5-Substituted 2-Aminopyridine *C*-Nucleosides as Protonated Cytidine Equivalents: Increasing Efficiency and Selectivity in DNA Triple-Helix Formation

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Abstract: The easily accessible *C*-nucleoside 2-amino-5-(2′-deoxy-β-D-ribofuranosyl)pyridine (**P**) and its 3-methyl (**MeP**) and 2′-*O*-methyl (**P_OMe**) derivatives were synthesized and incorporated as protonated cytidine equivalents in homopyrimidine oligodeoxynucleotides. T_m measurements indicate that oligonucleotides containing **P** or **MeP** have a higher affinity to double-stranded DNA over the pH range of 6−8 than 5-methylcytidine (**MeC**) containing oligonucleotides. This increase in stability is most pronounced above pH 7.0. The average increase in T_m/modification for the dissociation of oligonucleotide d(TTTTT^MePT^MePT^MePT^MePT^MePT) from a 21-mer target duplex at pH 7.5 is 2.3 °C relative to oligonucleotide d(TTTTT^MeCT^MeCT^MeCT^MeCT^MeCT). The pH dependence and sequence composition effects are much less pronounced for **MeP** (and also **P**) containing oligonucleotides than for **MeC** containing ones. While oligonucleotide d(TTT^MeC^MeC^MeC^MeCTTTT^MeCTTT) shows no longer any affinity to the target duplex above pH 6.5, oligonucleotide d(TTT^MeP^MeP^MeP^MePTTTT^MePTTT) displays preserved binding with a T_m of 32.5 °C at pH 7.0 and even binds with a T_m of 23.3 °C at pH 8.0. Oligonucleotides containing **P_OMe** show distinctly less stable triple helices. The average decrease in T_m/modification for oligonucleotide d(TTTTTP_OMeTP_OMeTP_OMeTP_OMeTP_OMeT) at pH 6.5 is 6.7 °C relative to the **MeC** containing oligonucleotide. DNase I footprint titration experiments indicate that d(TTTTT^MePT^MePT^MePT^MePT^MePT) binds not only five times stronger to a 229 base pair DNA fragment than d(TTTTT^MeCT^MeCT^MeCT^MeCT^MeCT) but also with higher selectivity. UV-melting experiments show that duplexes of d(TTTTTCTXTCTCTCT) (where X = **P**, **MeP**, or **P_OMe**) with their antiparallel Watson−Crick complement are dramatically less stable ($\Delta T_m < -12$ °C) at pH 8.0 than the corresponding natural duplex. Thus the new bases **P** and **MeP** show Hoogsteen specific pairing behavior.

Introduction

Oligonucleotide-directed sequence-specific recognition of double-helical DNA is of interest for the selective control of gene expression.[1] To date two structural motifs of such triple helices are known: those in which the third strand is primarily composed of pyrimidine bases (parallel binding motif), and those in which the third strand is primarily composed of purine bases (antiparallel binding motif). Triple-helix formation of oligonucleotides with duplex DNA in the parallel (pyrimidine−purine−pyrimidine) binding motif requires protonation of the cytosine units in the third strand.[2] This requirement, however, limits the formation of stable triple helices at physiological conditions due to the relatively low pK_a of deoxycytidine (pK_a = 4.3). In the past, attempts to overcome this obstacle mainly focused on the design and use of nonnatural nucleosides carrying charge-neutral bases which display the hydrogen-bonding pattern of protonated cytosine.[3]

An alternative approach to increase the affinity of oligonucleotides to target DNA at neutral pH consists in the replacement of the cytidine nucleosides by analogs that ideally were completely protonated at physiological conditions. Carbocyclic 5-methyldeoxycytidine for example was found to be more basic than 5-methyldeoxycytidine by 0.45 pK_a units.[4]

Triple helix-forming oligonucleotides containing this analog showed increased affinity to duplex DNA by 3.9 °C per modification relative to 5-methyldeoxycytidine. A more obvious way to increase the basicity consists in replacing cytosine by an intrinsically more basic heterocycle. In this context only 6-amino-2′-*O*-methylcytidine displaying a pK_a of 6.8 was investigated in triple-helix formation.[5] Oligonucleotides containing this analog, however, did not form stable triple helices with duplex DNA.

Our design of a more readily protonated cytidine equivalent started from the basic idea, that replacement of the nitrogen atom N(1) in the pyrimidine ring by carbon and concomitant removal of the 2-oxo function should result in the more basic pyridine *C*-nucleoside **P** without perturbing the hydrogen-

(3) Selected examples: (a) Ono, A.; Ts'o, P. O. P.; Kan, L.-S. *J. Am. Chem. Soc.* **1991,** *113,* 4032−4033. (b) Ono, A.; Ts'o, P. O. P.; Kan, L.-S. *J. Org. Chem.* **1992,** *57,* 3225−3230. (c) Koh, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1992,** *114,* 1470−1478. (d) Priestley, E. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1995,** *117,* 4761−4765. (e) Xiang, G.; Soussou, W.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1994,** *116,* 11155−11156. (f) Berressem, R.; Engels, J. W. *Nucleic Acids Res.* **1995,** *23,* 3465−3472. (g) Xiang, G.; Bogacki, R.; McLaughlin, L. W. *Nucleic Acids Res.* **1996,** *24,* 1963−1970. (h) Miller, P. S.; Bhan, P.; Cushman, C. D.; Trapane, T. L. *Biochemistry* **1992,** *31,* 6788−6793. (i) Jetter, M. C.; Hobbs, F. W. *Biochemistry* **1993,** *32,* 3249−3254. (k) Davison, E. C.; Johnsson, K. *Nucleosides Nucleotides* **1993,** *12,* 237−243. (l) Young, S. L.; Krawczyk, S. H.; Matteucci, M. D.; Toole, J. J. *Proc. Natl. Acad. Sci. USA* **1991,** *88,* 10023−10026. (m) Krawczyk, S. H.; Milligan, J. F.; Wadwani, S.; Moulds, C.; Froehler, B. C.; Matteucci, M. D. *Proc. Natl. Acad. Sci. USA* **1992,** *89,* 3761−3764. (n) Hunziker, J.; Priestley, E. S.; Brunar, H.; Dervan, P. B. *J. Am. Chem. Soc.* **1995,** *117,* 2661−2662. (o) von Krosigk, U.; Benner, S. A. *J. Am. Chem. Soc.* **1995,** *117,* 5361−5362.

(4) Froehler, B. C.; Ricca, D. J. *J. Am. Chem. Soc.* **1992,** *114,* 8320−8322.

(5) Pudlo, J. S.; Wadwani, S.; Milligan, J. F.; Matteucci, M. D. *Bioorg. Med. Chem. Lett.* **1994,** *4,* 1025−1028.

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.

(1) (a) Thuong, N. T.; Hélène, C. *Angew. Chem.* **1993,** *105,* 697−723; *Angew. Chem., Int. Ed. Engl.* **1993,** *33,* 666−690. (b) Soyfer, V. N.; Potaman, V. N. *Triple-Helical Nucleic Acids*; Springer-Verlag: New York, 1996.

(2) (a) Felsenfeld, G.; Davies, D. R.; Rich, A. *J. Am. Chem. Soc.* **1957,** *79,* 2023−2024. (b) Moser, H. E.; Dervan, P. B. *Science* **1987,** *238,* 645−650. (c) François, J.-C.; Saison-Behmoaras, T.; Hélène, C. *Nucleic Acids Res.* **1988,** *16,* 11431−11440.
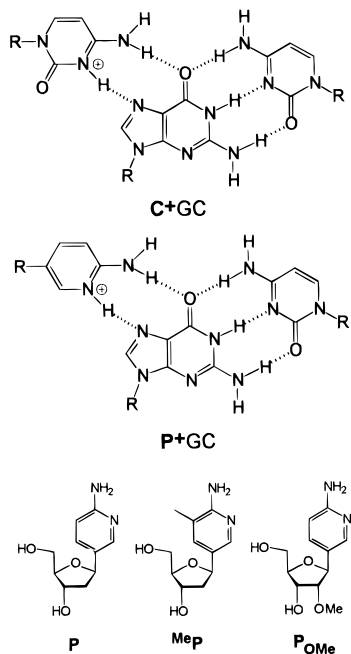
Figure 1. (top) The natural occurring base triplet C+GC (pyrimidine motif) and its isomorphous P+GC triplet. (bottom) The more basic cytidine analogs **P**, **MeP**, and **POMe**.

bonding pattern relevant for triple helix formation (Figure 1). The measured pK_a of free 2-aminopyridine is 6.86, thus almost within the neutral range.[6] We recently reported in a preliminary communication about the use and benefits of oligonucleotides containing the *C*-nucleoside **P** in triple-helix formation.[7] Independent of our work, this analog (as well as its α-anomer) was also prepared and used in triple helix-forming oligonucleotides by Neidle et al. with similar results.[8] On the basis of the positive results obtained with **P** as a cytidine substitute in triple-helix formation we looked for possible derivatives thereof, which were expected to additionally enhance triple-helix formation. Here we report on our synthesis of the pyridine *C*-nucleoside **P**[9] and of its two derivatives **MeP** and **POMe** (Figure 1), as well as on their incorporation into oligodeoxynucleotides and on the properties of these oligonucleotides with respect to binding to single- and double-stranded DNA.

Results

Synthesis of the *C*-Nucleosides. A scheme for the syntheses of the pyridine *C*-nucleosides **P**, **MeP**, and **POMe** is shown in Figure 2. As starting material for the syntheses of **P** and **MeP** the readily available ribonolactone **3**[11] and the stabase adducts of 2-amino-5-bromopyridine **2a** and its 3-methyl derivative **2b** were used. The coupling essentially followed the syntheses of *C*-glycosides by Kraus et al.[12] and Krohn et al.[13] Bromide-lithium exchange in **2** with *n*BuLi at −75 °C and *in situ* reaction with lactone **3** furnished a mixture of hemiacetals which were subsequently reduced with excess of Et₃SiH/BF₃·Et₂O to provide

4a and 4b (only the β-configured isomers).[14] The synthesis of **POMe** followed a slightly different way, in which the 2′-*O*-methylated precursor **11**, which could be readily obtained from D-ribonolactone **10** in two steps, was used as the sugar component. The analogous coupling reaction with **2a** yielded, however, after reduction an anomeric mixture **12** (β/α = 10:1), the α-anomer of which could be easily removed by column chromatography after protection of the exocyclic amino function with phenoxyacetic anhydride (Pac₂O). The anomeric configurations in **4a** and **6c** were assigned by ¹H NMR (NOE) experiments. Irradiation at the H−C(3′) atoms of **4a** and **6c** resulted in both cases in an enhancement of the signals of the base protons H−C(4) and H−C(6). The corresponding *N*-protected 2′-deoxynucleosides of **4a** and **4b** were obtained in five consecutive steps, representing standard transformations in nucleoside chemistry. Protection of the amino group with benzoyl chloride (BzCl) in the case of **4a** or phenoxyacetic anhydride in the case of **4b** followed by debenzylation with BBr₃ provided the *N*-protected ribo-*C*-nucleosides **5a** and **5b**. Selective protection of the 3′- and 5′-hydroxyl groups with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine afforded **6a** and **6b**. Reaction of **6a** with *p*-tolyl chlorothionoformate and **6b** with thiocarbonyl diimidazole[15] (→ **7a** and **7b**) followed by homolytic reductive cleavage of the C−O bond provided **8a** and **8b**. Desilylation of **8a** and **8b** (and also **6c**) with tetra-*n*-butylammonium fluoride gave the *N*-protected pyridine *C*-nucleosides **9a**−**c**. Compounds **9a**−**c** could then be converted into the free *C*-nucleosides **P**, **MeP**, and **POMe** by treatment with 40% aqueous MeNH₂ in the case of **9a** or 25% aqueous NH₃ in the case of **9b** and **9c**. The pK_a values of the cytidine nucleoside analogs are 6.26 for deoxyribonucleoside **9a** and 6.63 for its 3-methyl derivative **9b** as well as 6.15 for the ribonucleoside **9** (R₁ = R₂ = H, R₃ = OH)[7] as determined by potentiometric titration and were within the expected range.

Synthesis of the Oligonucleotides 16−22. The pyridine *C*-nucleosides **P**, **MeP**, and **POMe** were incorporated as cytidine equivalents in oligodeoxynucleotides. The phosphoramidite building blocks **14a**−**c** were obtained from the corresponding *N*-protected *C*-nucleosides **9a**−**c** by tritylation (4,4′-dimethoxytrityl chloride, pyridine) followed by reaction of **13a**−**c** with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and *N,N*-diisopropylethylamine (Hünig's base) (Figure 3).

The oligomers **16−22** (Figure 4) were synthesized on a DNA-Synthesizer using standard solid-phase β-cyanoethyl phosphoramidite chemistry.[16] Because of the poor lability of the benzoyl groups in residues of **P** an efficient deprotection of oligonucleotides containing the benzoyl protected *C*-nucleoside **9a** (→ **16**, **19**) could only be achieved by treatment with 40% aqueous methylamine (70 °C, 26 h). Therefore we decided to change the protective group strategy and to use the phenoxyacetyl (Pac) as the protective group for the exocyclic amino function in **MeP** and **POMe**. The Pac-protected amino groups in residues of **MeP** and **POMe**, however, were found to be partially acetylated during the capping step of the automated synthesis of the oligonucleotides **17**, **18**, **20**, and **21**. However these acetyl groups could then be removed by methylamine treatment (40% in water, 70

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(8) Bates, P. J.; Laughton, C. A.; Jenkins, T. C.; Capaldi, D. C.; Roselt, P. D.; Reese, C. B.; Neidle, S. *Nucleic Acids Res.* **1996**, *24*, 4176−4184.

(9) While a synthesis of **P** appeared recently,[10] **MeP** and **POMe** previously were unknown.

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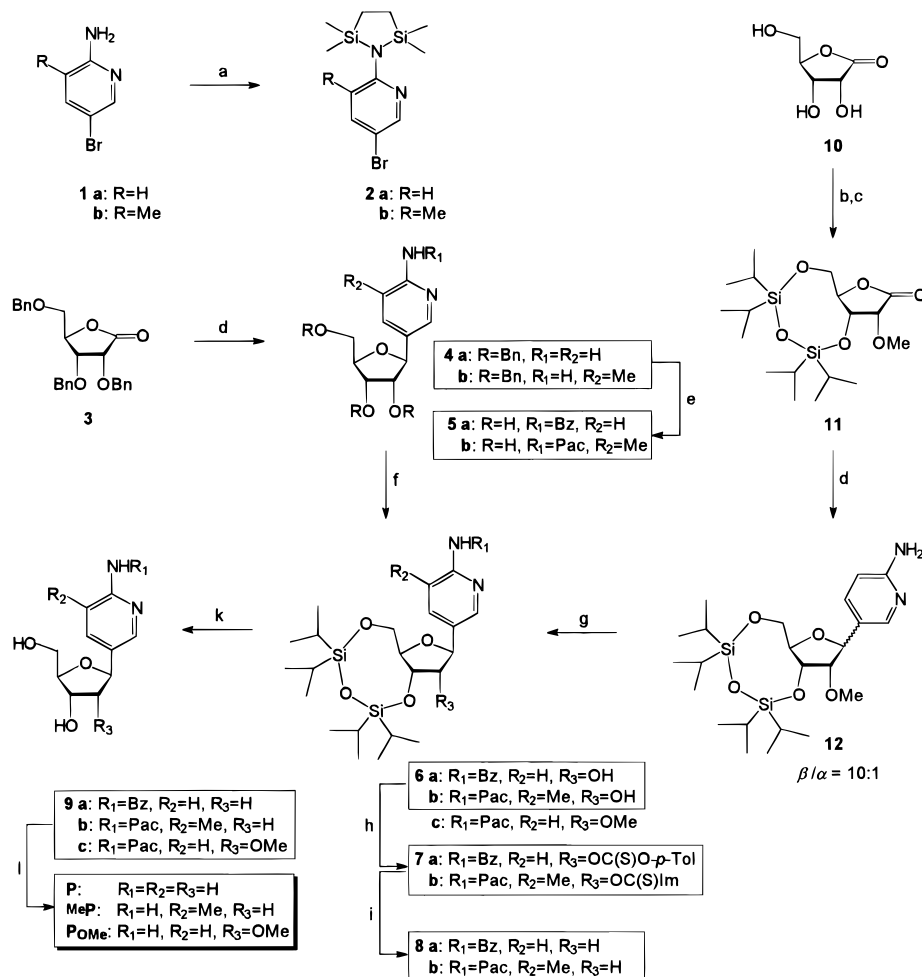(11) Timpe, W.; Dax, K.; Wolf, N.; Weidmann, H. *Carbohydr. Res.* **1975**, *39*, 53−60.

(12) Kraus, G. A.; Molina, M. T. *J. Org. Chem.* **1988**, *53*, 752−753.

(13) Krohn, K.; Heins, H.; Wielckens, K. *J. Med. Chem.* **1992**, *35*, 511−517.

(14) Attempts to extend this reaction to the 2-deoxy analog of lactone **3** were not successful, due to enolization at C(2) under the reaction conditions, as indicated by D₂O quenching after the addition step. See also: Piccirilli, J. A.; Krauch, T.; MacPherson, L. J.; Benner, S. A. *Helv. Chim. Acta* **1991**, *74*, 397−406.

(15) This reagent was used, because standard conditions for the introduction of the *p*-tolylthioformate residue resulted in a loss of the phenoxyacetyl group in **6b** during the reaction.

(16) (a) Sinha, N. D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843. (b) Users Manual (56-1111-56) "Gene Assembler Special / 4 Primers" from Pharmacia.

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Figure 2. Synthesis of the pyridine C-Nucleosides: (a) **1a**→**2a**: Cl(Me)$_2$SiCH$_2$CH$_2$Si(Me)$_2$Cl (1 equiv), nBuLi (2 equiv), THF, −75 °C, 2.5 h, 68%. **1b**→**2b**: 1,2-bis[(dimethylamino)dimethylsilyl]ethane (1 equiv), ZnI$_2$ (0.7 mol%), 140 °C, 15 h, 69%. (b) (Cl(iPr)$_2$Si)$_2$O (1.2 equiv), pyridine, room temperature, 5 h, 39%. (c) Ag$_2$O (3.4 equiv), MeI, 45 °C, 6 h, 67%. (d) **2a** (1.8 equiv) or **2b** (1.5 equiv), nBuLi (ca. 1.5 equiv), THF, −75 °C, 3 h, −75 °C → 0 °C, 4 h, then Et$_3$SiH (5 equiv), BF$_3$·Et$_2$O (5 equiv), CH$_2$Cl$_2$, −75°C → room temperature, over night, 64% (**4a**), 77% (**4b**) and 61% (**12**). (e) **4a**→**5a**: BzCl (1.3 equiv), pyridine, CH$_2$Cl$_2$, room temperature, 24 h, then 0.4 M BBr$_3$ in CH$_2$Cl$_2$, −75 °C, 4 h, 70%. **4b**→**5b**: (i) Pac$_2$O (3 equiv), pyridine, room temperature, 4.5 h, 67%; (ii) 0.4 M BBr$_3$ in CH$_2$Cl$_2$, −75 °C, 5 h, 81%. (f) (Cl(iPr)$_2$Si)$_2$O (1.2 equiv), pyridine, room temperature, 4 h, 75% (**6a**) and 84% (**6b**). (g) Pac$_2$O (3.6 equiv), pyridine, room temperature, 5 h, 85% (+ 8% α-anomer). (h) **6a**→**7a**: p-TolOC(S)Cl (1.6 equiv), N,N-dimethylaminopyridine (2.2 equiv), CH$_3$CN, room temperature, 25 h, 81%. **6b**→**7b**: (Im)$_2$CS (2.5 equiv), DMF, 40 °C, 4 h, 74%. (i) **7a**→**8a**: azobisisobutyronitrile (0.1 equiv), nBu$_3$SnH (1.5 equiv), toluene, 80 °C, 4 h, 84%. **7b**→**8b**: (Me$_3$Si)$_3$SiH (1.15 equiv), toluene, 80 °C, 5 h, 67%. (k) Bu$_4$NF (2 equiv), THF, room temperature, 1.5 h, 86% (**9a**), 93% (**9b**) and 87% (**9c**). (l) **9a**→**P**: MeNH$_2$ solution (40% in H$_2$O), 70 °C, 25 h, 83%. **9b**→**MeP**: NH$_3$ solution (25% in H$_2$O), 55 °C, 15 h, 63%. **9c**→**POMe**: NH$_3$ solution (25% in H$_2$O), 55 °C, 15 h, 91%.

°C, 24 h). In the subsequent synthesis of oligonucleotide **22**, acetylation was prevented by the use of phenoxy acetic anhydride as the capping agent. This allowed for efficient postsynthetic deprotection under standard ammonolysis conditions (25% NH$_3$, 55 °C, ~18 h) and is therefore the method of choice. The deprotected crude oligonucleotides were purified by DEAE (= (diethylamino)ethyl) ion exchange HPLC, their purity controlled by reversed-phase HPLC and their structural integrity analyzed by matrix-assisted laser desorption ionization−time of flight (MALDI−TOF) mass spectrometry. The oligonucleotides **15**, **23**, and **24** were prepared for comparison.

Binding of the Oligonucleotides 16−22 to Duplex DNA (Tm Measurements). To exclude any self-pairing of the pyridine C-nucleoside containing oligonucleotides, a UV-melting experiment of oligonucleotide **19** in 10 mM NaH$_2$PO$_4$ and 200 mM NaCl at pH 5.0 was performed. As expected no cooperative melting transition could be observed, indicating the absence of self-association. The binding of the oligonucleotides **16**−**22** to duplex DNA was monitored by UV melting curves in a pH range of 6.0−8.0 and salt concentrations of 140 mM KCl, 7 mM Na$_2$HPO$_4$, and 0.5 mM MgCl$_2$. These conditions were
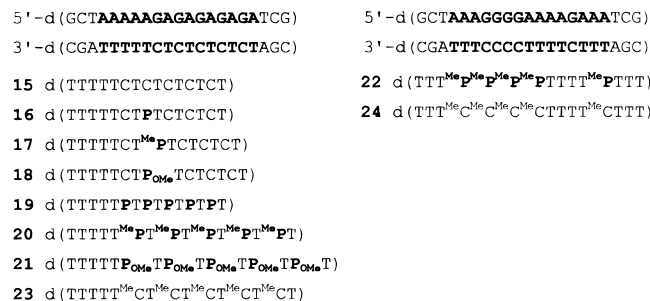
chosen to approximate the intracellular cationic environment as closely as possible.[17] The UV-melting curves were recorded for a consecutive heating−cooling−heating cycle with a linear gradient of 0.5 °C/min. While the heating cycles were superimposable the cooling cycles always showed slight hysteresis for the association of the third strands. Melting transition data (T_m values) for third strand dissociation of **15**−**24** from the chosen 21-mer target duplexes are summarized in Tables 1 and 2 and representative melting curves are reproduced in Figure 5.

From the data in Table 1 it appears, that the oligomers **19** and **20**, in which all cytidines were replaced by the 2′-deoxy-C-nucleosides **P** or **MeP**, show an increase in T_m relative to **15** (natural sequence) and **23** containing 5-methylcytidine (MeC). The increase in stability is most pronounced above pH 7.0. For example, the average increase in T_m/modification for **MeP** at pH 7.5 is 4.7 °C relative to cytidine and 2.3 °C relative to 5-methylcytidine. From the binding data of **16**, **17**, **19**, and **20**

(17) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. Molecular Biology of the Cell, 3rd ed.; Garland: New York, 1994; p 508.
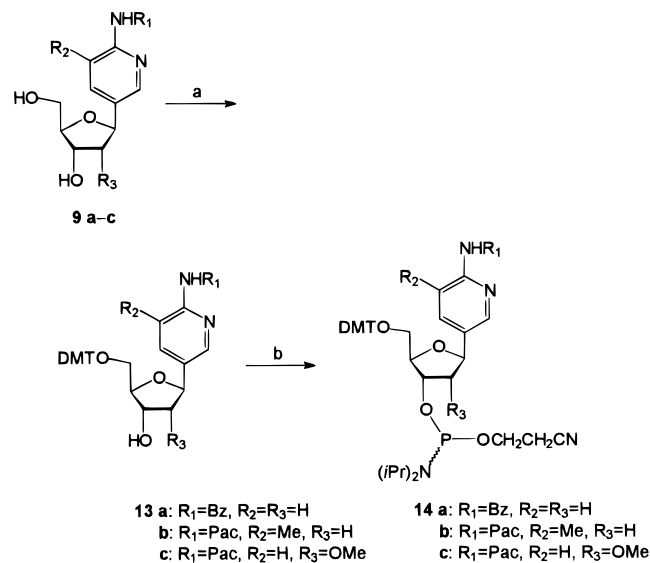
Figure 3. Synthesis of the phosphoramidites: (a) dimethoxytrityl chloride (DMTCl; 1.2 equiv), pyridine, room temperature, 3.5−8 h, 84% (**13a**), 74% (**13b**), and 81% (**13c**). (b) (NCCH$_2$CH$_2$O)(iPr$_2$N)PCl (1.5 equiv), Et(iPr)$_2$N (3 equiv) THF, room temperature, 2 h, 81% (**14a**), 81% (**14b**), and 86% (**14c**).

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5'-d(GCTAAAAAGAGAGAGAGATCG)        5'-d(GCTAAAGGGGAAAAGAAATCG)
3'-d(CGATTTTTCTCTCTCTCTAGC)        3'-d(CGATTTCCCCTTTTCTTTAGC)

15 d(TTTTTCTCTCTCTCT)              22 d(TTTMePMePMePMePTTTTMePTTT)
16 d(TTTTTCTPCTCTCT)               24 d(TTTMeCMeCMeCMeCTTTTMeCTTT)
17 d(TTTTTCTMePTCTCTCT)
18 d(TTTTTCTPOMeTCTCTCT)
19 d(TTTTTPTPTPTPTPT)
20 d(TTTTTMePTMePTMePTMePTMePT)
21 d(TTTTTPOMeTPOMeTPOMeTPOMeTPOMeT)
23 d(TTTTTMeCTMeCTMeCTMeCTMeCT)
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Figure 4. Sequences of the 21-mer target DNA duplexes (top) and the triple helix-forming oligonucleotides **15**−**24** (^{Me}C = 5-methyldeoxycytidine).

Table 1. T_m values (°C) and Hyperchromicity (%) (in Parentheses) of the Dissociation of the Third Strand **15**−**21** and **23** from the Target Duplex as Determined from UV-Melting Curves ($\lambda = 260$ nm)^a

| strand	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0
15	39.0 (7)	29.1 (4)	18.9 (2)	9.9 (1)	c
16	42.5 (10)	33.5 (7)	25.1 (4)	16.3 (1)	9.8 (1)
17	42.4 (8)	33.7 (5)	24.7 (3)	16.6 (2)	9.9 (1)
18	33.8 (8)	26.1 (5)	16.7 (2)	8.7 (1)	c
19	b	44.9 (9)	38.1 (6)	32.3 (5)	24.2 (3)
20	b	45.0 (10)	39.2 (8)	33.6 (7)	26.0 (5)
21	7.3 (2)	7.0 (2)	c	c	c
23	50.8 (8)	40.6 (6)	30.1 (3)	21.9 (2)	11.5 (1)

^{a} Triple-helix concentration 1.5−1.7 μM in 140 mM KCl, 7 mM Na$_2$HPO$_4$, 0.5 mM MgCl$_2$. T_m of the target duplex: 58.5 ± 1 °C. ^{b} Only one T_m value representing triplex → single strands transition (= 58.5 ± 1 °C) was observed. ^{c} Only one T_m value for duplex melting (= 58.5 ± 1 °C) was observed.

it can be seen, that the introduction of a methyl group in the base **P** in the position equivalent to the methyl group of thymine and 5-methylcytosine (→ ^{Me}**P**) has almost no effect on triplex stability. The oligomer **21**, in which all cytidines were replaced by the 2′-*O*-methyl-*C*-nucleoside **P$_{OMe}$**, has no longer any affinity to the target duplex above pH 6.5. Already the replacement of only one cytidine by **P$_{OMe}$** results in a loss of triplex stability relative to the controls (**15** and **23**) as can be seen from the binding data of **18**.

Table 2. T_m Values (°C) and Hyperchromicity (%) (in Parentheses) of the Dissociation of the Third Strand **22** and **24** from the Target Duplex as Determined from UV-Melting Curves ($\lambda = 260$ nm)^a

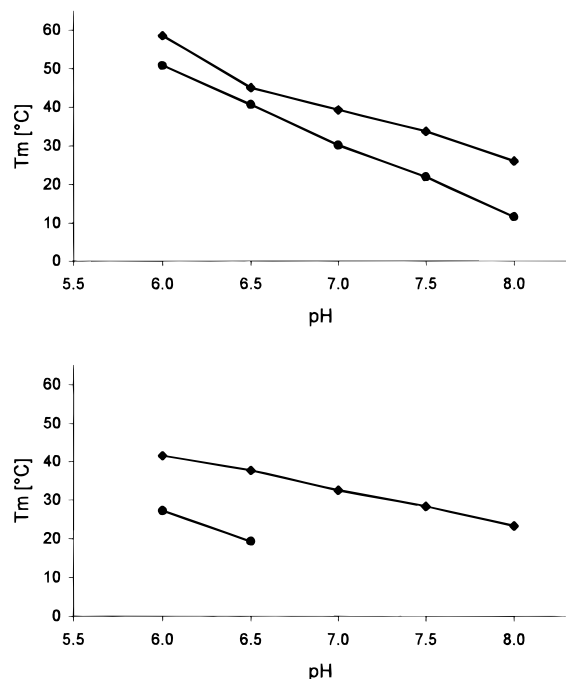| strand	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0
22	41.5 (10)	37.6 (5)	32.5 (7)	28.3 (5)	23.3 (3)
24	27.2 (8)	19.3 (5)	b	b	b

^{a} Triple-helix concentration 1.5−1.7 μM in 140 mM KCl, 7 mM Na$_2$HPO$_4$, 0.5 mM MgCl$_2$. T_m of the target duplex: 59.5 ± 1 °C. ^{b} Only one T_m value for duplex melting (= 59.5 ± 1 °C) was observed.
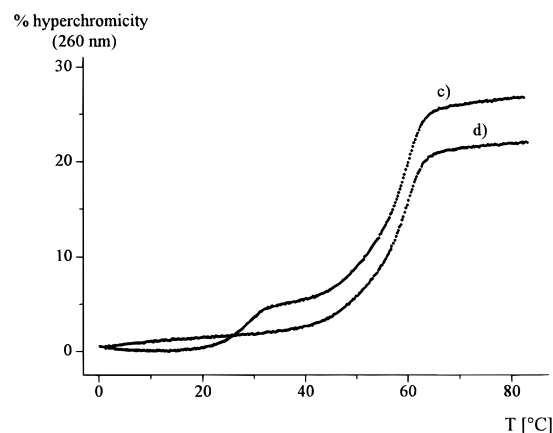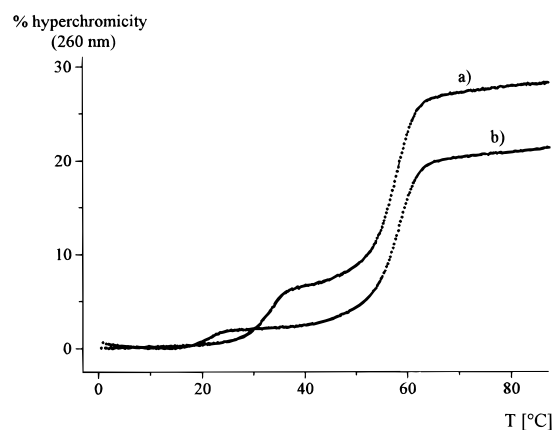
Figure 5. Representative UV-melting curves ($\lambda = 260$ nm) at pH 7.5 (triplex concentration 1.5−1.7 μM in 140 mM KCl, 7 mM HNa$_2$PO$_4$, 0.5 mM MgCl$_2$). (top) UV-melting curves of **20** (a) and **23** (b) with the corresponding target duplex sequence (T_m of the duplex 58 ± 1 °C). (bottom) UV-melting curves of **22** (c) and **24** (d) with the corresponding target duplex sequence (T_m of the duplex 59 ± 1 °C).

It is known that triple helix-forming oligonucleotides containing ^{Me}C units display serious sequence composition limitations with regard to targeting contiguous G-rich purine tracts.[18] To evaluate the sequence composition effect of the *C*-nucleoside ^{Me}**P**, oligonucleotide **22** containing four contiguous ^{Me}**P** units was prepared and compared with oligonucleotide **24** (which contains four contiguous ^{Me}C units) with respect to binding to the corresponding target duplex (Figure 4, right). From the binding data in Table 2 it becomes clear, that the affinity of oligonucleotide **24** to the target duplex decreases much more with increasing pH than that of oligonucleotide **22**, which contains four contiguous ^{Me}**P** units. While the oligomer **24** shows no longer any affinity to the target above pH 6.5, the oligomer **22** displays preserved binding with a T_m of 32.5 °C at pH 7.0 and even still binds at pH 8.0. Figure 6 shows a diagram

(18) Brunar, H.; Dervan, P. B. *Nucleic Acids Res.* **1996**, *24*, 1987−1991 and references cited therein.
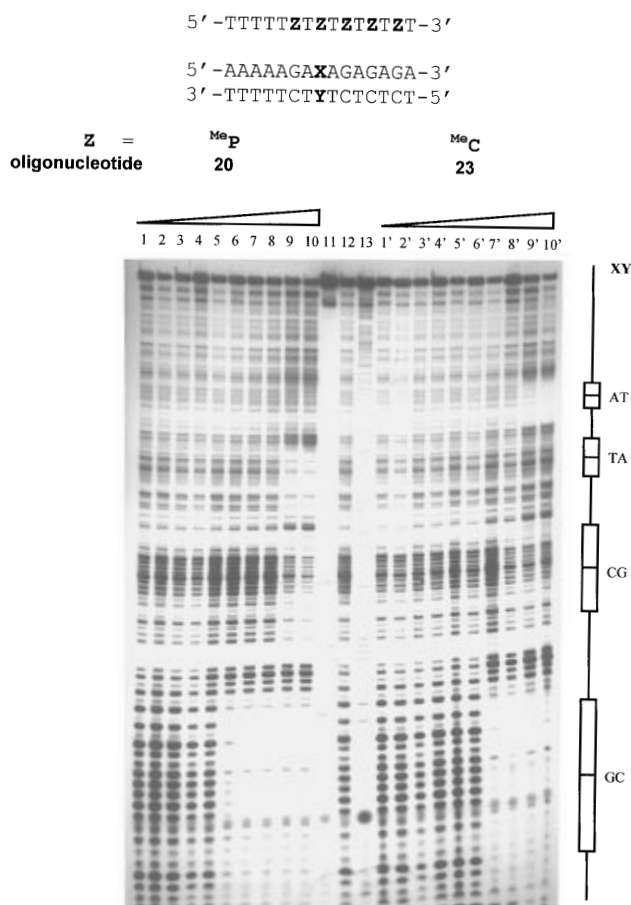
5-Substituted 2-Aminopyridine C-Nucleosides

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Figure 6. T_m values of third strand dissociation as a function of pH from UV-melting curves (260 nm) of **20** (◆), **23** (●) (top) and **22** (◆), **24** (●) (bottom) with the corresponding target duplex sequences. Triplex concentration $1.5-1.7$ μM in 140 mM KCl, 7 mM Na_2HPO_4, 0.5 mM $MgCl_2$.

of the triplex stability (T_m values) as a function of the pH for the oligonucleotides **20** and **23** (top) and **22** and **24** (bottom). As in the case of ^{Me}C also **P** or ^{Me}P containing oligonucleotides display pH dependent T_m values as expected. This pH dependence, however, is much less pronounced for ^{Me}P (and also **P**) than for ^{Me}C in the pH range $6.0-8.0$. The higher the pH the larger becomes the relative difference in T_m between ^{Me}C and ^{Me}P (and also **P**) containing oligonucleotides.

Selectivity of the Pyridine *C*-Nucleosides Containing Oligonucleotides to Duplex DNA. The selectivity in binding of oligonucleotides containing the base ^{Me}P was monitored by a DNase I footprint experiment. A $3'-{}^{32}P$-end-labeled (pyrimidine-rich strand) 229 base pair restriction fragment containing the four triplex target sites outlined in Figure 7, displaying each one of the four possible canonical base pairs in the center was incubated at 18 °C with increasing concentrations ranging from 500 pM to 10 μM of the triple helix-forming oligonucleotide **20** (and **23** as a control). The DNase I generated reaction products were separated by denaturing gel electrophoresis and visualized by storage phosphor autoradiography.

Figure 7 (bottom) shows a typical example of such an autoradiogram. On the left ^{Me}P-containing oligonucleotide **20** was used as triplex-forming strand and on the right the control oligonucleotide **23** (Z = ^{Me}C) was used. It can clearly be seen that oligonucleotide **20** (Z = ^{Me}P) prevents DNase I activity within the GC box at a concentration approximately five times lower than the ^{Me}C-containing oligonucleotide **23**. While the ^{Me}C-containing oligonucleotide **23** also binds to the AT-containing box at concentrations >1 μM, this is not observed for the ^{Me}P containing oligonucleotide **20**. Neither oligonucleotide (**20** and **23**) shows substantial binding to the TA or CG containing cassette at the concentration range investigated. Oligonucleotide **19** produced similar results as **20**.[19]

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(19) A systematic quantification according to published procedures[20] was not successful due to increased DNase I activity on either edges of the bound third strand, which may be due to sequence composition effects still not fully understood.

5′-TTTTT**Z**T**Z**T**Z**T**Z**T-3′

5′-AAAAAGA**X**AGAGAGA-3′
3′-TTTTTCT**Y**TCTCTCT-5′

| **Z** =	^{Me}**P**	^{Me}**C**
oligonucleotide	**20**	**23**

Figure 7. (top) Schematic representation of the oligonucleotides and duplex target sites used in the DNase footprint titrations. (bottom) Autoradiogram of a 8% denaturing polyacrylamide gel used to separate the fragments generated by DNase I digestion. The position of the binding sites within the 229-mer restriction fragment is indicated on the right. Lanes $1-10$ and $1'-10'$, DNA cleavage products produced by oligonucleotide **20** (left) resp. oligonucleotide **23** (right) at various concentrations (500 pM, lanes 1 and 1′; 1 nM, lanes 2 and 2′; 5 nM, lanes 3 and 3′; 10 nM, lanes 4 and 4′; 50 nM, lanes 5 and 5′; 100 nM, lanes 6 and 6′; 500 nM, lanes 7 and 7′; 1 μM, lanes 8 and 8′; 5 μM, lanes 9 and 9′; 10 μM, lanes 10 and 10′); lane 11, intact 3′ end-labeled duplex in the absence of a third strand; lane 12, DNA cleavage products produced in the absence of a third strand; lane 13, products of a A+G-specific sequencing reaction.

Affinity of the Pyridine *C*-Nucleosides Containing Oligonucleotides to Single-Stranded DNA. Binding of the oligonucleotides **15**–**19** to their antiparallel Watson–Crick complementary homopurine strand $d((AG)_5A_5)$ was monitored by UV-melting curves at two different salt concentrations (200 mM and 1 M NaCl) and pH 8.0. This pH value was chosen as to favor the deprotonated form of the 2-aminopyridine residues that is required for the formation of two hydrogen bonds to guanine (Figure 9). The duplex melting transition data are summarized in Table 3 and representative melting curves (200 mM NaCl, pH 8.0) are depicted in Figure 8. From the data in Table 3 it appears that already the replacement of one deoxycytidine residue by the pyridine *C*-nucleosides **P**, ^{Me}**P**, and **P$_{OMe}$** results in a dramatic decrease (more than 12 °C) of T_m. Consequently no cooperative melting transition could be observed in the case where all deoxycytidines were replaced by **P** (→**19**).

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(20) Brenowitz, M.; Senear, D. F.; Shea, M. A.; Ackers, G. K. *Methods Enzymol.* **1986**, *130*, 132–181.
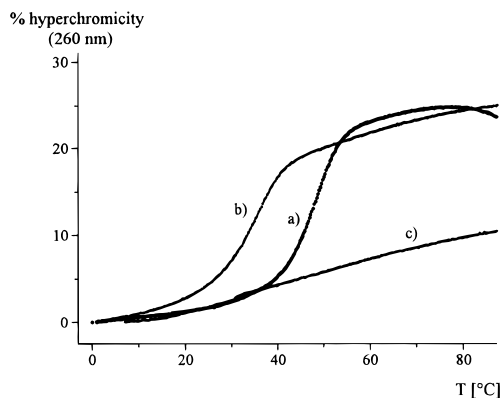
Figure 8. UV-melting curves ($\lambda = 260$ nm) of 1:1 mixtures of **15** (a), **16** (b) and **19** (c) with the Watson−Crick complement d((AG)$_5$A$_5$) in 10 mM NaH$_2$PO$_4$, 200 mM NaCl at pH 8.0 (concentration 2.2−2.4 μM).
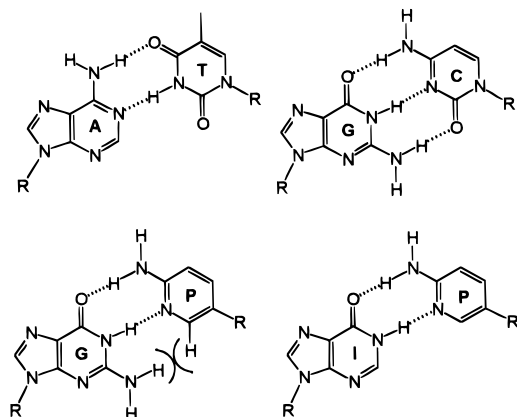
Figure 9. (top) Structures of the Watson−Crick base pairs adenine−thymine (A-T) and guanine−cytosine (G-C). (bottom) Structures of the 2-aminopyridine−guanine (P-G; expected steric repulsion between H−N(2) of G and H−C(6) of **P** indicated) and the 2-aminopyridine−inosine (P-I) base pairs.

Table 3. T_m Values (°C) of the Pyridine *C*-Nucleoside-Containing Duplexes as Determined from UV-Melting Curves ($\lambda = 260$ nm)[a]

duplex	200 mM NaCl	1 M NaCl
d((AG)$_5$A$_5$)/d(T$_5$(CT)$_5$) (**15**)	48.7	56.3
d((AG)$_5$A$_5$)/d(T$_5$CT**P**T(CT)$_3$) (**16**)	36.5	43.9
d((AG)$_5$A$_5$)/d(T$_5$CT^{Me}**P**T(CT)$_3$) (**17**)	31.2	40.3
d((AG)$_5$A$_5$)/d(T$_5$CT**P**$_{OMe}$T(CT)$_3$) (**18**)	36.9	46.4
d((AG)$_5$A$_5$)/d(T$_5$(**P**T)$_5$) (**19**)	*b*	*b*

[a] Duplex concentration: 2.2−2.4 μM in 10 mM NaH$_2$PO$_4$; pH 8.0. [b] No cooperative melting transition was observed.

Discussion

T_m vs DNase I Footprint Results. In contrast to duplex formation between two single strands of comparable length and sequence, where as a rule of thumb an increase in T_m by 3−5 °C corresponds to an increase in the association constant (K_{ass}) by a factor of 10,[21] there exists no clear correlation between T_m differences and K_{ass} in the case of triple-helix formation. From the data in Table 1 it appears that the replacement of all five ^{Me}C residues by ^{Me}**P** results in an increase of T_m by ~10 °C (at pH 7.2). On the other hand from the DNase I footprint results (Figure 7) it can be seen that the binding affinity of the ^{Me}**P**-containing oligonucleotide **20** is only five times stronger to the target than that of the ^{Me}C-containing oligonucleotide **23** and thus seems to contrast the results of the T_m measurements from

(21) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366−374.

which a higher K_{ass} would be expected. The differences may arise from several factors, as e.g. constant- vs variable-temperature experiments, differences in buffer conditions, secondary interactions of oligonucleotides with DNase I, and sequence length effects (21 vs 229 base pairs of the target duplex). Although it would be in general desirable to be able to correlate results from UV-melting experiments and DNase footprint experiments this seems not to be possible so far due to limited data available.

Effect of Enhanced pK_a on Triple-Helix Stability. From the T_m data in Table 1 it appears that the replacement of the cytidine (or 5-methylcytidine) residues in the third strand by the 2-amino-2′-deoxypyridine *C*-nucleosides **P** or ^{Me}**P**, which are by ~2 pK_a units more basic than cytidine, results in an enhanced affinity of these oligonucleotides to double-stranded DNA over a pH range of 6.0−8.0. Not only this increase in triple-helix stability but also the much less expressed pH dependence of the T_m values (in the pH range 6.0−8.0) for ^{Me}**P**, and **P**-containing oligonucleotides compared to ^{Me}C-containing oligonucleotides can be regarded as a direct consequence of the higher pK_a. This is especially pronounced for the sequence containing contiguous C units (Figure 6, bottom). Interestingly the replacement of **P** by ^{Me}**P** (which is by 0.4 pK_a units more basic than **P**) results in no further enhancement of triple-helix stability at neutral pH. It seems that the effect of the pK_a difference between ^{Me}**P** and **P** becomes only operative at pH values > 8.0.

Effect of the Methyl Group on Triple-Helix Stability. It is well documented that replacement of cytosine by 5-methyl-cytosine in the third strand extends the pH range of stable triple helix formation in the parallel binding motif although there exists no relevant difference in the pK_a of the two bases.[22] A thermodynamic analysis (differential scanning calorimetry) indicated that this enhanced stability imparted by 5-methylcy-tidine appears to be entropic in origin. A given explanation is that the methyl group fills a space in the major groove, causing a release of hydrating water molecules from the double helix to the bulk upon triplex formation.[22c] In contrast to this, oligonucleotides containing **P** or ^{Me}**P** residues show no differences in triplex stability as inferred from the almost equal T_m values for **19** and **20**, thus indicating that the "methyl effect" is not present or operative in this system. Since the two pairs C, ^{Me}C and **P**, ^{Me}**P** structurally diverge only in the presence or absence of a carbonyl group on the complexing side (opposite to the methyl groups) of the base, one is forced to reconsider the interpretation for the observed entropic effect in the former system. An alternative interpretation, consistent with the observed behaviour in both cases, would assign an indirect role to the methyl group in ^{Me}C, in that it favorably changes the hydration pattern of the carbonyl group by influencing the torsion around the nucleosidic bond. Clearly, this is speculative at present and more experiments directed to the determination of thermodynamic data with **P**- and ^{Me}**P**-containing oligonucleotides are necessary.

Effect of the 2′-*O*-Methyl Group on Triple-Helix Stability. It is known that triplexes formed with oligo(2′-*O*-methylribo-nucleotides) have increased affinity towards duplex DNA relative to oligo(2′-deoxynucleotides).[23] This enhanced stability

(22) (a) Lee, J. S.; Woodsworth, M. L.; Latimer, L. J. P.; Morgan, A. R. *Nucleic Acids Res.* **1984**, *12*, 6603−6614. (b) Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 3059−3061. (c) Xodo, L. E.; Manzini, G.; Quadrifoglio, F.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1991**, *19*, 5625−5631. (d) Plum, G. E.; Park, Y.-W.; Singleton, S. F.; Dervan, P. B.; Breslauer, K. J. *Proc. Natl. Acad. Sci. USA.* **1990**, *87*, 9436−9440.

(23) Shimizu, M.; Konishi, A.; Shimada, Y.; Inoue, H.; Ohtsuka, E. *FEBS Lett.* **1992**, *302*, 155−158.

is most probably due to the conformationally uniform sugar structure of the ribose units. By replacing **P** by **P_{OMe}** in the third strand no such stabilizing effect could be observed. The **P** → **P_{OMe}** replacement resulted in a distinct loss of triplex stability although there seems to be no relevant difference in the pK_a of these two nucleosides.[24] While the stabilizing effect of 2′-O-alkylated nucleosides was reported for oligonucleotides with a uniform ribose backbone there exists only limited data in the literature with oligonucleotides composed of both deoxy- and 2′-O-alkyl ribose units (heterogeneous backbone composition).[3a,f,5] However a clear comparison of the influence of partial sugar modification on thermal stability has not been done so far. Our data indicate that heterogeneous oligonucleotides may behave differently from their pure ribo and deoxyribo sequences, probably due to differences in the preferred conformation of the third strand or the triplex itself.

Sequence Composition Effects. Recognition of double-stranded DNA by homopyrimidine oligonucleotides containing charged cytidine or ^{Me}C residues is known to have serious sequence composition limitations with regard to targeting contiguous G-rich purine tracts at physiological pH.[18] It is believed that this destabilization is due to an intrastrand electrostatic repulsion between adjacent protonated C or ^{Me}C units in the third strand. From Table 2 (and Figure 6, bottom) it clearly appears that this destabilizing sequence effect is much less pronounced in oligonucleotides containing ^{Me}**P** compared to those containing ^{Me}C. This indicates that at neutral pH, oligonucleotides containing bases with intrinsically more basic heterocycles suffer less from intrastrand charge repulsion and take more energetic advantage from the reduced interstrand charge repulsion in the triplex.

Effect of the Missing Carbonyl Group—Selectivity in Triple-Helix Formation. While for obvious reasons the 2-oxo function of C and ^{Me}C is not involved in H-bond formation with its target guanine, it is unclear whether it has any significant influence on triplex stability and binding selectivity at all. The bases **P** and ^{Me}**P** represent excellent "deletion mutants" to assess the function of the 2-oxo group experimentally. From the data in Table 1 and 2 it can be deduced that the absence of this functional group has no vital effect on triplex stability. The DNase I footprinting studies, however, demonstrating higher binding selectivity of **P**- over C-containing oligonucleotides, underline the important role of this carbonyl group in secondary target site binding, ultimately being responsible for reduced pairing selectivity. The fact, that **23** has a much higher affinity to the AT containing cassette than **20**, specifically indicates that the 2-oxo function of cytidine is involved in H-bonding to adenine in this sequence context.

Watson—Crick Base-Pairing Properties. In its deprotonated form the base 2-aminopyridine can be considered as a cytosine equivalent that should still be able to bind to guanine via two hydrogen bonds (Figure 9). On the basis of the duplex sequence investigated it could be shown that these duplexes are distinctly less stable than the corresponding natural duplex (Table 3). Thus the incorporation of **P**, ^{Me}**P**, or **P_{OMe}** in oligonucleotides has nearly the same destabilizing consequences as is expected for a mismatch base pair. This dramatic duplex destabilization may be ascribed to steric repulsion between the 2-amino group of guanosine and the H—C(6) of **P** (violation of the van der Waals radii of the two H-atoms in the base pair; Figure 9).[25] This result suggests that if the 2-oxo function of cytosine is lost, the 2-amino group of guanine has also to

disappear in order to be able to form a stable base pair. Therefore one is tempted to predict the existence of a stable 2-aminopyridine—inosine (P-I) base pair (Figure 9).

Conclusion

The results presented here clearly demonstrate that the easily accessible pyridine C-nucleosides **P** and ^{Me}**P**, due to their enhanced basicity relative to natural cytidine are excellent replacements for ^{Me}C in recognition of double-stranded DNA at physiological pH. The superiority of **P** and ^{Me}**P** over ^{Me}C can be summarized as follows: (i) No self-association of oligonucleotides containing **P** and ^{Me}**P** could be detected at low pH. (ii) Oligonucleotides containing **P** or ^{Me}**P** have a higher affinity to double-stranded DNA over the pH range of 6—8 than ^{Me}C-containing oligonucleotides. (iii) The Hoogsteen association of the C-nucleosides **P** and ^{Me}**P** is more specific than for ^{Me}C. (iv) Sequence composition effects are much less pronounced for ^{Me}**P** than for ^{Me}C. (v) Contrary to ^{Me}C there is no stable Watson—Crick association mode to G possible with **P** and ^{Me}**P**.

Thus pyrimidine oligonucleotides containing the 2′-deoxy-pyridine C-nucleosides **P** and ^{Me}**P** are structurally preorganized to bind exclusively in the Hoogsteen association mode. This extreme selectivity in favor of triplex formation, together with their easy accessibility and the wide range of applicability in terms of base sequence composition within the parallel binding motif are the key features of this new cytidine analog.

Experimental Section

General Considerations. ^{1}H NMR (300 or 500 MHz respectively) spectra and NOE (500 MHz) experiments were recorded on Bruker AC-300 or DRX500 and ^{13}C NMR (75 MHz) on Bruker AC-300. ^{31}P NMR (202 MHz) spectra were measured on Bruker DRX500 using PPh$_3$ (= 0 ppm) as external standard. Infrared (IR) spectra were obtained using a Perkin-Elmer (782 or FT IR 1600) spectrometer. Optical rotations were determined at room temperature using a Perkin-Elmer 241 polarimeter. Melting points (mp) were recorded on Büchi 510. UV spectra were performed on a Cary 3E UV/vis spectrophotometer (Varian). Electron ionization (EI) mass spectral analyses were recorded on Varian MAT (CH-7A or 212) and fast-atom bombardment (FAB) mass spectra on AutoSpec Q VG. pK_a values were obtained by potentiometric titration at 25 °C with 0.1 M aqueous NaOH or HCl and were measured at Ciba-Geigy AG, Basel. Flash chromatography was performed using silica gel with an average particle size of 40 μm (Baker). Thin-layer chromatography was performed on ALUGRAM® SIL G/UV254 (Macherey-Nagel). Visualization by UV (254 nm) and/ or by dipping into a solution of 10.5 g of cerium(IV) sulfate, 21 g of phosphomolybdic acid, 60 mL of concentrated sulfuric acid, and 900 mL of H$_2$O, followed by heating with a heat gun. All reactions were carried out under argon, using purified and distilled solvents. Tetra-*n*-butylammonium fluoride (1.1 M in THF) was from Aldrich and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite from Sigma. All other reagents were obtained from Fluka (butyllithium (*n*BuLi) as a 1.6 M solution in hexane) and were used without further purification.

1-Aza-1-(5-bromo-2-pyridinyl)-2,2,5,5-tetrametyl-2,5-disilacyclo-pentane (2a). To a solution of 2-amino-5-bromopyridine **1a** (5.00 g, 28.9 mmol) in THF (80 mL) was added at −75 °C *n*BuLi (18.2 mL, 29.0 mmol). After 1 h at −75 °C a solution of 1,2-bis(chlorodimeth-ylsilyl)ethane (6.22 g, 28.90 mmol) in THF (15 mL) was added dropwise. After another 90 min at −75 °C *n*BuLi (18.2 mL, 29.0 mmol) was added and the mixture was warmed up to room temperature within 2 h and allowed to stir for additional 2 h at room temperature. Brine (50 mL) was added, and the mixture extracted with Et$_2$O (2 × 200 mL). The ether extracts were dried (MgSO$_4$) and concentrated. Kugelrohr distillation (150 °C/0.05 mbar) afforded 6.20 g (68%) of

(24) It can be expected that the 2′-O-methylated derivative **P_{OMe}** displays approximately the same pK_a value than 2-amino-5-(β-D-ribofuranosyl)-pyridine (pK_a = 6.15).[7]

(25) Although there exists a possibility to escape the steric repulsion by propeller twisting of the base pair this would most probably not be tolerated due to steric clash with the base pairs above and below in the stack.

the stabase adduct **2a** as a colorless solid, mp 64–66 °C. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.29 (s, 12 H), 0.82 (s, 4 H), 6.46 (dd, $J =$ 8.45, 0.75 Hz, 1 H), 7.46 (dd, $J =$ 8.85, 2.55 Hz, 1 H), 8.14 (dd, $J =$ 2.6, 0.8 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ −0.58 (4×), 8.54 (2×), 108.52, 113.26, 139.29, 148.67, 159.43. IR (film): 1465, 1370, 1310, 1250, 950, 815, 785 cm^{-1}. MS (EI): *m/z* (rel intensity) 317 (19, [M + 1]$^+$), 316 (77, M$^+$), 315 (64, [M + 1]$^+$), 314 (77, M$^+$), 288 (87), 286 (83), 273 (100), 271 (97).

1-Aza-1-(5-bromo-3-methyl-2-pyridinyl)-2,2,5,5-tetramethyl-2,5-disilacyclopentane (2b). To 2-amino-5-bromo-3-methylpyridine **1b** (2.22 g, 11.9 mmol) and ZnI$_2$ (26 mg, 0.08 mmol) was added dropwise 1,2-bis[(dimethylamino)dimethylsilyl]ethane (3.30 mL, 11.9 mmol) and the mixture was heated at 140 °C over night. Kugelrohr distillation (170 °C/0.02 mbar) of the reaction mixture yielded 2.70 g (69%) of **2b** as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.16 (s, 12 H), 0.88 (s, 4 H), 2.23 (s, 3 H), 7.53 (dd, $J =$ 2.6, 0.74 Hz, 1 H), 8.18 (dd, $J =$ 2.6, 0.74 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 0.66 (4×), 9.00 (2×), 19.39, 113.02, 129.12, 141.02, 146.38, 157.93. IR (film): 3040, 2952, 2915, 2891, 1575, 1545, 1461, 1416, 1293, 1273, 1251, 1154, 1144, 956, 927, 898, 886, 866, 812, 786, 663 cm^{-1}. MS (EI): *m/z* (rel intensity) 330 (36, M$^+$), 329 (13), 328 (31, M$^+$), 315 (100), 313 (95), 302 (57), 300 (53), 287 (83), 285 (82).

2-Amino-5-(2′,3′,5′-tri-*O*-benzyl-β-D-ribofuranosyl)pyridine (4a). To a solution of **2a** (4.07 g, 12.91 mmol) in THF (60 mL) was added at −75 °C *n*BuLi (7.6 mL, 12.2 mmol). After 1 h at −75 °C a solution of lactone **3**^{11} (3.00 g, 7.17 mmol) in THF (20 mL) was added at −75 °C. The mixture was stirred for 2 h at −75 °C and then allowed to warm up to 0 °C over 3 h. Saturated NaHCO$_3$ (75 mL) was added and the mixture extracted with Et$_2$O (4 × 100 mL). The organic layer was washed with brine (50 mL), dried over MgSO$_4$, and concentrated. The yellow residue was dried and dissolved in CH$_2$Cl$_2$ (20 mL), and triethylsilane (5.7 mL, 36 mmol) and BF$_3$·OEt$_2$ (4.5 mL, 36 mmol) were added dropwise at −75 °C. The reaction mixture was allowed to warm up to room temperature over night, quenched with 1 M HCl (20 mL) and stirred for 1 h at room temperature. The mixture was neutralized with 2% NaOH and extracted with ethyl acetate (5 × 100 mL). The organic layer was washed with brine (30 mL), dried over MgSO$_4$ and concentrated. Purification of the residue by flash chromatography (ethyl acetate) afforded 2.28 g (64%) of **4a** as a slightly yellow oil, R_f 0.35 (ethyl acetate). [α]$_D$ = −29.3 (c = 1.35, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 3.56 (dd, $J =$ 10.3, 4.1 Hz, 1 H), 3.61 (dd, $J =$ 10.3, 4.0 Hz, 1 H), 3.70 (s, broad, NH$_2$), 3.75 (dd, $J =$ 7.5, 5.1 Hz, 1 H), 3.99 (dd, $J =$ 5.0, 3.3 Hz, 1 H), 4.29 (q, $J \approx$ 3.7 Hz, 1 H), 4.45 (AB system, $J_{AB} =$ 12.0 Hz, 2 H), 4.54 (AB system, $J_{AB} =$ 12.0 Hz, 2 H), 4.61 (AB system, $J_{AB} =$ 12.5 Hz, 2 H), 4.86 (d, $J =$ 7.4 Hz, 1 H), 6.40 (d, $J =$ 8.8 Hz, 1 H), 7.13−7.38 (m, 15 H), 7.45 (dd, $J =$ 8.5, 2.25 Hz, 1 H), 7.95 (d, $J =$ 2.2 Hz, 1 H). NOE (500 MHz, CDCl$_3$): irradiation at δ 3.75 (H−C(2′)) produced NOE enhancements at δ 3.99 (H−C(3′)), 4.86 (H−C(1′)), 7.45 (H−C(4)), 7.95 ((H−C(6)); irradiation at δ 3.99 (H−C(3′)) produced NOE enhancements at δ 3.75 (H−C(2′)), 7.45 (H−C(4)), 7.95 ((H−C(6)). ^{13}C NMR (75 MHz, CDCl$_3$): δ 70.53, 71.91, 72.33, 73.47, 77.49, 80.40, 81.92, 83.29, 108.51, 125.44, 127.60, 127.66, 127.72, 127.80, 128.11, 128.32, 128.39, 128.41, 136.34, 137.66, 137.90, 138.05, 146.43, 158.30. IR (film): 3360, 3030, 2900, 2870, 1620, 1510, 1500, 1455, 1125, 1085, 1045, 1030, 785, 698 cm^{-1}. UV (CHCl$_3$): λ_{max} nm 243, 295. MS (EI): *m/z* (rel intensity) 496 (1, M$^+$), 213 (21), 121 (20), 92 (20), 91 (100), 45 (26), 43 (61), 19 (50).

2-Amino-3-methyl-5-(2′,3′,5′-tri-*O*-benzyl-β-D-ribofuranosyl)pyridine (4b). Nucleoside **4b** was prepared from **2b** (2.50 g, 7.59 mmol), lactone **3** (2.10 g, 5.02 mmol), and *n*BuLi (4.8 mL, 7.7 mmol) as described for **4a**. Flash chromatography (ethyl acetate) afforded 1.97 g (77%) of **4b** as a slightly yellow oil, R_f 0.36 (ethyl acetate). [α]$_D$ = −26.7 (c = 0.89, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.00 (s, 3 H), 3.60 (dd, $J =$ 10.3, 3.68 Hz, 1 H), 3.66 (dd, $J =$ 10.3, 4.05 Hz, 1 H), 3.82 (dd, $J =$ 7.35, 5.13 Hz, 1 H), 4.04 (dd, $J =$ 5.13, 3.66 Hz, 1 H), 4.31 (q, $J \approx$ 3.66 Hz, 1 H), 4.42 (broad, NH$_2$), 4.48 (AB system, $J_{AB} =$ 12.5 Hz, 2 H), 4.57 (AB system, $J_{AB} =$ 4.8 Hz, 2 H), 4.64 (AB system, $J_{AB} =$ 3.3 Hz, 2 H), 4.90 (d, $J =$ 7.35 Hz, 1 H), 7.15−7.39 (m, 16 H), 7.95 (d, $J =$ 2.22 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 17.00, 70.59, 71.95, 72.30, 73.48, 77.54, 80.50, 81.87, 83.14, 116.46, 125.97, 127.52, 127.63, 127.66, 127.77, 127.86, 128.06, 128.26, 128.39,

128.40, 136.08, 137.71, 137.98, 138.13, 144.31, 156.98. IR (film): 3482, 3378, 3029, 2864, 1621, 1486, 1454, 1125, 1091, 1048, 1028, 738, 698 cm^{-1}. MS (EI): *m/z* (rel intensity) 510 (10, M$^+$), 419 (26), 153 (31), 227 (50), 190 (40), 135 (46), 92 (42), 91 (100).

2-(*N*-Benzoylamino)-5-(β-D-ribofuranosyl)pyridine (5a). To an ice-cooled solution of **4a** (1.60 g, 3.22 mmol) and pyridine (0.30 mL) in CH$_2$Cl$_2$ (8 mL) was added benzoyl chloride (0.50 mL, 4.30 mmol) and the resulting mixture was stirred for 24 h at room temperature. HCl (20 mL, 1M) and H$_2$O (20 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (5 × 50 mL). The organic layer was washed with 2% NaOH (20 mL), dried over MgSO$_4$, and concentrated. The residue was filtered over silica gel using hexane/ethyl acetate 20:11 to afford a mixture (1.85 g) of mono- and dibenzoylated nucleoside (R_f 0.29 and 0.19) as a colorless solid, which was dissolved in CH$_2$Cl$_2$ (35 mL), cooled to −75 °C, and treated with a 1 M solution of BBr$_3$ in CH$_2$Cl$_2$ (13.0 mL). The resulting suspension was stirred for 4 h at −75 °C, quenched with MeOH (40 mL) and allowed to warm up to room temperature over night. The solvent was evaporated, the residue dissolved in H$_2$O (20 mL) and washed with CH$_2$Cl$_2$ (10 mL). NaOH (10%) was added to make the solution basic. A colorless solid precipitated slowly at 4 °C, which was filtered off after 48 h and dried to yield 0.75 g (70%) of *C*-nucleoside **5a** as a colorless solid, mp 192−194 °C. [α]$_D$ = −56.9 (c = 0.93, MeOH/0.5 M HCl 7:3). ^{1}H NMR (300 MHz, CD$_3$OD): δ 3.77 (dd, $J =$ 11.9, 4.6 Hz, 1 H), 3.85 (dd, $J =$ 11.9, 3.45 Hz, 1 H), 3.95 (dd, $J =$ 7.3, 5.5 Hz, 1 H), 4.05 (q, $J \approx$ 3.8 Hz, 1 H), 4.10−4.18 (m, 1 H), 4.78 (d, $J =$ 7.0 Hz, 1 H), 7.50−7.68 (m, 3 H), 7.93−8.06 (m, 3 H), 8.25 (d, $J =$ 8.4 Hz, 1 H), 8.46 (d, $J =$ 2.2 Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 63.86, 73.36, 79.34, 83.07, 87.29, 116.14, 129.01, 130.05, 133.64, 134.36, 135.88, 137.99, 147.64, 153.08, 168.83. IR (KBr): 3260, 1660, 1522, 1530, 1490, 1393, 1310, 1105 cm^{-1}. MS (EI): *m/z* (rel intensity) 331 (11, [M + 1]$^+$) 330 (29, M$^+$), 302 (61), 301 (100), 227 (38), 123 (26), 105 (72), 77 (31).

3-Methyl-2-[*N*-(phenoxyacetyl)amino]-5-(β-D-ribofuranosyl)pyridine (5b). A solution of *C*-nucleoside **4b** (4.09 g, 8.02 mmol) and phenoxyacetic anhydride (6.89 g, 24.0 mmol) in pyridine (40 mL) was stirred at room temperature for 4.5 h. The reaction mixture was quenched with H$_2$O (10 mL) and concentrated. The residue was diluted with ethyl acetate (250 mL), washed with 1 M Na$_2$CO$_3$ (200 mL), dried (MgSO$_4$), and concentrated. Flash chromatography (hexane/ethyl acetate 1:1) of the residue afforded 3.45 g (67%) of 3-methyl-2-(*N*-phenoxyacetylamino)-5-(2′,3′,5′-tri-*O*-benzyl-β-D-ribofuranosyl)pyridine as a yellow oil, R_f 0.33 (hexane/ethyl acetate 1:1). [α]$_D$ = −21.9 (c = 1.72, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.16 (s, 3 H), 3.62 (dd, $J =$ 10.3, 3.68 Hz, 1 H), 3.70 (dd, $J =$ 10.3, 4.05 Hz, 1 H), 3.83 (dd, $J =$ 7.0, 5.15 Hz, 1 H), 4.06 (dd, $J =$ 5.14, 4.04 Hz, 1 H), 4.38 (q, $J =$ 3.68 Hz, 1 H), 4.42−4.66 (m, 6 H), 4.70 (s, 2 H), 5.04 (d, $J =$ 6.98 Hz, 1 H), 6.98−7.10 (m, 3 H), 7.18−7.23 (m, 2 H), 7.25−7.39 (m, 15 H), 7.60 (d, $J =$ 2.21 Hz, 1 H), 8.31 (d, $J =$ 2.21, 1 H), 8.61 (broad, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 17.81, 67.46, 70.26, 71.95, 72.43, 73.45, 77.23, 79.82, 82.04, 83.43, 114.66, 122.23, 127.51, 127.66, 127.81, 127.88, 128.02, 128.32, 128.36, 128.38, 129.78, 134.41, 137.35, 137.62, 137.71, 137.89, 144.29, 147.78, 157.03, 166.58. IR (film): 3246, 2918, 2864, 1694, 1599, 1495, 1454, 1128, 1084, 753, 737, 698 cm^{-1}. MS (EI): *m/z* (rel intensity) 644 (12, M$^+$), 227 (41), 193 (75), 152 (88), 122 (87), 107 (98), 79 (76), 65 (81), 55 (73), 51 (100).

To a solution of this protected *C*-nucleoside (3.40 g, 5.27 mmol) in CH$_2$Cl$_2$ (60 mL) was added dropwise at −75 °C a 1 M solution of BBr$_3$ in CH$_2$Cl$_2$ (24 mL). After 5 h at −75 °C, MeOH (75 mL) was added and the mixture was allowed to warm up to room temperature over night. The mixture was concentrated and the residue diluted with H$_2$O (40 mL). The aqueous layer was washed with CH$_2$Cl$_2$ (15 mL) and neutralized with 10% NaOH. A colorless solid precipitated slowly at 4 °C, which was filtered off and dried to yield 1.60 g (81%) of **5b**, mp 170−171 °C. [α]$_D$ = −34.5 (c = 0.37, MeOH). ^{1}H NMR (300 MHz, CD$_3$OD): δ 2.32 (s, 3 H), 3.79 (dd, $J =$ 12.14, 4.42 Hz, 1 H), 3.87 (dd, $J =$ 12.13, 3.67 Hz, 1 H), 3.99 (dd, $J =$ 7.35, 5.51 Hz, 1 H), 4.10 (q, $J \approx$ 3.9 Hz, 1 H), 4.17 (dd, $J =$ 5.52, 3.31 Hz, 1 H), 4.83 (s, 2 H), 4.86 (d, $J =$ 7.35 Hz, 1 H), 7.05−7.15 (m, 3 H), 7.35−7.41 (m, 2 H), 7.93 (s, 1 H), 8.41 (s, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 17.68, 62.14, 66.88, 71.67, 77.68, 80.60, 85.77, 114.86, 121.36, 128.56,

129.66, 135.17, 137.32, 144.17, 148.87, 157.99, 167.23. IR (KBr): 3390, 2921, 1716, 1640, 1574, 1490, 1436, 1172, 1115, 1075, 760, 692 cm^{-1}. MS (EI): *m/z* (rel intensity) 374 (20, M$^+$), 187 (33), 137 (100), 136 (42), 107 (64), 94 (39), 77 (70).

2-*O*-Methyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-ribono-1,4-lactone (11). To a solution of D-ribono-1,4-lactone (**10**) (1.20 g, 8.10 mmol) in pyridine (18 mL) was added dropwise at room temperature 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.10 mL, 9.89 mmol) and the resulting solution was stirred for 5 h. Ethyl acetate (100 mL) was added and the mixture washed with 0.1 M HCl (2 × 50 mL). The aqueous layer was extracted with ethyl acetate (2 × 50 mL), the combined organic layers were washed with saturated NaHCO$_3$ (1 × 50 mL), dried (MgSO$_4$), and concentrated. Flash chromatography (hexane/ethyl acetate 5:1) afforded 1.23 g (39%) of 3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-D-ribono-1,4-lactone as a colorless oil, R_f 0.30 (hexane/ethyl acetate 5:1). [α]$_D$ = +28.9 (*c* = 0.5, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.00−1.13 (m, 28 H), 3.02 (d, *J* = 2.6, OH), 3.99 (dd, *J* = 12.5, 5.9 Hz, 1 H), 4.15 (dd, *J* = 12.5, 3.7 Hz, 1 H), 4.25 (dd, *J* = 5.9, 2.2 Hz, 1 H), 4.43 (td, *J* ≈ 6.4, 3.7 Hz, 1 H), 4.51 (dd, *J* = 6.6, 5.9 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.53, 12.81, 13.07, 13.25, 16.75, 16.84, 16.86, 17.02, 17.17, 17.18, 17.22, 17.34, 61.60, 68.45, 69.83, 82.64, 171.74. IR (film): 3500, 2945, 2870, 1792, 1090, 1040, 905, 885, 730 cm^{-1}. MS (EI): *m/z* (rel intensity) 389 (5, [M − 1]$^+$), 329 (32), 235 (44), 121 (47), 77 (39), 58 (96), 41 (32), 28 (100).

A suspension of this oil (1.05 g, 2.69 mmol) and silver(I) oxide (2.10 g, 9.06 mmol) in methyl iodide (17 mL) was heated at 45 °C for 6 h. The excess MeI was distilled off, the residue diluted with ethyl acetate and filtered through a pad of celite. The filtrate was concentrated and the residue chromatographed (hexane/ethyl acetate 10:1) to yield 0.73 g (67%) of **11** as a clear colorless oil, which solidified upon standing at −30 °C, R_f 0.30 (hexane/ethyl acetate 10:1), mp 38−39 °C. [α]$_D$ = +43.9 (*c* = 0.54, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.97−1.10 (m, 28 H), 3.59 (s, 3 H), 3.78 (d, *J* ≈ 4.8 Hz, 1 H), 4.00 (dd, *J* = 13.6, 2.6 Hz, 1 H), 4.13 (dd, *J* = 13.6, 1.85 Hz, 1 H), 4.37 (dd, *J* = 8.65, 4.6 Hz, 1 H), 4.43 (dt, *J* = 8.85, 2.2 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.47, 12.74, 12.94, 13.37, 16.76, 16.91, 17.01, 17.09, 17.16, 17.18, 17.32, 59.13, 59.29, 69.34, 77.69, 82.14, 170.60. IR (film): 2940, 2895, 2865, 1792, 1465, 1245, 1162, 1130, 1093, 1040, 980, 882, 695 cm^{-1}. MS (EI): *m/z* (rel intensity) 404 (1, M$^+$), 363 (58), 362 (79), 361 (97), 329 (73), 259 (70), 250 (73), 249 (100), 135 (73), 119 (72), 83 (83), 43 (71).

2-Amino-5-[2′-*O*-methyl-3′,5′-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α,β-D-ribofuranosyl]pyridine (12). *C*-Nucleoside **12** was prepared from protected lactone **11** (0.70 g, 1.73 mmol) and **2a** (1.12 g, 3.56 mmol) as already described for **4a**. Flash chromatography (ethyl acetate) afforded 0.51 g (61%) of coupling product **12** as an anomeric mixture (β/α ≈ 10:1) as a slightly yellow oil, R_f 0.36 (ethyl acetate). ^{1}H NMR (300 MHz, CDCl$_3$): δ β-anomer: 0.90−1.12 (m, 28 H), 3.50 (dd, *J* = 5.0, 2.0 Hz, 1 H), 3.53 (s, 3 H); 4.92−4.14 (m, 3 H), 4.0 (broad, NH$_2$), 4.32 (dd, *J* = 8.8, 5.15 Hz, 1 H), 4.80 (d, *J* = 2.2 Hz, 1 H), 6.45 (d, *J* = 8.45 Hz, 1 H), 7.51 (dd, *J* = 8.45, 2.2 Hz, 1 H), 8.00 (d, *J* = 2.55 Hz, 1 H); α-anomer: 0.90−1.12 (m, 28 H), 3.05 (dd, *J* = 9.55, 2.55 Hz, 1 H); 3.07 (s, 3 H), 3.71 (d, *J* = 8.1 Hz, 2 H), 4.00−4.10 (m, 1 H), 4.0 (broad, NH$_2$), 4.41 (*d*, *J* = 9.55 Hz, 1 H), 4.63 (t, *J* = 2.2 Hz, 1 H), 6.47 (d, *J* = 8.45 Hz, 1 H), 7.44 (dd, *J* = 8.65, 2.4 Hz, 1 H), 8.04 (d, *J* = 2.2 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl$_3$): δ β-anomer, 12.58, 12.85, 13.08, 13.50, 16.93, 17.07, 17.13, 17.20, 17.32, 17.43, 17.36, 17.47, 58.96, 60.73, 70.95, 81.08, 82.61, 86.71, 108.44, 126.44, 135.90, 145.74, 157.96; α-anomer, 12.61, 13.18, 13.85, 14.21, 17.08, 17.20, 17.23, 17.29, 17.35, 17.45, 17.55, 17.59, 57.60, 66.07, 70.08, 72.13, 73.55, 81.80, 108.25, 125.25, 137.23, 147.09, 152.73. IR (film): 3360, 2950, 2930, 2860, 1620, 1502, 1135, 1075, 1030, 988, 883, 690 cm^{-1}. MS (EI): *m/z* (rel intensity) 483 (8, [M + 1]$^+$), 482 (15, M$^+$), 440 (72), 439 (100), 249 (67), 247 (45), 173 (46), 135 (58), 121 (75), 119 (47), 107 (95).

2-(*N*-Benzoylamino)-5-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl)]pyridine (6a). To a solution of *C*-nucleoside **5a** (0.75 g, 2.27 mmol) in pyridine (20 mL) was added dropwise at 0 °C 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.85 mL, 2.7 mmol) and the resulting suspension was stirred for 4 h at room temperature. The solvent was evaporated, 1 M NaOH (20 mL) was

added, and the aqueous layer was extracted with CH$_2$Cl$_2$ (5 × 50 mL). The organic extracts were dried over MgSO$_4$ and concentrated. Flash chromatography (hexane/ethyl acetate 5:3) gave 0.97 g (75%) of **6a** as a colorless foam, R_f 0.35 (hexane/ethyl acetate 5:3). [α]$_D$ = −46.0 (*c* = 2.05, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.00−1.15 (m, 28 H), 3.11 (d, *J* = 4.0 Hz, OH), 3.90−3.97 (m, 1 H), 3.99−4.16 (m, 3 H), 4.38 (t, *J* = 6.25 Hz, 1 H), 4.81 (d, *J* = 4.0 Hz, 1 H), 7.44−7.60 (m, 3 H), 7.81 (dd, *J* = 8.8, 2.2 Hz, 1 H), 7.91−7.97 (m, 2 H), 8.28 (d, *J* = 2.2 Hz, 1 H), 8.39 (d, *J* = 8.1 Hz, 1 H), 8.93 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.67, 12.92, 13.21, 13.43, 16.97, 16.98, 17.08, 17.14, 17.32, 17.36, 17.39, 17.49, 62.46, 71.71, 77.07, 82.91, 83.16, 113.87, 127.28, 128.83, 131.73, 132.25, 134.26, 136.23, 145.64, 151.28, 165.73. IR (film): 3350, 2940, 2870, 1680, 1525, 1497, 1300, 1125, 1040, 700 cm^{-1}. MS (EI): *m/z* (rel intensity) 572 (4, M$^+$), 530 (34), 529 (100), 528 (45), 236 (16), 235 (70), 224 (14), 105 (16).

3-Methyl-2-(*N*-phenoxyacetylamino)-5-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]pyridine (6b). The protected *C*-nucleoside **6b** was prepared from **5b** (1.38 g, 3.7 mmol), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.4 mL, 4.5 mmol) and pyridine (30 mL) as described for **6a**. Flash chromatography (hexane/ethyl acetate 1:1) gave 1.90 g (84%) of **6b** as a colorless foam; R_f 0.34 (hexane/ethyl acetate 1:1). [α]$_D$ = −32.3 (*c* = 0.39, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.03−1.11 (m, 28 H), 2.28 (s, 3 H), 3.00 (d, *J* = 3.68 Hz, OH), 3.91−3.96 (m, 1 H), 4.00−4.06 (m, 1 H), 4.07−4.14 (m, 2 H), 4.36 (t, *J* = 6.25 Hz, 1 H), 4.70 (s, 2 H), 4.84 (d, *J* = 4.04 Hz, 1 H), 6.98−7.09 (m, 3 H), 7.33−7.39 (m, 2 H), 7.65 (d, *J* = 1.47 Hz, 1 H), 8.34 (d, *J* = 1.84 Hz, 1 H), 8.55 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.65, 12.87, 13.19, 13.41, 16.97, 16.99, 17.09, 17.15, 17.35, 17.40, 17.48, 18.00, 62.25, 67.51, 71.53, 76.98, 82.73, 83.00, 114.73, 122.33, 126.13, 129.87, 134.13, 137.37, 143.98, 147.85, 157.08, 166.73. IR (film): 3400, 2944, 2867, 1700, 1684, 1600, 1506, 1496, 1464, 1243, 1129, 1064, 1038, 913, 885, 748, 691 cm^{-1}. MS (EI): *m/z* (rel intensity) 617 (14, [M + 1]$^+$), 616 (22, M$^+$), 573 (57), 524 (34), 523 (90), 483 (36), 482 (100), 235 (31), 121 (31).

2-[*N*-(Phenoxyacetyl)amino]-5-[2′-*O*-methyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]pyridine (6c). A solution of **12** (0.33 g, 0.68 mmol) and phenoxyacetic anhydride (0.70 g, 2.45 mmol) in pyridine (7 mL) was stirred at room temperature for 5 h. The reaction was quenched with H$_2$O (10 mL) and stirred for another 1.5 h. The mixture was concentrated, and the residue was diluted with ethyl acetate (150 mL), washed with 0.1 M NaOH (2 × 20 mL), dried (MgSO$_4$), and concentrated. Flash chromatography (hexane/ethyl acetate 4:1) of the residue afforded 0.36 g (85%) of protected nucleoside **6c** and 35 mg (8%) of the corresponding α-anomer as colorless oils. Data of **6c** (β-anomer): R_f 0.25 (hexane/ethyl acetate 4:1). [α]$_D$ = −34.8 (*c* = 1.0, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.90−1.12 (m, 28 H); 3.52 (dd, *J* = 4.95, 2.0 Hz, 1 H), 3.56 (s, 3 H), 3.97−4.05 (m, 2 H), 4.14 (dd, *J* = 13.4, 3.1 Hz, 1 H), 4.34 (dd, *J* = 8.3, 4.95 Hz, 1 H), 4.61 (s, 2 H), 4.92 (d, *J* = 2.2 Hz, 1 H), 6.94−7.07 (m, 3 H), 7.26−7.37 (m, 2 H), 7.80 (dd; *J* = 8.5, 2.2 Hz, 1 H), 8.23 (d, *J* = 8.8 Hz, 1 H), 8.31 (d, *J* = 2.2 Hz, 1 H), 8.95 (s, NH). NOE (500 MHz, CDCl$_3$): irradiation at δ 3.52 (H−C(2′)) produced NOE enhancements at δ 4.34 (H−C(3′)), 4.92 (H−C(1′)), 7.80 (H−C(4)), 8.31 (H−C(6)); irradiation at δ 4.34 (H−C(3′)) produced NOE enhancements at δ 3.52 (H−C(2′)), 7.80 (H−C(4)), 8.31 (H−C(6)); irradiation at δ 7.80 (H−C(4)) produced NOE enhancements at δ 3.52 (H−C(2′)), 4.34 (H−C(3′)), 4.92 (H−C(1′)), 8.23 (H−C(3)); irradiation at δ 8.31 (H−C(6)) produced NOE enhancements at δ 3.52 (H−C(2′)), 4.34 (H−C(3′)), 4.92 (H−C(1′)). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.57, 12.83, 13.08, 13.47, 16.91, 17.05, 17.09, 17.18, 17.30, 17.34 (2×), 17.46, 59.06, 60.59, 67.39, 70.77, 81.31, 82.22, 86.73, 113.79, 114.80, 122.38, 129.83, 133.07, 136.06, 145.65, 149.91, 156.94, 166.79. IR (film): 3300, 2938, 2868, 1518, 1495, 1463, 1390, 1300, 1240, 1140, 1035, 882, 750, 689 cm^{-1}. MS (EI): *m/z* (rel intensity) 616 (15, M$^+$), 574 (54), 573 (100), 255 (18), 249 (31). Data of α-anomer: R_f 0.42 (hexane/ethyl acetate 4:1). [α]$_D$ = −22.7 (*c* = 1.16, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): δ 0.80−1.08 (m, 28 H); 2.97 (dd, *J* = 9.4, 2.0 Hz, 1 H), 3.00 (s, 3 H), 3.67−3.74 (m, 2 H), 4.03 (ddd, *J* = 10.0, 6.5, 2.5 Hz, 1 H), 4.48 (*d*, *J* = 9.4 Hz, 1 H), 4.56 (s, 2 H), 4.60 (t, *J* ≈ 2.0 Hz, 1 H), 6.89−7.01 (m, 3 H), 7.23−7.31 (m, 2 H), 7.71 (dd, *J* = 8.7, 2.0 Hz, 1 H), 8.19 (*d*, *J* = 8.7 Hz, 1 H), 8.26 (d, *J* = 2.0 Hz, 1 H), 8.89 (*s*, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.59, 13.18,

13.84, 14.21, 17.06, 17.21, 17.23, 17.27, 17.35, 17.44, 17.54, 17.57, 57.56, 66.04, 67.42, 69.85, 72.01, 73.19, 82.17, 113.44, 114.80, 122.35, 129.81, 132.13, 137.36, 147.20, 149.97, 156.98, 166.78. IR (film): 3395, 3015, 2940, 2870, 1700, 1590, 1520, 1495, 1462, 1212, 1168, 1118, 1080, 1030, 1000, 883, 750, 690, 665 cm^{-1}. MS (EI): *m/z* (rel intensity) 616(10, M$^+$), 575(45), 574(73), 573(100), 249(70), 247(80), 94(34).

2-(N-Benzoylamino)-5-[3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-[(p-tolyloxy)thiocarbonyl]-β-D-ribofuranosyl]pyridine (7a). A solution of **6a** (0.93 g, 1.62 mmol) and *N,N*-dimethylaminopyridine (0.43 g, 3.52 mmol) in acetonitrile (23 mL) was treated at room temperature with *O*-p-tolyl chlorothionoformate (0.40 mL, 2.59 mmol) and the resulting yellow suspension was stirred over 3 Å molecular sieves for 25 h. The mixture was concentrated, 1 M NaOH (30 mL) was added, and the aqueous layer was extracted with ethyl acetate (4 × 70 mL). The organic layer was dried over MgSO$_4$ and concentrated, and the residue was chromatographed (hexane/ethyl acetate 5:1) to afford 0.95 g (81%) of **7a** as a colorless foam, R_f 0.30 (hexane/ethyl acetate 5:1). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.96−1.19 (m, 28 H), 2.38 (s, 3 H), 4.03−4.14 (m, 2 H), 4.18−4.28 (m, 1 H), 4.58 (dd, J = 8.6, 5.0 Hz, 1 H), 5.23 (d, J = 1.1 Hz, 1 H), 5.64 (dd, J = 5.0, 1.7 Hz, 1 H), 7.00−7.06 (m, 2 H), 7.19−7.25 (m, 2 H), 7.46−7.60 (m, 3 H), 7.90−7.95 (m, 2 H), 7.97 (dd, J = 8.6, 2.4 Hz, 1 H), 8.41 (d, J = 8.5 Hz, 1 H), 8.44 (d, J = 2.2 Hz, 1 H), 8.87 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.77, 12.91, 13.08, 13.42, 16.96, 17.06, 17.08, 17.15, 17.30, 17.36, 17.38, 17.49, 20.98, 60.58, 69.02, 80.97, 81.88, 87.55, 113.81, 121.40, 127.26, 128.86, 130.10, 131.94, 132.30, 134.20, 136.43, 145.91, 151.25, 151.48, 165.75, 194.48. IR (film): 3200, 2945, 2925, 2880, 1680, 1505, 1492, 1390, 1300, 1270, 1220, 1195, 1150, 1125, 1038, 882, 700 cm^{-1}. MS (EI): *m/z* (rel intensity) 723 (<1, M$^+$), 557 (37), 556 (78), 555 (100), 514 (37), 513 (61), 512 (87), 278 (45), 277 (76), 105 (42).

3-Methyl-2-[N-(phenoxyacetyl)amino]-5-[3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(imidazolyl thiocarbonyl)-β-D-ribofuranosyl]pyridine (7b). A solution of *C*-nucleoside **6b** (1.68 g, 2.72 mmol) and thiocarbonyl diimidazole (1.22 g, 6.83 mmol) in DMF (7 mL) was stirred for 1 h at room temperature and then for another 3.5 h at 40 °C. The mixture was diluted with ethyl acetate (100 mL) and washed with H$_2$O (5 × 25 mL). The organic layer was dried (MgSO$_4$) and concentrated, and the residue was purified by flash chromatography (first ethyl acetate/hexane 1:1, then 2:1) to afford 1.47 g (74%) of **7b** as a colorless foam, R_f 0.32 (hexane/ethyl acetate 1:1). $[\alpha]_D = -11.2$ (*c* = 0.48, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.93−1.13 (m, 28 H), 2.31 (s, 3 H), 4.07−4.14 (m, 2 H), 4.24−4.29 (m, 1 H), 4.62 (q, J ≈ 4.78 Hz, 1 H), 4.71 (s, 2 H), 5.28 (s, 1 H), 5.74 (d, J = 4.78 Hz, 1 H), 6.98−7.10 (m, 4 H), 7.33−7.39 (m, 2 H), 7.70 (t, J = 1.48 Hz, 1 H), 7.81 (d, J = 2.20 Hz, 1 H), 8.40 (d, J = 1.10 Hz, 1 H), 8.52 (d, J = 1.83 Hz, 1 H), 8.58 (s, NH). ^{13}C-NMR (75 MHz, CDCl$_3$): δ 12.60, 12.70, 12.95, 13.29, 16.85, 16.93, 17.05, 17.24, 17.26, 17.33, 17.39, 17.99, 60.26, 67.49, 68.99, 80.91, 82.07, 86.86, 114.68, 118.02, 122.31, 129.82, 131.06, 132.61, 136.76, 137.44, 144.26, 148.38, 157.01, 166.70, 183.27. IR (film): 2945, 2867, 1694, 1600, 1494, 1464, 1392, 1324, 1287, 1231, 1154, 1137, 1065, 1038, 994, 884, 772, 691 cm^{-1}. MS (EI): *m/z* (rel intensity) 727 (5, M$^+$), 599 (47), 598 (83), 556 (52), 555 (100), 464 (53), 322 (40), 321 (80), 235 (55).

2-(N-Benzoylamino)-5-[3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-β-D-ribofuranosyl]pyridine (8a). A solution of **7a** (0.88 g, 1.22 mmol), azobisisobutyronitrile (20 mg, 0.12 mmol) and *n*Bu$_3$SnH (0.50 mL, 1.89 mmol) in toluene (40 mL) was flushed with argon for 50 min and then heated at 80 °C for 4 h. NaOH (30 mL, 1 M) was added, and the mixture was extracted with ethyl acetate (4 × 60 mL). The organic layer was dried over MgSO$_4$ and concentrated. Flash chromatography (hexane/ethyl acetate 5:1) of the residue yielded 0.57 g (84%) of **8a** as a colorless oil, R_f 0.22 (hexane/ethyl acetate 5:1). ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.91−1.20 (m, 28 H), 2.06 (dt, J = 12.9, 7.7 Hz, 1 H), 2.39 (ddd, J = 12.8, 6.7, 4.5 Hz, 1 H), 3.83−3.95 (m, 2 H), 4.07−4.18 (m, 1 H), 4.55 (dt, J = 7.7, 4.4 Hz, 1 H), 5.09 (t, J = 7.35, 1 H), 7.19−7.25 (m, 2 H), 7.46−7.61 (m, 3 H), 7.75 (dd, J = 8.7, 2.4 Hz, 1 H), 8.22 (d, J = 1.8 Hz, 1 H), 8.37 (d, J = 8.8 Hz, 1 H), 8.77 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.75, 13.04, 13.40, 13.52, 16.98, 17.07, 17.11, 17.27, 17.40, 17.44, 17.45, 17.58, 42.94, 63.55, 73.25, 76.69, 86.59, 113.82, 127.23, 128.84, 132.24,

133.69, 134.28, 136.26, 145.76, 151.09, 165.66. IR (film): 3100, 2940, 2870, 1680, 1518, 1305, 1085, 1030 cm^{-1}. MS (EI): *m/z* (rel intensity) 556 (17, M$^+$), 516 (30), 515 (83), 514 (100), 370 (22), 278 (36), 105 (93), 77 (51).

3-Methyl-2-[N-(phenoxyacetyl)amino]-5-[3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-β-D-ribofuranosyl]pyridine (8b). A solution of **7b** (1.23 g, 1.69 mmol), azobisisobutyronitrile (51 mg, 0.35 mmol), and tris(trimethylsilyl)silane (0.60 mL, 1.9 mmol) in toluene (60 mL) was flushed with argon for 45 min and then heated at 80 °C for 5 h. NaOH (40 mL, 1 M) was added, and the aqueous layer was extracted with ethyl acetate (5 × 80 mL). The organic layer was dried over MgSO$_4$ and concentrated. Flash chromatography (first hexane/ethyl acetate 4:1, then 1:1) of the residue yielded 0.68 g (67%) of **8b** as a colorless oil, R_f 0.57 (hexane/ethyl acetate 2:1). $[\alpha]_D = -2.1$ (*c* = 0.42, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.03−1.12 (m, 28 H), 2.05 (dt, J = 15.87, 7.35 Hz, 1 H), 2.28 (s, 3 H), 2.40 (dd, J = 12.87, 6.99, 4.78 Hz, 1 H), 3.85−3.94 (m, 2 H), 4.08−4.18 (m, 1 H), 4.54 (dt, J = 7.73, 4.78 Hz, 1 H), 4.69 (s, 2 H), 5.11 (t, J = 7.35 Hz, 1 H), 6.98−7.09 (m, 3 H), 7.32−7.38 (m, 2 H), 7.59 (d, J = 2.21 Hz, 1 H), 8.25 (d, J = 2.20 Hz, 1 H), 8.57 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 12.54, 12.97, 13.34, 13.46, 16.94, 17.04, 17.07, 17.22, 17.37, 17.39, 17.43, 17.54, 17.96, 42.80, 63.33, 67.49, 72.95, 76.35, 86.49, 114.70, 122.29, 129.83, 136.12, 137.37, 143.97, 147.65, 157.06, 166.69, 171.09. IR (film): 3250, 2944, 2893, 2867, 1698, 1600, 1495, 1464, 1244, 1142, 1119, 1089, 1063, 1040, 886, 754, 691 cm^{-1}. MS (EI): *m/z* (rel intensity) 601 (10, [M + 1]$^+$), 600 (23, M$^+$), 558 (40), 557 (97), 507 (20), 482 (18), 466 (19), 135 (16).

2-(N-Benzoylamino)-5-(2′-deoxy-β-D-ribofuranosyl)pyridine (9a). To a solution of **8a** (570 mg, 1.02 mmol) in THF (15 mL) was added at room temperature Bu$_4$NF (1.85 mL, 2.0 mmol), and the mixture was stirred for 90 min at room temperature. H$_2$O (0.1 mL) followed by 10% NaOH (0.1 mL) was added, and the mixture was concentrated. Flash chromatography (ethyl acetate/MeOH 9:1) of the residue afforded 277 mg (86%) of **9a** as a colorless solid, R_f 0.34 (ethyl acetate/MeOH 9:1), mp 174−175 °C. $[\alpha]_D = +38.0$ (*c* = 0.85, MeOH). ^{1}H NMR (300 MHz, CD$_3$OD): δ 2.04 (ddd, J = 13.0, 10.5, 5.9 Hz, 1 H), 2.28 (ddd, J = 13.0, 5.3, 1.65 Hz, 1 H), 3.73 (d, J = 4.8, 2 H), 4.01 (td, J = 4.9, 2.3 Hz, 1 H), 4.40 (dt, J = 5.9, 1.9 Hz, 1 H), 5.20 (dd, J = 10.95, 5.35 Hz, 1 H), 7.53−768 (m, 3 H), 7.94 (dd, J = 8.65, 2.35 Hz, 1 H), 8.99−8.04 (m, 2 H), 8.25 (d, J = 8.45 Hz, 1 H), 8.42 (d, J = 2.2 Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 45.01, 64.25, 74.72, 79.38, 89.67, 116.18, 129.02, 130.05, 133.63, 135.60, 135.90, 137.95, 147.51, 153.00, 168.82. IR (KBr): 3460, 1663, 1531, 1310, 708 cm^{-1}. MS (EI): *m/z* (rel intensity) 314 (18, M$^+$), 286 (32), 285 (85), 107 (29), 105 (100), 77 (50), 43 (31).

3-Methyl-2-[N-(phenoxyacetyl)amino]-5-(2′-deoxy-β-D-ribofuranosyl)pyridine (9b). Compound **9b** was prepared from *C*-nucleoside **8b** (0.63 g, 1.04 mmol) and Bu$_4$NF (1.9 mL, 2.1 mmol) as described for **9a**. Flash chromatography (ethyl acetate/MeOH 10:1) afforded 0.35 g (93%) of nucleoside **9b** as a colorless foam, R_f 0.29 (ethyl acetate/MeOH 10:1). $[\alpha]_D = +18.7$ (*c* = 0.93, CHCl$_3$). ^{1}H NMR (300 MHz, CD$_3$OD): δ 1.99 (ddd, J = 13.24, 10.67, 5.88 Hz, 1 H), 2.27 (s, 3 H), 2.28 (ddd, J = 13.24, 5.51, 1.84 Hz, 1 H), 3.72 (d, J = 5.15 Hz, 2 H), 4.00 (dt, J = 4.78, 2.58 Hz, 1 H), 4.39 (dt, J = 5.51, 1.84 Hz, 1 H), 4.78 (s, 2 H), 5.20 (dd, J = 10.67, 5.52 Hz, 1 H), 7.02−7.13 (m, 3 H), 7.33−7.40 (m, 2 H), 7.83 (d, J = 2.21 Hz, 1 H), 8.34 (d, J = 2.20 Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 17.93, 45.01, 64.16, 68.64, 74.57, 79.02, 89.66, 116.18, 123.20, 130.95, 131.37, 138.69, 139.45, 145.18, 149.36, 159.39, 170.58. IR (film): 3392, 3011, 2930, 1686, 1599, 1589, 1495, 1427, 1231, 1175, 1084, 1053, 998, 755, 691 cm^{-1}. MS (EI): *m/z* (rel intensity) 359 (36, [M + 1]$^+$), 358 (99, M$^+$), 266 (32), 265 (88), 224 (86), 175 (40), 147 (36), 135 (69), 134 (32), 121 (55), 108 (32).

2-[N-(Phenoxyacetyl)amino]-5-(2′-O-methyl-β-D-ribofuranosyl)pyridine (9c). Compound **9c** was prepared from *C*-nucleoside **6c** (330 mg, 0.53 mmol) and Bu$_4$NF (1.0 mL, 1.1 mmol) as described for **9a**. Flash chromatography (first ethyl acetate, then ethyl acetate/MeOH 10:1) yielded 175 mg (87%) of **9c** as a colorless solid, mp 117−118 °C, R_f 0.56 (ethyl acetate/MeOH 20:1). $[\alpha]_D = -5.8$ (*c* = 0.35, CHCl$_3$). ^{1}H NMR (300 MHz, CD$_3$OD): δ 3.44 (s, 3 H), 3.64 (dd, J = 7.15, 5.35 Hz, 1 H), 3.75 (dd, J = 12.1, 4.4 Hz, 1 H), 3.83 (dd, J = 12.1, 3.7 Hz, 1 H), 4.05 (q, J ≈ 3.9 Hz, 1 H), 4.30 (dd, J = 5.15, 3.7 Hz, 1

H), 4.76 (s, 2 H), 4.85 (d, $J = 7.0$ Hz, 1 H), 7.00−7.13 (m, 3 H), 7.29−7.42 (m, 2 H), 7.96 (dd, $J = 8.6$, 2.4 Hz, 1 H), 8.21 (d, $J = 8.5$ Hz, 1 H), 8.41 (d, $J \approx 2$ Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 58.95, 63.70, 68.68, 71.69, 81.16, 87.68, 88.53, 115.50, 116.21, 123.37, 131.05, 134.63, 138.27, 147.58, 152.02, 159.30, 169.79. IR (film): 3390, 2930, 1692, 1592, 1525, 1492, 1400, 1302, 1232, 1115, 1080, 1058, 750, 688 cm^{-1}. MS (EI): m/z (rel intensity) 375 (17, [M + 1]$^+$), 374 (43, M$^+$), 281 (82), 240 (83), 163 (58), 135 (58), 123 (100), 107 (71), 87 (90), 77 (68).

2-Amino-5-(2′-deoxy-β-D-ribofuranosyl)pyridine (P). A solution of *C*-nucleoside **9a** (61 mg, 0.19 mmol) in 40% aqueous Me$_2$NH (6 mL) was heated in a closed flask at 70 °C for 25 h. The reaction mixture was concentrated, and the residue was adsorbed onto silica gel and chromatographed (CH$_2$Cl$_2$/MeOH 4:1) to afford 34 mg (83%) of free nucleoside **P** as a colorless oil, R_f 0.35 (CH$_2$Cl$_2$/MeOH 4:1), pK_a 6.26. ^{1}H NMR (300 MHz, CD$_3$OD): δ 2.01 (ddd, $J = 13.2$, 10.7, 5.9 Hz, 1 H), 2.14 (ddd, $J = 13.2$, 5.4, 1.8 Hz, 1 H), 3.69 (d, $J = 4.8$ Hz, 2 H), 3.94 (td, $J = 4.9$, 2.5 Hz, 1 H), 4.37 (dt, $J = 5.9$, 1.8 Hz, 1 H), 5.03 (dd, $J = 10.7$, 5.5 Hz, 1 H), 6.61 (d, $J = 8.8$ Hz, 1 H), 7.57 (dd, $J = 8.8$, 2.2 Hz, 1 H), 7.92 (d, $J = 1.8$ Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 44.42, 64.25, 74.73, 79.71, 89.32, 110.51, 127.30, 138.34, 146.46, 160.87. IR (film): 3340, 1630, 1510, 1045, 1025. UV (H$_2$O): λ_{max} nm (ϵ) 234 (9300), 260 (650) 292 (3000). MS (EI): m/z (rel intensity) 210 (54, M$^+$), 179 (32), 123 (24), 121 (72), 120 (35), 107 (100), 94 (36), 31 (22).

2-Amino-3-methyl-5-(2′-deoxy-β-D-ribofuranosyl)pyridine (^{Me}P). A solution of **9b** (70.6 mg, 0.20 mmol) in 25% aqueous NH$_3$ (3 mL) was heated in a closed flask at 55 °C overnight. The reaction mixture was concentrated, and the residue was dissolved in H$_2$O (10 mL) and made acidic (ca. pH 1) with 1 M HCl. The aqueous layer was washed with ethyl acetate (5 × 20 mL) and CH$_2$Cl$_2$ (2 × 20 mL), made basic by adding 1 M NaOH, concentrated, and purified by flash chromatography (CH$_2$Cl$_2$/MeOH 4:1) to yield 27.6 mg (63%) of ^{Me}P as a colorless oil, R_f 0.31 (CH$_2$Cl$_2$/MeOH 4:1). [α]$_D$ = +43.4 ($c = 0.08$, H$_2$O). ^{1}H NMR (300 MHz, CD$_3$OD): δ 2.00 (ddd, $J = 13.24$, 10.66, 5.88 Hz, 1 H), 2.19 (ddd, $J = 13.24$, 5.52, 1.84 Hz, 1 H), 2.23 (s, 3 H), 3.70 (d, $J = 4.78$ Hz, 2 H), 3.96 (dt, $J = 4.78$, 2.58 Hz, 1 H), 4.37 (dt, $J = 5.89$, 1.84 Hz, 1 H), 5.04 (dd, $J = 10.30$, 5.52 Hz, 1 H), 7.50 (s, 1 H), 7.80 (s, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 17.34, 44.46, 64.24, 74.69, 79.53, 89.38, 119.61, 128.06, 138.71, 142.43, 158.75. IR (KBr): 3343, 2918, 1666, 1630, 1489, 1436, 1380, 1094, 1050, 1000, 863 cm^{-1}. UV (H$_2$O): λ_{max} nm (ϵ) 232 (10 400), 260 (950), 293 (5250). MS (EI): m/z (rel intensity) 225 (17, [M + 1]$^+$), 224 (100, M$^+$), 223 (22), 193 (22), 137 (21), 135 (97), 134 (32), 121 (80), 108 (28).

A sample of ^{Me}P was treated with a saturated solution of HCl in MeOH to afford after flash chromatography (CH$_2$Cl$_2$/MeOH 4:1) its hydrochloride salt ^{Me}P·HCl as a colorless foam, pK_a 6.63. ^{1}H NMR (300 MHz, CD$_3$OD): δ 1.99 (ddd, $J = 12.87$, 10.306, 5.88 Hz, 1 H), 2.25 (ddd, $J = 12.87$, 5.52, 1.84 Hz, 1 H), 2.31 (s, 3 H), 3.69 (dd, $J = 12.14$, 4.78 Hz, 1 H), 3.74 (dd, $J = 11.77$, 4.04 Hz, 1 H), 3.98 (dt, $J = 4.78$, 2.58 Hz, 1 H), 4.39 (dt, $J = 5.88$, 1.84 Hz, 1 H), 5.08 (dd, $J = 10.30$, 5.52 Hz, 1 H), 7.83 (s, 1 H), 7.94 (s, 1 H).

2-Amino-5-[2′-O-methyl-β-D-ribofuranosyl)pyridine (P$_{OMe}$). A solution of **9c** (75 mg, 0.20 mmol) in 25% NH$_3$ (4 mL) was heated in a closed flask at 57 °C for 14 h. The reaction mixture was concentrated, the residue diluted with 0.1 M HCl (10 mL) and washed with ethyl acetate (5 × 40 mL). The organic layer was extracted with 0.1 M HCl (10 mL). The combined aqueous layers were made basic (ca. pH 9) with 2 M NaOH and concentrated. Flash chromatography (ethyl acetate/MeOH 6:1) yielded 42 mg (91%) of free nucleoside P$_{OMe}$ as a colorless oil, R_f 0.20 (ethyl acetate/MeOH 6:1). [α]$_D$ = −41.9 ($c = 0.36$, H$_2$O). ^{1}H NMR (300 MHz, CD$_3$OD): δ 3.42 (s, 3 H), 3.62 (dd, $J = 7.35$, 5.15 Hz, 1 H), 3.72 (d, $J = 12.15$, 4.4 Hz, 1 H), 4.00 (q, $J \approx 3.7$ Hz, 1 H), 4.27 (dd, $J = 5.15$, 3.7 Hz, 1 H), 4.70 (d, $J = 7.35$ Hz, 1 H), 6.71 (d, $J = 8.8$ Hz, 1 H), 7.70 (dd, $J = 8.8$, 2.2 Hz, 1 H), 7.93 (d, $J = 2.2$ Hz, 1 H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 58.89, 63.73, 71.71, 81.31, 87.39, 87.97, 111.39, 126.41, 139.44, 144.14, 160.03. IR (KBr): 3350, 2920, 2900, 1625, 1570, 1505, 1410, 1349, 1129, 1115, 1080, 1050, 1030, 980, 821 cm^{-1}. UV (H$_2$O): λ_{max} nm (ϵ) 235 (14450), 292 (4570), 260 (850). MS (EI): m/z (rel intensity) 240 (28, M$^+$), 208 (15), 150 (17), 124 (19), 123 (100), 107 (26), 87 (45).

2-(N-Benzoylamino)-5-[5′-O-[(4,4′-dimethoxytriphenyl)methyl]-2′-deoxy-β-D-ribofuranosyl]pyridine (13a). To a solution of **9a** (105 mg, 0.33 mmol) in pyridine (1.5 mL) was added at room temperature (in 3 portions within 2 h) 4,4′-dimethoxytrityl chloride (DMTCl; 136 mg, 0.40 mmol). After another 2 h at room temperature, toluene (3 mL) was added, and the reaction mixture was concentrated. The residue was dissolved in CH$_2$Cl$_2$ (40 mL) and washed with 0.4 M NaOH (10 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (5 × 40 mL), the combined organic layers were dried (Na$_2$SO$_4$) and concentrated. Flash chromatography (ethyl acetate/hexane 5:1) yielded 173 mg (84%) of **13a** as a colorless foam, R_f 0.35 (ethyl acetate/hexane 5:1). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.04 (ddd, $J = 13.7$, 9.6, 6.0 Hz, 1 H), 2.16 (broad, OH), 2.26 (ddd, $J = 13.2$, 5.5, 1.8 Hz, 1 H), 3.26 (dd, $J = 9.7$, 5.3 Hz, 1 H), 3.36 (dd, $J = 9.7$, 4. Hz, 1 H), 3.79 (s, 6 H), 4.04−4.12 (m, 1 H), 4.41−4.50 (m, 1 H), 5.17 (dd, $J = 10.3$, 5.5 Hz, 1 H), 6.79−6.85 (m, 4 H), 7.17−7.36 (m, 7 H), 7.41−7.60 (m, 5 H), 7.75 (dd, $J = 8.6$, 2.4 Hz, 1 H), 7.89−7.95 (m, 2 H), 8.26 (d, $J = 1.8$ Hz, 1 H), 8.35 (d, $J = 8.8$ Hz, 1 H), 8.74 (broad, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 43.63, 55.24 (2×), 64.41, 74.61, 77.61, 86.33, 86.51, 113.18, 113.81, 126.88, 127.22, 127.88, 128.17, 128.85, 130.05, 130.08, 132.25, 133.45, 134.24, 135.93, 135.95, 136.44, 144.74, 145.87, 151.07, 158.54, 158.56, 165.67. IR (film): 3400, 1672, 1600, 1585, 1510, 1300, 1247, 1170, 1078, 1030, 903, 825, 725, 700 cm^{-1}. MS (EI): m/z (rel intensity) 616 (1, M$^+$), 304 (20), 303 (62), 286 (17), 285 (48), 225 (13), 105 (100), 77 (39).

3-Methyl-2-[N-(phenoxyacetyl)amino]-5-[5′-O-[(4,4′-dimethoxytriphenyl)methyl]-2′-deoxy-β-D-ribofuranosyl]pyridine (13b). Nucleoside **13b** was prepared from **9b** (133 mg, 0.37 mmol), DMTCl (257 mg, 0.76 mmol), and pyridine (2 mL) as described for **13a**. Flash chromatography (ethyl acetate/hexane 5:1) of the residue yielded 181 mg (74%) of **13b** as a slightly yellow foam, R_f 0.18 (ethyl acetate/hexane 5:1). [α]$_D$ = +17.6 ($c = 1.09$, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.05 (ddd, $J = 13.24$, 10.29, 5.88 Hz, 1 H), 2.21 (s, 3 H), 2.27 (ddd, $J = 13.24$, 5.52, 1.84 Hz, 1 H), 3.29 (dd, $J = 9.92$, 5.15 Hz, 1 H), 3.36 (dd, $J = 9.92$, 4.05 Hz, 1 H), 3.80 (s, 6 H), 4.09 (dt, $J = 5.15$, 2.58 Hz, 1 H), 4.41−4.53 (m, 1 H), 4.69 (s, 2 H), 5.19 (dd, $J = 9.92$, 5.52 Hz, 1 H), 6.80−6.87 (m, 4 H), 6.96−7.10 (m, 3 H), 7.19−7.40 (m, 9 H), 7.43−7.49 (m, 2 H), 7.67 (d, $J = 2.20$, 1 H), 8.28 (d, $J = 2.21$ Hz, 1 H), 8.55 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 17.90, 43.81, 55.21, 64.32, 67.50, 74.58, 77.37, 86.33, 86.54, 113.16, 114.72, 122.31, 126.85, 127.85, 128.14, 129.85, 130.05, 135.92, 137.65, 144.13, 144.74, 147.69, 157.07, 158.53, 166.71. IR (film): 3392, 2931, 1684, 1607, 1508, 1442, 1300, 1249, 1175, 1083, 1034, 829, 754, 692, 668 cm^{-1}. MS (FAB): (rel intensity) 661 (61, [M + 1]$^+$), 304 (27), 303 (100), 269 (10), 135 (10).

2-[N-(Phenoxyacetyl)amino]-5-[5′-O-[(4,4′-dimethoxytriphenyl)-methyl]-2′-O-methyl-β-D-ribofuranosyl]pyridine (13c). Nucleoside **13c** was prepared from **9c** (170 mg, 0.45 mmol), DMTCl (180 mg, 0.53 mmol) and pyridine (2.5 mL) as described for **13a**. Flash chromatography (first ethyl acetate/hexane 1:1, then 3:1) of the residue yielded 294 mg (81%) of DMT-protected nucleoside **13c** as a colorless foam, R_f 0.23 (ethyl acetate/hexane 1:1). [α]$_D$ = +4.0 ($c = 0.6$, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.57 (d, $J = 4.1$, OH), 3.24 (dd, $J = 10.3$, 4.05 Hz, 1 H), 3.36 (dd, $J = 10.3$, 3.3 Hz, 1 H), 3.36 (s, 3 H), 3.64 (dd, $J = 7.0$, 5.5 Hz, 1 H), 3.72 (s, 6 H), 4.11 (q, $J = 3.7$ Hz, 1 H), 4.15−4.21 (m, 1 H), 4.57 (s, 2 H), 4.79 (d, $J = 7.0$ Hz, 1 H), 6.73−6.79 (m, 5 H), 6.91−7.03 (m, 4 H), 7.11−7.41 (m, 9 H), 7.78 (dd, $J = 8.6$, 2.4 Hz, 1 H), 8.19 (d, $J = 8.8$ Hz, 1 H), 8.32 (d, $J = 2.2$ Hz, 1 H), 8.90 (s, NH). ^{13}C NMR (75 MHz, CDCl$_3$): δ 55.21 (2×), 58.66, 63.92, 67.41, 70.85, 79.33, 84.39, 86.32, 86.85, 113.15, 113.87, 114.80, 122.42, 126.84, 127.84, 128.17, 129.87, 130.08, 130.10, 132.50, 135.85, 135.89, 136.32, 144.72, 146.02, 158.51, 166.81. IR (film): 3400, 1600, 1510, 1490, 1300, 1248, 1172, 725 cm^{-1}. MS (FAB): m/z (rel intensity) 676 (3, M$^+$), 304 (40), 303 (100), 285 (12), 135 (14), 109 (12).

2-(N-Benzoylamino)-5-[5′-O-[(4,4′-dimethoxytriphenyl)methyl]-2′-deoxy-β-D-ribofuranosyl]pyridine-3′-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (14a). 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (75 μL, 0.34 mmol) was added dropwise at room temperature to a solution of **13a** (135 mg, 0.22 mol) and *N,N*-diisopropylethylamine (110 μL, 0.65 mmol) in THF (7 mL). After 2 h at room temperature, CH$_2$Cl$_2$ (50 mL) was added and the organic

Table 4. Purification, Characterization, and Yields of Oligonucleotides **16−22**

	HPL−chromatography		MALDI−TOF MS		
strand	DEAE−HPLC[a]	RP−HPLC[b]	calcd[c]	found[c]	OD[d] (yield (%))
16	35−65% B in 35 min (t_R 17.9)	t_R 16.0	4407.9	4407.9	51 (33)
17	40−50% B in 30 min (t_R 15.1)	t_R 9.8	4422.0	4428.0	68 (45)
18	40−60% B in 40 min (t_R 14.5)	t_R 14.8	4438.0	4439.4	59 (39)
19	35−65% B in 35 min (t_R 11.5)	t_R 16.9	4340.0	4340.4	37 (32)
20	35−40% B in 30 min (t_R 11.3)	t_R 8.8	4410.1	4414.7	55 (47)
21	41−46% B in 40 min (t_R 10.4)	t_R 16.6	4490.1	4489.3	34 (30)
22	35−40% B in 30 min (t_R 12.5)	t_R 8.9	4410.1	4412.5	39 (33)

[a] Purification was performed by elution with a linear gradient of B. Mobile phase A: 20 mM NaH_2PO_4 in H_2O/CH_3CN 4:1 (pH 3.9 for **16**, **19** and 4.1 for **17**, **18**, **20−22**), B: A + 1 M NaCl; flow 1 mL/min (t_R in min); UV detection (260 nm). [b] The purity of the isolated oligomers **16−22** was proved using always the same linear gradient: 10−35% B in 30 min; mobile phase A: 0.1 M triethylammonium acetate in H_2O (pH 7.0), B: 0.1 M triethylammonium acetate in H_2O/CH_3CN 1:4 (pH 7.0); flow 1 mL/min (t_R in min); UV detection (260 nm). [c] Monoanionic form. [d] Total optical density measured at 260 nm.

layer was washed with saturated $NaHCO_3$ (2 × 10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL), the combined organic layers were dried (Na_2SO_4) and concentrated. Flash chromatography (ethyl acetate/hexane 1:1) gave 145 mg (81%) of **14a** as a colorless foam consisting of a 1:1 mixture of diastereoisomers, R_f 0.64, 0.52 (ethyl acetate/hexane 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.10 (d, J = 6.8, 3 H), 1.16−1.21 (m, 9 H), 1.99−2.09 (m, 1 H), 2.34 (dd, J = 13.0, 5.1, 0.5 H), 2.44 (dd, $J \approx$ 13.0, 4.9, 0.5 H), 2.46 (t, J = 6.5, 1 H), 2.62 (t, J = 6.5, 1 H), 3.23−3.36 (m, 2 H), 3.54−3.67 (m, 2 H), 3.67−3.90 (m, 2 H), 3.784, 3.786, 3.789, 3.792 (4s, 6 H), 4.22−4.29 (m, 1 H), 4.51−4.58 (m, 1 H), 5.14−5.19 (m, 1 H), 6.80−6.85 (m, 4 H), 7.18−7.24 (m, 1 H), 7.26−7.31 (m, 2 H), 7.33−7.37 (m, 4 H), 7.44−7.52 (m, 4 H), 7.54−7.59 (m, 1 H); 7.78−7.82 (m, 1 H), 7.92 (s, 1 H), 7.93 (s, 1 H), 8.29−8.32 (m, 1 H), 8.359, 8.364 (2d, J = 8.5, 1 H), 8.71, 8.72 (2s, NH). ^{31}P NMR (202 MHz, $CDCl_3$): δ 154.00, 154.12. IR (film): 2970, 2930, 1675, 1605, 1580, 1510, 1692, 1460, 1443, 1390, 1360, 1340, 1300, 1248, 1175, 1070, 1030, 973, 905, 830, 728 cm^{-1}. MS (FAB): m/z (rel intensity) 817 (2, M$^+$), 304 (40), 303 (100), 135 (10), 119 (13), 105 (30).

3-Methyl-2-[N-(phenoxyacetyl)amino]-5-[5′-O-[(4,4′-di-methoxytriphenyl)methyl]-2′-deoxy-β-D-ribofuranosyl]pyridine-3′-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (14b). Phosphoramidite **14b** was prepared from 2-cyanoethyl N,N-diisopropyl-chlorophosphoramidite (95 μL, 0.43 mmol), **13b** (135 mg, 0.20 mmol) and N,N-diisopropylethylamine (110 μL, 0.65 mmol) in THF (8 mL) as described for **14a**. Flash chromatography (ethyl acetate/hexane 1:1) afforded 143 mg (81%) of **14b** as a colorless foam consisting of a 1:1 mixture of diastereoisomers, R_f 0.29 and 0.19 (ethyl acetate/hexane 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (d, J = 6.57 Hz, 3 H), 1.16−1.22 (m, 9 H), 2.01−2.10 (m, 1 H), 2.20 (s, 3 H), 2.35 (dd, J = 13.0, 5.3 Hz, 0.55 H), 2.44 (dd, J = 13.0, 5.3 Hz, 0.45 H), 2.47 (t, J = 6.57 Hz, 1 H), 2.63 (t, J = 6.42 Hz, 1 H), 3.23−3.38 (m, 2 H), 3.56−3.66 (m, 2 H), 3.67−3.76 (m, 1 H), 3.79 (s, 3 H), 3.80 (s, 3 H), 3.80−3.88 (m, 1 H), 4.22−4.28 (m, 1 H), 4.53−4.59 (m, 1 H), 4.69 (s, 2 H), 5.16−5.20 (m, 1 H), 6.80−6.84 (m, 4 H), 6.98−7.02 (m, 2 H), 7.04−7.08 (m, 1 H), 7.19−7.24 (m, 1 H), 7.25−7.31 (m, 2 H), 7.32−7.38 (m, 6 H), 7.45−7.48 (m, 2 H), 7.68−7.74 (m, 1 H), 8.28−8.34 (m, 1 H), 8.53 (s, NH). ^{31}P NMR (202 MHz, $CDCl_3$): δ 164.69, 164.85. IR (film): 2965, 2930, 1696, 1607, 1508, 1364, 1300, 1249, 1178, 1033, 977, 829, 754 cm^{-1}. MS (FAB): m/z (rel intensity) 861 (6, M$^+$), 304 (23), 303 (100).

2-[N-(Phenoxyacetyl)amino]-5-[5′-O-[(4,4′-dimethoxytriphenyl)-methyl]-2′-O-methyl-β-D-ribofuranosyl]pyridine-3′-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (14c). Phosphoramidite **14c** was prepared from **13c** (0.22 g, 0.33 mmol), 2-cyanoethyl N,N-diisopropyl-pylchlorophosphoramidite (110 μL, 0.49 mmol) and N,N-diisopropyleth-ylamine (170 μL, 0.99 mmol) in THF (10 mL) as described for **14a**. Flash chromatography (hexane/ethyl acetate 15:14) yielded 246 mg (86%) of **14c** as a colorless foam consisting of a 1:1 mixture of diastereoisomers, R_f 0.40 and 0.35 (hexane/ethyl acetate 3:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.02 (d, J = 7.0 Hz, 3 H), 1.13−1.22 (m, 9 H), 2.31 (t, J = 6.6 Hz, 1 H), 2.61−2.69 (m, 1 H), 3.21−3.28 (m, 1 H), 3.33−3.71 (m, 5 H), 3.34 (s, 1.5 H), 3.37 (s, 1.5 H), 3.77 (s, 3 H), 3.78 (s, 3 H), 3.83−3.97 (m, 1 H), 4.23−4.29 (m, 0.5 H), 4.30−4.45 (m, 1.5 H), 4.61 (s, 2 H), 4.85 (d, J = 8.1 Hz, 0.5 H), 4.86 (d, J = 8.1

Hz, 0.5 H), 6.76−6.81 (m, 4 H), 6.95−7.07 (m, 3 H), 7.15−7.37 (m, 9 H), 7.39−7.47 (m, 2 H), 7.83 (dd, J = 8.8, 2.2 Hz, 0.5 H), 7.87 (dd, J = 9.4, 2.0 Hz, 0.5 H), 8.23 (d, J = 8.5 Hz, 1 H), 8.39 (d, J = 2.2 Hz, 0.5 H), 8.40 (d, J = 2.2 Hz, 0.5 H), 8.95 (s, broad, NH). ^{31}P NMR (202 MHz, $CDCl_3$): δ 150.74, 151.33. IR (film): 3400, 2964, 2928, 1702, 1602, 1510, 1300, 1245, 1175, 1080, 1030, 750 cm^{-1}. MS (FAB): m/z (rel intensity) 877(6, M$^+$), 304 (23), 303 (100).

Oligonucleotide Synthesis and UV-Melting Experiments. Oligonucleotides **16−21** were synthesized on a Pharmacia Gene-Assembler Special (connected to a Compaq ProLinea 3/25 ZS personal computer) using standard β-cyanoethyl phosphoramidite chemistry. Reagents and concentrations were as for the synthesis of natural DNA oligomers. Syntheses were performed on a 1.3 μmol scale, according to the manufacturer's protocol.[16b] The only change made to the usual synthesis cycle was the prolongation of the coupling time of the synthetic nucleosides to 6 min. Coupling efficiencies were estimated from trityl assays and were >97% per step. After chain assembly and final detritylation (trityl off mode) the oligomers were removed from the support and deprotected by treatment with ca. 4 mL 25% NH_3 (55 °C, 12−22 h) followed by ca. 4 mL 40% $MeNH_2$ (70 °C, 20−26 h). The crude oligonucleotides were purified by ion exchange HPLC using a Nucleogen DEAE 60-7 (125 × 4.0 mm) column (Macherey & Nagel). The isolated oligonucleotides were desalted over SEP-PAK C-18 cartridges (Waters) according to a standard procedure.[26] The purity of the oligomers were determined by reversed-phase HPLC using a Aquapore Rp-300 (220 × 4.6 mm) column (Brownlee). The synthesis of oligonucleotide **22** essentially followed the protocol described, however, phenoxyacetic anhydride was used instead of acetic anhydride as the capping agent. This change in the synthesis simplified the deprotection step in that the $MeNH_2$ treatment was not necessary. The control oligomers **15**, **23**, and **24** were prepared by standard oligonucleotide chemistry. Details for syntheses, HPLC purification, and characterization by MALDI−TOF mass spectrometry are given in Table 4.

UV melting experiments were performed on a Cary 3E UV/vis spectrophotometer (Varian). Melting curves (λ = 260 nm) were recorded for a consecutive heating (0 → 90 °C) cooling−heating protocol with a linear gradient of 0.5 °C/min. All measurements were conducted in buffer solutions. The final pH was always adjusted to the desired value using 0.1 M HCl or 0.1 M NaOH.

Materials for DNase I Footprinting. All aqueous solutions for DNA manipulations were prepared with Milli-Q water. DNA and cell manipulations followed standard protocols.[26] The construction of plasmid pJM4C1 was performed by standard methods and is published elsewhere.[27] dGTP, dCTP, sonicated calf thymus DNA, AvaI restriction enzyme, Klenow fragment of the DNA polymerase I and DNase I were from Pharmacia. PvuII restriction enzyme was from Boehringer Mannheim and was used according to the suppliers protocol in the buffer provided. [α-^{32}P]-dCTP (≥3000 Ci/mmol) was obtained from Amersham. Bis-tris was from Sigma and the salts used for buffers were from Fluka (MicroSelect grade).

(26) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Plainview, NY, 1989.

(27) Marfurt, J.; Parel, S. P.; Leumann, C. *Nucleic Acids Res.* **1997**, *25*, 1875−1882.

5-Substituted 2-Aminopyridine C-Nucleosides

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3′-End-Labeling of the Restriction Fragment. Approximately 2 μg plasmid pJM4C1 were subsequently digested with the AvaI and PvuII restriction enzymes and the products separated by agarose gel electrophoresis. The desired band was excised and extracted with a QiaexII extraction kit (Qiagen). The restriction fragment was ethanol precipitated and resuspended in water. 3′-End-labeling was performed with [α-^{32}P]dCTP using the Klenow fragment of the DNA polymerase I. Excess unlabeled nucleotide triphosphates were added after labeling to ensure complete fill in. Unincorporated nucleotides were removed on a gel filtration column (NICK column, Pharmacia). The labeled fragment was precipitated with ethanol. From 2 μg of plasmid DNA a total Cerenkov radioactivity of $\sim$3 500 000 cpm was achieved.

DNase I Footprint Titration. These experiments were performed essentially according to a published protocol.[3d] Triplex forming oligonucleotide was placed in 10 microcentrifuge tubes to span a concentration range of 25 μM to 2.5 nM (final concentration) and adjusted to a volume of 18 μL with H_2O. Two more tubes containing only 18 μL H_2O were used for the reference lanes. A stock solution (27 μL) containing 5X physiological association buffer (50 mM NaCl, 700 mM KCl, 2.5 mM MgCl$_2$, 5 mM spermine, 50 mM bis-tris, pH 7.2, 50 μM base pairs sonicated calf thymus DNA; 9 μL), the labeled restriction fragment, and H_2O in appropriate amounts were added to each tube. The tubes were vortexed, shortly centrifuged and then allowed to equilibrate for 7 days at 18 °C (final solution conditions, 10 mM NaCl, 140 mM KCl, 0.5 mM MgCl$_2$, 1 mM spermine, 10 mM bis-tris, 10 μM bp calf thymus DNA, $\sim$20000 cpm labeled restriction fragment), and then 5 μL of a solution containing DNase I (5.9 u, 0.45 u/μL), CaCl$_2$ (50 mM), MgCl$_2$ (50 mM), non-specific oligonucleotide (10 μM, 5′-d(TATAATTTAA)-3′), bis-tris (10 mM, pH 7.0) and glycerol (5%) were added and digestion was allowed to proceed for 2 min at room temperature. The reaction was stopped by the addition of 8.3 μL of a solution containing 1.4 M NaCl, 0.14 M EDTA (pH 8.0) and 0.35 μg/μL of glycogen, followed by 120 μL of ethanol. Precipitation was performed at -20 °C for 3 h, followed by centrifugation at 16000g for 30 min at 0 °C. The pellets were washed with cold 70% ethanol, resuspended in 20 μL of H_2O, lyophilized to dryness, and dissolved in 7 μL of 80% formamide loading buffer. After denaturation (10 min at 85 °C), the samples were loaded on a 8% denaturing polyacrylamide gel and separated by electrophoresis in 1X TBE buffer at 1800V for 75 min. After drying on a slab drier, the gel was exposed to a storage Phosphor-Imager screen (Molecular Dynamics) overnight.

Supporting Information Available: ^{1}H NMR spectra of all compounds described (**2a**−**14c**) (14 pages). See any current masthead page for ordering and Internet access instructions.

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