Synthesis and Structure of 1-Deoxy-1-phenyl-β-D-ribofuranose and Its Incorporation into Oligonucleotides

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Hammerhead ribozymes[1] are one of the smallest catalytic RNAs with sequence-specific endoribonuclease activity. Their highly specific cleavage activity suggests their use as therapeutic agents for the inhibition of gene expression. As part of our studies[2−5] on the molecular mechanism of action of hammerhead ribozymes, we were interested in the effect of incorporation of base-modified nucleosides into the hammerhead domain as well as into the RNA substrate. In particular, we were interested in universal base analogs[6] which behave indiscriminately toward its opposite base, as well as in hydrophobic base analogs and/or base analogs lacking hydrogen bonding capability. We and others reported the incorporation of abasic nucleoside analogs into ribozymes.[3,7] Surprisingly, several ribozymes containing 1-deoxy-D-ribofuranose instead of uridine at the U4 and/or U7 position of the catalytic core had cleavage activity.[4] By designing additional analogs that retain the close structural and steric relationship of the natural bases, but are not likely to form hydrogen bonds, we wanted to study their effect on structure and activity in the hammerhead ribozyme.

The synthesis of *C*-aryl glycosides has recently received increasing attention; however, good methods for attaching fully carbocyclic aromatic moieties to a deoxyribofuranosyl and ribofuranosyl sugar are lacking. Recently, Chaudhuri and Kool[8] reported the high-yield synthesis of deoxyribo-*C*-glycosides from 1-chloro sugars using diarylcadmium and diarylzinc reagents. While the method proved to be useful in the preparation of deoxyribo-*C*-nucleosides, yields for the preparation of *C*-riboside derivatives were poor.[8,9] Millican and co-workers[10] incorporated 1,2-dideoxy-1-phenyl-β-D-ribofuranose into oligonucleotides and paired them against the natural bases; in that study weak pairing was observed, the result was attributed to poor base stacking. The three-step synthesis of 1-deoxy-1-phenyl-β-D-ribofuranose was reported[11] from 2,3,5-tri-*O*-benzyl-D-ribose and phenylmagnesium bromide. Recently Schweitzer and Kool[12] reported the synthesis and incorporation of hydrophobic isosteres of the natural bases adenine and thymine in DNA and examined their base-pairing properties. They found that hydrophobic base analogs are significantly selective for pairing with hydrophobic partners (by ∼20-fold) rather than natural bases. In addition, they attributed destabilization of the duplex upon introduction of the hydrophobic base to the cost of desolvation of the hydrophobic partner and not to the weak stacking propensity of hydrophobic bases.

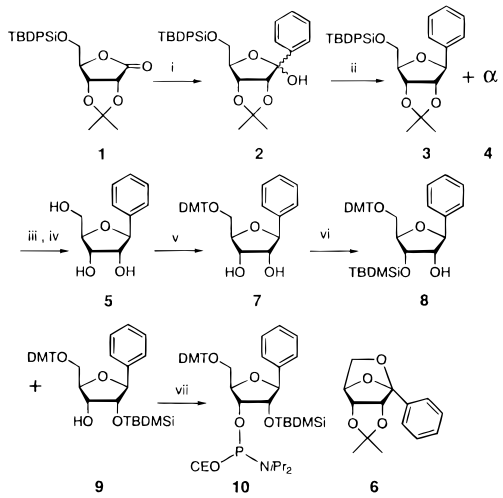

Figure 1. Synthesis of 2′-*O*-*tert*-butyldimethylsilyl-5′-*O*-dimethoxytrityl-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite)-1′-deoxy-1′-phenyl-β-D-ribofuranose. Reagents and conditions: (i) PhLi/THF, −78 °C, (ii) $Et_3SiH/BF_3{\cdot}Et_2O/CH_3CN$, −40 °C, (iii) 1 M TBAF/THF, (iv) 70% aqueous CH_3COOH, (v) DMT-Cl/DMAP/Et_3N/Pyr, (vi) TBDMS-Cl/$AgNO_3$/Pyr/THF, (vii) P(OCE)(N-*i*-Pr_2)Cl/ DIPEA/1-methylimidazole.

Our synthetic approach to 1-deoxy-1-phenyl-β-D-ribofuranose (**5**) (Figure 1) was based on the work of Czernecki and Ville[13] for the highly stereoselective preparation of 1-deoxy-1-phenyl-β-D-glucopyranose from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranolactone and phenyllithium.

The choice of protecting groups was crucial for this approach:[14] they must be compatible with a very reactive organometallic reagent and at the same time withstand the strongly acidic conditions during reduction with $Et_3SiH/BF_3{\cdot}Et_2O$. Easily accessible 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone (**1**)[15] provided a suitable starting material for these transformations. The addition of PhLi to **1** using a slightly modified procedure[13] afforded after silica gel column chromatography a 1:3 α/β[16] mixture of lactols **2** in 71% yield.[17] The above mixture was subjected to reduction using Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_3CN at −40 °C to yield the anomeric

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(16) It should be noted that for hemiacetals **2** the prefix α refers to the position of the glycosidic OH group relative to the configuration at the reference C-atom (C4′ in **2**; *i.e.*, the phenyl moiety is in the β-position).

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mixture of protected phenyl ribosides. These anomers were easily separated by flash silica gel chromatography to yield the faster moving β-anomer **3** in 51% yield and the slower moving α-anomer **4** in 13% yield. Some cleavage of the TBDPSi group was observed under the above reaction conditions. We believe that this cleavage could be completely avoided by further lowering the reaction temperature and/or shortening the reaction time. It is worth noting that when 5-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone was used as the starting material reduction as described above led to the exclusive formation of 1,5-anhydro derivative **6**.[13] In this case, because of the greater acid lability of the TBDMSi ether compared to that of the TBDPSi ether, the cleavage of the 5′-*O*-TBDMSi group occurred first, followed by cyclization to give **6**. Fully protected **3** was first treated with 1.5 equiv of TBAF in THF to afford after silica gel column chromatography the 5′-OH derivative quantitatively. The cleavage of the isopropylidene group using boiling 70% aqueous AcOH for 30 min proceeded in a quantitative manner to give the known 1-deoxy-1-phenyl-β-D-ribofuranose (**5**).[9]

Recent studies of the anomeric effect in a series of pyrimidine *C*-nucleosides[18,19] demonstrated the stronger equatorial preference of the *C*-nucleoside base (the absence of anomeric effect) in these compounds compared to *N*-nucleosides. This feature makes phenyl riboside, which might be considered a *C*-nucleoside analog, an attractive analog to study the effect of sugar conformation on ribozyme activity.

The structure of **5** was confirmed by X-ray crystallography[20,27] and an ORTEP representation is shown in Figure 2. The sugar pucker is in the C2′-*endo* conformation (pseudorotation angle *P*, 175.7°) and the backbone torsion angles are γ −174.2° and δ 149.8°. The small difference of 0.015 Å for the C4′−O4′ (1.422 Å) and O4′−C1′ (1.427 Å) bond lengths is consistent with the absence of an anomeric effect between the furanose oxygen and the nucleobase. Similar differences were observed in the crystal structures of other *C*-nucleosides.[21] The S-type sugar pucker puts the phenyl ring in a pseudoequatorial conformation as expected for a sterically driven pseudorotational equilibrium. The glycosidic torsion angle χ (O4′−C1′−C1−C2, termed in analogy to pyrimidine bases) in **5** is −171.7°. This generates a short 1···4 contact between O4′ and the phenyl C6 of 2.76 Å. The corresponding O4′−C1′−C1−C6 torsion angle is 8°. Thus, there may be a weak C6−H6···O4′ hydrogen-bonding

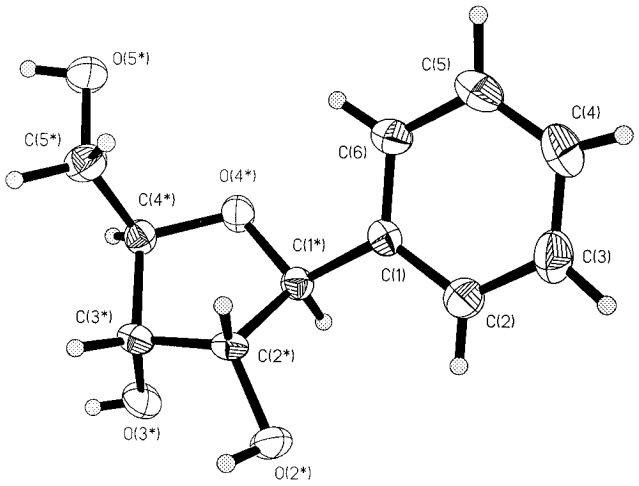

Figure 2.

interaction. The H6···O4′ distance is 2.37 Å, the angle at the hydrogen atom is 104°, and the angle C1′−O4′−H6 is 85°.

To incorporate the phenyl nucleoside into oligonucleotides, we prepared phosphoramidite **10** in three steps from **5**. Protection of the 5′-hydroxyl group by dimethoxytritylation was achieved under standard conditions (DMT-Cl, DMAP, Et_3N, Pyr)[22] to afford, after silica gel column purification, 5′-*O*-DMT-1′-deoxy-1′-phenyl-β-D-ribofuranose (**7**) in 75% yield. Selective protection of the 2′-hydroxyl using *tert*-butyldimethylsilyl chloride proceeded in the presence of $AgNO_3$ and pyridine in THF[23] to afford a mixture of 2′-*O*-TBDMSi, 3′-*O*-TBDMSi, and some 2′,3′-bis-*O*-TBDMSi derivatives. Careful separation of these products using flash silica gel chromatography afforded a faster running 3′-*O*-TBDMSi isomer **8** in 32% yield and 2′-*O*-TBDMSi isomer **9** in 35% yield. It is noteworthy that the 3′-*O*-TBDMSi isomer is faster moving than the 2′-*O*-TBDMSi isomer, unlike the majority of silylated nucleoside regioisomers. The 3′-isomer **8**, when subjected to 1% Et_3N in MeOH, isomerizes to a 1:1 mixture of 2′- and 3′-isomers from which an additional amount of the desired 2′-isomer could be obtained. Phosphitylation of **9** using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in the presence of *N*,*N*-diisopropylethylamine and 1-methylimidazole[24] yielded the desired 3′-phosphoramidite **10**, ^{31}P NMR in $CDCl_3$ δ 149.1 & 146.6 ppm for two *P*-diastereoisomers, respectively. No migration of the TBDMSi group, under these conditions, was observed.

Phosphoramidite **10** was incorporated, instead of U, into the cleavage site of a 17-mer RNA substrate 5′-CAG GGA UU**U** AUG GAG AU-3′ (cleavage site in bold). The method of oligoribonucleotide synthesis, deprotection, purification, and testing has been described.[25,26] The average stepwise coupling yields for all nucleotides were

(17) ^1H NMR ($CDCl_3$) resonances for 2′,3′-*O*-isopropylidene groups of **2**: δ 1.68 (s) and 1.40 (s), $\Delta\delta = 0.28$, α-isomer; 1.38 (s) and 1.25 (s), $\Delta\delta = 0.13$, β-isomer. For the assignment of the anomeric configuration of nucleosides based on $\Delta\delta$ values, see: Tapiero, C.; Imbach, J.-L. In *Nucleic Acid Chemistry*, Part 1; Townsend, L. B., Tipson, R. S., Eds.; J. Wiley & Sons, Inc.: New York, 1978; pp 1055−1059. Ohrui, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602−4613.

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(20) Crystals were obtained from a saturated toluene solution containing 5−10% EtOAc, and cooling to −18 °C. The space group was $P2_1$ and the cell constants were $a = 6.736$ Å, $b = 6.780$ Å, $c = 11.085$ Å, $\beta = 99.64°$. Data were collected on an Enraf-Nonius CAD4 diffractometer (Mo Kα) and reduced to 1582 unique reflections ($I \geq 2\sigma(I)$). The structure was solved with direct methods using the program SHELXS-86 and refined with the program SHELXL-93 (Sheldrick, G. SHELXS-86, Universität Göttingen, Germany, 1986. Sheldrick, G. SHELXL-93, Universität Göttingen, Germany, 1993). The final *R*-factor was 5.45%.

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(27) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

~97−98%. The presence of the phenyl nucleotide was confirmed by nucleoside compositional analysis.[5] The substrate containing the phenyl nucleoside, 5′-CAG GGA UU**P** AUG GAG AU-3′, was cleaved by the hammerhead ribozyme, 5′-UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU-3′, *ca.* 10 times slower than the U-containing substrate. Contributions of altered sugar conformations, anomeric effect, and hydrophobic interactions to the change of the cleavage rate in a series of substrates containing pyrimidine analogs incorporated at the cleavage site are under investigation and will be reported in due course.

Experimental Section

General. All reactions were carried out under a positive pressure of argon in anhydrous solvents. Commercially available reagents and anhydrous solvents were used without further purification. 2,3-*O*-Isopropylidene-D-ribono-1,4-lactone was purchased from Aldrich. ^{1}H (400.075 MHz) and ^{31}P (161.947 MHz) NMR spectra were recorded in $CDCl_3$, unless stated otherwise, and chemical shifts in ppm refer to TMS and H_3PO_4, respectively. *J* values are in hertz. Analytical thin-layer chromatography (TLC) was performed with Merck Art. 5554 kieselgel 60 F_{254} plates and flash column chromatography using Merck 0.040−0.063 mm silica gel 60. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ.

5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-*O*-isopropylidene-1′-deoxy-1′-phenyl-β-D-ribofuranose (3). To a stirred solution of 5-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribono-1,4-lactone (**1**)[15] (12 g, 28 mmol) in dry THF (70 mL) cooled to −78 °C was added phenyllithium (2 M solution in cyclohexane/ether 70/30, 15 mL, 30 mmol) dropwise. The reaction mixture was stirred at −78 °C for 2 h and at rt for 4 h, then quenched with cold water, and extracted with ether (×3). The combined organic layers were dried (Na_2SO_4) and evaporated to a syrup under reduced pressure. Silica gel column chromatographic purification using a 3−10% gradient of ethyl acetate in hexanes afforded **2** as a syrup (α/β[16] 1:3 mixture) (10 g, 71%): ^{1}H NMR for α-anomer δ 7.80−7.38 (m, Ph), 4.95 (d, $J_{2',3'}$ = 5.2, H-2′), 4.63 (d, $J_{3',4'}$ = 7.3, H-3′), 4.48 (s, OH-1′), 4.34 (m, H-4′), 3.99 (dd, $J_{5',4'}$ = 3.4, $J_{5',5''}$ = 11.4, H-5′), 3.91 (dd, $J_{5'',4'}$ = 3.4, $J_{5'',5'}$ = 11.4, H-5″), 1.68 (s, Me), 1.40 (s, Me), 1.08 (s, *t*-Bu), β-anomer δ 7.80−7.38 (m, Ph), 4.93 (d, $J_{2',3'}$ = 5.7, H-2′), 4.68 (d, $J_{3',2'}$ = 5.7, H-3′), 4.42 (s, OH-1′), 4.45 (m, H-4′), 3.96 (dd, $J_{5',4'}$ = 3.8, $J_{5',5''}$ = 11.2, H-5′), 3.78 (dd, $J_{5'',4'}$ = 3.6, $J_{5'',5'}$ = 11.2, H-5″), 1.38 (s, Me), 1.25 (s, Me), 1.12 (s, *t*-Bu).

Compound **2** (9 g, 17.8 mmol) was dissolved in dry acetonitrile (170 mL) and the solution cooled to −40 °C. Et_3SiH (5.7 mL, 36 mmol) was added followed by the dropwise addition of BF_3·Et_2O (2.49 mL, 20 mmol). The mixture was stirred at −40 °C for 1 h and, after warming to rt, quenched with saturated aqueous K_2CO_3 (18 mL). The aqueous mixture was extracted with ether and the organic layer dried (Na_2SO_4) and evaporated to a syrup. Silica gel column chromatography (0.5−3% gradient of ethyl acetate in hexanes) afforded **3** as a white foam (4.4 g, 51% yield): ^{1}H NMR δ 7.82−7.36 (m,15 H, Ph), 4.99 (d, $J_{1',2'}$ = 5.4, 1H, H-1′), 4.89 (dd, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 4.0, 1H, H-3′), 4.61 (dd, $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 6.4, 1H, H-2′), 4.29 (m, 1H, H-4′), 4.03 (dd, $J_{5',4'}$ = 3.4, $J_{5',5''}$ = 11.2, 1H, H-5′), 3.97 (dd, $J_{5'',4'}$ = 3.9, $J_{5'',5'}$ = 11.2, 1H, H-5″), 1.71 (s, 3H, Me), 1.44 (s, 3H, Me), 1.15 (s, 9H, *t*-Bu). Anal. Calcd for $C_{30}H_{36}O_4Si$: C, 73.73; H, 7.42. Found: C, 73.68; H, 7.45.

The slower moving α-anomer **4** was isolated as a syrup (1.1 g, 13% yield): ^{1}H NMR δ 7.77−7.34 (m,15 H, Ph), 5.39 (d, $J_{1'2'}$ = 4.1, 1H, H-1′), 5.07 (d, $J_{3',2'}$ = 6.0, 1H, H-3′), 4.95 (dd, $J_{2',1'}$ = 4.1, $J_{2',3'}$ = 6.0, 1H, H-2′), 4.39 (m, 1H, H-4′), 4.00 (dd, $J_{5',4'}$ = 3.8, $J_{5',5''}$ = 11.1, 1H, H-5′), 3.88 (dd, $J_{5'',4'}$ = 3.6, $J_{5'',5'}$ = 11.1, 1H, H-5″), 1.53 (s, 3H, Me), 1.38 (s, 3H, Me), 1.18 (s, 9H, *t*-Bu). Anal. Calcd for $C_{30}H_{36}O_4Si$: C, 73.73; H, 7.42. Found: C, 73.73; H, 7.30.

1′-Deoxy-1′-phenyl-β-D-ribofuranose (5). Compound **3** (1 g, 2.1 mmol) was dissolved in THF (20 mL), and 1 M TBAF in THF (3 mL, 3 mmol) was added. The reaction mixture was stirred at rt for 30 min and then evaporated to a syrup. The residue was applied to the silica gel column and eluted with toluene followed by a 5−10% gradient of ethyl acetate in hexanes. The 5′-*O*-desilylated product was obtained as a colorless foam (0.68 g, 96% yield). This material was dissolved in 70% aqueous acetic acid and heated at 100 °C (oil bath) for 30 min. Evaporation to dryness under reduced pressure and crystallization of the residual syrup from toluene afforded **5** (0.49 g, 94% yield), mp 120−121 °C (lit.[9] mp 120−121 °C): ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.47−7.31 (m, 5H, Ph), 4.63 (d, $J_{1',2'}$ = 7.0, 1H, H-1′), 3.95 (dd, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 3.7, 1H, H-3′), 3.89 (dd, $J_{4',5'}$ = 4.4, 1H, H-4′), 3.76 (dd, $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 5.4, 1H, H-2′), 3.61 (m, 2H, H-5′, H-5″).

3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-dimethoxytrityl-1′-deoxy-1′-phenyl-β-D-ribofuranose (8) and 2′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-dimethoxytrityl-1′-deoxy-1′-phenyl-β-D-ribofuranose (9). Compound **5** (770 mg, 3.7 mmol) was 5′-*O*-dimethoxytritylated according to the standard procedure[22] to yield, after silica gel column chromatography (0.5−2% gradient of ethyl acetate in hexanes), 1.4 g (75% yield) of 5′-*O*-dimethoxytrityl derivative **7** as a yellowish foam: ^{1}H NMR δ 7.43−6.81 (m,18 H, Ph), 4.80 (d, $J_{1',2'}$ = 6.3, 1H, H-1′), 4.18 (dd, $J_{3',OH}$ = 3.9, $J_{3',4'}$ = 8.7, 1H, H-3′), 4.13 (dd, $J_{4',5'}$ = 4.2, $J_{4',3'}$ = 8.7, 1H, H-4′), 4.08 (dd, $J_{2',1'}$ = 6.3, $J_{2',OH}$ = 5.8, 1H, H-2′), 3.79 (s, 6H, OMe), 3.47 (dd, $J_{5',4'}$ = 4.2, $J_{5',5''}$ = 10.1, 1H, H-5′), 3.37 (dd, $J_{5'',4'}$ = 4.0, $J_{5'',5'}$ = 10.1, 1H, H-5″), 2.48 (d, $J_{OH,2'}$ = 5.8, 1H, 2′-OH), 2.43 (d, $J_{OH,3'}$ = 3.9, 1H, 3′-OH). Anal. Calcd for $C_{32}H_{32}O_6$: C, 74.98; H, 6.29. Found: C, 75.17; H, 6.15.

Compound **7** was treated with *tert*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*,[23] and the reaction mixture was purified by the silica gel column chromatography (1−2% gradient of ethyl acetate in hexanes) to afford faster moving 3′-*O*-TBDMSi isomer **8** as a foam (0.55 g, 32%): ^{1}H NMR ($CDCl_3$ + D_2O) δ 7.62−6.89 (m, 18 H, Ph), 4.85 (d, $J_{1',2'}$ = 6.2, 1H, H-1′), 4.30 (dd, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 4.2, 1H, H-3′), 4.13 (m, 1H, H-4′), 4.01 (app t, $J_{2',1'}$ = 6.2, 1H, H-2′) 3.86 (s, 6H, OMe), 3.56 (dd, $J_{5',4'}$ = 3.2, $J_{5',5''}$ = 10.3, 1H, H-5′), 3.27 (dd, $J_{5'',4'}$ = 3.9, $J_{5'',5'}$ = 10.3, 1H, H-5″), 0.93 (s, 9H, *t*-Bu), 0.09 (s, 3H, Me), 0.00 (s, 3H, Me). Anal. Calcd for $C_{38}H_{46}O_6Si$: C, 72.81; H, 7.40. Found: C, 72.77; H, 7.26.

The slower migrating 2′-*O*-TBDMSi isomer **9** was then eluted to give, upon evaporation, a white foam (0.60 g, 35%): ^{1}H NMR ($CDCl_3$ + D_2O) δ 7.59−6.88 (m,18 H, Ph), 4.81 (d, $J_{1',2'}$ = 7.2, 1H, H-1′), 4.27 (m, 1H, H-4′), 4.22 (dd, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.4, 1H, H-2′), 4.15 (dd, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 2.6, 1H, H-3′), 3.87 (s, 6H, OMe), 3.60 (dd, $J_{5',4'}$ = 3.0, $J_{5',5''}$ = 10.3, 1H, H-5′), 3.35 (dd, $J_{5'',4'}$ = 3.6, $J_{5'',5'}$ = 10.3, 1H, H-5″), 0.93 (s, 9H, *t*-Bu), 0.12 (s, 3H, Me), 0.05 (s, 3H, Me). Anal. Calcd for $C_{38}H_{46}O_6Si$: C, 72.81; H, 7.40. Found: C, 72.97; H, 7.22.

2′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-dimethoxytrityl-3′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite)-1′-deoxy-1′-phenyl-β-D-ribofuranose (10). Compound **9** (0.87 g, 1.4 mmol) was phosphitylated under the conditions described by Tuschl *et al.*,[24] and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et_3N) for elution (0.85 g, 74% yield): ^{31}P NMR δ 149.1 (s), 146.6 (s). Anal. Calcd for $C_{47}H_{63}N_2O_7PSi$: C, 68.25; H, 7.68; N, 3.39. Found: C, 68.21; H, 7.49; N, 3.26.

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Supporting Information Available: Coordinates for the structure of 1-deoxy-1-phenyl-β-D-ribofuranose (**5**) (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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