Synthesis of 5′-*O*-Phosphoramidites with a Photolabile 3′-*O*-Protecting Group

In recent years, DNA microarrays have become a diagnostic assay system of ever-increasing importance to a wide range of biotechnical and biomedical applications. Among the various technologies used for their production, the light-controlled in situ synthesis of oligonucleotide arrays has proved to be especially versatile. This methodology has permitted oligonucleotide synthesis in only the 3′→5′ direction, however, thus linking the 3′ terminus to the solid support (Beier and Hoheisel, 1999). For several enzymatic reactions, and especially for polymerase extension, the availability of the 3′-hydroxyl is a prerequisite. Thus, reversing the direction of synthesis is advantageous or even essential to various chip-based applications, such as highly parallel DNA sequencing or the creation of microarrays containing double-stranded DNA probes. This unit describes the chemical synthesis of phosphoramidite building blocks that carry a photolabile protecting group at the 3′ position. These inversely oriented synthons expose a 2-(2-nitrophenyl)propoxycarbonyl (NPPOC) group as the photolabile protecting group of choice. Among other applications, the building blocks can be employed for in situ synthesis of DNA microarrays in which each oligonucleotide is immobilized at the 5′ terminus, leaving the 3′ terminus available as a polymerase substrate. This unit describes the synthesis of 3′-*O*-NPPOC-protected nucleosides (see Basic Protocol 1) and 3′-*O*-NPPOC-5′-*O*-phosphoramidites (see Basic Protocol 2), as well as the synthesis of the required acylating reagent (see Support Protocol).

NOTE: All reactions should be performed using dry reagent-grade solvents and should be carried out under an inert atmosphere unless otherwise specified.

NOTE: All reactions described involve photolabile compounds. All reactions should be performed and all photolabile derivatives should be stored in the dark (e.g., by covering reaction flasks with aluminum foil).

INTRODUCTION OF THE PHOTOLABILE 2-(2-NITROPHENYL)PROPOXYCARBONYL GROUP INTO 5′-*O*-DIMETHOXYTRITYLATED NUCLEOSIDES

BASIC PROTOCOL 1

The reaction scheme for the introduction of the photolabile 2-(2-nitrophenyl)propoxycarbonyl (NPPOC) group into the 3′-*O* position is depicted in Figure 12.3.1. Chemical synthesis starts from commercially available 5′-*O*-dimethoxytrityl (DMTr) nucleosides (**S.1a**-**S.1d**). The photolabile NPPOC protecting group is attached to the 3′-*O* position by a mild acylation reagent that is generated in situ from 2-(2-nitrophenyl)propoxycarbonyl chloride and *N*-methylimidazole (see Support Protocol). Subsequently, the 5′-*O*-DMTr moiety is removed by acid treatment without the need to isolate the 5′-*O*-DMTr-3′-*O*-NPPOC-protected intermediate nucleoside compounds. The 3′-*O*-NPPOC-protected derivatives (**S.2a**-**S.2d**) are purified by flash chromatography.

Materials

Nitrogen source
2-(2-Nitrophenyl)propoxycarbonyl-*N*-methylimidazolium chloride solution (**S.6**; see Support Protocol), prepare fresh
Dichloromethane, anhydrous

Contributed by Markus Beier and Jörg D. Hoheisel
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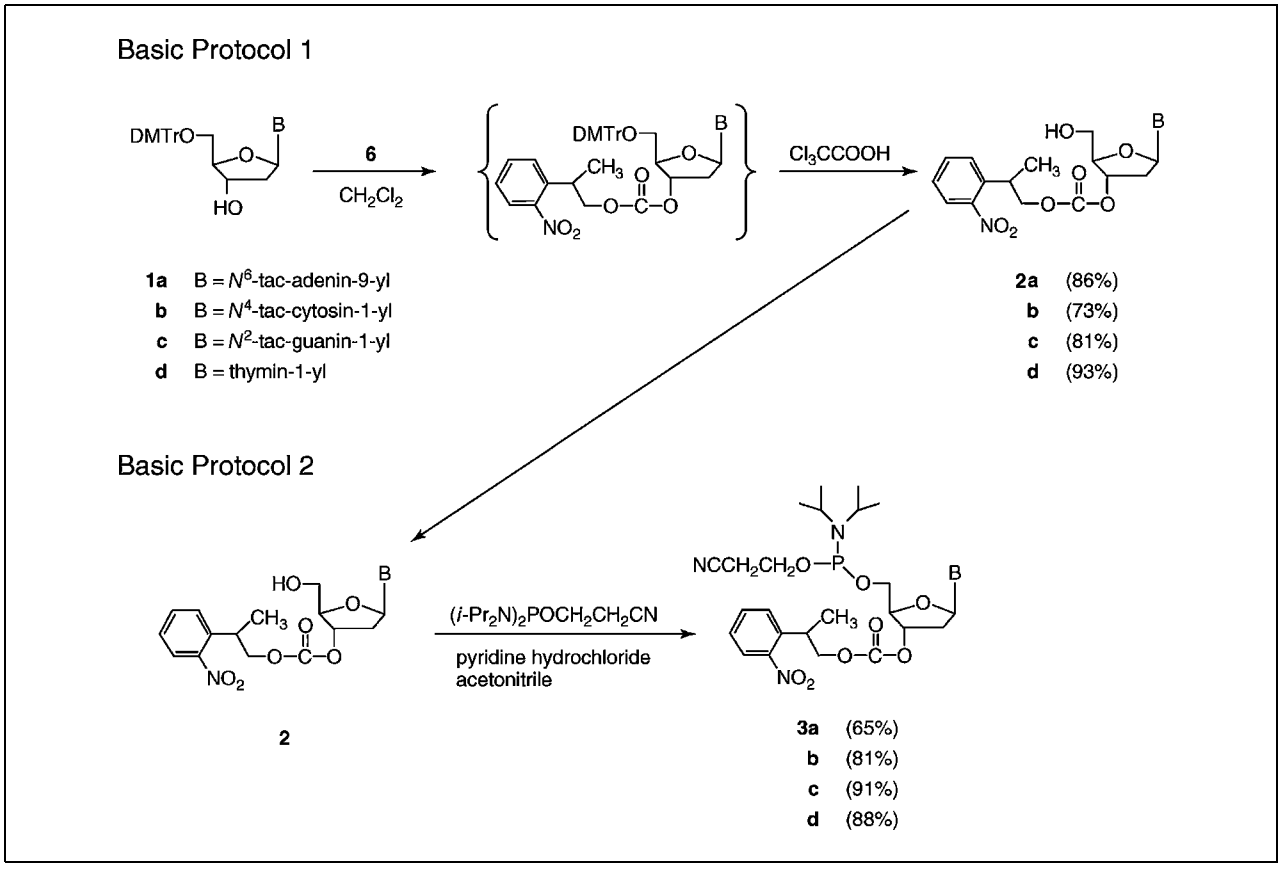

Figure 12.3.1 Synthesis of 2-(2-nitrophenyl)propoxycarbonyl (NPPOC)-protected nucleosides (**S.2a**-**S.2d**) and phosphoramidites (**S.3a**-**S.3d**). **S.6** is the acylating reagent shown in Figure 12.3.2. Average yields are given in parentheses. DMTr, 4,4′-dimethoxytrityl.

N-[(4-*tert*-Butylphenoxy)acetyl]-5′-*O*-(4,4′-dimethoxytrityl) deoxyribonucleosides:
  N^6-tac-5′-*O*-DMTr-2′-deoxyadenosine (**S.1a**; e.g., Proligo, ChemGenes; see Background Information)
  N^4-tac-5′-*O*-DMTr-2′-deoxycytidine (**S.1b**; e.g., Proligo, ChemGenes; see Background Information)
  N^2-tac-5′-*O*-DMTr-2′-deoxyguanosine (**S.1c**; e.g., Proligo, ChemGenes; see Background Information)
  5′-*O*-DMTr-thymidine (**S.1d**; e.g., Proligo)
Toluene
Ethyl acetate
5% (v/v) aqueous HCl
Sodium sulfate (Na_2SO_4)
10% (v/v) trichloroacetic acid in dichloromethane
Methanol
Saturated aqueous sodium bicarbonate ($NaHCO_3$) solution
Silica gel (30 to 60 μm; e.g., Baker) for flash chromatography

100-mL three-neck round-bottom flask with drying tube
Dropping funnel
Balloons
100-mL two-neck flask
Thin-layer chromatography (TLC) silica gel 60 plates (Merck)
254-nm UV lamp

500-mL separatory funnels
Rotary evaporator equipped with a water aspirator

Additional reagents and equipment for TLC (*APPENDIX 3D*), flash chromatography (*APPENDIX 3E*), nuclear magnetic resonance (NMR), and mass spectrometry (MS)

Prepare 3′-O-NPPOC-protected deoxyribonucleosides

1. Equip a 100-mL three-neck round-bottom flask with a drying tube, a dropping funnel, and a balloon filled with nitrogen. Under a positive flow of nitrogen, transfer the entire **S.6** solution to this flask.
2. Chill solution in an ice bath to 0°C.
3. In a 100-mL two-neck flask, also under a positive flow of nitrogen, dissolve one of the following compounds in 40 mL dry dichloromethane:

   2.73 g (3.67 mmol) **S.1a**
   2.65 g (3.67 mmol) **S.1b**
   2.79 g (3.67 mmol) **S.1c**
   2.00 g (3.67 mmol) **S.1d**.

4. Connect the flask to the dropping funnel of the chilled **S.6** and add the deoxyribonucleoside dropwise over 10 min, while stirring. Stir overnight at 0°C.
5. Check the reaction progress by TLC (*APPENDIX 3D*) on a silica gel 60 plate. Co-spot the starting material for comparison. For solvents and R_f values, see step 13 (analytical data). Visualize by exposing the plate to a 254-nm UV lamp.

   If the reaction is not complete, more reagent should be added, and the reaction should be allowed to continue. The amount to add must be determined empirically, although with experience this shouldn't be necessary.

Work up product

6. Wash the reaction mixture with 100 mL of 5% aqueous HCl and separate the phases using a 500-mL separatory funnel.
7. Dry the organic phase with ~2 g Na_2SO_4.
8. Add 70 mL of 10% (v/v) trichloroacetic acid in dichloromethane to the organic phase and stir for 2 min.
9. Check the reaction progress by TLC (step 5).

   If the reaction is not complete, more reagent should be added, and the reaction should be allowed to continue.

10. Wash the red reaction mixture with 100 mL saturated aqueous $NaHCO_3$ solution and separate the phases using a 500-mL separatory funnel.
11. Dry the organic phase with ~2 g Na_2SO_4 and evaporate the organic phase to dryness on a rotary evaporator equipped with a water aspirator.

Purify nucleosides

12. Dissolve the residue in a minimal amount of dichloromethane and purify by flash chromatography (*APPENDIX 3E*). Prepare a 3 × 30–cm column using 90 g silica gel, and elute (50-mL fractions) with the following solvents (~2 liters total):

Adenosine derivative: 1:1 (v/v) toluene/ethyl acetate with 0% to 4% (v/v) methanol
Cytidine derivative: 1:1 (v/v) toluene/ethyl acetate with 0% to 4% (v/v) methanol
Guanosine derivative: 3:1 (v/v) ethyl acetate/methanol
Thymidine derivative: 5:4 (v/v) toluene/ethyl acetate with 0% to 10% (v/v) methanol.

13. Identify fractions containing product by TLC and check purity by NMR and MS.

*N^6-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyadenosine (**S.2a**): Yield, 86%. TLC (toluene/ethyl acetate, 1:1 [v/v]): R_f = 0.17. ^{1}H NMR (DMSO, δ): 10.78 (br, NH), 8.66 (m, H-C(2), H-C(8)), 7.83 (m, 1H o to NO_2), 7.70 (m, 1H m to NO_2, 1H p to NO_2), 7.49 (m, 1H m to NO_2), 7.30 (m, 2H o to tert-butyl), 6.89 (m, 2H m to tert-butyl), 6.43 (m, H-C(1′)), 5.28 (m, H-C(3′)), 5.14 (m, HO-C(5′)), 4.98 (s, $\underline{CH_2}O$), 4.34 (m, $\underline{OCH_2}CH$), 4.12 (m, H-C(4′)), 3.60 (m, 2 H-C(5′), $\underline{CH}CH_3$), 3.02 (m, H-C(2′)), 2.57 (m, H-C(2′)), 1.31 (d, $CH\underline{CH_3}$), 1.24 (s, $C(\underline{CH_3})_3$). HRMS (FAB, M + H^+): calcd. for $C_{32}H_{36}N_6O_9$, 649.2621; found, 649.2644. ESI-MS: 649 (M + H^+), 671 (M + Na^+), 1297 (2M + H^+), 1319 (2M + Na^+).*

*N^4-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxycytidine (**S.2b**): Yield, 73%. TLC (toluene/ethyl acetate, 1:4 [v/v]): R_f = 0.50. ^{1}H NMR (DMSO, δ): 10.87 (br, NH), 8.29 (d, H-C(6)), 7.82 (m, 1H o to NO_2), 7.69 (m, 1H m to NO_2, 1H p to NO_2), 7.48 (m, 1H m to NO_2), 7.29 (m, 2H m to tert-butyl), 7.13 (d, H-C(5)), 6.84 (m, 2H o to tert-butyl), 6.08 (m, H-C(1′)), 5.10 (m, H-C(3′), HO-C(5′)), 4.77 (s, CH_2O), 4.33 (m, $\underline{OCH_2}CH$), 4.10 (m, H-C(4′)), 3.62 (m, 2 H-C(5′)), 3.53 (m, $\underline{CH}CH_3$), 2.48 (m, H-C(2′)), 2.21 (m, H-C(2′)), 1.29 (d, $CH\underline{CH_3}$), 1.24 (s, $C(\underline{CH_3})_3$). HRMS (FAB, M + H^+): calcd. for $C_{31}H_{36}N_4O_{10}$, 625.2509; found, 625.2495. ESI-MS: 625 (M + H^+), 647 (M + Na^+), 1249 (2M + H^+), 1271 (2M + Na^+).*

*N^2-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyguanosine (**S.2c**): Yield, 81%. TLC (ethyl acetate/methanol, 3:1 [v/v]): R_f = 0.18. ^{1}H NMR (DMSO, δ): 11.74 (br, 2 NH), 8.23 (2s, H-C(8)), 7.83 (m, 1H o to NO_2), 7.70 (m, 1H m to NO_2, 1H p to NO_2), 7.49 (m, 1H m to NO_2), 7.30 (m, 2H o to tert-butyl), 6.89 (m, 2H m to tert-butyl), 6.18 (m, H-C(1′)), 5.13 (m, H-C(3′)), 5.06 (m, HO-C(5′)), 4.81 (2s, $\underline{CH_2}O$), 4.34 (m, $\underline{OCH_2}CH$), 4.04 (m, H-C(4′)), 3.55 (m, 2 H-C(5′), $\underline{CH}CH_3$), 2.83 (m, H-C(2′)), 2.49 (m, H-C(2′)), 1.29 (d, $CH\underline{CH_3}$), 1.25 (s, $C(\underline{CH_3})_3$). HRMS (FAB, M + H^+): calcd. for $C_{32}H_{36}N_6O_{10}$, 665.2570; found, 665.2582. ESI-MS: 665 (M + H^+), 687 (M + Na^+), 1329 (2M + H^+), 1351 (2M + Na^+).*

*3′-O-[2-(2-Nitrophenyl)propoxycarbonyl]thymidine (**S.2d**): Yield, 93%. TLC (toluene/ethyl acetate, 1:2 [v/v]): R_f = 0.21. ^{1}H NMR (DMSO, δ): 11.26 (br, NH), 7.82 (m, 1H o to NO_2), 7.69 (m, H-C(6), 1H m to NO_2, 1H p to NO_2), 7.49 (m, 1H m to NO_2), 6.11 (m, H-C(1′)), 5.09 (m, H-C(3′), HO-C(5′)), 4.33 (m, $CH\underline{CH_2}O$), 3.96 (m, H-C(4′)), 3.59 (2m, 2 H-C(5′)), 3.52 (m, $\underline{CH}CH_2O$), 2.24 (m, 2 H-C(2′)), 1.77 (2s, CH_3), 1.29 (d, $CH\underline{CH_3}$). HRMS (FAB, M + H^+): calcd. for $C_{20}H_{23}N_3O_9$, 450.1512; found, 450.1524. ESI-MS: 450 (M + H^+), 472 (M + Na^+), 899 (2M + H^+), 921 (2M + Na^+).*

BASIC PROTOCOL 2

PREPARATION OF 3′-*O*-[2-(2-NITROPHENYL)PROPOXYCARBONYL]-PROTECTED 5′-*O*-PHOSPHORAMIDITES

The reaction scheme for synthesis of the 3′-*O*-NPPOC-protected phosphoramidite building blocks (**S.3a**-**S.3d**) is depicted in Figure 12.3.1. For conversion of the 3′-*O*-NPPOC nucleosides into the corresponding phosphoramidites, a bifunctional phosphitylating reagent is utilized in combination with pyridine hydrochloride. Alternatively, other activators (e.g., tetrazole) may be employed.

Synthesis of 5′-*O*-Phosphoramidites with a Photolabile 3′-*O*-Protection

Materials

Nitrogen source
3′-*O*-NPPOC-protected nucleosides (**S.2a**-**S.2d**; see Basic Protocol 1)
Acetonitrile, anhydrous
2-Cyanoethyl-*N,N,N,N*-tetraisopropylphosphorodiamidite
0.5 M pyridine hydrochloride in anhydrous acetonitrile, dried over 4 Å molecular sieves
Toluene
Ethyl acetate
Dichloromethane, anhydrous
Saturated aqueous sodium bicarbonate ($NaHCO_3$) solution
Sodium sulfate (Na_2SO_4)
Silica gel for flash chromatography (30 to 60 μm; Baker)

100-mL three-neck round-bottom flask with drying tube
Dropping funnel
Balloons
100-mL two-neck flask
Thin-layer chromatography (TLC) silica gel 60 plates (Merck)
254-nm UV lamp
500-mL separatory funnel
Rotary evaporator equipped with a water aspirator

Additional reagents and equipment for TLC (*APPENDIX 3D*), flash chromatography (*APPENDIX 3E*), nuclear magnetic resonance (NMR), and mass spectrometry (MS)

1. Connect a 100-mL three-neck round-bottom flask equipped with a drying tube to a dropping funnel and a balloon filled with nitrogen. Under a positive flow of nitrogen, dissolve one of the following 3′-*O*-NPPOC-protected nucleosides in 15 mL dry acetonitrile:

   1.90 g (2.93 mmol) **S.2a**
   1.51 g (2.41 mmol) **S.2b**
   1.0 g (1.5 mmol) **S.2c**
   1.53 g (3.4 mmol) **S.2d**.

2. Chill solution in an ice bath to 0°C.

3. In a 100-mL two-neck flask, also under a positive flow of nitrogen, mix the following amounts of 2-cyanoethyl-*N,N,N,N*-tetraisopropylphosphorodiamidite (A) and a dry solution of 0.5 M pyridine hydrochloride in acetonitrile (B):

   for **S.2a** derivative: 1.2 mL A plus 2.9 mL B
   for **S.2b** derivative: 0.9 mL A plus 2.6 mL B
   for **S.2c** derivative: 0.9 mL A plus 1.75 mL B
   for **S.2d** derivative: 1.2 mL A plus 3.4 mL B.

4. Connect the flask, under a positive stream of nitrogen, to the dropping funnel of the three-neck round-bottom flask. Add the phosphitylating solution within 10 min dropwise to the chilled nucleoside reaction solution. Stir 1 hr at 0°C.

5. Check the reaction progress by TLC (*APPENDIX 3D*) on a silica gel 60 plate. Visualize by exposing the plate to a 254-nm UV lamp. For solvents and R_f values, see step 9 (analytical data).

If the reaction is not complete, more reagent should be added, and the reaction should be allowed to continue. The amount to add must be determined empirically, although with experience this shouldn't be necessary.

6. Dilute reaction mixture with 100 mL dichloromethane. Wash with 100 mL saturated aqueous $NaHCO_3$ solution and separate the phases using a 500-mL separatory funnel.

7. Dry the organic phase with ~2 g Na_2SO_4 and evaporate the organic phase to dryness on a rotary evaporator equipped with a water aspirator.

8. Dissolve the residue in a minimal amount of dichloromethane and purify by flash chromatography (*APPENDIX 3E*). Prepare a 2 × 20–cm column using 25 g silica gel, and elute with (20- to 25-mL fractions) 0% to 30% (v/v) ethyl acetate/toluene (~500mL total).

9. Identify fractions containing product by TLC and check purity by NMR and MS.

*N^6-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyadenosine-5′-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**S.3a**): Yield, 65%. TLC (toluene/ethyl acetate, 1:1 [v/v]) $R_f = 0.53$. ^1H NMR (DMSO, δ): 11.70 (br, NH), 8.64 (m, H-C(2), H-C(8)), 7.83 (m, 1H o to NO_2), 7.71 (m, 1H m to NO_2, 1H p to NO_2), 7.48 (m, 1H m to NO_2), 7.29 (m, 2H o to tert-butyl), 6.88 (m, 2H m to tert-butyl), 6.45 (m, H-C(1′)), 5.33 (m, H-C(3′)), 4.98 (s, $O\underline{CH_2}$), 4.36 (m, $O\underline{CH_2}CH_2$), 4.24 (m, H-C(4′)), 4.78 (m, 2 H-C(5′), $\underline{OCH_2}CH_2CN$), 3.53 (m, 3 $CHCH_3$), 3.12 (m, H-C(2′)), 2.74 (m, $CH_2\underline{CH_2}CN$), 2.60 (m, H-C(2′)), 1.30 (d, CH_3), 1.11 (m, 7 CH_3). ^{31}P NMR (DMSO, δ): 149.24, 149.20, 149.16. HRMS (FAB, $M + H^+$): calcd. for $C_{41}H_{53}N_8O_{10}P$, 849.3700; found, 849.3723. ESI-MS: 849 ($M + H^+$), 871 ($M + Na^+$), 1697 ($2M + H^+$), 1719 ($2M + Na^+$).*

*N^4-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxycytidine-5′-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**S.3b**): Yield, 81%. TLC (toluene/ethyl acetate, 1:1[v/v]) $R_f = 0.43$. ^1H NMR (DMSO, δ): 10.90 (br, NH), 8.14 (m, H-C(6)), 7.82 (m, 1H o to NO_2), 7.69 (m, 1H m to NO_2, 1H p to NO_2), 7.48 (m, 1H m to NO_2), 7.29 (m, 2H o to tert-butyl), 7.14 (m, H-C(5)), 6.83 (m, 2H m to tert-butyl), 6.07 (m, H-C(1′)), 5.10 (m, H-C(3′)), 4.77 (s, $O\underline{CH_2}$), 4.30 (m, $O\underline{CH_2}CH_2$, H-C(4′)), 3.75 (m, 2 H-C(5′),$\underline{OCH_2}CH_2CN$), 3.55 (m, 3 $\underline{CH}CH_3$), 2.73 (m, $CH_2\underline{CH_2}CN$), 2.55 (m, H-C(2′)), 2.25 (m, H-C(2′)), 1.29 (m, CH_3), 1.15 (m, 7 CH_3). ^{31}P NMR (DMSO, δ): 149.35. HRMS (FAB, $M + H^+$): calcd. for $C_{40}H_{53}N_6O_{11}P$, 825.3587; found, 825.3568. ESI-MS: 825 ($M + H^+$), 847 ($M + Na^+$), 1649 ($2M + H^+$), 1671 ($2M + Na^+$).*

*N^2-[(4-tert-Butylphenoxy)acetyl]-3′-O-[2-(2-nitrophenyl)propoxycarbonyl]-2′-deoxyguanosine-5′-O-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] (**S.3c**): Yield, 91%. TLC (toluene/ethyl acetate/methanol, 5:4:1 [v/v/v]) $R_f = 0.39$. ^1H NMR (DMSO, δ): 11.45 (br, 2 NH), 8.15 (m, H-C(8)), 7.83 (m, 1H o to NO_2), 7.70 (m, 1H m to NO_2, 1H p to NO_2), 7.49 (m, 1H m to NO_2), 7.29 (m, 2H o to tert-butyl), 6.88 (m, 2H m to tert-butyl), 6.19 (m, H-C(1′)), 5.23 (m, H-C(3′)), 4.79 (m, $O\underline{CH_2}$), 4.36 (m, $O\underline{CH_2}CH_2$), 4.20 (m, H-C(4′)), 3.73 (m, 2 H-C(5′), $\underline{OCH_2}CH_2CN$), 3.55 (m, 3 $\underline{CH}CH_3$), 2.87 (m, $CH_2\underline{CH_2}CN$), 2.76 (m, H-C(2′)), 2.57 (m, H-C(2′)), 1.30 (d, CH_3), 1.15 (m, 7 CH_3). ^{31}P NMR (DMSO, δ): 149.46, 149.41. HRMS (FAB, $M + H^+$): calcd. for $C_{41}H_{53}N_8O_{11}P$, 865.3649; found, 865.3660. ESI-MS: 865 ($M + H^+$), 887 ($M + Na^+$), 1751 ($2M + Na^+$).*

*3′-O-[2-(2-Nitrophenyl)propoxycarbonyl]thymidine-5′-O-[(2-cyanoethyl)-N,N-diisopropyl phosphoramidite] (**S.3d**): Yield, 88%. TLC (toluene/ethyl acetate, 1:1 [v/v]) $R_f = 0.37$. ^1H NMR (DMSO, δ): 11.26 (br, NH), 7.82 (m, 1H o to NO_2), 7.69 (m, 1H m to NO_2, 1H p to NO_2), 7.55-7.47 (m, H-C(5), 1H m to NO_2), 6.08 (m, H-C(1′)), 5.09 (m, H-C(3′)), 4.35-4.27 (m, $O\underline{CH_2}CH_2$), 4.12 (m, H-C(4′)), 3.83-3.70 (m, 2 H-C(5′), $O\underline{CH_2}CH_2CN$), 3.59-3.49 (m, 3 $\underline{CH}\underline{CH}CH_3$), 2.75 (m, $CH_2\underline{CH_2}CN$), 2.29 (m, 2 H-C(2′)), 1.78 (m, CH_3), 1.28 (d, CH_3), 1.24-1.09 (m, 7 CH_3). ^{31}P NMR (DMSO, δ): 149.36, 149.33, 149.29. HRMS (FAB, $M + H^+$): calcd. for $C_{29}H_{40}N_5O_{10}P$, 650.2590; found, 650.2576. ESI-MS: 649 ($M + H^+$), 672 ($M + Na^+$), 1321 ($2M + Na^+$).*

PREPARATION OF 2-(2-NITROPHENYL)PROPOXYCARBONYL-*N*-METHYLIMIDAZOLIUM CHLORIDE

The preparation of the acylating reagent used in Basic Protocol 1 is illustrated in Figure 12.3.2.

CAUTION: Trichloromethyl chloroformate (diphosgene) is a hazardous reagent; upon reaction, it decomposes into phosgene gas, which is extremely toxic. Therefore, all preparations should be performed in a well-ventilated fume hood, and extreme precautions should be taken to avoid inhalation of diphosgene or phosgene fumes.

NOTE: **S.4** and subsequent compounds are light sensitive and thus require special handling to prevent photolysis.

Materials

Nitrogen source
Tetrahydrofuran (THF), dry
Trichloromethyl chloroformate (diphosgene) (e.g., Fluka, Sigma)
2-(2-Nitrophenyl)propanol (**S.4**; see Uhlmann and Pfleiderer, 1981)
N-Methylmorpholine
Toluene
Ethyl acetate
Liquid nitrogen
Methanolic KOH solution: 5% (w/v) KOH in MeOH, prepare fresh
N-Methylimidazole
Dichloromethane, dry
Molecular sieves, 4 Å

100- and 250-mL three-neck round-bottom flasks
Drying tubes and septa for 100- and 250-mL flasks
Dropping funnel for 250-mL flasks
Balloons
20-mL syringes and 18- to 22-G needles
Thin-layer chromatography (TLC) silica gel 60 plates (Merck)
Glass frit
250- and 500-mL two-neck Schlenk-type flasks with glass stoppers (neoLab)

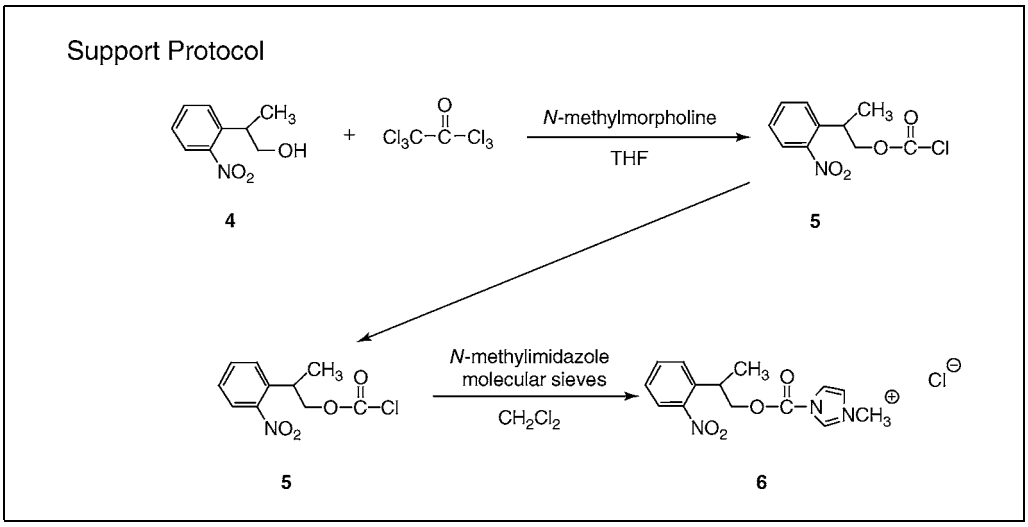

Figure 12.3.2 Synthesis of the acylating reagent 2-(2-nitrophenyl)propoxycarbonyl-*N*-methylimidazolium chloride. THF, tetrahydrofuran.

Vacuum tubing
Gas inlets
Vacuum pump with appropriate tubing
2-mL gas-tight syringe with disposable needles

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

Prepare S.5

1. Connect a 250-mL three-neck round-bottom flask with a drying tube, a dropping funnel, and a septum. Flush the apparatus with nitrogen.
2. Under a positive flow of nitrogen from a balloon filled with nitrogen, add 25 mL dry THF and cool to 0°C in an ice bath.
3. Using a 20-mL syringe and an 18- to 22-G needle, add 14.2 mL (118 mmol) diphosgene through the septum.
4. In a separate flask, under a positive flow of nitrogen, dissolve 20.8 g (114 mmol) **S.4** and 12.5 mL (114 mmol) *N*-methylmorpholine in 25 mL dry THF.
5. Connect this flask, under a positive flow of nitrogen, to the dropping funnel and add solution dropwise over 30 min to chilled diphosgene solution. Stir 1 hr at 0°C.

   A white solid precipitate is formed.

6. Check the reaction progress by TLC (*APPENDIX 3D*) on silica gel 60 plates using 1:1 (v/v) toluene/ethyl acetate (**S.4** $R_f = 0.2$; **S.5** $R_f = 0.7$)

   If the reaction is not complete, allow to continue for an additional 30 min and check again. If it is still not complete, add more diphosgene. The amount to add must be determined empirically, although with experience this shouldn't be necessary.

7. Remove ice bath and let the reaction slowly warm to ambient temperature while stirring. Continue stirring 1 hr at ambient temperature.
8. Attach a glass frit to a 250-mL two-neck Schlenk-type flask and add a magnetic stir-bar. Connect this flask with vacuum tubing to a 500-mL two-neck Schlenk flask (with stir bar) connected to a gas inlet. Using a vacuum pump attached by appropriate tubing, apply a slight vacuum to the second Schlenk flask and cool this flask in liquid nitrogen.
9. Filter the reaction mixture into the first Schlenk flask by extending the slight vacuum to the first Schlenk flask.
10. Wash precipitate on the glass frit with dry THF.
11. Remove glass frit and replace with a glass stopper.
12. Apply an initial modest vacuum to the whole apparatus to remove liquid from the filtrate, while stirring, until the vigorous bubbling ceases.

    CAUTION: *If the initial vacuum is too high, most of the phosgene gas will not be condensed within the second Schlenk flask, but will be sucked into the vacuum pump.*

13. Increase the vacuum and remove all liquid from the filtrate.
14. Finally, apply high vacuum overnight to remove the last remaining solvent residues.

    *The product, 2-(2-nitrophenyl)propyl carbonochloridate (**S.5**) is a brown oil (23.3 g; 84%) that can be used without further purification.*

15. To destroy the remaining phosgene gas, which was condensed in the second flask by cooling with liquid nitrogen, slowly add methanolic KOH solution to the condensate while stirring vigorously.

Convert to S.6

16. Connect a 100-mL three-neck round-bottom flask to a gas inlet, a drying tube, and a septum. Flush the apparatus with a positive flow of nitrogen.

17. Under a continuous flow of nitrogen, dissolve in the flask 1.24 mL (15.5 mmol) *N*-methylimidazole in 40 mL dry dichloromethane over 1 to 2 g of 4 Å molecular sieves. Chill to 0°C in an ice bath.

18. Load a 2-mL gas-tight syringe with 1.07 mL (4.4 mmol) **S.5** and add dropwise within 10 min through the septum to the chilled reaction solution. Stir an additional 30 min at 0°C.

*This reaction solution, 2-(2-nitrophenyl)propoxycarbonyl-N-methylimidazolium chloride (**S.6**), is obtained in quantitative yield and is used directly to introduce the photolabile protecting group to the 3′-O position as described (see Basic Protocol 1). It must be used immediately.*

COMMENTARY

Background Information

Photolabile 3′-*O*-[2-(2-nitrophenyl)propoxycarbonyl]-protected 5′-*O*-phosphoramidites (**S.3a**-**S.3d**) are synthesized mainly for an alternative mode of light-directed production of oligonucleotide arrays (Beier et al., 2001). Because of their characteristics, light-controlled in situ DNA synthesis—performed by photolithography (Pease et al., 1994) or micro-mirror control (Singh-Gasson et al., 1999; Baum et al., 2003)—occurs in a 5′→3′ direction, conforming to the orientation of enzymatic synthesis. The resulting oligonucleotides are attached to the surface via their 5′ termini, leaving the 3′-hydroxyls available as substrates for enzymatic reactions, such as primer extension upon hybridization of a DNA template. The production of such oligonucleotide chips adds new procedural avenues to the growing number of applications of DNA microarrays.

The starting monomers are commercially available 5′-*O*-DMTr-protected nucleosides. The derivatives described here utilize a labile 4-(*tert*-butylphenoxy)acetyl (tac) protecting group for protection of the exocyclic amino groups (Fig. 12.3.3; Sinha et al., 1993). This results in a shorter and milder deprotection procedure when the 3′-*O*-NPPOC-protected 5′-*O*-phosphoramidites are employed for in situ synthesis of DNA microarrays (Beier and Hoheisel, 2000). N-tac-5′-O-DMTr-protected nucleosides are available from Proligo and ChemGenes (under the name tBPAC instead of tac). The authors have also had excellent success using the phenoxyacetyl (pac) protecting group to synthesize standard NPPOC-protected 3′-*O*-phosphoramidites for synthesis in the standard direction. It is reasonable to conclude that working with pac protection would give good results with the reverse 5′-*O*-phosphoramidites, although this has not been tested. The *N*-pac-5′-*O*-DMTr-protected nucleosides are available from ChemGenes.

The synthesis of the photolabile NPPOC protecting group (see Support Protocol) starts out from 2-(2-nitrophenyl)propanol (**S.4**). The 2-(2-nitrophenyl)propyl carbonochloridate (**S.5**) is preferentially synthesized employing the less-dangerous liquid diphosgene reagent (Giegrich et al., 1998) instead of phosgene gas (Hasan et al., 1997). The mild acylating reagent 2-(2-nitrophenyl)propoxycarbonyl-*N*-methylimidazolium chloride (**S.6**) is

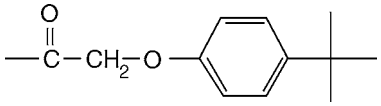

Figure 12.3.3 Structure of the 4-(*tert*-butylphenoxy)acetyl group used for N protection.

generated from **S.5** and *N*-methylimidazole. The introduction of the NPPOC protecting group into the 3′-*O* position is best performed by employing **S.6**. When the carbonochloridate (**S.5**) is employed directly, the reaction produces several side products and the yields drop significantly. The addition of molecular sieves to the reaction increases the yields further.

Having installed the NPPOC group, the DMTr group is cleaved by a short acid treatment. The straightforward synthesis of the phosphoramidites (**S.3a–d**) utilizes a mild bifunctional phosphitylating reagent combined with an acidic activator. Instead of employing tetrazole, the authors prefer to use pyridine hydrochloride as the activator of choice because it is easier to handle and gives slightly higher yields (Beier and Pfleiderer, 1999). In the analysis, the phosphoramidites showed additional peaks in their nuclear magnetic resonance (NMR) spectra. These were due to the additional chiral center within the NPPOC moiety.

Critical Parameters

Keeping moisture from entering the reactions is the most critical factor that can significantly affect yield and quality.

S.6 (see Support Protocol) must be prepared immediately before use.

Anticipated Results

Average yields of the various products are shown in Figure 12.3.1. All products are seen as single spots by TLC, with no detectable sideproducts (peaks) in ^{1}H NMR or mass spectra. If the products are not sufficiently pure, use flash chromatography (*APPENDIX 3E*) to purify them further.

Time Considerations

Each of the intermediates and products described can be prepared and isolated within 1 or 2 days.

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