Synthesis of 1,5-Anhydrohexitol Building Blocks for Oligonucleotide Synthesis

Hexitol nucleic acids (HNA) represent a new oligomeric structure able to hybridize as well with DNA and RNA as with itself, and in a sequence-specific manner. In addition, HNA seems to be superior to its DNA and RNA analogs as an antisense construct. This is supported by a study demonstrating that HNA forms highly selective and exceptionally stable duplexes with RNA (Hendrix et al., 1997a) and that these HNA:RNA duplexes are stable towards nuclease degradation (Hendrix et al., 1997b). Furthermore, a high potential for antiviral activities has been reported (Verheggen et al., 1995).

Hexitol nucleic acids are made up of phosphorylated 1,5-anhydro-D-arabino-2,3-dideoxy-hexitol building blocks with a base moiety positioned in the 2 position. According to the Westheimer model, the base moiety of the hexitol nucleosides is axially oriented, avoiding the sterically unfavorable 1,3-diaxial repulsions (De Winter et al., 1998).

This unit describes in detail the preparation of 1,5-anhydrohexitol (see Basic Protocol 1 and Fig. 1.9.1) and the 1,5-anhydrohexitol building blocks for oligonucleotide synthesis (*h*G, *h*A, *h*C, *h*T; see Basic Protocols 2 to 5 and Figs. 1.9.2 to 1.9.5, respectively).

NOTE: Carry out reactions with anhydrous solvents and dried glassware (i.e., 2 hr at 70°C).

NOTE: All chemicals are commercially available (e.g., ACROS, Fluka, Aldrich). All starting materials can be synthesized but are also available commercially. The sugar intermediate **S.5** is now also commercially available. All reactions can be carried out with standard laboratory equipment and glassware (e.g., round-bottom and Erlenmeyer flasks, consensers, dropping funnels, separatory funnels, desiccators, ultrasonic baths, oil baths, and magnetic stirrers).

PREPARATION OF 1,5-ANHYDRO-4,6-*O*-BENZYLIDINE-3-DEOXY-D-GLUCITOL

BASIC PROTOCOL 1

In this protocol, synthesis of the sugar building block starting from tetra-*O*-acetyl-α-D-bromoglucose is given, as outlined in Figure 1.9.1 (Verheggen et al., 1993). The starting sugar, tetra-*O*-acetyl-α-D-bromoglucose, can be used to make both tolylsulfonyl and toluoyl intermediates (**S.4a** and **S.4b**, respectively). The latter is used to make **S.5** (described below), which is then used to make the *h*G, *h*T, and *h*C nucleoside monomers (see Basic Protocols 2, 4, and 5, respectively). The former is used directly to make the *h*A monomer (see Basic Protocol 3). The chemical reactions described give good yields, and purification of the intermediates is straightforward.

Materials

2,3,4,6-Tetra-*O*-acetyl-α-D-bromoglucose
Diethyl ether: reflux overnight on sodium (Na, $FeCl_2$, Et_2O) and distill
Azoisobutyronitrile [2,2′-azobis(2-methylpropionitrile); AIBN]
Tri-*n*-butyltin hydride
Precoated silica gel TLC plates (Alugram Sil G/UV254)
Dichloromethane
Anisaldehyde/sulfuric acid spray (*UNIT 1.3*)
Potassium fluoride dihydrate
Sodium sulfate
Silica gel (0.060 to 0.200 nm)
Methanol

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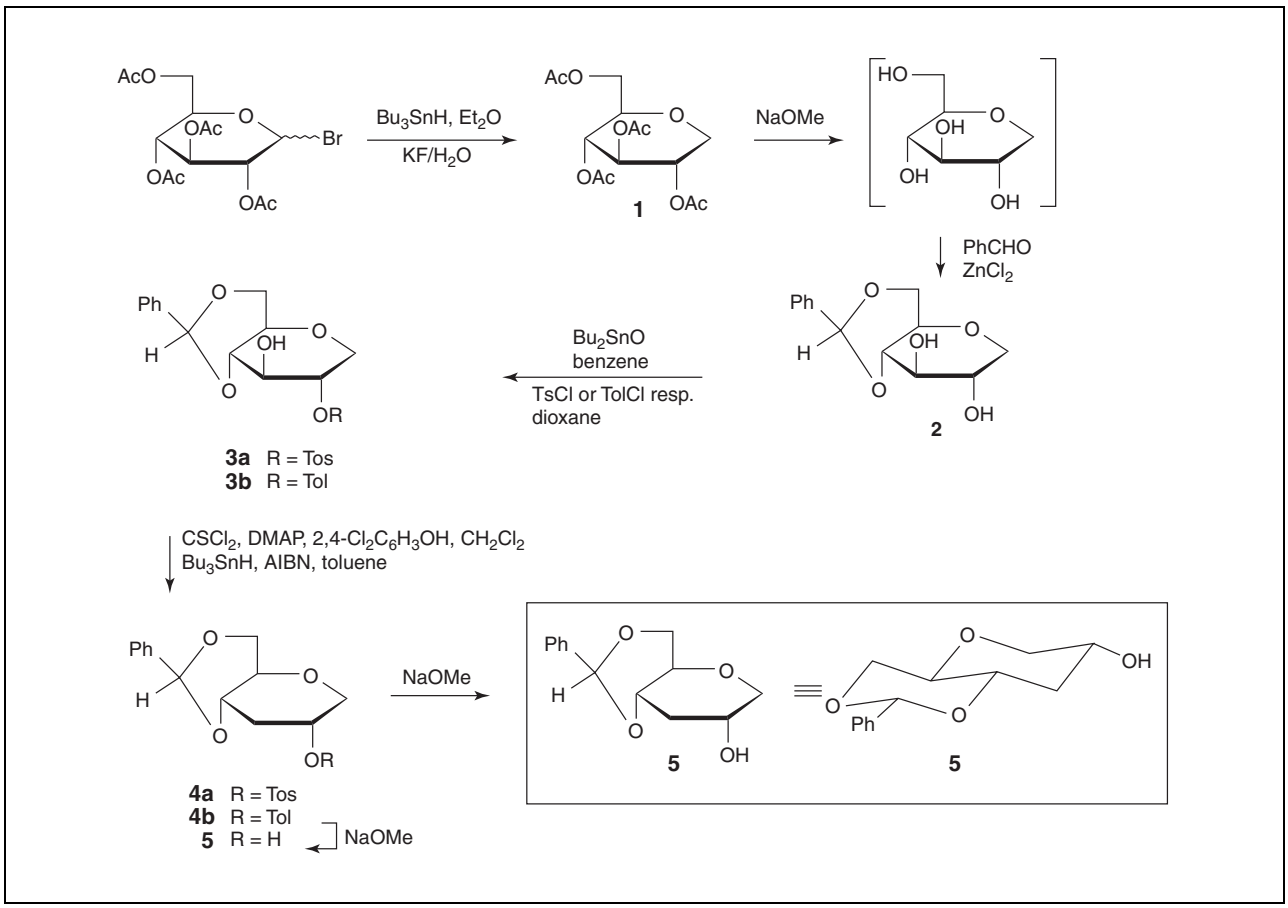

Figure 1.9.1 Preparation of 1,5-anhydro-4,6-*O*-benzylidine-3-deoxy-D-glucitol (**S.5**). Abbreviations: AIBN, azoisobutyronitrile; Bu_2SnO, dibutyltinoxide; Bu_3SnH, tributyltinhydride; DMAP, 4-(dimethylamino)pyridine; NaOMe, sodium methoxide; PhCHO, benzaldehyde; TolCl, toluoyl chloride; TsCl, tosyl chloride.

0.1 N sodium methoxide, freshly prepared from sodium and dry methanol
Acetic acid (glacial not necessary)
Toluene
Zinc chloride (dry fresh before use)
Benzaldehyde
Ethyl acetate
n-Hexane
Dibutyltinoxide
Benzene
Dioxane (reflux overnight on lithium aluminum hydride and distill)
p-Toluenesulfonyl chloride (for **S.3a**) *or p*-toluoyl chloride (for **S.3b**)
Delite Celite
4-(Dimethylamino)pyridine (DMAP)
Dry ice/isopropanol
Thiophosgene
2,4-Dichlorophenol
1 M potassium dihydrogenphosphate solution, pH 5
Nitrogen gas

Oil bath and magnetic stirrer
Rotary evaporator equipped with a vacuum pump and cooling trap
5 × 35–, 6 × 50–, and 5 × 20–cm chromatography columns

2×18–cm test tubes
1-L round-bottom flask with rubber stopper
Dropping funnel
Glass funnel
Dean-Stark condenser
UV lamp, 254 nm

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

NOTE: The ^{1}H and ^{13}C NMR spectra given as examples were determined with a JEOL FX 90Q spectrometer or 400 MHz Bruker AMX with tetramethylsilane as internal standard. Electron-impact mass spectra (EIMS) and chemical-ionization mass spectra (CIMS) were obtained using a KRATOS Concept ^{1}H mass spectrometer. Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad signal; m, multiplet; ddd, double doublet of doublet; and dm, double multiplet.

Prepare S.1

1. Dissolve 25.03 g (60.9 mmol) of 2,3,4,6-tetra-*O*-acetyl-α-D-bromoglucose in 375 mL diethyl ether.
2. Add 0.99 g (6.1 mmol) of AIBN and 25 mL (93.1 mmol) of tri-*n*-butyltin hydride.
3. Stir the mixture 1 hr at 35°C and then at room temperature until conversion is complete (2 to 12 hr). Monitor by TLC (*APPENDIX 3D*) by spotting the reaction mixture (100 µL) between two spots of starting material (1 mg diluted to 200 µL) as a reference. Use precoated silica gel plates and develop with dichloromethane. Visualize under a UV lamp (254 nm) and then spray the plate with anisaldehyde/sulfuric acid spray and dry at 150°C (also see Critical Parameters).
4. Dissolve 8.21 g potassium fluoride dihydrate in 40 mL water, add to the suspension, and stir 15 min.
5. Filter off the precipitated tri-*n*-butyltin hydride and wash the organic retentate (on top of the funnel) with 120 mL water. Set aside the aqueous layer and wash the organic layer two more times with 120 mL water. Combine all of the aqueous layers and wash three times with 60 mL diethyl ether.
6. Dry the combined organic layers from step 5 over sodium sulfate, filter, and evaporate to dryness using a rotary evaporator equipped with a vacuum pump and a cooling trap.
7. Divide the oily residue into two fractions and purify each by column chromatography (*APPENDIX 3E*) on 350 g of 0.060- to 0.200-nm silica gel in a 5×35–cm column. Use a step gradient from 1.5 L of 100% dichloromethane to 1.5 L of 99:1 (v/v) dichloromethane/methanol to elute. Collect fractions in 2×18–cm test tubes.

   See Critical Parameters for additional discussion of column chromatography.

8. Draw a roster of the fraction collector on a TLC plate and apply a drop of each fraction onto the corresponding field of the plate. Spray with anisaldehyde/sulfuric acid spray and dry at 150°C. Combine the product-containing fractions (blue) and remove solvents in vacuo on a rotary evaporator.

   *The resulting product, 1,5-anhydro-2,3,4,6-tetra-O-acetyl-D-glucitol (**S.1**), should be obtained in a 93% yield (18.82 g, 56.9 mmol). The spectroscopic properties are identical with those previously described (Kocienski and Pant, 1982).*

Prepare S.2

9. Weigh 30.06 g (90.5 mmol) **S.1** into a 1-liter round-bottom flask. Add 400 mL of 0.1 N sodium methoxide (freshly prepared from 0.92 g sodium and 400 mL dry methanol) and stir the reaction 2 hr at room temperature.
10. Neutralize the reaction mixture with ~2 mL acetic acid (confirm with pH paper) and evaporate the solvent.
11. Coevaporate the residue with 20 mL toluene four times. Add 12.40 g (91.0 mmol) zinc chloride and 46.50 mL (455.0 mmol) benzaldehyde. Close the reaction flask with a rubber stopper and vigorously stir the suspension for two days at room temperature.
12. Pour the reaction mixture into 250 mL ice-water and extract with 100 mL ethyl acetate four times. Dry the combined organic layers over sodium sulfate.
13. Remove excess benzaldehyde on a rotary evaporator in vacuo (bath temperature 70°C) and wash the obtained solid residue with 100 mL *n*-hexane on a glass funnel.
14. Purify by column chromatography using 700 g silica gel in a 6 × 50–cm column. Elute with a step gradient of 1.5 L each:

    1:1 (v/v) *n*-hexane/dichloromethane
    100% dichloromethane
    98:2 (v/v) dichloromethane/methanol.

    Collect fractions in 2 × 18–cm test tubes and monitor by TLC as in step 8. Combine product-containing fractions and remove solvent in vacuo on a rotary evaporator.

    *The resulting product, 1,5-anhydro-4,6-O-benzylidene-D-glucitol (**S.2**), is obtained in 75% yield (17.1 g, 68.0 mmol). CIMS (iC_4H_{10}): m/e 253 (MH^+); ^{1}H NMR (DMSO-d_6): δ = 3.00-3.90 (m, 7H) and 4.10-4.30 (m, 1H) (H-1′, H-1″, H-2′, H-3′, H-4′, H-5′, H-6′, H-6″), 5.03-5.31 (dd, 2H, 2′-OH, 3′-OH), 5.55 (s, 1H, PhCH), 7.20-7.57 (m, 5H, aromatic H); ^{13}C NMR (DMSO-d_6): δ = 68.0 (C-6′), 70.2, 70.4 (C-1′, C-5′), 71.0 (C-2′), 74.4 (C-3′), 81.1 (C-4′), 100.7 (PhCH), 126.3, 127.9, 128.7, 137.8 (arom. C).*

Prepare S.3a or S.3b

15. Suspend 8.50 g (33.7 mmol) **S.2** and 8.38 g (33.7 mmol) dibutyltinoxide in 250 mL benzene.
16. Reflux the mixture 16 hr with azeotropic removal of water using a Dean-Stark condenser until the volume is reduced to ~100 mL. Add 150 mL dioxane.
17. For **S.3a**, add 7.06 g (37.0 mmol) *p*-toluenesulfonyl chloride and heat the mixture 6 hr at 50°C, until a quantitative conversion to a less polar product (higher R_f value) is demonstrated by TLC. For **S.3b**, add 4.44 mL (33.7 mmol) *p*-toluoyl chloride and stir the mixture 5 hr at room temperature. For both, perform TLC using 1:2 (v/v) *n*-hexane/dichloromethane and visualize the products under a UV lamp.
18. Concentrate the mixture in vacuo using a rotary evaporator, absorb on Delite Celite, and purify by column chromatography using a step gradient from 1:1 (v/v) *n*-hexane/dichloromethane to 100% dichloromethane. Combine product-containing fractions and evaporate solvent on a rotary evaporator.

    *1,5-Anhydro-4,6-O-benzylidene-2-O-(p-tolylsulfonyl)-D-glucitol (**S.3a**) is obtained in 82% yield (11.22 g, 27.6 mmol). EIMS: m/e 406 (MH^+); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s, 3H, CH_3), 3.35-3.42 (m, 2H, H-4′, H-5′), 3.49 (t, 1H, J = 11 Hz, H-1′α), 3.61 (m, 1H, H-6′α), 3.67 (m, 1H, H-3′), 3.87 (dd, J = 5.5 and 11 Hz, 1H, H-1′β), 4.14-4.25 (m, 2H, H-2′, H-6′β), 5.05 (s, 1H, PhCH), 5.12 (d, J = 5.5 Hz, 1H, OH), 7.35-7.50 (m, 7H, H-aromatic), 7.85 (m, 2H, H-aromatic); ^{13}C NMR (90 MHz, DMSO-d_6): δ = 21.0 (CH_3), 66.9, 67.6 (C-1, C-6), 70.7, 70.8 (C-3, C-5), 79.2, 80.4 (C-2, C-4), 100.7 (PhCH), + aromatic C.*

*1,5-Anhydro-4,6-O-benzylidene-2-O-(p-toluoyl)-D-glucitol (**S.3b**) is obtained in 78% yield (9.73 g, 26.3 mmol). CIMS (iC_4H_{10}): m/e 371 (MH^+); ^1H NMR (DMSO-d_6): δ = 2.40, (s, 3H, CH_3), 3.19-4.51 (m, 8H, H-1′, H-1″, H-2′, H-3′, H-4′, H-5′, H-6′, H-6″), 4.93-5.50 (br, s, 3′-OH), 5.55 (s, 1H, PhCH), 7.05-8.03 (m, 9H, H-aromatic); ^{13}C NMR (DMSO-d_6): δ = 21.5 (CH_3), 67.2, 68.4 (C-1, C-6),70.9, 71.9, (C-3, C-5), 72.6 (C-2), 80.9(C-4), 101.9 (PhCH), 165.9 (C=O), + aromatic C.*

Prepare S.4a or S.4b

19. Dissolve 23.60 g (193.0 mmol) DMAP and either 11.22 g (27.6 mmol) **S.3a** or 10.21 g (27.6 mmol) **S.3b** in 400 mL dichloromethane.
20. Cool the reaction mixture to –40°C with a dry ice/isopropanol mixture and add 2.53 mL (33.1 mmol) thiophosgene under vigorous stirring.
21. Allow the mixture to come slowly to room temperature and continue stirring 1 hr at room temperature.
22. Add 6.30 g (38.6 mmol) 2,4-dichlorophenol and continue stirring another 2 hr. Monitor by TLC using 1:5 (v/v) *n*-hexane/dichloromethane and visualize with a UV lamp.
23. Pour the mixture into 300 mL of 1 M potassium dihydrogenphosphate solution, pH 5, and extract with 300 mL dichloromethane twice.
24. Dry the organic layers over sodium sulfate, filter, and evaporate the solvent.
25. Purify the residue using a 5 × 35–cm flash column and a step gradient from 1.5 L of 2:8 (v/v) *n*-hexane/dichloromethane to 1.5 L of 100% dichloromethane. Combine product-containing fractions and evaporate solvent.
26. Dissolve the obtained thiocarbonyl compound in 300 mL toluene.
27. Bubble nitrogen gas through the solution for 10 min and then add 7.84 mL (29.1 mmol) tri-*n*-butyltin hydride and 0.33 g (2.0 mmol) AIBN.
28. Heat the reaction mixture overnight at 80°C.
29. Evaporate the mixture and purify by column chromatography as in step 25.

*1,5-Anhydro-4,6-O-benzylidene-3-deoxy-2-O-(p-tolylsulfonyl)-D-ribohexitol (**S.4a**) is obtained in 64% yield (6.90 g, 17.7 mmol). CIMS (NH_3): m/e 391 (MH^+); ^1H NMR ($CDCl_3$): δ = 1.50-2.10 (m, 2H, H-3′, H-3″), 2.48 (s, 3H, CH_3), 3.06-4.84 (m, 7H, H-1′, H-1″, H-2′, H-4′, H-5′, H-6′, H-6″), 5.50 (s, 1H, PhCH), 7.04-7.98 (m, 9H, H aromat.); ^{13}C NMR ($CDCl_3$): δ = 21.4 (CH_3), 35.3 (C-3′), 68.7, 69.0 (C-1′, C-6′), 72.9, 73.1 (C-4′, C-5′), 75.7 (C-2′), 101.5 (PhCH) + aromatic C.*

*1,5-Anhydro-4,6-O-benzylidene-3-deoxy-2-O-(p-toluoyl)-D-ribohexitol (**S.4b**) is obtained in 75% yield (7.12 g, 20.7 mmol). CIMS (iC_4H_{10}): m/e 355 (MH^+); ^1H NMR ($CDCl_3$): δ = 1.42-2.12 (m, 2H, H-3′, H-3″), 2.39 (s, 3H, CH_3), 3.12-3.92 (m, 4H) and 4.02-4.49 (m, 2H) (H-1′, H-1″, H-4′, H-5′, H-6′, H-6″), 4.95-5.43 (m, 1H, H-2′), 5.54 (s, 1H, PhCH), 7.10-8.08 (m, 9H, H aromat.); ^{13}C NMR ($CDCl_3$): δ = 21.5 (CH_3), 34.8 (C-3′), 66.9 (C-5′), 69.0, 69.1 (C-1′, C-6′), 73.4 (C-2′), 76.0 (C-4′), 101.5 (PhCH), 165.3 (C=O), + aromatic C.*

Prepare S.5

30. Weigh 6.79 g (19.7 mmol) **S.4b** into a 1-liter round-bottom flask.
31. Add 300 mL of 0.1 N sodium methoxide (freshly prepared from 0.70 g sodium and 300 mL dry methanol) and stir the reaction 4 hr at room temperature.
32. Neutralize the reaction mixture with acetic acid (~6 mL), confirming with pH paper, and evaporate the solvent.
33. Purify by flash chromatography using a 5 × 20–cm column and eluting with 99:1 (v/v) dichloromethane/methanol. Combine product-containing fractions and remove solvent in vacuo on a rotary evaporator.

*The resulting product of 1,5-anhydro-4,6-O-benzylidene-3-deoxy-D-glucitol (**S.5**) is obtained in 80% yield (3.72 g, 15.8 mmol). CIMS (iC_4H_{10}): m/e 237 (MH^+); ^{1}H NMR (DMSO-d_6): δ = 1.20-1.66 (m, 1H, H-3′), 2.06-2.42 (m, 1H, H-3″), 2.99-3.98 (m, 6H) and 4.05-4.30 (m, 1H) (H-1′, H-1″, H-2′, H-4′, H-5′, H-6′, H-6″), 5.08 (d, 1H, 2′-OH), 5.57 (s, 1H, PhCH), 7.17-7.67 (m, 5H, H aromatic); ^{13}C NMR (DMSO-d_6): δ = 38.3 (C-3′), 65.4 (C-5′), 69.1 (C-6′), 72.3, 73.0 (C-1′, C-2′), 76.3 (C-4′), 101.6 (PhCH), 126.1, 128.2, 129.0, 137.2 (arom. C).*

BASIC PROTOCOL 2

SYNTHESIS OF 1′,5′-ANHYDRO-2′,3′-DIDEOXY-2′-(N^2-ISOBUTYRYL-GUANIN-9-YL)-6′-*O*-MONOMETHOXYTRITYL-D-ARABINOHEXITOL

This protocol details the synthesis of the *h*G 1,5-anhydrohexitol building block **S.9** from **S.5** (see Fig. 1.9.2 and DeBouvere et al., 1997).

Materials

6-Chloro-9*H*-purine-2-amine
1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (**S.5**; see Basic Protocol 1)
Triphenylphosphine
Dioxane (reflux overnight on lithium aluminum hydride and distill)
Nitrogen gas
Diisopropyl azodicarboxylate (DIAD)
n-Hexane
Ethyl acetate
10% (v/v) HCl
Dichloromethane (store over phosphorous pentoxide and distill before use)
Phenolpthalein solution
4 N sodium hydroxide
Phosphorous pentoxide
Pyridine (reflux overnight over potassium hydroxide distill before use)
Bis(trimethylsilyl)acetamide (BSA)
Isobutyric anhydride
25% (v/v) ammonia
Diethyl ether
Dimethylformamide (DMF; remove water by distillation with benzene followed by distillation under vacuum)
4-Monomethoxytrityl chloride (MMTr·Cl)
Methanol
Saturated sodium bicarbonate solution
Sodium sulfate
Toluene

Dropping funnel
Rotary evaporator equipped with a vacuum pump and cooling trap

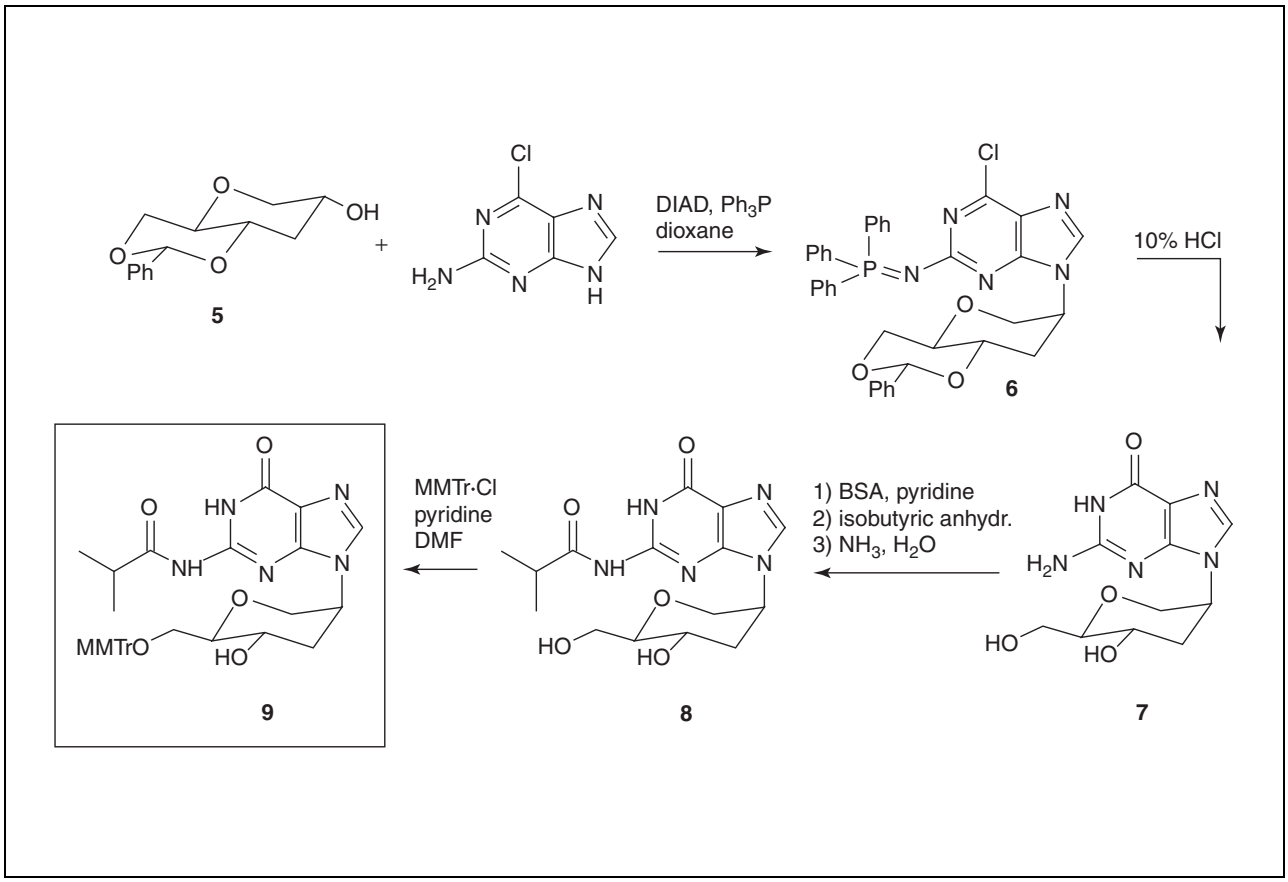

Figure 1.9.2 Preparation of protected *h*G (**S.9**). Abbreviations: BSA, bis(trimethylsilyl)acetamide; DIAD, diisopropyl azodicarboxylate; DMF, dimethylformamide; MMTr·Cl, 4-monomethoxytrityl chloride; Ph_3P, triphenylphosphine.

Oil bath and magnetic stirrer
5 × 35– and 4 × 25–cm chromatography columns

Additional reagents and equipment for TLC and column chromatography (see Basic Protocol 1 and Critical Parameters)

NOTE: The ^{1}H NMR and ^{13}C NMR spectra were determined with a 200 MHz Varian Gemini spectrometer with tetramethylsilane as internal standard. Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad signal; m, multiplet; ddd, double doublet of doublet; dm, double multiplet. Liquid secondary-ion (LSIMS) mass spectra were obtained using a KRATOS Concept ^{1}H mass spectrometer.

Prepare S.6

1. Suspend 6.78 g (40.0 mmol) 6-chloro-9*H*-purine-2-amine, 4.72 g (20.0 mmol) **S.5**, and 13.12 g (50.0 mmol) triphenylphosphine in 200 mL dioxane. Carry out the reaction under a nitrogen gas atmosphere.
2. Add a solution of 9.84 mL (50.0 mmol) DIAD in 25 mL dioxane via a dropping funnel over a period of 200 min.
3. Stir the mixture overnight at room temperature. Monitor by TLC (see Basic Protocol 1, step 3) using 3:2 (v/v) *n*-hexane/ethyl acetate. Visualize product using a UV lamp (254 nm).
4. Remove volatiles by evaporation under vacuum using rotary evaporator equipped with a vacuum pump and cooling trap.

5. Absorb the crude product on 15.0 g of 0.060- to 0.200-nm silica gel in a 5 × 35–cm chromatography column and elute with a gradient from 1.5 L of 3:2 (v/v) to 1.5 L of 2:3 (v/v) *n*-hexane/ethyl acetate. Collect fractions in 2 × 18–cm test tubes.
6. Draw a roster of the fraction collector on a TLC plate and apply a drop of each fraction to the corresponding field on the plate. Visualize product-containing fractions using a UV lamp (254 nm). Combine the product-containing fractions and remove solvents in vacuo on a rotary evaporator.

*The resulting product, 1′,5′-anhydro-4′,6′-O-benzylidene-2′-6-chloro-2[(triphenylphosphoranylidene)amino]-9H-purin-9-yl-2′,3′-dideoxy-D-arabinohexitol (**S.6**) is obtained in 64% yield (8.31 g, 12.8 mmol). mp: 158°C; LSIMS (thygly): m/z: 648 $[M+H]^+$; ^1H NMR ($CDCl_3$): δ = 2.00 (dt ,1H, 3′ax-H, J = 11.6 Hz, J = 4.5 Hz), 2.29 (br, d, 1H, 3′-eq-H, J = 13.0 Hz), 3.57 (m, 2H, 5′-H, 4′-H), 3.76 (t, 1H, 6′ax-H, J = 9.7 Hz), 4.10 (dd, 1H, 1′ax-H, J = 13.2 Hz, J = 2.8 Hz), 4.36 (m, 2H, 1′eq-H, 6′eq-H), 4.73 (br, s, 1H, 2′-H), 5.49 (s, 1H, PhCH), 7.19-7.72 (m, 21H, H aromat.), 8.15 (s, 1H, 8-H); ^{13}C NMR ($CDCl_3$): δ = 32.9 (C-3′), 50.2 (C-2′), 68.9 (C-6′), 69.5 (C-1′), 73.9 (C-4′), 74.5 (C-5′), 101.8 (PhCH), 122.8 (C-5), 125.9 (,2,6-C Ph), 127.5-133.4 (C, CH Ar),137.2 (C-6), 140.2 (C-8), 149.6 (C-4), 158.7 (C-2).*

Prepare S.7

7. Suspend 8.20 g (12.6 mmol) **S.6** in 160 mL of 10% HCl. Heat the reaction mixture 2 hr at 100°C.
8. Cool to room temperature and wash the yellow-brown solution with 60 mL dichloromethane to remove benzaldehyde and triphenylphosphine.
9. Add a drop of phenolphthalein solution as an indicator and neutralize the aqueous layer with 120 mL of 4 N sodium hydroxide.

At pH 7 the product begins to precipitate.

10. Concentrate the suspension in vacuo using a rotary evaporator.
11. Dissolve the obtained white product in 745 mL boiling water and filter hot.
12. Cool to room temperature and incubate the mixture overnight at 4°C.
13. Filter off the obtained crystals and dry over phosphorus pentoxide.

*The resulting product, 1′,5′-anhydro-2′,3′-dideoxy-2′-(guanin-9-yl)-D-arabinohexitol (**S.7**) is obtained in 83% yield (2.95 g, 10.5 mmol). mp: >300°C; UV (H_2O): λ_{max} ε = 253 nm (9100); LSIMS (thgly): m/z: 282 $[M+H]^+$; ^1H NMR (DMSO-d_6): δ = 1.80 (m, 1H, 3′ax-H), 2.17 (br, 1H, 3′eq-H), 3.20-3.70 (m, 2H, 5′-H, 4′-H, 6A-H), 3.79 (dd, 1H, 1′ax-H, J = 12.5 Hz, J = 2.2 Hz), 4.05-4.15 (m, 2H, 1′eq-H, 6B-H), 4.52 (s, br, 1H, 2′-H), 4.63 (t, 1H, 6′-OH, J = 6.0 Hz), 4.91 (d, 1H, 4′-OH, J = 5.3 Hz), 6.46 (br, s, 2H, NH_2), 7.87 (s, 1H, 8-H); ^{13}C NMR (DMSO-d_6): δ = 36.3 (3′-C), 50.2 (2′-C), 61.0 (6′-C), 61.2 (4′-C), 68.4 (1′-C), 83.2 (5′-C), 116.3 (C-5), 136.9 (C-8), 151.5 (C-4), 154.1 (C-2), 157.9 (C-6).*

Prepare S.8

14. Suspend 3.58 g (12.7 mmol) **S.7** in 160 mL pyridine.
15. Add 16.57 mL (63.7 mmol) BSA and reflux 8 hr.
16. Stir the dark-red solution overnight (16 hr) at room temperature.
17. Add 10.00 g (63.7 mmol) isobutyric anhydride and stir 24 hr.
18. Cool the mixture to 0°C and add 20 mL water.
19. After 15 min, add 20 mL of 25% ammonia.

20. Continue stirring 2 hr at room temperature.

21. Evaporate the volatiles under vacuum and add 200 mL water.

22. Stir the suspension 10 min, then filter off the precipitate. Wash once with 50 mL water followed by three washes of 100 mL of 1:1 (v/v) ethyl acetate/diethyl ether.

*The resulting product, 1′,5′-anhydro-2′,3′-dideoxy-2′-(N^2-isobutyrylguanin-9-yl)-D-arabinohexitol (**S.8**) is obtained in 90% yield (3.74 g, 10.7 mmol). mp: 258°C; LSIMS (thgly): m/z: 352 $[M+H]^+$; ^1H NMR (DMSO-d_6): δ = 1.13 (d, 6H, 2 × CH_3, J = 7 Hz), 1.87 (dt, 1H, 3′ax-H, J = 12.3 Hz, 4.2 Hz), 2.23 (br, d, 1H, 3′eq-H, J = 12.8 Hz), 2.78 (sept, 1H, CH, J = 7 Hz), 3.18 (m, 2H, 5′-H, 4′-H), 3.45-3.75 (m, 2H, 6′-H), 3.85 (dd, 1H, 1′ax-H, J = 12.5 Hz, J = 2.7 Hz), 4.17 (d, 1H, 1′eq-H, J = 12.4 Hz), 4.65 (m, 2H, 2′-H, 6′-OH), 5.01 (br, s, 1H, 4′-OH), 8.15 (s, 1H, 8-H), 10.2 (br, s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 19.0 (2 × CH_3), 34.8 (C-3′), 36.2 (CH), 50.4 (C-2′), 60.6 (C-6′, C-4′), 68.0 (C-1′), 83.1 (C-5′), 119.7 (C-5), 138.6 (C-8), 148.0 (C-2), 148.7 (C-4), 155.1 (C-6), 180.2 (HNC=O).*

Prepare S.9

23. Suspend 4.03 g (11.5 mmol) **S.8** in a mixture of 60 mL DMF and 60 mL pyridine.

24. Heat the suspension to 120°C until a clear red-brown solution is obtained.

25. Cool to room temperature and add 4.6 g (14.92 mmol, 1.3 eq) MMTr·Cl.

26. Stir the reaction overnight at room temperature.

27. Monitor the reaction by TLC using 94:6 (v/v) dichloromethane/methanol and visualize the product using a UV lamp (the product has a higher R_f value than the starting material).

28. Quench the reaction with 200 mL saturated sodium bicarbonate solution.

29. Extract with 100 mL dichloromethane four times.

30. Dry the organic layer over sodium sulfate.

31. Remove the solvent and coevaporate with 10 mL toluene three times.

32. Purify by flash chromatography on a 4 × 25–cm column using a step gradient from 500 mL of 100% dichloromethane to 99:1 (1 L), 98:2 (1 L), and 97:3 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate solvents in vacuo on a rotary evaporator.

*The resulting product, 1′,5′-anhydro-2′,3′-dideoxy-2′-(N^2-isobutyrylguanin-9-yl)-6′-O-monomethoxytrityl-D-arabinohexitol (**S.9**) is obtained in 93% yield (6.65 g, 10.7 mmol). mp: 165°C; LSIMS (thgly): m/z: 646 $[M+Na]^+$; ^1H NMR ($CDCl_3$): δ = 1.19 (d, 6H, 2 x CH_3, J = 6.8 Hz), 1.80 (t, 1H, 3′ax-H), J = 12.6 Hz), 2.20 (s, 1H, 4′-OH), 2.32 (br, d, 1H, 3′eq-H, J = 12.1 Hz), 2.70 (sept, 1H, CH, J = 6.9 Hz), 3.35-3.50 (m, 4H, 5′-H, 4′-H, 6A-H, 6B-H), 3.75 (m, 4H, 1′ax-H, OCH_3), 4.20 (d, 1H, 1′eq-H, J = 13.4 Hz), 4.52 (br, s, 1H, 2′-H), 6.80 (d, 2H, aromatic H), 7.10-7.50 (m, 12H, aromatic H), 8.10 (s, 1H, 8-H), 9.66 (br, s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 18.8 (CH_3), 18.9 (CH_3), 35.8 (3′-C), 36.0 (CH), 50.7 (2′-C), 55.1 (OCH_3), 63.3 (4′-C), 63.9 (6′-C), 68.5 (1′-C), 81.1 (5′-C), 86.7 (O-C-Tr), 113.1 (m′-C, 2C), 120.0 (C-5), 126.9 (p-C, 2C), 127.8 (o-C, 4C), 128.3 (m-C, 4C), 130.2 (o′-C, 2C), 135.2 (i′C), 138.6 (C-8), 144.1 (iC, 2C), 147.8 (C-2), 148.8 (C-4), 156.0 (C-6), 158.5 (p′C), 180.0 (HNC=O).*

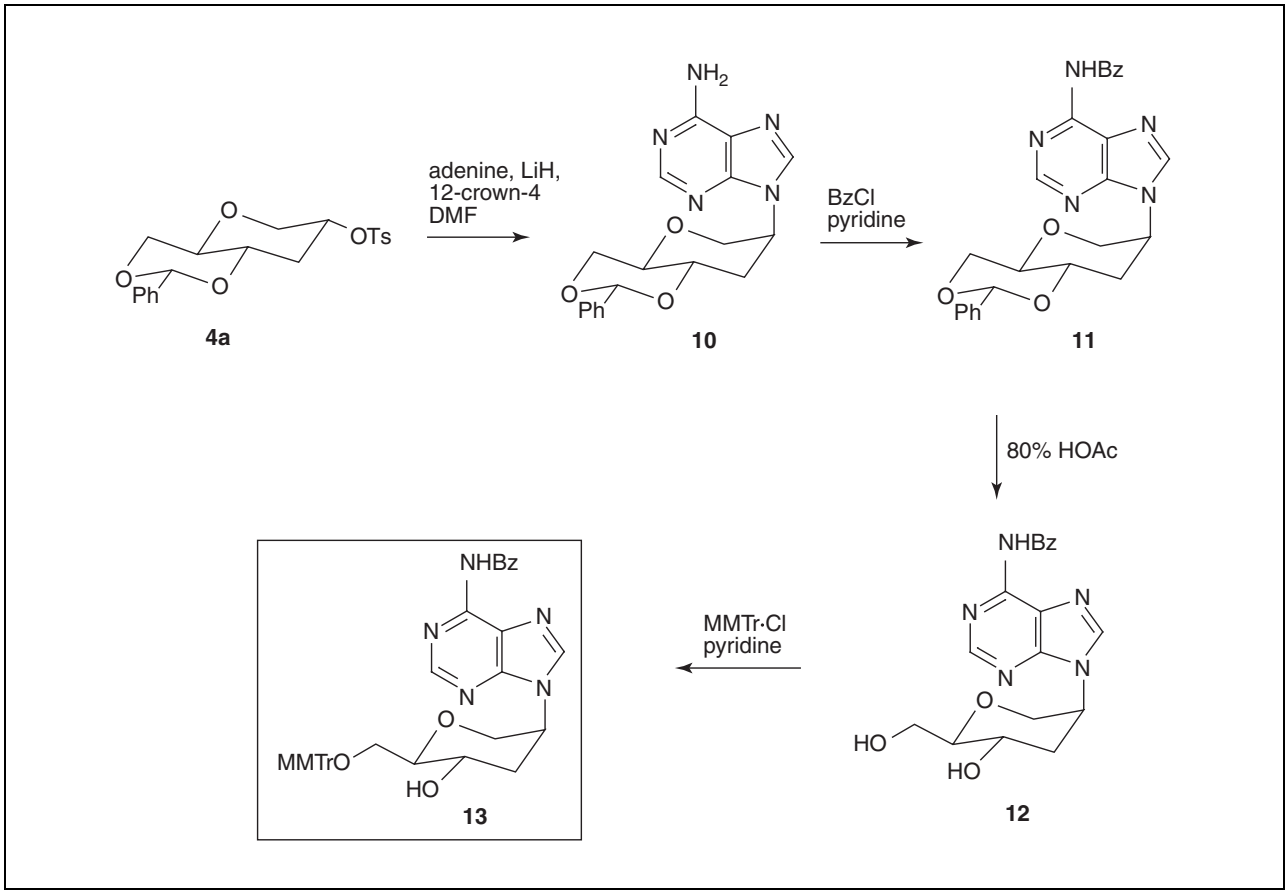

Figure 1.9.3 Preparation of protected *h*A (**S.13**). Abbreviations: BzCl, benzoyl chloride; DMF, dimethylformamide; HOAc, acetic acid; MMTr·Cl, monomethoxytrityl chloride.

BASIC PROTOCOL 3

SYNTHESIS OF 1′,5′-ANHYDRO-6′-MONOMETHOXYTRITYL-2′,3′-DIDEOXY-2′-(N^6-BENZOYLADENIN-9-YL)-D-ARABINOHEXITOL

This protocol details the synthesis of the *h*A 1,5-anhydrohexitol building block **S.13** from **S.4a** (see Fig. 1.9.3 and DeBouvere et al., 1997).

Materials

Adenine
Lithium hydride
12-Crown-4
Dimethylformamide (DMF; remove water by distillation with benzene followed by distillation under vacuum)
Nitrogen gas
1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-2-*O*-(*p*-tolylsulfonyl)-D-ribohexitol (**S.4a**; see Basic Protocol 1)
n-Hexane
Ethyl acetate
Dichloromethane
Saturated sodium bicarbonate solution
Sodium sulfate
Methanol
Pyridine (reflux over potassium hydroxide overnight and distill)
Benzoyl chloride
25% (v/v) ammonia

Toluene
80% (v/v) acetic acid
Diethyl ether: reflux overnight on sodium (Na, $FeCl_2$, Et_2O) and distill
Pyridine (reflux over potassium hydroxide overnight and distill)
4-Monomethoxytrityl chloride (MMTr·Cl)

Oil bath and magnetic stirrer
Rotary evaporator equipped with a vacuum pump and cooling trap
3 × 30–, 5 × 40–, and 4 × 30–cm chromatography columns

Additional reagents and equipment for TLC and column chromatography (see Basic Protocol 1 and Critical Parameters)

NOTE: The ^{1}H NMR and ^{13}C NMR spectra were determined with a 200 MHz Varian Gemini spectrometer with tetramethylsilane as internal standard. Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad signal; m, multiplet; ddd, double doublet of doublet; dm, double multiplet. Liquid secondary-ion (LSIMS) mass spectra were obtained using a KRATOS Concept ^{1}H mass spectrometer.

Prepare S.10

1. Suspend 1.35 g (10.0 mmol) adenine, 0.08 g (9.6 mmol) lithium hydride, and 0.32 mL of 12-crown-4 (2.0 mmol) in 60 mL DMF. Carry out the reaction under a nitrogen gas atmosphere.
2. Heat the reaction mixture 1 hr at 110°C.
3. Add a solution of 1.95 g (5.0 mmol) of **S.4a** in 13 mL DMF with stirring.
4. Continue stirring at 110°C for 8 hr.
5. Test the reaction by TLC (see Basic Protocol 1, step 3) using 8:2 (v/v) hexane/ethyl acetate as solvent.
6. Cool the reaction mixture to room temperature and quench with 0.09 mL (5.0 mmol) water.
7. Concentrate the mixture under reduced pressure using a rotary evaporator with a vacuum pump.
8. Dissolve the residue in 100 mL dichloromethane and wash with 200 mL saturated sodium bicarbonate followed by two washes with 100 mL water.
9. Dry over sodium sulfate, filter, concentrate, and purify the residue by flash chromatography on a 3 × 30–cm column using a step gradient of 99:1 (1 L), 98:2 (1 L), 97:3 (1 L), and 96:4 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate solvent in vacuo on a rotary evaporator.

*The resulting product, 2′-(adenin-9-yl)-1′,5′-anhydro-4′,6′-O-benzylidene-2′,3′-dideoxy-D-arabinohexitol (**S.10**) is obtained in 82% yield (1.45 g, 4.1 mmol). mp: 227°C; UV (MeOH): λ_{max} (ε) = 262 nm (11300); EIMS: m/z: 353 [M]$^+$; ^{1}H NMR (DMSO-d_6): δ = 2.17 (dt, 1H, H- 3′ax, J = 12 Hz, J = 1.3 Hz, J = 4 Hz), 2.46 (m, 1H, H-3′eq), 3.53 (ddd, 1H, H-5′, J = 9 Hz, J = 10 Hz, J = 5 Hz), 3.73 (ddd, 1H, H-4′, J = 12.9 Hz, J = 4 Hz), 3.80 (t, 1H, H-6′ax, J = 10 Hz), 4.10 (dd, 1H, H-1′ax, J = 13 Hz, J = 2.5 Hz), 4.22 (dd, 1H, H-6′eq, J = 5 Hz), 4.44 (d, 1H, H-1′eq, J = 13 Hz), 4.90 (br, s, 1H, H-2′), 5.62 (s, 1H, PhCH), 7.30-7.40 (m, 7H, aromatic H, NH_2), 8.18 (s, 1H, H-2), 8.27 (s, 1H, H-8); ^{13}C NMR (DMSO-d_6): δ = 32.2 (C-3′), 50.5 (C-2′), 68.1, 68.9 (C-1′, C-6′), 73.4, 73.8 (C-4′, C-5′), 100.9 (PhCH), 118.6 (C-5), 126.2 (2,6-C), 128.1 (3,5-C), 128.9 (4-C), 137.8 (1-C), 139.3 (C-8), 149.5 (C-4), 152.6 (C-2), 156.2 (C-6).*

Prepare S.11

10. Dissolve 5.90 g (16.7 mmol) **S.10** in 100 mL pyridine and coevaporate three times.
11. Add 160 mL pyridine and then cool to 0°C.
12. Add 9.7 mL (83.57 mmol) benzoyl chloride.
13. Allow the mixture to come to room temperature and continue stirring overnight.
14. Cool the orange-brown solution in an ice-bath and add 18 mL water.
15. After 5 min add 35 mL of 25% ammonia and continue stirring 2 hr at room temperature.
16. Remove the volatiles under reduced pressure and coevaporate three times with 20 mL toluene.
17. Dilute the resulting solid with 250 mL dichloromethane and wash with 200 mL saturated sodium bicarbonate solution.
18. Dry the organic layer over sodium sulfate, remove the solvent, and purify by column chromatography using a 5 × 40–cm silica gel column and a step gradient from 1.5 L of 2:8 (v/v) *n*-hexane/ethyl acetate to 1 L of 1:9 *n*-hexane/ethyl acetate, to 1 L of 100% ethyl acetate. Combine product-containing fractions and evaporate solvent.

*The resulting product, 1′,5′-anhydro-2′-(N^6-benzoyladenin-9-yl)-4′,6′-O-benzylidene-2′,3′-dideoxy-D-arabinohexitol (**S.11**) is obtained in 94% yield (7.20 g, 15.8 mmol). mp: 150°C; LSIMS (thygly): m/z: 458 $[M+H]^+$; ^{1}H NMR ($CDCl_3$): δ = 2.25 (m, 1H, 3′ax-H, 2.66 (br, d, 1H, 3′eq-H, J = 12 Hz), 3.65 (m, 2H, 5′-H, 4′-H), 3.78 (t, 1H, 6′ax-H, J = 9.8 Hz), 4.16 (dd, 1H, 1′ax-H, J = 13.5 Hz, J = 2.5 Hz), 4.38 (dd, 1H, 6′eq-H, J = 10.2 Hz, J = 3.6 Hz), 4.48 (br, d, 1H, 1′eq-H, J = 13.3 Hz), 5.07 (br, s, 1H, 2′-H, 5.49 (s, 1H, PhCH), 7.50 (m, 8H, aromatic H), 8.04 (d, 2H, aromatic H, J = 6.7 Hz), 8.55 (s, 1H, 8-H), 8.77 (s, 1H, 2-H), 9.30 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 33.1 (C-3′), 51.0 (C-2′), 68.8 (C-6′), 69.4 (C-1′), 73.8 (C-4′), 74.5 (C-5′), 102.0 (PhCH), 122.8 (C-5), 125.9 (2,6-C Ph), 127.9 (3,5C Ph), 128.2 (3,5-C Bz), 128.8 (2,6-C Bz), 129.1 (4-C Ph), 132.7 (4-C Bz), 133.5 (1-C Bz), 136.9 (1-C Ph), 142.0 (C-8), 149.6 (C-4), 152.0 (C-2), 152.5 (C-6), 164.7 (HNC=O).*

Prepare S.12

19. Dissolve 8.06 g (17.6 mmol) **S.11** in 450 mL of 80% (v/v) acetic acid.
20. Heat the reaction mixture 6 hr at 60°C.
21. Evaporate the solvent under reduced pressure using a rotary evaporator with a vacuum pump and coevaporate with 25 mL toluene three times.
22. Dissolve the residue in a minimal volume of 1:1 (v/v) dichloromethane/methanol.
23. Slowly add 500 mL diethyl ether while stirring.
24. Filter off the precipitate, wash with diethyl ether, and dry over phosphorous pentoxide.

*The resulting product, 1′,5′-anhydro-2′-(N^6-benzoyladenin-9-yl)-2′,3′-dideoxy-D-arabinohexitol (**S.12**) is obtained in 82% yield (5.35 g, 14.5 mmol). mp: 220°C; LSIMS (thygly): m/z: 370 $[M+H]^+$; ^{1}H NMR (DMSO-d_6): δ = 1.97 (dt, 1H, 3′ax-H, J = 13 Hz, J = 3.9 Hz), 2.36 (br, d, 1H, 3′eq-H, J = 13.2 Hz), 3.23 (m, 1H, 5′-H), 3.50-3.80 (m, 3H, 6′-H, 4′H), 3.93 (dd, 1H, 1′ax-H, J = 12.7 Hz, J = 2.1 Hz), 4.30 (br, d, 1H, 1′eq-H, J = 12.5 Hz), 4.7 (t, 1H, 6′-OH, J = 6.2 Hz), 4.98 (br, s, 1H, 2′-H), 5.00 (d, 1H, 4′-OH, J = 5.5 Hz), 7.58 (m, 3H, aromatic H), 8.05 (d, 2H, aromatic H, J = 6.9 Hz), 8.62 (s, 1H, 8-H), 8.75 (s, 1H, 2-H), 11.18 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 35.8 (C-3′), 50.7 (C-2′), 60.5 (C-6′, C-4′), 67.9 (C-1′), 83.1 (C-5′)125.1 (C-5), 128.6 (2,3,5,6-C Bz), 132.5 (4-C Bz), 133.5 (1-C Bz), 143.5 (C-8), 150.2 (C-4), 151.5 (C-2), 152.4 (C-6), 165.7 (HNC=O).*

Prepare S.13

25. Dissolve 5.35 g (14.5 mmol) **S.12** in 100 mL pyridine and coevaporate three times.
26. Add 340 mL pyridine and dissolve.
27. Add 7.85 g (24.6 mmol) MMTr·Cl.
28. Stir the reaction 2 days at room temperature.
29. Monitor by TLC using 94:6 (v/v) dichloromethane/methanol (the product has a higher R_f value than the starting material).
30. Quench the reaction with 300 mL saturated sodium bicarbonate solution.
31. Extract with 400 mL dichloromethane twice.
32. Dry the organic layer over sodium sulfate, evaporate the solvent, and then coevaporate with 25 mL toluene three times.
33. Purify the residue by flash chromatography on a 4 × 30–cm column using a step gradient from 1.5 L of 100% dichloromethane to 99:1 (1 L), 98:2 (1 L), and 97:3 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate the solvent.

*The resulting product, 1′,5′-anhydro-6′-monomethoxytrityl-2′,3′-dideoxy-2′-(N^6-benzoyladenin-9-yl)-D-arabinohexitol (**S.13**) is obtained in 84% yield (7.79 g, 12.2 mmol). mp: 125°C; LSIMS (thygly/NaOAc): m/z: 664 $[M+Na]^+$; ^{1}H NMR ($CDCl_3$): δ = 1.98 (dt, 1H, 3′ax-H, J = 11.6 Hz, J = 4.4 Hz), 2.54 (br, d, 1H, 3′eq-H, J = 12.6 Hz), 2.88 (br, s, 1H, 4′-OH), 3.47 (m, 3H, 5′-H, 4′-H, 6A-H), 3.77 (br, s, 4H, 6B-H, OCH_3), 3.96 (dd, 1H, 1′ax-H, J = 12.9 Hz, J = 2.5 Hz), 4.34 (br, d, 1H, 1′eq-H, J = 12.8 Hz), 4.98 (br, s, 1H, 2′-H), 6.84 (d, 2H, aromatic H, J = 8.9 Hz), 7.38 (m, 15H, aromatic H), 8.02 (d, 2H, aromatic H, J = 8.3 Hz), 8.54 (s, 1H, 8-H), 8.77 (s, 1H, 2-H), 9.30 (br, s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 35.7 (C-3′), 50.6 (C-2′), 55.2 (OCH_3), 64.3 (C-4′, C-6′), 69.0 (C-1′), 80.6 (C-5′), 87.2 (O-C-MMTr), 113.1 (3′5′C MMTr), 120.0 (C-5), 127.1 (4C MMTr 2x), 127.9 (3,5C Bz), 128.0 (2,6C MMTr, 2×), 128.2 (3,5C MMTr, 2x), 128.8 (2,6C Bz), 130.2 (2′6′C MMTr), 132.7 (4C Bz), 133.7 (1C Bz), 134.9 (1′C MMTr), 142.4 (C-8), 143.8 (1C MMTr, 2×), 149.5 (C-4), 152.5 (C-2, C-6), 158.7 (HNC=O).*

SYNTHESIS OF 1′,5′-ANHYDRO-6′-*O*-MONOMETHOXYTRITYL-2′,3′-DIDEOXY-2′-(THYMIN-1-YL)-D-ARABINOHEXITOL

BASIC PROTOCOL 4

This protocol details the synthesis of the *h*T 1,5-anhydrohexitol building block **S.16** from **S.5** (see Fig. 1.9.4 and DeBouvere et al., 1997).

Materials

N^3-Benzoylthymine
1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (**S.5**; see Basic Protocol 1)
Triphenylphosphine
Tetrahydrofuran (THF; reflux overnight on lithium aluminum hydride and distill)
Nitrogen gas
Diethyl azodicarboxylate (DEAD)
n-Hexane
Ethyl acetate
Saturated ammonia in methanol
Dichloromethane
Toluene
80% (v/v) acetic acid
Methanol

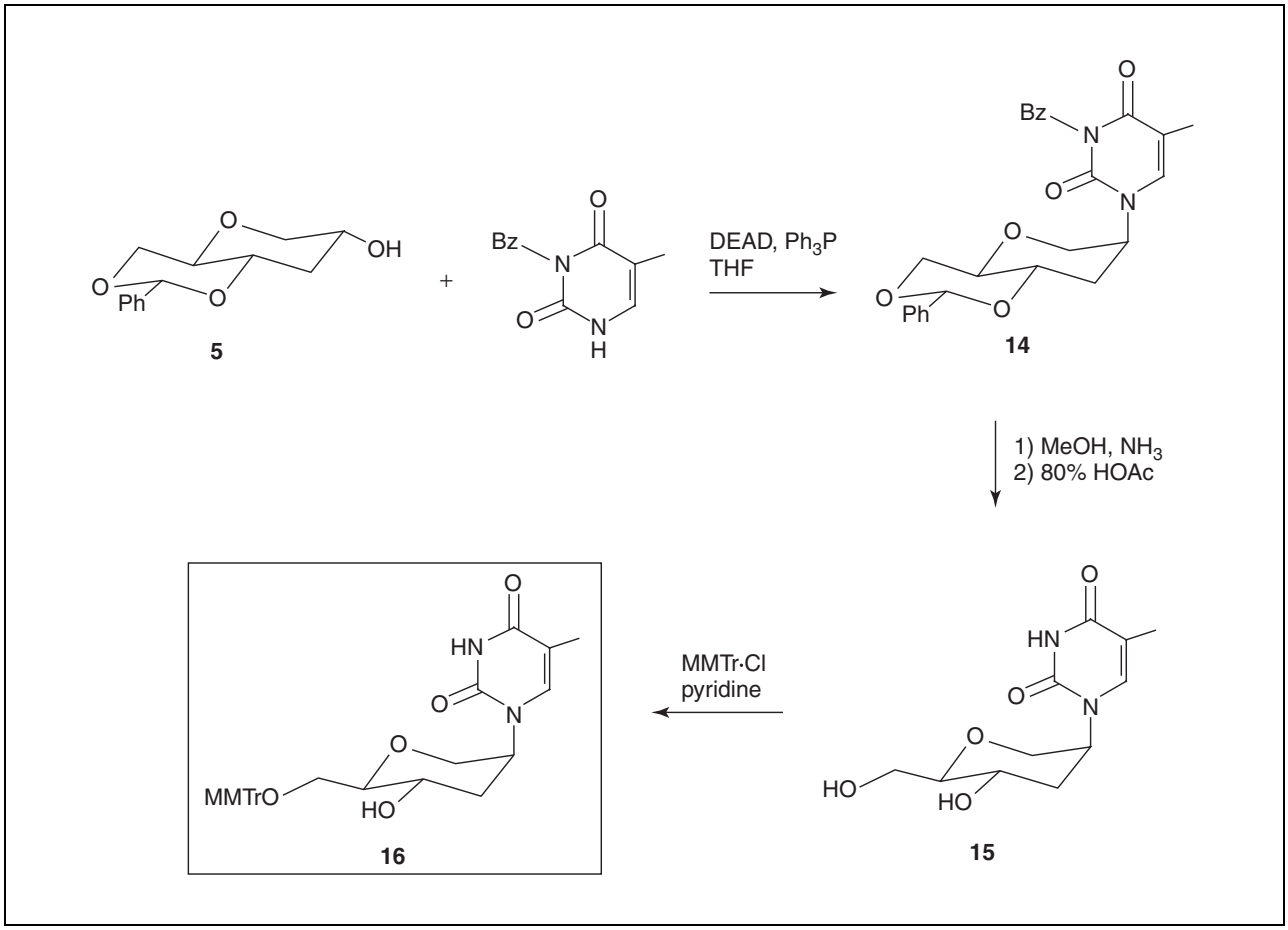

Figure 1.9.4 Preparation of protected *h*T (**S.16**). Abbreviations: DEAD, diethyl azodicarboxylate; THF, tetrahydrofuran; Ph_3P, triphenylphosphine; HOAc, acetic acid; MMTr·Cl, monomethoxytrityl chloride.

Pyridine (reflux over potassium hydroxide overnight and distill)
Monomethoxytrityl chloride (MMTr·Cl)
Saturated sodium bicarbonate solution
Sodium sulfate

Oil bath and magnetic stirrer
Dropping funnel
Rotary evaporator equipped with a vacuum pump and cooling trap
3×35– and 4×35–cm chromatography columns

Additional reagents and equipment for TLC and column chromatography (see Basic Protocol 1 and Critical Parameters)

NOTE: The ^{1}H NMR and ^{13}C NMR spectra were determined with a 200-MHz Varian Gemini spectrometer with tetramethylsilane as internal standard. Liquid secondary-ion (LSIMS) mass spectra were obtained using a KRATOS Concept ^{1}H mass spectrometer. Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad signal; m, multiplet; ddd, double doublet of doublet; dm, double multiplet.

Prepare S.14

1. Dissolve 2.40 g (10.5 mmol) N^3-benzoylthymine, 1.23 g (5.2 mmol) **S.5**, and 3.43 g (13.1 mmol) triphenylphosphine in 100 mL THF. Carry out the reaction under a nitrogen atmosphere.
2. Add a solution of 2.06 mL (13.1 mmol) DEAD in 15 mL THF via a dropping funnel over a period of 60 min.
3. Stir the mixture at room temperature overnight.
4. Remove the volatiles under vacuum using a rotary evaporator with a vacuum pump.
5. Absorb the crude product on 20 g of 0.060- to 0.200-nm silica gel and purify by flash chromatography in a 3 × 35–cm column using a step gradient of 1 L each of 8:2, 7:3, and 6:4 (v/v) *n*-hexane/ethyl acetate. Combine product-containing fractions and evaporate solvent in vacuo on a rotary evaporator.

   *The resulting product, 1′,5′-anhydro-2′-(N^3-benzoylthymin-1-yl)-4′,6′-O-benzylidene-2′,3′-dideoxy-D-arabinohexitol (**S.14**) is obtained in 80% yield (1.88 g, 4.2 mmol). mp: 200°C; LSIMS (thygly): m/z: 449 $[M+H]^+$; ^1H NMR ($CDCl_3$): δ = 2.02 (s, 3H, CH_3), 2.07 (m, 1H, 3′ax-H), 2.47 (d, br, 1H, 3′eq-H, J = 13.9 Hz), 3.53 (dt, 1H, 5′-H, J = 9.6 Hz, J = 4.7 Hz), 3.8 (m, 1H, 4′-H), 3.80 (t, 1H, 6′ax-H, J = 10.2 Hz), 4.02 (dd, 1H, 1′ax-H, J = 13.7 Hz, J = 3.5 Hz), 4.28 (d, br, 1H, 1′eq-H, J = 13.9 Hz), 4.37 (dd, 1H, 6′eq-H, J = 10.5 Hz, J = 4.7 Hz), 4.73 (s, br, 1H, 2′-H), 5.64 (s, 1H, PhCH), 7.30-7.90 (m, 8H, aromatic H), 7.95 (s, + d, 3H, 6-H + aromatic 2H); ^{13}C NMR ($CDCl_3$): δ = 32.9 (C-3′), 51.7 (C-2′), 68.8 (C-6′, C-1′), 73.6 (C-4′), 74.2 (C-5′), 102.0 (PhCH), 110.7 (C-5), 126.0 (2,6C Ph), 128.3 (4C Ph), 129.1 (2,3,56C Bz), 130.4 (3,5 C Ph), 131.5 (1C Bz), 135.0 (4C Bz), 137.0 (1C Ph), 137.7 (C-6), 149.7 (C-2), 162.6 (C-4), 168.9 (HNC = O).*

Prepare S.15

6. Dissolve 1.83 g (4.1 mmol) **S.14** in 100 mL saturated ammonia in methanol.
7. Once a precipitate has formed, add 50 mL dichloromethane and continue stirring 90 min at room temperature.
8. Check the reaction by TLC (see Basic Protocol 1, step 3) using 1:1 (v/v) *n*-hexane/ethyl acetate (the starting material has the higher R_f value).
9. Evaporate the volatiles and coevaporate three times with 15 mL toluene.
10. Dissolve the crude product (1.8 g) in 75 mL of 80% (v/v) acetic acid.
11. Heat the reaction mixture 3 hr at 60°C.
12. Evaporate the volatiles on a rotary evaporator and coevaporate three times with 15 mL toluene.
13. Purify the residue by column chromatography using a 3 × 25–cm column and a gradient from 1 L of 95:5 to 1 L of 93:7 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate the solvent.

    *The resulting product, 1′,5′-anhydro-2′,3′-dideoxy-2′-(thymin-1-yl)-D-arabinohexitol (**S.15**) is obtained in 82% yield (0.86 g, 3.4 mmol). mp: 170°C; UV (MeOH): λ_{max} (ε) = 272 nm (9500); LSIMS (thygly): m/z: 357 $[M+H]^+$; ^1H NMR (DMSO-d_6): δ = 1.75 m, 1H, 3′ax-H), 1.76 (s, 3H, CH_3), 2.08 (d, br, 1H, 3′eq-H, J = 13.8 Hz), 3.13 (m, 1H, 5′-H), 3.35 (m, 1H, 4′-H), 3.60 (m, 2H, 6′-H), 3.73 (dd, 1H, 1′ax-H, J = 12.9 Hz, J = 3.4 Hz), 3.99 (d,br, 1H, 1′eq-H, J = 12.8 Hz), 4.50 (s br,, 1H, 2′-H), 4.65 (s, br, 1H, 6′-OH), 4.91 (s, br, 1H, 4′-OH), 7.88 (s, 1H, 6-H), 11.25 (s, 1H, 3-H); ^{13}C NMR (DMSO-d_6): δ = 12.5 (CH_3), 35.2 (C-3′), 50.1 (C-2′), 60.3 (C′-6′), 60.7 (C-4′), 67.0 (C-1′), 82.4 (C-5′), 108.4 (C-5), 139.0 (C-6), 151.0 (C-2), 163.9 (C-4).*

Prepare S.16

14. Dissolve 2.77 g (10.8 mmol) **S.15** in 40 mL pyridine and coevaporate three times.
15. Dissolve in 100 mL pyridine.
16. Add 5.85 g (18.4 mmol) MMTr·Cl.
17. Stir the reaction at room temperature overnight.
18. Check by TLC using 9:1 (v/v) dichloromethane/methanol as the solvent (the reaction mixture has the higher R_f value).
19. Quench the reaction with 200 mL saturated sodium bicarbonate solution.
20. Extract twice with 200 mL dichloromethane.
21. Dry the organic layer over sodium sulfate, evaporate the solvent, and coevaporate with 15 mL toluene three times.
22. Purify the residue by column chromatography on a 4 × 35–cm column using a step gradient from 500 mL of 100% dichloromethane to 1 L each of 99:1, 98:2, and 95:5 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate solvents.

*The resulting product, 1′,5′-anhydro-6′-O-monomethoxytrityl-2′,3′-dideoxy-2′-(thymin-1-yl)-D-arabinohexitol (**S.16**) is obtained in 86% yield (4.90 g, 9.3 mmol). mp: 125°C; LSIMS (thygly / NaOAc): m/z: 551 [M+Na]$^+$; ^{1}H NMR ($CDCl_3$): δ = 1.85 (m, 1H, 3′ax-H), 1.86 (s, 3H, CH_3), 2.28 (d, 1H, 4′-OH, J = 3.8 Hz), 2.38 (d, br, 1H, 3′eq-H, J = 14 Hz), 3.31 (m, 1H, 5′-H), 3.45 (m, 2H, 4′H, 6′ax-H), 3.78 (s, 3H, OCH_3), 3.81 (dd, 1H, 1′ax-H, J = 13Hz, J = 3.4 Hz), 3.98 (m, 1H, 6′eq-H), 4.18 (d, br, 1H, 1′eq-H, J = 13.2 Hz), 4.67 (s, br, 1H, 2′-H), 6.83 (d, 2H, aromatic H, J = 8.7 Hz), 7.20-7.50 (m, 12H, aromatic H), 8.00 (s, 1H, 6-H), 9.20 (s, br, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 12.8 (CH_3), 35.5 (C-3′), 51.0 (C-2′), 55.2 (OCH_3), 63.1 (C-4′), 63.3 (C-6′), 68.5 (C-1′), 80.9 (C-5′), 86.8 (OC MMTr), 110.4 (C-5), 113.2 (3′5′C MMTr), 127.1 (4 MMTr, 2×), 128.0 (2,6C MMTr, 2×), 128.2 (3,5C MMTr, 2×), 130.2 (2′6′C MMtr), 135.0 (1′C MMTr), 138.6 (C-6), 143.9 (1C MMTr, 2×), 151.0 (C-2), 158.7 (4′C MMTr), 163.8 (C-4).*

BASIC PROTOCOL 5

SYNTHESIS OF 1′,5′-ANHYDRO-2′-(N^4-BENZOYLCYTOSIN-1-YL)-2′,3′-DIDEOXY-6′-MONOMETHYOXYTRITYL-D-ARABINOHEXITOL

This protocol details the synthesis of the *h*C 1,5-anhydrohexitol building block **S.20** from **S.5** (see Fig. 1.9.5 and DeBouvere et al., 1997).

Materials

N^4-Benzoyluracil
1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (**S.5**; see Basic Protocol 1)
Triphenylphosphine
Dioxane (reflux overnight on lithium aluminum hydride and distill)
Nitrogen gas
Diethyl azodicarboxylate (DEAD)
Saturated ammonia in methanol
Dichloromethane
Methanol
Toluene
Triazole
Pyridine (reflux overnight over potassium hydroxide and distill)
Phosphoroxy chloride
25% ammonia

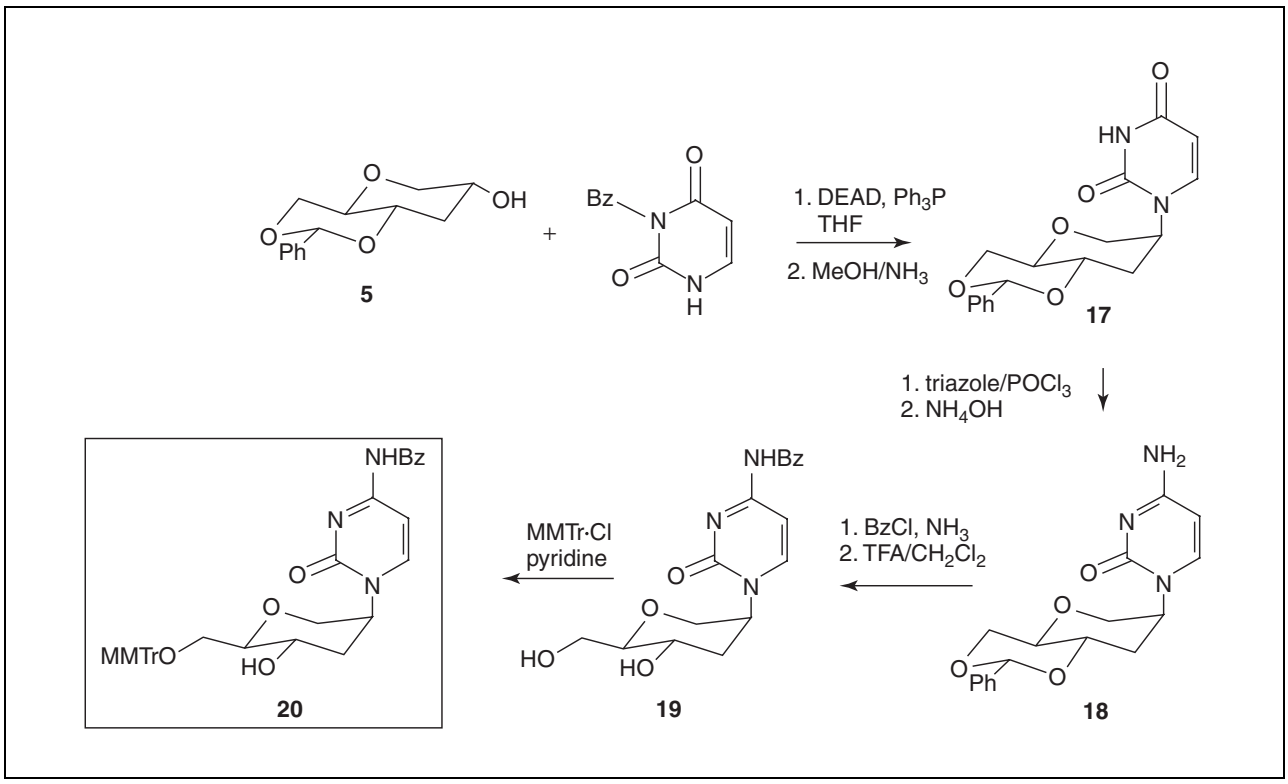

Figure 1.9.5 Preparation of protected *h*C (**S.20**). Abbreviations: BzCl, benzoylchloride; DEAD, diethyl azodicarboxylate; MMTr·Cl, 4-monomethoxy chloride; $POCl_3$, phosphoroxy chloride; TFA, trifluoroacetic acid.

Sodium sulfate
Benzoyl chloride
Saturated sodium bicarbonate solution
Trifluoroacetic acid (TFA)
Diethyl ether: reflux overnight on sodium (Na, $FeCl_2$, Et_2O) and distill
Monomethoxytrityl chloride (MMTr·Cl)

Dropping funnel
Rotary evaporator equipped with a vacuum pump and cooling trap
4×30– and 3×35–cm chromatography columns
Oil bath and magnetic stirrer
Drying tube

Additional reagents and equipment for TLC and column chromatography (see Basic Protocol 1 and Critical Parameters)

NOTE: The ^{1}H NMR and ^{13}C NMR spectra were determined with a 200 MHz Varian Gemini spectrometer with tetramethylsilane as an internal standard. Abbreviations: s, singlet; d, doublet; dd, double doublet; t, triplet; br s, broad signal; m, multiplet; ddd, double doublet of doublet; dm, double multiplet. Liquid secondary-ion (LSIMS) mass spectra were obtained using a KRATOS Concept ^{1}H mass spectrometer.

Prepare S.17

1. Suspend 6.48 g (30.0 mmol) N^3-benzoyluracil, 4.72 g (20 mmol) **S.5**, and 7.87 g (30.0 mmol) triphenylphosphine in 200 mL dioxane. Carry out the reaction under a nitrogen atmosphere.
2. Add a solution of 5.90 mL (30.0 mmol) DEAD in 30 mL dioxane via a dropping funnel over a period of 200 min.
3. Stir the mixture overnight at room temperature.
4. Filter off the precipitate formed during the reaction and wash the precipitate with 50 mL dioxane.
5. Remove the volatiles of the filtrate by evaporation under vacuum in a rotary evaporator with a vacuum pump.
6. Dissolve the obtained residue in 100 mL saturated ammonia in methanol.
7. Stir the mixture 90 min at room temperature.
8. Check the reaction by TLC (see Basic Protocol 1, step 3) using 97.5:2.5 (v/v) dichloromethane/methanol.
9. Remove the volatiles and coevaporate with 20 mL toluene three times.
10. Purify the residue by column chromatography on a 4 × 30–cm column using a gradient from 1 L of 100% dichloromethane to 1 L of 97:3 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate solvents in vacuo on a rotary evaporator.

    *The resulting product, 1′,5′-anhydro-4′,6′-O-benzylidene-2′,3′-dideoxy-2′-(uracil-1-yl)-D-arabinohexitol (**S.17**) is obtained in 67% yield (4.24 g, 12.8 mmol). mp: 200°C; LSIMS (thygly): m/z: 331 $[M+H]^+$; ^{1}H NMR ($CDCl_3$): δ = 2.12 (dt, 1H, 3′ax-H, J = 11.9 Hz, J = 4.8 Hz), 2.52 (d ,br, 1H, 3′eq-H, J = 12.9 Hz), 3.48-3.74 (m, 2H, 5′-H, 4′-H), 3.79 (t, 1H, 6′ax-H, J = 10.2 Hz), 4.06 (dd, 1H, 1′ax-H, J = 13.7 Hz, J = 3.0 Hz), 4.37 (d, 1H, 1′eq-H, J = 13.8 Hz), 4.36 (dd, 1H, 6′eq-H, J = 10 Hz, J = 4.7 Hz), 4.78 (s, br, 1H, 2′-H), 5.59 (s, 1H, PhCH), 5.80 (d, 1H, J = 8.0 Hz,5-H), 7.34-7.51 (m, 5H, Ph H), 8.09 (d, 1H, 6-H, J = 8.0 Hz), 9.30 (s, br, 1H, NH); ^{13}C NMR ($CDCl_3$): δ = 32.8 (C-3′), 51.5 (C-2′), 68.8 (C-6′), 68.8 (C-1′), 73.6 (C-4′), 74.3 (C-5′), 102.0, 102.2 (PhCH, C-5), 126.0 (2,6-C Ph), 128.4 (3,5-C Ph), 129.3 (4-C Ph), 137.1 (1-C Ph), 142.4 (C-6), 150.9 (C-2), 163.2 (C-4).*

Prepare S.18

11. Dissolve 6.23 g (96.0 mmol) triazole in 120 mL pyridine.
12. Add 2.61 mL (28.0 mmol) phosphoroxy chloride and continue stirring at room temperature for 25 min.
13. Add 2.64 g (8 mmol) **S.17**.

    The solution should turn yellow.

14. Continue stirring 45 min at room temperature.
15. Remove the volatiles in vacuo on a rotary evaporator and coevaporate with 15 mL toluene three times.
16. Dissolve the brown residue in 80 mL dioxane and add 40 mL of 25% ammonia.
17. Continue stirring another 30 min.
18. Evaporate to dryness, dissolve in 100 mL dichloromethane, and wash with 200 mL water.

19. Dry the organic layer over sodium sulfate and remove the solvent.

20. Purify the crude product by column chromatography using 95:5 (v/v) dichloromethane/methanol as a solvent. Combine product-containing fractions and evaporate solvent.

*The resulting product, 1′,5′-anhydro-4′,6′-O-benzylidene-2′,3′-dideoxy-2′-(cytosin-1-yl)-D-arabinohexitol (**S.18**) is obtained in 71% yield (1.87 g, 5.7 mmol). mp: 200°C; LSIMS (thygly): m/z: 330 $[M+H]^+$; ^1H NMR ($CDCl_3$): δ = 2.05 (dt, 1H, 3′ax-H, J = 11.9 Hz, J = 4.8 Hz), 2.58 (d, br 1H, 3′eq-H, J = 12.9 Hz), 3.43-3.69 (m, 2H, 5′-H, 4′-H), 3.74 (t, 1H, 6′ax-H, J = 10.2 Hz), 4.00 (dd, 1H, 1′ax-H, J = 13.7 Hz, J = 3.0 Hz), 4.20 (d, 1H, 1′eq-H, J = 13.8 Hz), 4.30 (dd, 1H, 6′eq-H, J = 10 Hz, J = 4.7 Hz), 4.82 (s, br, 1H, 2′-H), 5.54 (s, 1H, PhCH), 5.86 (d, 1H, 5-H, J = 7.4 Hz, 7.30-7.47 (m, 5H, aromatic H), 8.05 (d, 1H, 6-H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$): δ = 32.5 (C-3′), 51.7 (C-2′), 68.8 (C-6′), 69.2 (C-1′), 73.8 (C-4′), 74.1 (C-5′), 94.5 (C-5), 101.9 (PhCH), 126.0 (2,6-C Ph), 128.3 (3,5-C Ph), 129.1 (4-C Ph), 137.2 (1-C Ph), 143.5 (C-6), 156.3 (C-2), 162.0 (C-4), 165.7 (HNC=O).*

Prepare S.19

21. Dissolve 2.31 g (7.0 mmol) **S.18** in 70 mL pyridine in a 250-mL round-bottom flask equipped with a drying tube.

22. Add 4.1 mL (35.0 mmol, 5 eq) benzoyl chloride and stir the reaction mixture overnight.

23. Analyze by TLC using 95:5 (v/v) dichloromethane/methanol as a solvent.

24. Cool the reaction mixture to 0°C and add 10 mL of 25% ammonia.

25. Continue stirring 1 hr at room temperature.

26. Remove the volatiles, coevaporate with 15 mL toluene three times, and dissolve the residue in 100 mL dichloromethane.

27. Wash with 100 mL saturated sodium bicarbonate solution, dry the organic layer over sodium sulfate, and remove the solvent in vacuo on a rotary evaporator.

28. Dissolve the resulting brownish foam in 70 mL dichloromethane.

29. Slowly add 30 mL TFA and continue stirring 45 min at room temperature.

30. Monitor by TLC using 9:1 (v/v) dichloromethane/methanol.

31. Evaporate the volatiles and coevaporate with 15 mL toluene three times.

32. Purify the resulting foam by column chromatography using a 3 × 35–cm column and a step gradient from 1.5 L of 95:5 to 1.5 L of 93:7 (v/v) dichloromethane/methanol. Collect product-containing fractions.

33. Reduce volatiles in vacuo on a rotary evaporator to a volume of 10 mL. Precipitate by adding 50 mL diethyl ether.

*The resulting product, 1′,5′-anhydro-2′-(N^4-benzoylcytosin-1-yl)-2′,3′-dideoxy-D-arabino-hexitol (**S.19**) is obtained in an overall yield of 67% (1.08 g, 3.1 mmol). mp: 130°C; LSIMS (thygly): m/z: 346 $[M+H]^+$; ^1H NMR (DMSO-d_6): δ = 1.79 (dt, 1H, 3′ax-H, J = 13.6 Hz, J = 4.4 Hz), 2.20 (d, br, 1H, 3′eq-H, J = 13.7 Hz), 3.14 (m, 1H, 5′-H), 3.50-3.70 (m, 3H, 6′-H, 4′-H), 3.81 (dd, 1H, 1′ax-H, J = 12.9 Hz, J = 2.9 Hz), 4.14 (d, br, 1H, 1′eq-H, J = 13.2 Hz), 4.60 (m, 2H, 6′-OH, + 2′-H), 4.95 (d, 1H, 4′-H, J = 5.1 Hz), 7.31 (d, 1H, 5-H, J = 7.3 Hz), 7.57 (m, 3H, aromatic H), 8.01 (d, 2H, aromatic H, J = 7 Hz), 8.50 (d, 1H, 6-H, J = 7.4 Hz), 11.19 (s, 1H, HNC=O); ^{13}C NMR (DMSO-d_6): δ = 34.8 (C-3′), 52.3 (C-2′), 60.5 (C-6′, C-4′), 67.2 (C-1′), 82.8 (C-5′), 95.9 (C-5), 128.5 (2,3,5,6-C Bz), 132.8 (1,4-C Bz), 148.1 (C-6), 155.0 (C-4), 162.6 (C-4), 167.5 (HNC=O).*

Prepare S.20

34. Dissolve 2.72 g (7.9 mmol) **S.19** in 40 mL pyridine and coevaporate three times (40 mL each).
35. Dissolve in 100 mL pyridine.
36. Add 4.26 g (13.4 mmol) of MMTr·Cl.
37. Stir the reaction at room temperature overnight.
38. Monitor by TLC using 95:5 (v/v) dichloromethane/methanol.
39. Quench the reaction with 200 mL saturated sodium bicarbonate solution.
40. Extract twice with 200 mL dichloromethane.
41. Dry the organic layer over sodium sulfate, evaporate the solvent, and coevaporate three times with 20 mL toluene.
42. Purify the residue by column chromatography on a 3 × 35–cm column using a step gradient of 1 L each 99:1, 98:2, 97:3, and 96:4 (v/v) dichloromethane/methanol. Combine product-containing fractions and evaporate solvents.

*The resulting product, 1′,5′-anhydro-2′-(N^4-benzoylcytosin-1-yl)-2′,3′-dideoxy-6′-monomethoxytrityl-D-arabinohexitol (**S.20**) is obtained in 80% yield (3.88 g, 6.3 mmol). mp: 140°C; LSIMS (thygly / NaOAc): m/z: 640 $[M+Na]^+$; ^1H NMR ($CDCl_3$): δ = 1.90 (m, 1H, 3′ax-H), 2.55 (br, d, 1H, 3′eq-H, J = 12.2 Hz), 3.08 (s, br, 1H, 5′-H), 3.42 (m, 3H, 4′H, 6′-H), 3.79 (s, 3H, OCH_3), 3.89 (dd, 1H, 1′ax-H, J = 13.5 Hz, J = 2.9 Hz), 4.01 (s, br, 1H, 4′-OH), 4.25 (d, br, 1H, 1′eq-H, J = 13.5 Hz), 4.83 (s, br, 1H, 2′-H), 6.85 (d, 2H, aromatic H, J = 8.8 Hz), 7.20-7.70 (m, 18H, aromatic H + 5-H), 8.73 (br, d, 2H, NH + 6-H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$): δ = 35.0 (C-3′), 52.7 (C-2′), 55.1 (OCH_3), 62.1 (C-4′), 62.7 (C-6′), 68.4 (C-1′), 80.9 (C-5′), 86.8 (OC Tr), 96.4 (C-5), 113.2 (3′5′C), 127.1 (4C, 2×), 127.9 (3,5C Bz), 128.0 (2,6C 2×), 128.4 (3,5C 2×), 128.9 (2,6C Bz), 130.0 (2′6′C), 132.7 (4C Bz), 132.9 (1C Bz), 135.0 (1′C), 143.9 (1C 2×), 148.0 (C-6), 155.5 (C-2), 158.6 (4′C), 161.9 (C-4), 166.3 (HNC=O).*

COMMENTARY

Background Information

Hexitol nucleic acid (HNA) is the first example of an oligonucleotide with a six-membered carbohydrate moiety that hybridizes with natural nucleic acids. The base moiety is connected to the 2′-position of the sugar, which means that the nucleic acids have no anomeric center. These nucleic acids are stable against enzymatic and chemical degradation (Hendrix et al., 1997a,b). HNA can be synthesized by the phosphoramidite method (Hendrix et al., 1997a,b; also see *UNIT 3.3*) starting from the building blocks described in this protocol.

HNAs are RNA-selective oligonucleotides, which means that they hybridize more strongly to RNA as the complement than to DNA (Hendrix et al., 1997a,b). The HNA-RNA duplex is thermally more stable than the DNA-RNA duplex, making HNA suitable as a steric blocking agent for antisense purposes (ΔT_m/mod +1° to + 5°C). This higher stability is mainly based on its preorganization (entropy factor).

The NMR structure of an HNA-RNA duplex has been solved (Lescrinier et al., 2000a,b) and it shows typical A-form geometry. The hexitol nucleoside itself is a good mimic of a furanose nucleoside in its 2′-exo,3′-endo conformation (northern type). In a qualitative model, cellular uptake of HNA seems to be similar to that of phoshorothioate oligonucleotides, using cationic lipids as a transfecting agent (Atkins et al., 2000).

Hybridization of HNA with RNA is more sequence specific than between DNA and RNA, which is demonstrated by the larger difference in T_m between match and mismatch duplexes of HNA-RNA versus DNA-RNA (Hendrix et al., 1997b).

HNA functions as a very efficient template for nonenzymatic oligomerization of activated ribonucleoside monophosphates (Kozlov et al., 1999a,b). HNA templates are chiral-discriminating in that they selectively accept D-nucleotides over L-nucleotides for primer extension

(Kozlov et al., 1999a,b). Two HNA codons (6 nucleotides) incorporated in an otherwise intact RNA are accepted as messenger in the translation process (Lavrik et al., 2001). Hexitol nucleoside triphosphates may be used by polymerases for enzymatic incorporation into oligodeoxyribonucleotides (Vastmans et al., 2000).

Critical Parameters

Overall, it is important that for each step of the syntheses the starting sugar- and base-building blocks or nucleosides, respectively, are thoroughly dried either by coevaporation with anhydrous pyridine or in a desiccator over phosphorus pentoxide. The MMtr-protected building blocks (**S.9**, **S.13**, **S.16**, and **S.20**) are dried over silica blue (not over phosphorous pentoxide) and can be stored at 4°C.

For all reactions using anhydrous solvents, the glassware must be predried at 70°C for at least 2 hr.

All solvents must be distilled before use. Anhydrous solvents are very important. They should either be freshly distilled and stored under nitrogen or argon, or be taken from a freshly opened bottle of commercially prepared anhydrous solvent.

For evaporation of solvents, it is helpful if the rotary evaporator is equipped with a dry ice condenser.

For all compounds, a sample of 100 mg should be kept as a reference.

In all cases, the reaction progress is followed by TLC. The starting material (1 mg diluted to 200 µL) as well as the reaction mixture (100 µL) are prepared. A baseline is marked and the spots of the solutions are placed at equal distances, with the reaction mixture in the middle surrounded by starting material. After developing in the appropriate solvent, the end line of the TLC is marked, and spots are identified first under a UV lamp (254 nm) and then by spraying the plates with anisaldehyde/sulfuric acid spray and heating to 150°C. The sugar-containing products turn blue. The monomethoxytritylated products (**S.9**, **S.13**, **S.16**, and **S.20**) turn yellow immediately after spraying (deprotection). After heating, the product-containing spots turn green.

Before each column purification, TLC is performed on the reaction mixture to evaluate the completeness of the reaction and the presence of starting materials to be recovered. Silica gel (0.060 to 0.200 nm) is suspended in the first solvent and loaded into the column, which is tightly packed by gentle tapping. The silica layer is topped by a 1-cm layer of sand. The crude product is dissolved in a minimum volume of solvent and applied to the column. The fractions are collected with a fraction collector and a roster of the collector is drawn on a TLC plate. A drop from each fraction is applied to the corresponding field on the plate, and the plates are evaluated under a UV lamp and by applying anisaldehyde/sulfuric acid spray. The fraction-containing products (or unreacted starting material) are tested for homogeneity by TLC. Elution of the column is continued with the final solvent of the indicated gradient until all product has been isolated. Product-containing fractions are combined and solvents are removed in vacuo on a rotary evaporator.

Troubleshooting

Preparation of hC

The identical product can be obtained by Mitsunobu reaction of N^4-benzoylcytosine with the hexitol building block **S.5** as demonstrated in De Bouvere et al. (1997). In this case the yield was rather modest (34%) and turned out to be less reproducible. Furthermore, in some attempts the 1′,5′-anhydro-2′-(N^4-benzoylcytosin-2-yl)-4′,6′-*O*-benzylidene-2′,3′-dideoxy-D-arabinohexitol (*O*-nucleoside) instead of the desired 1′,5′-anhydro-2′-(N^4-benzoylcytosin-1-yl)-4′,6′-*O*-benzylidene-2′,3′-dideoxy-D-arabinohexitol (*N*-nucleoside) was obtained.

Preparation of hG

Due to the high price of 6-iodo-9*H*-purine-2-amine, the synthesis of *h*G may be performed using the Mitsunobu reaction of 6-chloro-9*H*-purine-2-amine with the hexitol building block **S.5**. If 6-iodo-9*H*-purine-2-amine is available, another approach for obtaining *h*G is described in De Bouvere et al. (1997).

Anticipated Results

Preparation of appropriately protected anhydrohexitol building blocks is straightforward using the described protocols. Automated oligomer assembly using standard phosphoramidite chemistry cycles—as provided by the manufacturers of automated DNA synthesizers—will yield anhydrohexitol oligomers.

Time Considerations

In the planning of the syntheses it has to be considered that most of reactions have to be stirred overnight. Normally each step (includ-

ing purification and spectroscopic analysis) can be performed in 1.5 to 2 working days.

Literature Cited

Atkins, D., Miller, M., De Bouvere, B., Van Aerschot, A., and Herdewijn, P. 2000. Evaluation of the cellular uptake of hexitol nucleic acids in HeLa cells. *Pharmazie* 55:615-617.

De Bouvere, P., Kerremans, L., Rozenski, J., Janssen, G., Van Aerschot A., Claes, P., Busson, R., and Herdewijn, P. 1997. Improved synthesis of anhydrohexitol building blocks for oligonucleotide synthesis. *Liebigs Ann./Recueil* 1453-1461.

De Winter, H., Lescrinier, E., Van Aerschot, A., and Herdewijn, P. 1998. Molecular dynamics simulation to investigate differences in minor groove hydration of HNA/RNA hybrids as compared to HNA/DNA complexes. *J. Am. Chem. Soc.* 120:5381-5394.

Hendrix, C., Verheggen, I., Rosemeyer, H., Seela, F., Van Aerschot, A., and Herdewijn, P. 1997a. 1′,5′-Anhydrohexitol oligonucleotides: Synthesis, base pairing and recognition by regular oligodeoxyribonucleotides and oligoribonucleotides. *Chem. Eur. J.* 3:110-119.

Hendrix, C., Rosemeyer, H., De Bouvere, B., Van Aerschot A., Seela, F., and Herdewijn, P. 1997b. 1′,5′-Anhydrohexitol oligonucleotides: Hybridization and strand displacement with oligoribonucleotides, interaction with RNase H and HIV reverse transcriptase. *Chem. Eur.J.* 3:1513-1520.

Kocienski, P. and Pant, C. 1982. A convenient preparation of some 2,3,4,6-tetraacetyl-1,5-anhydro-D-hexitols. *Carbohydr. Res.* 110:330–332.

Kozlov, I.A., Politis, P.K., Pitsch, S., Herdewijn, P., and Orgel, L.E. 1999a. A highly enantio-selective hexitol nucleic acid template for nonenzymatic oligoguanylate synthesis. *J. Am. Chem. Soc.* 121:1108-1109.

Kozlov, I.A., Politis, P.K., Van Aerschot, A., Busson, R., Herdewijn, P., and Orgel, L.E. 1999b. Nonenzymatic synthesis of RNA and DNA oligomers on hexitol nucleic acid templates: The importance of the A structure. *J. Am. Chem. Soc.* 121:2613-2656.

Lavrik, I.N., Avdeeva, O.N., Dontsova, O.A., Froeyen, M., and Herdewijn, P.A. 2001. Translational properties of mHNA, a messenger RNA containing anhydrohexitol nucleotides. *Biochemistry* 40:11777-11784.

Lescrinier, E., Esnouf, R.M., Schraml, J., Busson, R., and Herdewijn, P. 2000a. Solution structure of a hexitol nucleic acid duplex with four consecutive T·T base pairs. *Helv. Chim. Acta* 83:1291-1310.

Lescrinier, E., Esnouf, R.M., Schraml, J., Busson, R., Heus, H.A., Hilbers, C.W., and Herdewijn, P. 2000b. Solution structure of a HNA-RNA hybrid. *Chem. & Biol.* 7:719-731.

Vastmans, K., Pochet, S., Peys, A., Kerremans, L., Van Aerschot, A., Hendrix, C., Marlière, P., and Herdewijn, P. 2000. Enzymatic incorporation in DNA of 1,5-anhydrohexitol nucleotides. *Biochemistry* 39:12757-12765.

Verheggen, I., Van Aerschot, A., Toppet, S. Snoeck, R., Janssen, G., Balzarini, J., De Clercq, E., and Herdewijn, P. 1993. Synthesis and antiherpes virus activity of 1,5-anhydrohexitol nucleosides. *J. Med. Chem.* 36:2033-2039.

Verheggen, I., Van Aerschot, A., Van Meervelt, L., Rozenski, J., Wiebe, L., Snoek, R., Andrei, G., Balzarini, J., Claes, P., De Clercq, E., and Herdewijn, P. 1995. Synthesis, biological evaluation, and structure analysis of a series of new 1,5-anhydrohexitol nucleosides *J. Med. Chem.* 38:826-835.

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