1 mL THF. Reaction time: 1 hr.*

14. Evaporate the reaction mixture on a rotary evaporator.

    *Skip this step for synthesis of compounds **S.4f**, **S.8b**, and **S.12b**, and go directly to step 16.*

Step two of the two-step etherification process: Reaction with alcohol or phenol and Cs_2CO_3

15. For **S.4a-e**: add 106 mg (0.326 mmol) Cs_2CO_3 and the appropriate alcohol (quantities given below) to the mixture from step 13, and stir the reaction mixture at room temperature (reaction times given below).

    S.4a: 132 μL (3.26 mmol) methanol, 10 min
    S.4b: 222 μL (3.26 mmol) allyl alcohol, 20 min
    S.4c: 188 μL (3.26 mmol) propargyl alcohol, 10 min
    S.4d: 251 μL (3.26 mmol) isopropyl alcohol, 7 hr
    S.4e: 182 μL (3.26 mmol) ethylene glycol, 2 hr
    For **S.8a**: add 52 mg (0.159 mmol) Cs_2CO_3, 72 μL (1.59 mmol) methanol, and stir the reaction mixture at room temperature for 2 hr
    For **S.12a**: add 65 mg (0.200 mmol) Cs_2CO_3, 81 μL (2 mmol) methanol, and stir the reaction mixture at room temperature for 3.5 hr.

16. For **S.4f**, **S.8b**, and **S.12b**: add Cs_2CO_3 and the appropriate phenol (quantities given below) to the mixture from step 13, and stir the reaction mixture at 70°C (reaction times given below).

    S.4f: 106 mg (0.326 mmol) Cs_2CO_3, 45 mg (0.326) *p*-nitrophenol, 4 hr
    S.8b: 52 mg (0.0.159 mmol) Cs_2CO_3, 20 mg (0.159 mmol) *p*-methoxyphenol, 1.5 hr
    S.12b: 65 mg (0.200 mmol) Cs_2CO_3, 19 mg (0.200 mmol) phenol, 1.5 hr.

Workup of the reaction mixture, isolation of the crude products, and their purification

17. Transfer the reaction mixture to a 60-mL separatory funnel. Dilute the reaction mixture with water (10 mL) and extract with EtOAc (3 × 10 mL). Separate and combine the organic layers, dry over Na_2SO_4, and evaporate to dryness on a rotary evaporator.

18. Purify the crude product by silica gel column chromatography, using the appropriate solvent system given below:

    For **S.4a**: 20% (v/v) EtOAc in hexanes
    For **S.4b**: 20% (v/v) EtOAc in hexanes
    For **S.4c**: 20% (v/v) EtOAc in hexanes
    For **S.4d**: 15% (v/v) EtOAc in hexanes
    For **S.4e**: 70% (v/v) EtOAc in hexanes (15% of a dimer arising from reaction with both hydroxyl groups of ethylene glycol was also isolated as an early eluting material)
    For **S.4f**: 20% (v/v) EtOAc in hexanes
    For **S.5a**: 20% (v/v) EtOAc in hexanes
    For **S.5b**: 20% (v/v) EtOAc in hexanes
    For **S.12a**: 20% (v/v) EtOAc in hexanes
    For **S.12b**: 20% (v/v) EtOAc in hexanes.

19. Analyze fractions obtained from the column by TLC, combine fractions containing product, and remove the solvent on a rotary evaporator. Finally dry the product under vacuum.

20. Characterize the products by TLC, ^{1}H NMR, ^{13}C NMR, and HRMS.

    *2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-methylinosine (**S.4a**).*

    Yield of a yellowish solid 96 mg (94%). R_f (SiO_2/20% v/v EtOAc in hexanes) = 0.4. ^{1}H NMR: δ 8.52 (s, 1H, purinyl-H), 8.32 (s, 1H, purinyl-H), 6.08 (d, 1H, H-1′, J = 4.8 Hz), 4.62 (t, 1H, H-2′, J = 4.6 Hz), 4.31 (t, 1H, H-3′, J = 4.1 Hz), 4.18 (s, 3H, OCH_3), 4.13 (q, 1H, H-4′, J = 3.1 Hz), 4.03 (dd, 1H, H-5′, J = 3.9, 11.2 Hz), 3.79 (dd, 1H, H-5′, J = 2.6, 11.2 Hz), 0.95, 0.93, and 0.75 (3s, 27H, tert-Bu), 0.14, 0.13, 0.10, 0.09, –0.04, and –0.22 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 161.2, 152.2, 151.9, 141.3, 122.2, 88.6, 85.6, 76.3, 72.1, 62.7, 54.3, 26.3, 26.0, 25.8, 18.7, 18.3, 18.0, –4.1, –4.4, –4.8, –5.1. HRMS (ESI) calcd for $C_{29}H_{57}N_4O_5Si_3$ $[M + H]^+$ 625.3631, found 625.3636.

    *O^6-(Allyl)-2′,3′,5′-tri-O-(tert-butyldimethylsilyl)inosine (**S.4b**).*

    Yield of a white solid 96 mg (90%). R_f (SiO_2/30% v/v EtOAc in hexanes) = 0.7. ^{1}H NMR: δ 8.50 (s, 1H, purinyl-H), 8.30 (s, 1H, purinyl-H), 6.17 (m, 1H, −CH=), 6.08 (d, 1H, H-1′, J = 4.8 Hz) 5.45 (d, 1H, $=CH_{trans}$, J = 17.0 Hz), 5.29 (d, 1H, $=CH_{cis}$, J = 10.2 Hz), 5.15 (br d, 2H, OCH_2, J = 5.8 Hz), 4.63 (t, 1H, H-2′, J = 8.3 Hz), 4.31 (t, 1H, H-3′, J = 3.4 Hz), 4.13 (br d, 1H, H-4′, J = 2.4 Hz), 4.02 (dd, 1H, H-5′, J = 3.4, 11.2 Hz), 3.79 (d, 1H, H-5′, J = 11.2 Hz), 0.95, 0.93, and 0.78 (3s, 27H, tert-Bu), 0.14, 0.13, 0.10, 0.09, –0.04, and –0.23 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 160.5, 152.1, 141.3, 132.6, 122.1, 118.7,

88.5, 85.7, 76.3, 72.1, 67.7, 62.7, 26.3, 26.0, 25.8, 18.7, 18.3, 18.0, –4.1, –4.4, –4.8, –5.1. HRMS (ESI) calcd for $C_{31}H_{59}N_4O_5Si_3$ $[M + H]^+$ 651.3788, found 651.3787.

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-propargylinosine (**S.4c**).*

Yield of a white solid 91 mg (86%). R_f (SiO_2/30% v/v EtOAc in hexanes) = 0.7. ^{1}H NMR: δ 8.54 (s, 1H, purinyl-H), 8.36 (s, 1H, purinyl-H), 6.08 (d, 1H, H-1′, J = 4.8 Hz), 5.23 (dd, 2H, OCH_2, J = 1.4, 2.4 Hz), 4.59 (t, 1H, H-2′, J = 4.4 Hz), 4.32 (t, 1H, H-3′, J = 4.1 Hz), 4.13 (q, 1H, H-4′, J = 3.4 Hz), 4.00 (dd, 1H, H-5′, J = 3.6, 11.4 Hz), 3.79 (dd, 1H, H-5′, J = 2.4, 11.7 Hz), 2.49 (t, 1H, ≡C-H, J = 2.4 Hz), 0.95, 0.92, and 0.79 (3s, 27H, tert-Bu), 0.14, 0.13, 0.10, 0.09, –0.04, and –0.21 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 159.5, 152.3, 151.9, 141.7, 122.1, 88.7, 85.6, 78.3, 76.4, 75.3, 71.9, 62.6, 54.3, 26.3, 26.0, 25.8, 18.7, 18.2, 18.0, –4.1, –4.5, –4.7, –5.1. HRMS (ESI) calcd for $C_{31}H_{57}N_4O_5Si_3$ $[M + H]^+$ 649.3631, found 649.3634.

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-isopropylinosine (**S.4d**).*

Yield of clear, gummy material 80 mg (75%). R_f (SiO_2/30% v/v EtOAc in hexanes) = 0.7. ^{1}H NMR: δ 8.48 (s, 1H, purinyl-H), 8.26 (s, 1H, purinyl-H), 6.08 (d, 1H, H-1′, J = 4.8 Hz), 5.66 (septet, 1H, OCH, J = 6.3 Hz), 4.63 (t, 1H, H-2′, J = 4.6 Hz), 4.31 (t, 1H, H-3′, J = 3.6 Hz), 4.12 (br d, 1H, H-4′, J = 2.4 Hz), 4.01 (dd, 1H, H-5′, J = 3.6, 11.4 Hz), 3.79 (dd, 1H, H-5′, J = 1.9, 11.2 Hz), 1.47 (d, 6H, $(CH_3)_2$, J = 6.3 Hz), 0.94, 0.92, and 0.78 (3s, 27H, tert-Bu), 0.13, 0.12, 0.10, 0.09, –0.04, and –0.22 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 160.7, 152.2, 152.1, 141.0, 122.3, 88.5, 85.6, 76.2, 72.1, 70.4, 62.7, 26.3, 26.0, 25.8, 22.1, 18.7, 18.3, 18.0, –4.1, –4.4, –4.8, –5.1. This compound has been previously reported (Bae and Lakshman, 2007).

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-(2-hydroxyethyl)inosine (**S.4e**).*

Yield of a white solid 68 mg (63%). R_f (SiO_2/EtOAc) = 0.3. ^{1}H NMR: δ 8.49 (s, 1H, purinyl-H), 8.35 (s, 1H, purinyl-H), 6.87 (d, 1H, H-1′, J = 4.4 Hz), 4.72 (m, 2H, OCH_2), 4.61 (t, 1H, H-2′, J = 4.4 Hz), 4.31 (m, 1H, H-3′), 4.13 (br s, 1H, H-4′), 4.02 (m, 3H, H-5′and OCH_2), 3.79 (d, 1H, H-5′, J = 11.2 Hz), 3.52 (s, 1H, OH), 0.95, 0.92, and 0.79 (3s, 27H, tert-Bu), 0.14, 0.13, 0.09, 0.08, –0.04, and –0.22 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 160.8 152.2, 152.0, 141.6, 122.0, 88.6, 85.7, 76.4, 72.0, 69.8, 62.6, 61.9, 26.3, 26.0, 25.8, 18.7, 18.2, 18.0, –4.1, –4.5, –4.8, –5.1. HRMS (ESI) calcd for $C_{30}H_{59}N_4O_6Si_3$ $[M + H]^+$ 655.3737, found 655.3739.

The reaction with ethylene glycol also gave a product arising from reaction of both hydroxyl groups (data below).

Ethylene glycol bis-O^6-[9-(2′,3′,5′-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)] purinyl ether.

*Minor product obtained as a white solid (32 mg, 15%) in the reaction of **S.2** with ethylene glycol. R_f (SiO_2/20% v/v EtOAc in hexanes) = 0.4. ^{1}H NMR: δ 8.49 (s, 2H, purinyl-H), 8.29 (s, 2H, purinyl-H), 6.09 (d, 2H, H-1′, J = 4.8 Hz), 5.06 (m, 4H, OCH_2), 4.61 (t, 2H, H-2′, J = 4.4 Hz), 4.31 (t, 2H, H-3′, J = 2.9 Hz), 4.13 (br d, 2H, H-4′, J = 2.4 Hz), 4.02 (dd, 2H, H-5′, J = 3.4, 11.2), 3.79 (d, 2H, H-5′, J = 11.7 Hz), 0.94, 0.92, and 0.79 (3s, 54H, tert-Bu), 0.13, 0.12, 0.10, 0.09, –0.04, and –0.22 (6s, 36H, $SiCH_3$). ^{13}C NMR: δ 160.5, 152.2, 152.0, 141.3, 122.0, 88.6, 85.7, 76.3, 72.1, 64.9, 62.7, 26.3, 26.0, 25.9, 18.7, 18.3, 18.0, –4.1, –4.5, –4.7, –5.1. HRMS (ESI) calcd for $C_{58}H_{111}N_8O_{10}Si_6$ $[M + H]^+$ 1247.7033, found 1247.7032.*

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-(4-nitrophenyl)inosine (**S.4f**).*

Yield of a white solid 102 mg (85%). R_f (SiO_2/20% v/v EtOAc in hexanes) = 0.5. ^{1}H NMR: δ 8.52 (s, 1H, purinyl-H), 8.50 (s, 1H, purinyl-H), 8.33 (d, 2H, Ar-H, J = 8.7 Hz), 7.47 (d, 2H, Ar-H, J = 8.7 Hz), 6.14 (d, 1H, H-1′, J = 4.7 Hz), 4.60 (t, 1H, H-2′, J = 4.4 Hz), 4.33 (t, 1H, H-3′, J = 3.9 Hz), 4.17 (m, 1H, H-4′), 4.04 (dd, 1H, H-5′, J = 3.3, 11.4), 3.82 (d, 1H, H-5′, J = 11.3 Hz), 0.97, 0.93, and 0.81 (3s, 27H, tert-Bu), 0.16, 0.15, 0.11, 0.10, –0.01, and –0.18 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 159.0, 157.5, 153.5, 151.9, 145.3, 143.0, 125.6, 122.6, 122.3, 88.8, 85.7, 76.6, 71.9, 62.5, 26.3, 26.0, 25.8, 18.7, 18.2, 18.0, –4.1, –4.4, –4.7, –5.1. This compound has been previously reported (Bae and Lakshman, 2007).

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-methylguanosine (**S.8a**).*

Yield of an orange solid 37 mg (73%). R_f (SiO_2/20% v/v EtOAc in hexanes) = 0.3. ^1H NMR: δ 7.97 (s, 1H, purinyl-H), 5.91 (d, 1H, H-1′, J = 5.3 Hz), 5.12 (s, 2H, NH_2), 4.46 (t, 1H, H-2′, J = 4.9 Hz), 4.26 (t, 1H, H-3′, J = 4.3 Hz), 4.08 (m, 1H, H-4′), 4.04 (s, 3H, OCH_3), 3.96 (dd, 1H, H-5′, J = 3.9, 11.2 Hz), 3.76 (dd, 1H, H-5′, J = 2.4, 11.2 Hz), 0.94, 0.92, and 0.79 (s, 27H, tert-Bu), 0.13, 0.12, 0.11, 0.09, –0.04, and –0.18 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 161.6, 159.5, 153.8, 137.9, 116.0, 87.7, 85.4, 76.4, 72.2, 62.8, 53.9, 26.2, 26.0, 25.9, 18.7, 18.2, 18.1, –4.1, –4.5, –4.8, –5.2. This compound has been previously reported (Lakshman and Frank, 2009).

*2′,3′,5′-Tri-O-(tert-butyldimethylsilyl)-O^6-(4-methoxyphenyl)guanosine (**S.8b**).*

Yield of a white solid 44 mg (75%). R_f (SiO_2/20% v/v EtOAc in hexanes) = 0.4. ^1H NMR: δ 8.08 (s, 1H, purinyl-H), 7.16 (d, 2H, Ar-H, J = 8.8 Hz), 6.91 (d, 2H, Ar-H, J = 8.8 Hz), 5.92 (d, 1H, H-1′, J = 4.5 Hz), 4.79 (s, 2H, NH_2), 4.51 (t, 1H, H-2′, J = 4.4 Hz), 4.31 (t, 1H, H-3′, J = 4.0 Hz), 4.16 (m, 1H, H-4′), 3.99 (dd, 1H, H-5′, J = 3.5, 11.4 Hz), 3.82 (s, 3H, OCH_3), 3.79 (dd, 1H, H-5′, J = 1.9, 11.4 Hz), 0.95, 0.92, and 0.83 (3s, 27H, tert-Bu), 0.14, 0.13, 0.10, 0.09, 0.00, and –0.10 (6s, 18H, $SiCH_3$). ^{13}C NMR: δ 160.9, 159.3, 157.0, 154.7, 146.2, 138.9, 122.9, 116.1, 114.4, 88.2, 85.1, 76.3, 71.9, 62.6, 55.7, 26.3, 26.0, 25.9, 18.7, 18.7, 18.2, 18.1, –4.1, –4.4, –4.6, –5.1. HRMS (ESI) calcd for $C_{35}H_{62}N_5O_6Si_3$ $[M + H]^+$ 732.4002, found 732.4007.

*3′,5′-Di-O-(tert-butyldimethylsilyl)-O^6-methyl-2′-deoxyguanosine (**S.12a**).*

Yield of a yellowish solid 38 mg (75%). R_f (SiO_2/40% v/v EtOAc in hexanes) = 0.3. ^1H NMR: δ 7.70 (s, 1H, purinyl-H), 6.31 (t, 1H, H-1′, J = 6.3 Hz), 4.89 (s, 2H, NH_2), 4.57 (m, 1H, H-3′), 4.05 (s, 3H, OCH_3), 3.89 (m, 1H, H-4′), 3.80 (dd, 1H, H-5′, J = 4.4, 11.2 Hz), 3.74 (dd, 1H, H-5′, J = 2.9, 11.2 Hz), 2.55 (app quint, 1H, H-2′, $J_{app} \sim 6.3$ Hz), 2.34 (ddd, 1H, H-2′, J = 2.2, 6.0, 13.1 Hz), 0.90 (br s, 18H, tert-Bu), 0.08, 0.07, and 0.06 (3s, 12H, $SiCH_3$). ^{13}C NMR: δ 161.7, 159.5, 153.6, 137.7, 116.1, 87.8, 83.7, 72.0, 63.0, 53.9, 41.1, 26.1, 25.9, 18.6, 18.1, –4.4, –4.5, –5.2, –5.3. This compound has been previously reported (Lakshman and Frank, 2009).

*3′,5′-Di-O-(tert-butyldimethylsilyl)-O^6-phenyl-2′-deoxyguanosine (**S.12b**).*

Yield of a yellowish solid 42 mg (73%). R_f (SiO_2/40% v/v EtOAc in hexanes) = 0.3. ^1H NMR: δ 8.00 (s, 1H, purinyl-H), 7.40 (t, 2H, Ar-H, J = 7.7 Hz), 7.25 (m, 3H, Ar-H), 6.34 (t, 1H, H-1′, J = 6.4 Hz), 4.77 (s, 2H, NH_2), 4.60 (m, 1H, H-3′), 3.99 (m, 1H, H-4′), 3.83 (dd, 1H, H-5′, J = 4.1, 11.1 Hz), 3.77 (dd, 1H, H-5′, J = 2.6, 11.1 Hz), 2.59 (app quint, 1H, H-2′, $J_{app} \sim 6.4$ Hz), 2.37 (ddd, 1H, H-2′, J = 1.4, 5.8, 12.9 Hz), 0.923 and 0.920 (2s, 18H, tert-Bu), 0.10 and 0.09 (2s, 12H, $SiCH_3$). ^{13}C NMR: δ 160.6, 159.3, 154.7, 152.7, 138.7, 129.4, 125.4, 122.1, 116.2, 87.9, 83.9, 72.1, 63.0, 41.2, 26.1, 25.9, 18.6, 18.2, –4.4, –4.5, –5.1, –5.2. This compound has been previously reported (Lakshman and Frank, 2009).

COMMENTARY

Background Information

In 2000, Lin and Robins reported that activation of the C-6 amide functionality in inosine and 2′-deoxyinosine can be accomplished by treatment of the acetyl-protected nucleosides with $PPh_3/I_2/(iso\text{-}Pr)_2NEt$ (Lin and Robins, 2000). Addition of morpholine, piperidine, or imidazole to the reaction mixtures led to the formation of C-6 morpholinyl, piperidinyl, and imidazolyl derivatives, respectively (Lin and Robins, 2000). Subsequently, in 2004, a similar procedure was applied to the synthesis of C-6 imidazolyl derivatives from guanosine and 2′-deoxyguanosine (Janeba et al., 2004). A year later, Wan et al. (2005) reported a one-pot synthesis of adenosine analogs by reaction of acetyl-protected inosine with 1*H*-benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and amines, in the presence of $(iso\text{-}Pr)_2NEt$ in DMF as solvent. They also demonstrated that sugar-unprotected inosine and 2′-deoxyinosine (a single example) could be utilized for this conversion (Wan et al., 2005). In this work, they reported the formation of a C-6 phosphonium ion as a putative intermediate (such an intermediate was reported to be observed by liquid chromatography/mass spectrometry). The plausible mechanisms of the

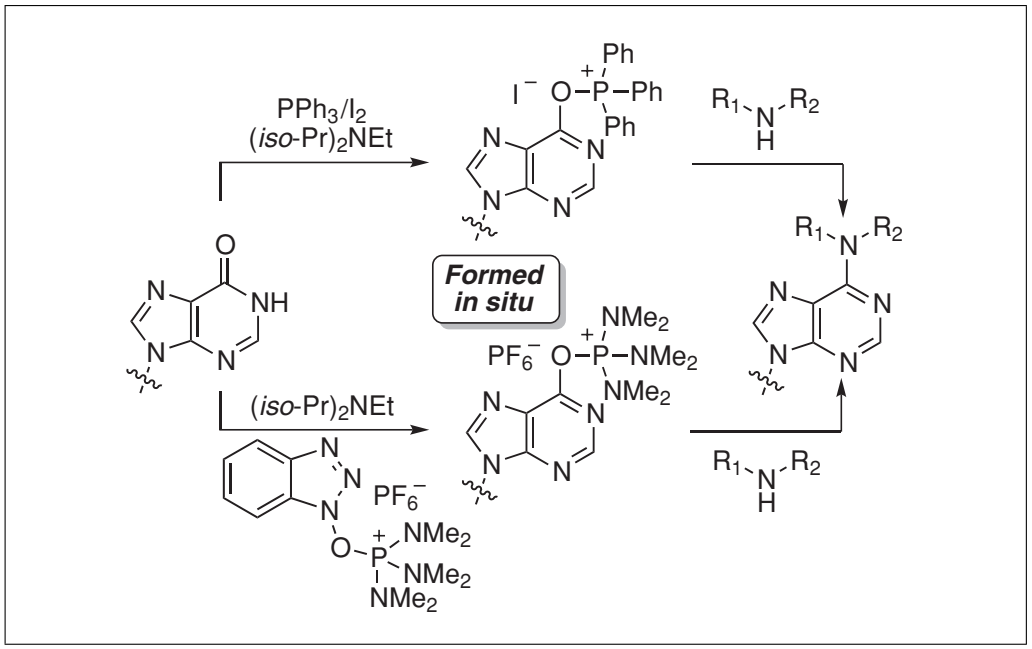


Figure 1.26.2 Plausible mechanism for the activation of the C-6 amide in inosine nucleosides and conversion to adenosine derivatives.

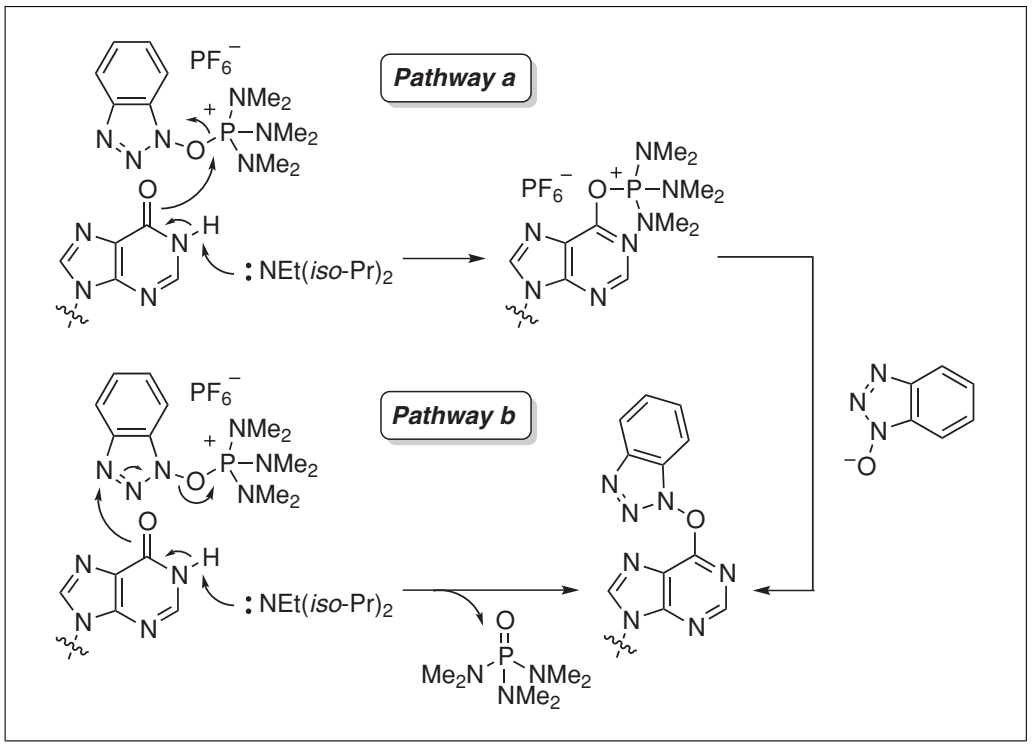


Figure 1.26.3 Two possible modes by which the C-6 amide could react with BOP leading to O^6-(benzotriazol-1-yl)inosine derivatives.

reactions described above are shown in Figure 1.26.2.

We became interested in the mechanism by which BOP reacts at the C-6 amide bond since we believed that two possible mechanisms could operate. This is shown in Figure 1.26.3. In principle, as reported by Wan et al. (2005), the reaction could proceed via the phosphonium salt as a result of deprotonation at N1 by the base, followed by attack of the amide oxygen atom at the phosphorus atom of BOP (pathway **a** in Figure 1.26.3). The alternative is deprotonation at N1 by base and attack of the amide oxygen at the nitrogen atom in BOP, in an S_N2'-like fashion, with expulsion of HMPA (pathway **b** in Figure 1.26.3).

We therefore investigated the mechanism of this reaction by $^{31}P\{^1H\}$ NMR and discovered that the reaction does not instantly produce HMPA, and that the nucleoside phosphonium ion does make an appearance (Bae and Lakshman, 2007). This was evidenced by a singlet at $\delta = 35$ ppm in the $^{31}P\{^1H\}$ NMR in addition to the PF_6^- resonance at δ –143 ppm. Also, the ^1H NMR spectrum of the isolated intermediate indicated the presence of the $-P(NMe)_2$ with a doublet at 2.92 ppm ($J_{H-P} = 11.2$ Hz). Thus, pathway **a** is definitely operational in these types of transformations, although pathway **b** cannot be excluded.

On the basis of these results, we then developed a second-generation synthesis of O^6-(benzotriazol-1-yl)inosine as well as the 2′-deoxy analog (Bae and Lakshman, 2008a), and also a "catch-and-release" approach for high-throughput-type applications, using polymer-supported 1-hydroxy-1*H*-benzotriazole (Bae and Lakshman, 2008b). In addition, we evaluated the use of BOP for modifying the C-2 position. For this purpose, reactions of disilyl O^6-benzyl-2′-deoxyxanthosine were studied (Bae and Lakshman, 2008c). Here we discovered that a O^2-(benzotriazol-1-yl) derivative is not formed, but that the reactions terminate at the stage of the phosphonium ion. The C-2 phosphonium derivative is an excellent reagent for synthesis of 2′-deoxyguanosine derivatives, and its applicability was exemplified via rapid access to an acrolein adduct of 2′-deoxyguanosine (Bae and Lakshman, 2008c).

We then developed a method for the functionalization at the C-6 position of guanine nucleosides, via reaction of silylated guanosine and 2′-deoxyguanosine with BOP, and isolation of the O^6-(benzotriazol-1-yl) derivatives (Lakshman and Frank, 2009). In the cases of the hypoxanthine (inosine and 2′-deoxyinosine) and guanine nucleosides, the O^6-(benzotriazol-1-yl) derivatives showed extremely good reactivity towards amine, alcohol, phenol, and thiol nucleophiles.

Building on these developments, we next discovered a method for the etherification of the C-6 amide linkage in inosine, guanosine, and 2′-deoxyguanosine (Kokatla and Lakshman, 2010). The present protocol describes this methodology, and there are some notable differences when compared with the one-pot amination reactions (Wan et al., 2005; Lakshman and Frank 2009). Importantly, these etherification reactions are successful as a *two-step, one-pot operation*. This is shown in Figure 1.26.4.

S_NAr, or nucleophilic aromatic substitution, is a two-step process where a nucleophile first adds to an sp^2 carbon atom of an aromatic ring that bears a good leaving group. The ensuing Meisenheimer complex then undergoes expulsion of the leaving group with concomitant rearomatization. Such a process is quite facile with nucleosides, and therefore this reaction has found wide application for modifying the heterocyclic moieties of nucleosides.

For the S_NAr reaction, several leaving groups have been used at the C-6 position of purines, and the references below provide some examples:

a. Chloro (Robins and Basom, 1973; Robins and Uznański, 1981a; Véliz and Beal, 2000; Francom et al., 2002; Francom and Robins, 2003).

b. Bromo (Nair and Richardson, 1980; Véliz and Beal, 2001; Lagisetty et al., 2006).

c. Iodo (Cosstick and Douglas, 1991; van der Wenden et al., 1995; Liu et al., 2004).

d. Fluoro (Robins and Basom, 1973; Robins and Uznański, 1981b).

e. Phenoxy (Gao et al., 1992; Ferentz and Verdine, 1992; Lakshman and Zajc, 1996; Allerson et al., 1997).

f. Aryl sulfonyl (Hayakawa et al., 1993; Allerson et al., 1997; Lakshman et al., 2005).

g. Alkyl sulfonyl (Nagatsugi et al., 1997), pyridyl (Adamiak et al., 1985; Fathi et al., 1990).

h. Sulfone (Lin and Robins, 2000).

i. Imidazolyl (Lin and Robins, 2000; Janeba et al., 2004).

j. 1,2,4-Triazol-4-yl (Samano et al., 1994; Miles et al., 1995).

Many of the abovementioned derivatives have been utilized for the synthesis of C-6 ethers of purine nucleoside. As examples, a C-6 chloride has been displaced with alkoxide (Nair et al., 1988; Aso et al., 2000), a C-6 pentafluorophenyl ether of 2′-deoxyguanosine was converted to the methyl ether (Gao et al., 1992), we (Lakshman et al., 2000) and others (Kaloudis et al., 2009) have converted C-6 arylsulfonyl guanine nucleosides to O^6-ethers via a quaternary ammonium salt, and a C-6 imidazolyl guanosine derivative was converted to the methyl ether (Janeba et al., 2004), as was a C-6 1,2,4-triazol-4-yl analog (Samano et al., 1994; Miles et al., 1995).

In all of these cases, the methodology typically requires the synthesis and isolation of a discrete reactive nucleoside derivative prior to the etherification step. Another approach is the generation of a reactive

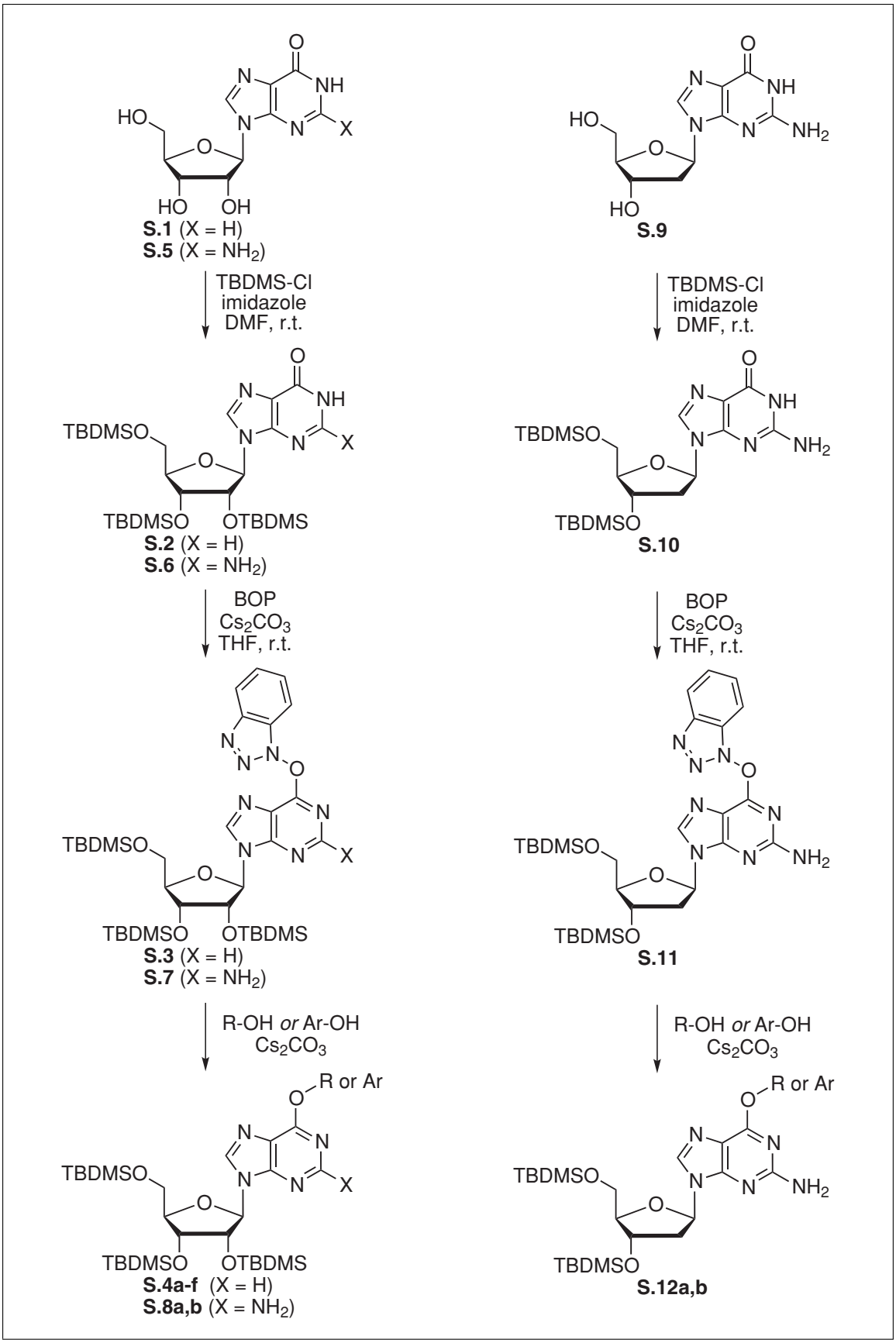


Figure 1.26.4 Synthesis of silylated nucleoside precursors, their conversion to the O^6-(benzotriazol-1-yl) derivatives (which are not isolated), and conversion to the O^6-alkyl and O^6-aryl ethers.

intermediate in situ, which then undergoes reaction with an alcohol. A classic example within the area of purine nucleoside modification is the Mitsunobu reaction. Pfleiderer and colleagues developed the Mitsunobu reaction as a method to introduce the base-labile *p*-nitrophenylethyl group (Himmelsbach et al., 1984). This method was then modified and applied to the synthesis of silyl-protected, reactive nucleoside derivatives such as 2-fluoro-2′-deoxyinosine (Zajc et al., 1992), 2-bromo-2′-deoxyinosine (Harwood et al., 1999), 2-bromoinosine (Qian and Glaser, 2005), and 8-bromo-2′-deoxyguanosine (Meier and Gräsl, 2002).

The protocol described herein is a bridge between the actual isolation of the reactive nucleoside derivatives and in situ formation of a reactive species that undergoes further transformation. That is, as described earlier in our original publications (Bae and Lakshman 2007; Lakshman and Frank 2009) and a previous protocol (Bae et al., 2009), isolation of the reactive nucleoside derivatives **S.3**, its 2′-deoxy analog, **S.7**, and **S.11**, followed by displacement with alcohols as a separate, second step, is eminently possible. However, as described herein, isolation is not specifically necessary, and the etherification can be conducted as a two-step, one-pot operation, ensuring that **S.3**, **S.7**, or **S.11** are pre-formed at the end of the first step.

Critical Parameters and Troubleshooting

The silylation reactions of inosine **S.1**, guanosine **S.5**, and 2′-deoxyguanosine **S.9** are reasonably simple and are not typically expected to produce any problems. Methods for the conversion of the silylated nucleosides to the O^6-(benzotriazol-1-yl) derivatives **S.3**, **S.7**, and **S.11** are also relatively simple. In these procedures, it is important to ensure that the equipment and solvents used for the reaction are dry. Some important noteworthy points are as follows. It appears that BOP and an alcohol do not react, since exposure of BOP to methanol *in the absence of a base* led to no discernable reaction over 1 hr (as judged by $^{31}P\{^1H\}$ NMR; Kokatla and Lakshman, 2010). However, in the presence of Cs_2CO_3, a reaction between BOP and methanol was observed, leading to the formation of hexamethylphosphoramide (HMPA) and 1-methoxy-1*H*-1,2,3-benzotriazole. It appears, from preliminary experiments, that 1,8-diazabicycloundec-7-ene (DBU) can also be used in place of Cs_2CO_3, although the reaction may be slower (Kokatla and Lakshman, 2010). It is also possible that reactions of alcohols, like those of phenols, can be conducted without evaporation of the solvent after step one (this has been demonstrated with methanol; Kokatla and Lakshman, 2010). However, evaporation of the reaction mixture after step one ensures good reactivity of alcohols and produces generally faster reactions. In unpublished experiments, attempts at preparing the acetyl-protected O^6-allyl derivative of 2′-deoxyguanosine (acetyl-protected 2′-deoxy analog of **S.4b**) were unsuccessful. This is possibly due to the reactivity of acetate esters to nucleophilic conditions.

The labile nature of the nucleosides needs to be recognized, and these compounds can be intolerant of certain conditions. Typically, glassware and other equipment that nucleosides are expected to come into contact with should be acid free. Routinely, glassware is washed with 10% aqueous ammonium hydroxide, water, and acetone, followed by drying in an oven. Use of high temperatures for the water bath during rotary evaporation procedures should be avoided. Typically, evaporations were conducted at 50°C or less.

Generally, the procedures contained in this protocol can be considered simple. Nevertheless, familiarity with standard operating procedures of a chemical synthesis laboratory is essential. These include but are not limited to the ability to set up, monitor, and work up reactions and perform extraction procedures and thin-layer as well as column chromatography, evaporation, and vacuum drying. The ability to use NMR and mass spectrometry instrumentation typically requires training, and the ability to interpret data obtained by spectroscopic means requires adequate knowledge. The authors suggest that the methods outlined in this protocol be adhered to as closely as possible.

Compound characterization

For monitoring reactions, aluminum foil–backed, silica gel TLC plates of 200 μm thickness were used. For purifications by column chromatography, 200–300 mesh silica gel was used. Please refer to the individual compound headings for eluting solvents used for chromatography, and for R_f values. In this protocol, the ^1H NMR spectra reported were recorded at 500 MHz, and are referenced to the residual protonated solvent. ^{13}C NMR spectra reported here were recorded at 125 MHz and

are referenced to the ^{13}C resonance of the deuterated solvent. Chemical shifts (δ) are reported in parts per million, and coupling constants (J) are in hertz (Hz). It is expected that lower-field-strength instruments (400 MHz for ^{1}H/100 MHz for ^{13}C or 300 MHz for ^{1}H/75 MHz for ^{13}C) will provide adequate resolution for compound characterization. The purine is numbered 1 to 9 by the accepted convention, and the sugar carbon atoms are numbered 1′ to 5′ starting at the anomeric carbon atom and proceeding via the carbon chain to the primary carbinol carbon. Multiplicities in the ^{1}H NMR are reported using standard abbreviations (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, app = apparent), based upon the apparent signal multiplicities observed.

Anticipated Results

Yields for all products are indicated in Table 1.26.1 and in the characterization data for each compound. Following the procedures presented here, it is anticipated that comparable or better results should be obtainable for the synthesis of the various O^6-alkyl and O^6-aryl ethers of inosine, guanosine, and 2′-deoxyguanosine. The method should also be applicable to silyl-protected 2′-deoxyinosine (not described here). Some of the nucleoside ethers described herein could have additional uses. As examples, the propargyl derivative can be used in Cu-catalyzed azide-alkyne ligation chemistry (Tornøe et al., 2002; Rostovtsev et al., 2002), the ethylene glycol derivative with a free terminal hydroxyl group can be used for additional functionalization or to append other molecules, and the *p*-nitrophenoxy, *p*-methoxyphenoxy, and phenoxy derivatives can be used in subsequent S_NAr reactions, and may be amenable to oligonucleotide assembly procedures. Such an approach had been demonstrated previously (Ferentz and Verdine, 1992; Allerson et al., 1997). *p*-Nitrophenoxy derivatives of nucleosides are attractive for processes wherein the released *p*-nitrophenoxide can be assessed by UV-Vis spectrometry (Zemlicka and Endo, 1996).

Time Considerations

Step 1, the conversion of silyl-protected inosine (**S.2**) to the O^6-(benzotriazol-1-yl) ether **S.3**, takes 10 to 15 min to reach completion with silyl-protected inosine. This step takes about 1 hr with silyl-protected guanosine (**S.6** → **S.7**) and 2′-deoxyguanosine (**S.10** → **S.11**). These reactions can be monitored for completion by TLC, using a UV lamp. Evaporation of the reaction mixtures should take a few minutes, depending upon the volume of the relatively volatile THF (65° to 67°C). Reactions with alcohols take anywhere from 10 min to 7 hr, at room temperature, for the alcohols reported in this protocol. Reaction times with other alcohols may vary, but completion of reaction can again be monitored by TLC, using a UV lamp. Reactions with phenols reported herein take 1.5 to 4 hr at 70°C. It should be noted that the reported reactions include an electron-deficient as well as an electron-rich phenol, and thus the reaction times may represent general times for reactions with other phenols. Nevertheless, reactions with other phenols will need to be monitored by TLC for completion. The entire chemical process from start to finish, which includes isolation of the crude products and chromatographic purification, should typically take no more than 2 days. Characterization of the products by NMR and mass spectrometry will take additional time depending upon the experiments being performed. Multiple reactions can be conducted in parallel, with appropriate purifications performed upon completion of the reactions. However, this may require greater skills, time management, and equipment availability.

In their pure form, products **S.4a-f**, **S.8a,b**, and **S.12a,b** are all likely to be quite stable to prolonged storage in a freezer at −20°C, although individual stability assays have not been performed for each product.

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