Synthesis and Pairing Properties of Oligodeoxynucleotides Containing N^7-(Purin-2-amine Deoxynucleosides)

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Dedicated to Professor *Albert Eschenmoser* on the occasion of his 75th birthday

A general synthesis of the four isomeric N^7-α-D-, N^7-β-D-, N^9-α-D-, and N^9-β-D-(purin-2-amine deoxynucleoside phosphoramidite) building blocks for DNA synthesis is described (*Scheme*). The syntheses start with methyl 3′,5′-di-*O*-acetyl-2′-deoxy-D-ribofuranoside (**2**) as the sugar component and the N^2-acetyl-protected 6-chloropurin-2-amine **1** as the base precursor. N^7-Selectivity was achieved by kinetic control, and N^9-selectivity by thermodynamic control of the nucleosidation reaction. The two N^7-(purin-2-amine deoxynucleosides) were introduced into the center of a decamer DNA duplex, and their pairing preferences were analyzed by UV-melting curves. Both the N^7-α-D- and N^7-β-D-(purin-2-amine nucleotide) units preferentially pair with a guanine base within the *Watson-Crick* pairing regime, with ΔT_ms of -6.7 and -8.7 K, respectively, relative to a C·G base pair (*Fig. 3* and *Table 1*). Molecular modeling suggests that, in the former base pair, the purinamine base is rotated into the *syn*-arrangement and is able to form three H-bonds with O(6), N(1), and NH_2 of guanine, whereas in the latter base pair, both bases are in the *anti*-arrangement with two H-bonds between the N(3) and NH_2 of guanine, and NH_2 and N(1) of the purin-2-amine base (*Fig. 4*).

1. Introduction. – The development of new antigene agents with improved pairing properties towards double-stranded nucleic acids is of importance for potential applications in the fields of human therapy and diagnosis [1]. However, the success in obtaining novel oligonucleotides with improved chemical and biological properties by design has been rather limited so far. This is well-illustrated by the fact that a solution to the problem of targeting any desired base sequence of a DNA double helix by a third oligonucleotide strand to form a triple-helix structure is still elusive, despite almost 15 years of research efforts [2].

In this context, we set out to study the triplex-forming properties of third-strand oligonucleotides containing the four isomeric forms of the purin-2-amine nucleotides depicted in *Fig. 1*. More specifically, we intended to use the configuration at the anomeric center (α-D- *vs.* β-D) and the constitution of the glycosidic bond (N^7 *vs.* N^9) as structural switches for alternate-strand purine recognition in the major groove of a given DNA double helix [3]. To the best of our knowledge, however, only the anomeric pair of the N^9-(purin-2-amine deoxyribonucleosides) have been described so far. The β-D anomer is particularly well-characterized because it corresponds to a DNA-lesion base that induces transition mutations (A-T → C-G and C-G → A-T) [4]. Furthermore, its inherent fluorescent properties makes it an attractive analytical probe for sensing local changes in nucleic-acid structure [5].

Here we present a general synthetic procedure leading selectively to either the N^7- or N^9-(purin-2-amine nucleotides). Furthermore, we describe the incorporation of both

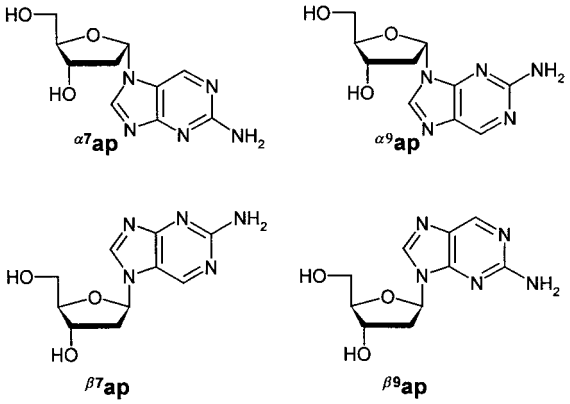


Fig. 1. *Structures of the* N^7- *and* N^9-*(purin-2-amine nucleosides)* $^{\alpha 7}$**ap**, $^{\beta 7}$**ap**, $^{\alpha 9}$**ap**, *and* $^{\beta 9}$**ap**

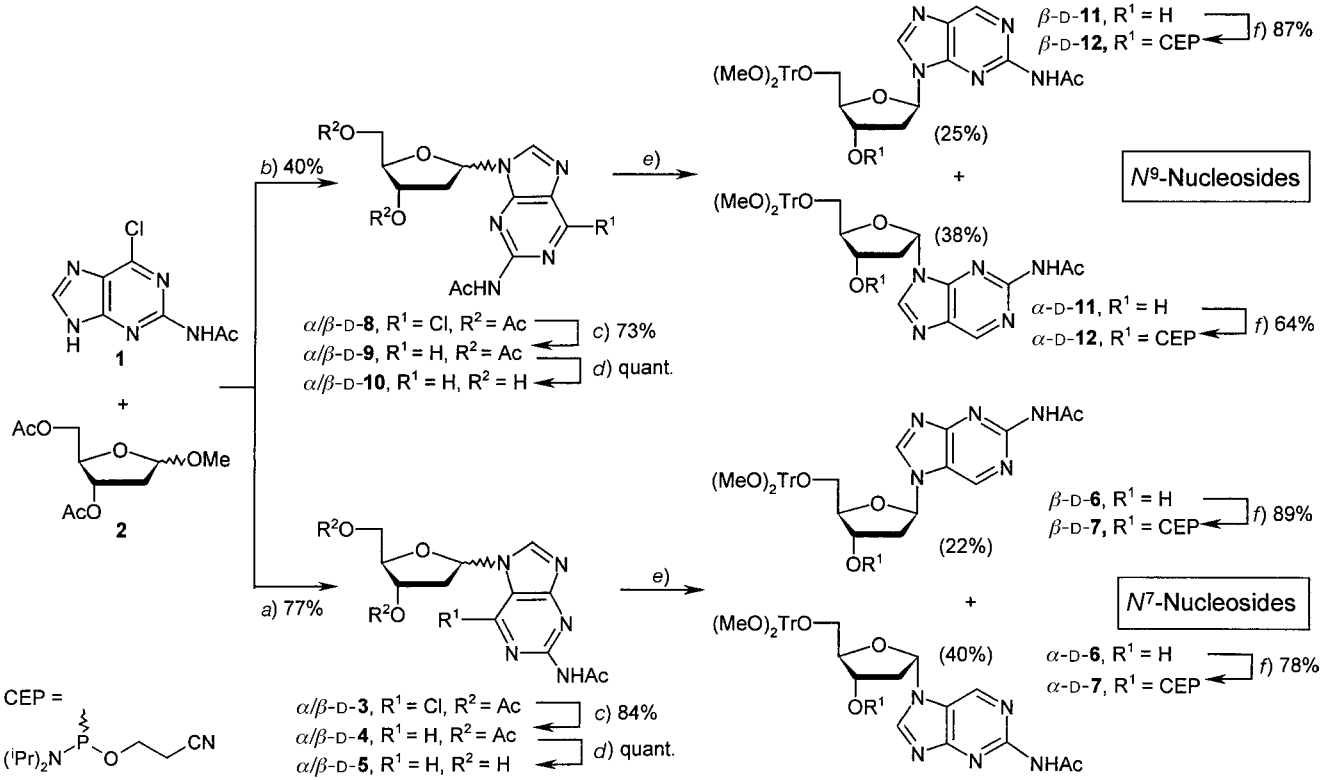
N^7-isomers $^{\alpha 7}$**ap** and $^{\beta 7}$**ap** into oligonucleotides and their pairing properties with a complementary DNA strand. The latter effort was undertaken to screen the *Watson-Crick* base-pair-formation properties of these nucleosides in DNA duplex formation.

2. Synthesis of Building Blocks for DNA Synthesis. – A series of syntheses of N^9-purin-2-amine nucleosides) have been known since the late 1950s [6][7]. Most of them start from naturally occurring guanosine, which is converted to 6-thioguanosine with a thiolating agent like P_4S_{10} [7], the *Lawesson* reagent [8], or H_2S [9], followed by reductive desulfurization. Other methods involved chlorination at the C(6) position of guanosine with $POCl_3$ [10] followed by reductive dechlorination *via* hydrogenolysis [11] or by photochemistry [12].

Since we were interested in N^7- and N^9-purinamine nucleotides, a more general synthetic approach was necessary. *Garner et al.* [8] showed that the regioselectivity of the nucleosidation (N^7 *vs.* N^9) of N^2-acetyl-protected 6-chlorpurin-2-amine with peracetylated glucopyranose can be controlled by careful tuning of the *Vorbrüggen* [13] nucleosidation conditions. Especially, the choice of the *Lewis* acid, the solvent, and the reaction temperature are critical for the N^9/N^7 regioselectivity of the reaction. We thus investigated the performance of these procedures for the regioselective synthesis of the corresponding deoxyribonucleosides (*Scheme*).

The synthesis started with α/β-D-ribofuranoside **2**, which was prepared as described in [14]. To obtain, under kinetic control, the N^7-isomers, the N^2-acetyl-protected base 6-chloropurin-2-amine **1** was persilylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and subsequently condensed with the protected sugar **2** in the presence of *ca.* 7 equiv. of $SnCl_4$ in MeCN at room temperature to give the corresponding readily separable anomer pairs of nucleosides α/β-D-**3** and α/β-D-**8** in a ratio of 10:1.5. The amount of $SnCl_4$ seems to be crucial for good N^7/N^9 selectivity. The anomer mixture α/β-D-**3** (*ca.* 1:1 by ^{1}H-NMR) was dechlorinated with H_2/Pd, yielding the protected N^7-(purin-2-amine deoxynucleosides) α/β-D-**4**. At this stage, both acetate groups could selectively and almost quantitatively be removed by ammonolysis. The α/β-D-**5** thus obtained were then tritylated at the 5′-position with 4,4′-dimethoxytrityl chloride (=chlorobis(4-

Scheme. *Synthesis of the* N^7*-and* N^9*-(Purin-2-amine Phosphoramidites)* α-D- *and* β-D-**7** *and* α-D- *and* β-D-**12**



a) **1**, BSA (3 equiv.), MeCN, 60°, 1 h; then **2**, $SnCl_4$ (7 equiv.), 0° → r.t., 2.5 h. *b*) **1**, BSA (3 equiv.), MeCN, 60°, 1 h; then **2**, $Me_3SiOSO_2CF_3$ (2 equiv.), 60°, 3 h. *c*) 10% Pd/C, Et_3N, H_2, 1 bar, AcOEt, 3–4 h. *d*) Conc. NH_3/MeOH, −20° → r.t., 18 h. *e*) $(MeO)_2TrCl$ (1.3 equiv.), Py, 18 h. *f*) $(^iPr)_2NEt$ (5 equiv.), $Cl[P(OCH_2CH_2CN)(N^iPr_2)]$ (2–3 equiv.), THF, 0°, 15 min, then r.t., 1.5–3 h.

methoxyphenyl)(phenyl)methane; $(MeO)_2TrCl$) in pyridine to give α-D- and β-D-**6**, which could be separated by column chromatography. Both anomers were then separately converted to the corresponding phosphoramidites α-D- and β-D-**7** by standard methods. The synthesis of the N^9-isomers was performed under reaction conditions leading to thermodynamic control of product distribution. In this case, the silylated base was coupled to the sugar with trimethylsilyl triflate as *Lewis* acid in boiling MeCN to afford the anomer mixture of the N^9-isomers α/β-D-**8**, though in moderate yield (40%). No N^7-isomers could be isolated. This mixture was then converted *via* α/β-D-**9**, α/β-D-**10**, and α/β-D-**11**, into the phosphoramidites α-D- and β-D-**12** by applying the same string of transformations described previously for the N^7-series. In this case, again anomer separation was possible by chromatography of the trityl derivatives α/β-D-**11**.

3. Structural Assignments. – Unambiguous confirmation of the constitution and configuration of the two N^7-(purin-2-amine deoxyribonucleosides) was achieved on the level of the tritylated nucleosides α-D- and β-D-**6** by T-ROESY-NMR analysis [15]. *Fig. 2,a* shows the H–C(1′), H–C(3′), H–C(4′) *vs.* the aromatic portion of a T-ROESY-NMR spectrum of β-D-**6**. H–C(1′) gives a clear ROE signal with both protons H–C(6) and H–C(8). Only the N^7-isomers are expected to produce a cross-peak for H–C(1′)/H–C(6). The signal between the anomeric proton and H–C(8) is due to the population of both the *syn*- and *anti*-conformations around the nucleosidic bond. Furthermore, a ROE cross-peak for H–C(1′)/H–C(4′) confirms the β-D-configuration of nucleoside β-D-**6**. Cross-peaks for H–C(1′)/H–C(6) and H–C(1′)/H–C(8) in the T-ROESY spectrum of α-D-**6** (*Fig. 2,b*) again confirm the N^7-constitution of the nucleosidic bond. In this case, the signal between H–C(8) and H–C(4′) corroborates the α-D-configuration.

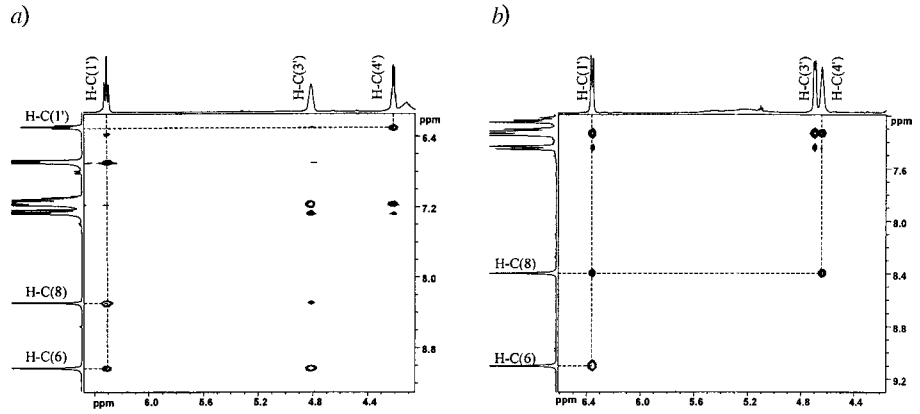


Fig. 2. *Sections of the T-ROESY-NMR spectra* (t_m 220 ms) *of* a) β-D-**6** *and b)* α-D-**6** *in* $CDCl_3$ *showing the cross-peaks between the aromatic H–C(6) and H–C(8) and the sugar H–C(1′) and H–C(4′)*

4. Synthesis and Pairing Behavior of Oligodeoxynucleotides Containing N^7-(Purin-2-amine Deoxynucleosides). – The syntheses of the two decamers **13** and **14** containing an α-D- and β-D-N^7-(purin-2-amine nucleoside) residue in the center of the sequence,

respectively, as well as the synthesis of the natural oligodeoxynucleotide complements **16**–**19** were carried out by automated DNA synthesis on the 1.3-μmol scale by standard protocols of DNA synthesis (see *Exper. Part*). Electrospray mass spectrometry confirmed the constitution of the oligonucleotides.

		X	Y
	13	$^{\alpha 7}$**ap**	
	14	$^{\beta 7}$**ap**	
5′-d(G-C-T-A-**X**-G-T-C-G-A)-3′	**15**		C
3′-d(C-G-A-T-**Y**-C-A-G-C-T)-5′	**16**		A
	17		C
	18		G
	19		T

Complementary duplex formation was followed by UV-melting-curve analysis (*Fig. 3*) (for the corresponding melting temperatures T_m, see *Table 1*). From the data it can be seen that, within the *Watson-Crick* pairing regime of oligonucleotides, both anomeric forms of the N^7-(purin-2-amine nucleotides) selectively pair with a guanine base, although the pairing efficiency is significantly reduced with respect to a reference duplex displaying a C·G base pair. The $^{\alpha 7}$**ap** unit leads to a decrease in the melting temperature of 6.7°, whereas the $^{\beta 7}$**ap** unit causes a reduction of 8.7°. All other base·base arrangements with both anomeric forms of purin-2-amine show T_ms that are in the region of that of a duplex with a mismatched natural base pair.

Without invoking rare tautomeric forms of the bases guanine and purin-2-amine, we propose the following $^{\beta 7}$**ap**·G and the $^{\alpha 7}$**ap**·G base pairs (*Fig. 4*), that conform with the general structure of a *Watson-Crick* base-paired duplex. In the former, the purinamine base is rotated into the *syn*-arrangement and is able to form three H-bonds with O(6), N(1), and NH_2 of guanine, whereas in the latter base pair, both bases are in the *anti*-

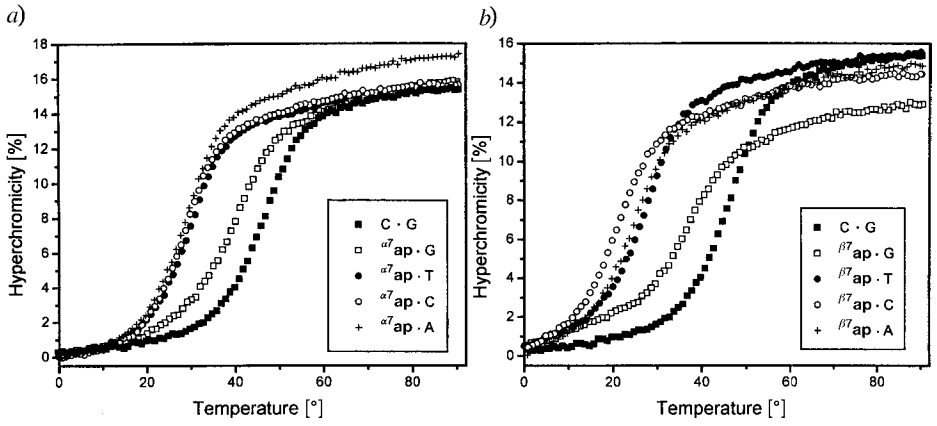
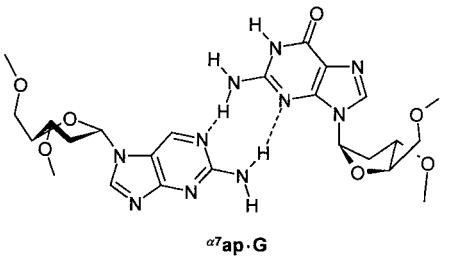


Fig. 3. *UV-Melting curves* (λ 260 nm) *of 1:1 mixtures of* a) **13** (**X** = $^{\alpha 7}$**ap**) *and* b) **14** (**X** = $^{\beta 7}$**ap**), *each with* **16** (**Y** = A), **17** (**Y** = C), **18** (**Y** = G), *and* **19** (**Y** = T). In both cases, the curve of the reference duplex **15**·**18** (see C·G) is also shown. Buffer: 10 mM NaH_2PO_4, 150 mM NaCl, pH 7; c = 4 μM.

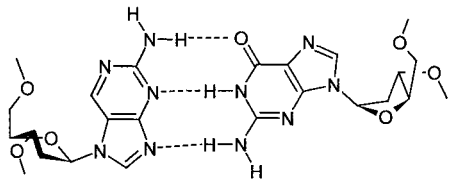
Table 1. *Melting Temperatures* T_m *for Duplexes between 5′-d(GCTA***X***GTCGA)-3′* **13**–**15** *and 5′-d(TCGAC***Y***-TAGC)* **16**–**19**. Buffer: 10 mM NaH_2PO_4, 150 mM NaCl, pH 7, $c = 4$ μM.

	T_m [°]			
	16 (**Y** = A)	**17** (**Y** = C)	**18** (**Y** = G)	**19** (**Y** = T)
13 (**X** = $^{\alpha 7}$**ap**)	30.1	29.5	40.5	31.0
14 (**X** = $^{\beta 7}$**ap**)	20.8	24.3	38.5	28.2
15 (**X** = C)	24.6	16.9	47.2	24.5

arrangement with two H-bonds between the N(3) and NH_2 of guanine, and NH_2 and N(1) of the purin-2-amine base. The *syn*-arrangement of the base in $^{\beta 7}$**ap** is in agreement with the ^{1}H-ROE observations on the monomeric unit β-D-**6** described above.



$^{\alpha 7}$**ap**·**G**



syn-$^{\beta 7}$**ap**·**G**

Fig. 4. *Postulated base pairs between* $^{\alpha 7}$**ap** *and G and between* $^{\beta 7}$**ap** *and G*

5. Molecular Modeling. – The proposed base pairs (*Fig. 4*) were constructed within the duplexes **13**·**18** and **14**·**18** in the B-conformation, and a molecular-dynamics calculation on a 200-ps trajectory at 300 K was performed (for details, see *Exper. Part*). The average MD structure of the duplex **13**·**18** containing the $^{\alpha 7}$**ap**·G base pair is shown in *Fig. 5*,*a*, and the corresponding structure of the reference duplex **15**·**18** containing C·G in *Fig. 5*,*b*. One clear difference between both structures is the strong bend of the duplex induced by a large propeller twist of the $^{\alpha 7}$**ap**·G base pair. The time evolution of the H-bond lengths between the purin-2-amine base and guanine (*Fig. 7*) suggests the N*H*$_2$(G)–N(1)(ap) H-bond ($d_{average} = 2.04$ Å) to contribute more to the base-pair stability than the N(3)(G)–N*H*$_2$(ap) H-bond ($d_{average} = 2.75$ Å). For the duplex **14**·**18** containing the *syn*-$^{\beta 7}$**ap**·G base pair (*Fig. 5*,*c*), the main structural change observed during the dynamics simulation, as compared to the reference duplex **15**·**18**, is a strong widening of the minor groove. This is the consequence of the position that the guanine base needs to adopt to form three H-bonds with $^{\beta 7}$**ap** and can clearly be

demonstrated by aligning the $^{\beta 7}$**ap** · G base pair with the corresponding C · G base pair of the reference duplex **15** · **18** (see *Fig. 6*). Interpretation of the time evolution of the length of the three H-bonds between $^{\beta 7}$**ap** and G (*Fig. 8*) suggests that the $^{\beta 7}$**ap** base only forms two strong H-bonds with the opposite guanine base between N(9)(ap) and NH_2(G) ($d_{\text{average}} = 2.18$ Å), and between N(3)(ap) and *H*N(1)(G) ($d_{\text{average}} = 2.38$ Å). The third H-bond between NH_2(ap) and O(6)(G) ($d_{\text{average}} = 3.02$ Å) is very loose, and probably does not contribute significantly to stability.

6. Discussion and Conclusions. – The general convergent approach for the synthesis of purin-2-amine nucleosides and of the corresponding phosphoramidites presented here allows a rapid and efficient access to the four possible isomers, in particular to the hitherto unknown N^7-isomers. The preliminary UV denaturation experiments with

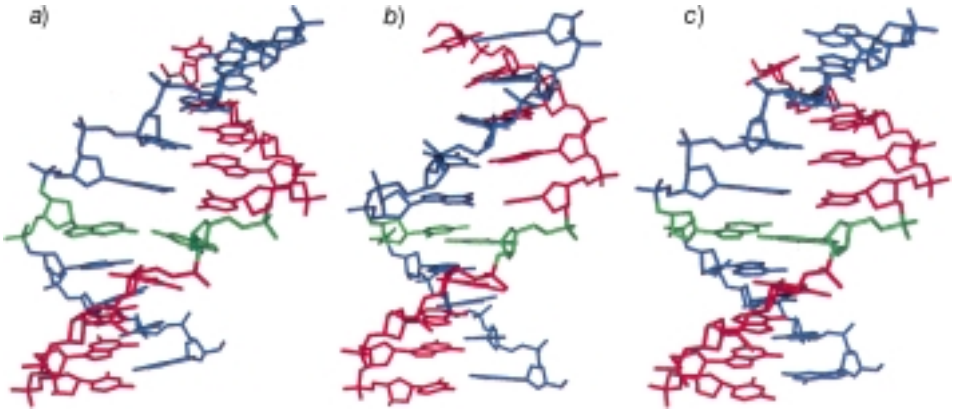


Fig. 5. *Time-averaged structures over the last 50 ps of the duplexes* a) **13** · **18** *and* c) **14** · **18** *containing the* **ap** · *G base-pairs of* Fig. 4, *and* b) *of the reference duplex* **15** · **18** *containing the* Watson-Crick *C* · *G base pair*

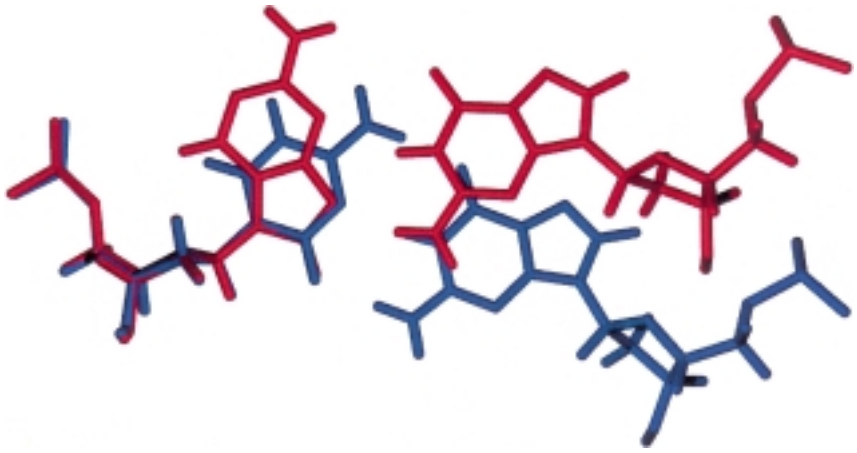


Fig. 6. *Superposition of* $^{\beta 7}$**ap** · *G and C* · *G base pairs from the duplexes* **14** · **18** *and* **15** · **18**

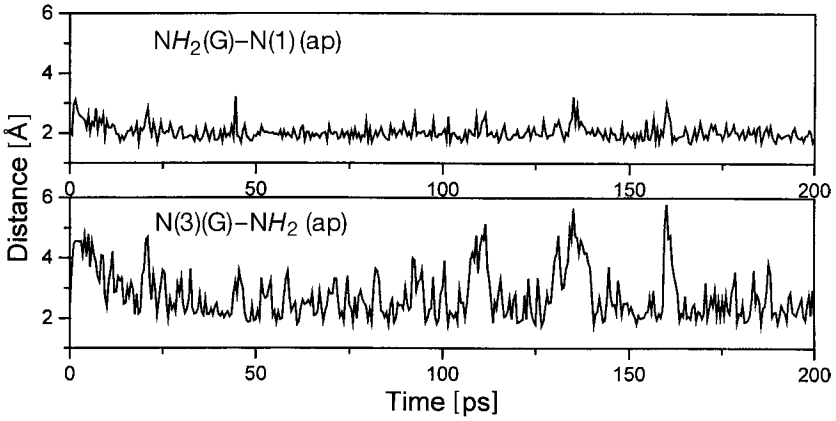


Fig. 7. *Time evolution of the H-bond distances between* $^{\alpha 7}$**ap** *and G during the MD simulation*

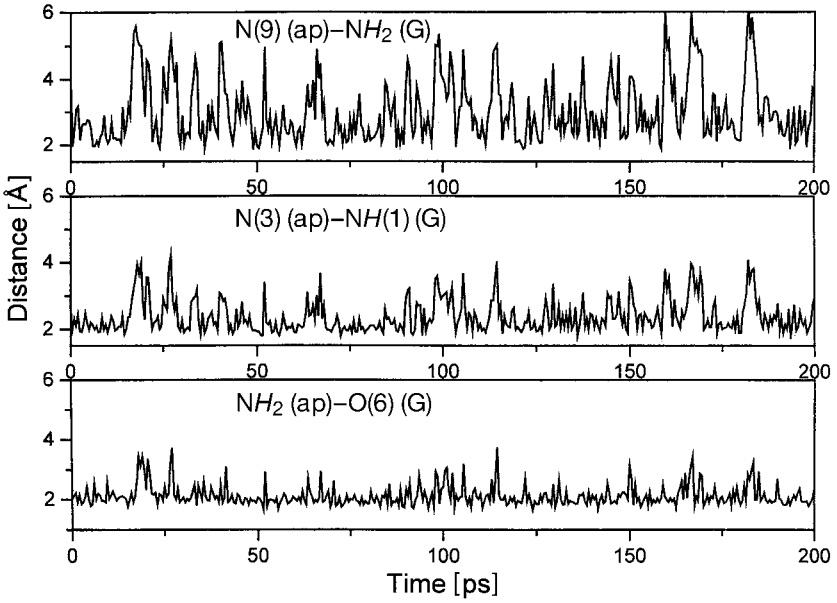


Fig. 8. *Time evolution of the H-bond distances between* $^{\beta 7}$**ap** *and G during the MD simulation*

both N^7-isomers showed a clear preference of these nucleotides for a guanine base within a *Watson-Crick* DNA duplex, although pairing with thymine and cytosine could also have been expected. With this, potential uses as a 'universal' base in PCR-based diagnostic assays can be ruled out. It remains, however, open whether this base may prove useful as a constituent of a novel unnatural DNA base pair for the extension of the genetic alphabet [16].

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Experimental Part

General. FC = Flash chromatography. Solvents for extraction: technical grade, distilled. Solvents for reactions: reagent grade, distilled over CaH_2 (MeCN) or Na (THF, Py). Reagents were from *Fluka*, highest quality available, except for 2-cyanoethyl diisopropyl phosphoramidochloridite, which was from *Sigma*, and for 6-chloro-9*H*-purin-2-amine, which was a gift from *Lonza Group*. N^2-Acetyl-protected-6-chloro-9*H*-purin-2-amine **1** (*N*-(6-chloro-9*H*-purin-2-yl)acetamide) was prepared according to [8] and methyl 3,5-di-*O*-acetyl-2-deoxy-α/β-D-ribofuranoside (**2**) according to [14]. HPLC: *Äkta Basic 10* system from *APBiotech*; t_R in min. NMR: δ in ppm, ^{13}C multiplicities from DEPT spectra, J in Hz; δ(P) rel. to PPh_3 as external standard; T-ROESY: *Bruker AMX-400* with 220 ms mixing time; 1024 (ω_2) × 512 (ω_1) data points acquired, 16 scans per FID, 4789.3 Hz spectral width in both dimensions; phase-sensitive (TPPI); transformed after multiplication with a squared sine-bell function shifted by $\pi/2$ in ω_2 and ω_1 to give a 512 × 512 data matrix. Liquid secondary-ion mass spectrometry (LSI-MS): Cs^+ (25 keV); matrix: 3-nitrobenzyl alcohol, unless otherwise indicated. ESI-MS: *Fisons Instruments VG* platform.

N-*[6-Chloro-7-(3′,5′-di*-O-*acetyl-2′-deoxy-α/β-*D-*ribofuranosyl)-7*H-*purin-2-yl]acetamide* (α/β-D-**3**). To a suspension of **1** (500 mg, 2.4 mmol) in abs. MeCN (20 ml), *N*,*O*-bis(trimethylsilyl)acetamide (BSA; 1.67 ml, 7.15 mmol, 3 equiv.) was added. The mixture was stirred for 1 h at 60°, upon which **1** dissolved completely. After cooling at 0°, **2** (500 mg, 2.15 mmol) was added, followed by $SnCl_4$ (1.95 ml, 16.7 mmol, *ca*. 7 equiv.) in four portions within 30 min. The cooling bath was removed and the soln. stirred for 2.5 h. CH_2Cl_2 (100 ml) was added and the mixture washed with sat. NaCl (50 ml), sat. $NaHCO_3$ (50 ml) and again sat. NaCl soln. (50 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the residue (822 mg) purified by FC (silica gel (50 g) AcOEt/MeOH 20:1): α/β-D-**3** 1:1 (685 mg, 77%). White foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.40. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.92, 2.08, 2.13, 2.13 (4*s*, 2 AcO); 2.53, 2.75, 2.94 (3*m*, 2 H–C(2′)); 2.59 (*s*, AcN); 4.27 (*m*, H–C(5′)); 4.38 (*m*, 1 H–C(5′)); 4.41, 4.69 (2*m*, H–C(4′)); 5.30 (*m*, H–C(3′)); 6.70, 6.75 (*t*, J = 6.3, *dd*, J = 1.2, 6.9, H–C(1′)); 8.04 (br., NH); 8.42, 8.54 (2*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.7, 20.8 (2*q*, *Me*COO)); 25.1 (*q*, *Me*CON); 40.3, 40.3 (2*t*, C(2′)); 63.2, 63.4, (2*t*, C(5′)); 73.4, 74.2 (2*d*, C(3′)); 83.1, 85.3 (2*d*, C(1′)); 86.6, 88.2 (2*d*, C(4′)); 118.0 (*s*, C(5)); 143.0 (*s*, C(4)); 146.1, 146.8 (2*d*, C(8)); 152.7 (*s*, C(6)); 163.8 (*s*, C(2)); 169.6, 169.9, 170.2, 171.0 (4*s*, C=O). HR-LSI-MS: 412.10245 ($C_{16}H_{19}N_5O_6Cl^+$, $[M+H]^+$; calc. 412.10239).

N-*[7-(3′,5′-Di*-O-*acetyl-2′-deoxy-α/β-*D-*ribofuranosyl)-7*H-*purin-2-yl]acetamide (α/β-D-**4**). To a soln. of α/β-D-**3** (685 mg, 1.65 mmol) in AcOEt (25 ml), Et_3N (300 µl) and 10% Pd/C (800 mg) were added. The suspension was stirred at r.t. under H_2 for 3 h and then filtered through a *Celite* pad, the filtrate evaporated, and the residue submitted to FC (silica gel (40 g), AcOEt/MeOH 10:1): α/β-D-**4** 1:1 (526 mg, 84%). White foam. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.39. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.99, 2.02, 2.05, 2.14, 2.12 (5*s*, 2 AcO); 2.58 (*s*, AcN); 2.63–2.74, 2.90–3.00 (2*m*, 2 H–C(2′)); 4.28, 4.33 (2*m*, 2 H–C(5′)); 4.37, 4.47 (2*m*, H–C(4′)); 5.34 (*m*, H–C(3′)); 6.26, 6.37 (*dd*, J = 5.8, 7.9, *dd*, J = 1.9, 7.0, H–C(1′)); 8.31, 8.33 (2*s*, H–C(8)); 8.78 (br. *s*, NH)); 8.99, 9.01 (2*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.7, 20.8, 20.9 (3*q*, *Me*COO); 25.2 (*q*, *Me*CON); 37.9, 38.2 (2*t*, C(2′)); 63.4 (*t*, C(5′)); 73.7, 74.0 (2*d*, C(3′)); 82.8, 84.2 (2*d*, C(1′)); 86.3, 86.9 (2*d*, C(4′)); 121.0, 120.7 (2*s*, C(5)); 142.1, 142.2 (2*d*, C(6)); 146.0, 146.1 (2*d*, C(8)); 153.9 (*s*, C(2)); 162.4, 163.2 (2*s*, C(4)); 170.3 (*s*, C=O). HR-LSI-MS: 378.14120 ($C_{16}H_{20}N_5O_6^+$, $[M+H]^+$; calc. 378.14136).

N-*[7-(2′-Deoxy-α/β-*D-*ribofuranosyl)-7*H-*purin-2-yl]acetamide* (α/β-D-**5**). At −20°, a soln. of α/β-D-**4** (526 mg, 1.4 mmol) in conc. NH_3 in MeOH (20 ml) was stirred overnight, allowing the temperature to increase to r.t. Evaporation furnished a white product, which was lyophilized from H_2O (20 ml): α/β-D-**5** (447 mg, *ca*. 95–100%), contaminated with 10% (*w*/*w*) of acetamide (by NMR). White powder. TLC (CH_2Cl_2/MeOH 5:1): R_f 0.32. ^{1}H-NMR (300 MHz, CD_3OD): 1.98 (*s*, *ca*. 1.5 H, *Me*$CONH_2$); 2.33 (*s*, 3 H, AcN); 2.54–2.91 (*m*, 2 H, 2 H–C(2′)); 3.70–3.82 (*m*, 2 H, 2 H–C(5′)); 4.08–4.10, 4.23–4.30 (2*m*, 1 H, H–C(4′)); 4.51–4.61 (2*m*, 1 H, H–C(3′)); 6.48 (*m*, 1 H, H–C(1′)); 8.78, 8.80 (2*s*, 1 H, H–C(8)); 9.15, 9.20 (2*s*, 1 H, H–C(6)). ^{13}C-NMR (100 MHz, CD_3OD, long relaxation time): 24.7 (*q*, *Me*CO); 41.8, 42.0 (2*t*, C(2′)); 63.0, 63.6 (2*t*, C(5′)); 72.4, 72.8 (2*d*, C(3′)); 88.4, 89.0 (2*d*, C(1′)); 89.2, 89.8 (2*d*, C(4′)); 122.9, 123.2 (2*s*, C(5)); 144.1, 144.5 (2*d*, C(6)); 149.3, 149.6 (2*d*, C(8)); 154.9, 155.0 (2*s*, C(2)); 163.2 (*s*, C(4)); 172.7 (*s*, C=O). HR-LSI-MS: 294.12045 ($C_{12}H_{16}N_5O_4^+$, $[M+H]^+$; calc. 294.12023).

N-*{7-[5′*-O-*(Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-α,β-*D-*ribofuranosyl}-7*H-*purin-2-yl}acetamide* (α/β-D-**6**). The mixture α/β-D-**5** (234 mg, 0.8 mmol) was co-evaporated twice with abs. Py (10 ml), dissolved in abs. Py (5 ml), and cooled to 0°. $(MeO)_2$TrCl (360 mg, 1.05 mmol, 1.3 eq.) was added portionwise within 3 h. The mixture was allowed to warm to r.t. and stirred overnight. H_2O (5 ml) was added, the mixture evaporated, the residue partitioned between sat. NaCl soln. (100 ml) and AcOEt (3 × 100 ml), and the combined org. phase

dried (Na_2SO_4) and evaporated. The two anomers were separated by two consecutive FCs (1st column: silica gel (100 g), CH_2Cl_2/MeOH 19:1; 2nd column: silica gel (50 g), CH_2Cl_2/MeOH 25:1): α-D-**6** (190 mg, 40%) and β-D-**6** (104 mg, 22%), both as slightly yellow foams.

Data of α-D-**6**: TLC (CH_2Cl_2/MeOH 10:1): R_f 0.43. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.61 (*s*, AcN); 2.95 (*m*, 2 H–C(2′)); 3.27 (*m*, 2 H–C(5′)); 3.80 (*s*, 2 MeO); 4.61 (*m*, H–C(4′)); 4.68 (*m*, H–C(3′)); 6.35 (*m*, H–C(1′)); 6.85 (*d*, *J* = 8.9, 4 arom. H); 7.23–7.45 (*m*, 9 arom. H); 8.40 (*s*, H–C(8)); 9.09 (*s*, H–C(6)); 10.9 (br., NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.5 (*q*, *Me*CON); 41.4 (*t*, C(2′)); 55.2 (*q*, MeO); 64.0 (*t*, C(5′)); 72.6 (*d*, C(3′)); 86.6 (*s*, Ar_2C(Ph)); 87.8 (*d*, C(1′)); 89.4 (*d*, C(4′)); 113.3 (*d*, arom. CH); 122.6 (*s*, C(5)); 127.0, 128.0, 128.1, 130.0 (4*d*, arom. CH); 135.6, 135.7 (2*s*, arom. C); 141.9 (*d*, C(6)); 144.9 (*s*, arom. C); 147.1 (*d*, C(8)); 153.8 (*s*, C(2)); 158.6 (*s*, arom. C); 160.7 (*s*, C(4)); 172.1 (*s*, C=O). HR-LSI-MS: found: 596.25085 ($C_{33}H_{34}N_5O_6^+$, $[M+H]^+$; calc. 596.25091).

Data of β-D-**6**: TLC (CH_2Cl_2/MeOH 10:1): R_f 0.37. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.47 (*s*, AcN); 2.68 (*m*, 2 H–C(2′)); 3.34 (*m*, 2 H–C(5′)); 3.69 (*s*, 2 MeO); 4.21 (*m*, H–C(4′)); 4.29 (br., 0.5 H, OH–C(3′)); 4.83 (*m*, H–C(3′)); 6.31 (*t*, *J* = 6.2, H–C(1′)); 6.68 (*d*, *J* = 8.8, 4 arom. H); 7.09–7.28 (*m*, 9 arom. H); 8.30 (*s*, H–C(8)); 9.05 (*s*, H–C(6)); 9.66 (*s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.1 (*q*, *Me*CON); 40.6 (*t*, C(2′)); 55.1 (*q*, MeO); 63.3 (*t*, C(5′)); 71.4 (*d*, C(3′)); 86.2 (*d*, C(1′)); 86.3 (*d*, C(4′)); 86.4 (*s*, Ar_2C(Ph)); 113.1 (*d*, arom. CH); 120.8 (*s*, C(5)); 126.9, 127.8, 127.9, 129.9 (4*d*, arom. CH); 135.4, 135.7 (2*s*, arom. C); 142.5 (*d*, C(6)); 144.3 (*s*, arom. C); 146.7 (*d*, C(8)); 153.7 (*s*, C(2)); 158.5 (*s*, arom. C); 162.1 (*s*, arom. C); 171.7 (*s*, C=O). HR-LSI-MS: 596.25140 ($C_{33}H_{34}N_5O_6^+$, $[M+H]^+$; calc. 596.25091).

N-*{7-[5′*-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-*α-D-*ribofuranosyl]-7*H-*purin-2-yl}acetamide 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (α-D-**7**). Compound α-D-**6** (200 mg, 0.34 mmol) was co-evaporated twice with abs. THF (10 ml), dissolved in abs. THF (10 ml), and cooled to 0°. *Hünig*'s base (288 µl, 1.68 mmol, 5 equiv.) was added, followed by 2-cyanoethyl diisopropylphosphoramidochloridite (149 µl, 0.67 mmol, 2 equiv.). The soln. was stirred for 15 min at 0° and then allowed to warm to r.t. After 1.5 h, the reaction was quenched with MeOH (3 ml), the mixture evaporated, the residue dissolved in AcOEt (20 ml), the soln. washed twice with sat. $NaHCO_3$ soln. (30 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the crude product (321 mg) purified by FC (silica gel (30 g), CH_2Cl_2 containing 2% MeOH): α-D-**7** (240 mg, 89%). White foam after precipitation from hexane. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.47. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.98 (*dd*, *J* = 4.1, 6.8, 1 Me_2CH); 1.08 (*dd*, *J* = 3.9, 6.7, 1 Me_2CH); 2.36, 2.54 (2*t*, *J* = 6.2, CH_2CH_2CN); 2.57 (*s*, AcN); 2.70–3.05 (*m*, 2 H–C(2′)); 3.18 (*m*, 1 Me_2CiH); 3.27–3.65 (*m*, 1 Me_2CH, 2 H–C(5′), CH_2CH_2CN); 3.77, 3.78 (2*s*, 2 MeO); 4.35, 4.50 (2*m*, H–C(4′)); 4.65 (*m*, H–C(3′)); 6.35 (*dd*, *J* = 2.3, 7.0, H–C(1′)); 6.81–6.85 (2*d*, *J* = 3.3, 4 arom. H); 7.18–7.42 (*m*, 9 arom. H); 8.43, 8.50 (2*s*, H–C(8)); 8.61 (br., NH); 8.98 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.1, 20.2, 20.3, 20.4 (4*t*, CH_2CH_2CN); 24.3, 24.3, 24.4, 24.4, 24.5 (5*q*, Me_2CH); 25.1 (*q*, *Me*CON); 39.7, 40.5 (2*t*, C(2′)); 43.1, 43.3 (2*d*, Me_2CH); 55.2 (*q*, MeO); 57.7, 57.8, 57.9, 58.1 (*t*, CH_2CH_2CN); 63.4, 63.6 (2*t*, C(5′)); 74.3, 75.0 (2*d*, C(3′)); 86.6 (*s*, Ar_2C(Ph)); 87.3, 87.5, 87.6, 87.7 (4*d*, C(1′), C(4′)); 113.2 (*d*, arom. C); 117.3, 117.6 (2*s*, C(5)); 121.2, 121.3 (2*s*, CN); 126.9, 127.0, 127.9, 128.0, 130.0, 130.0 (6*d*, arom. C); 135.5, 135.6, 135.6 (3*s*, arom. C); 141.9, 142.1 (2*d*, C(6)); 144.4 (*s*, arom. C); 146.9, 147.1 (2*d*, C(8)); 153.4, 153.5 (2*s*, arom. C), C(2)); 158.6 (*s*, arom. C); 162.3, 162.3 (2*s*, C(4)); 171.4 (*s*, C=O). ^{31}P-NMR (81 MHz, $CDCl_3$): 149, 150. HR-LSI-MS: 796.35944 ($C_{42}H_{51}N_7O_7P^+$, $[M+H]^+$; calc. 796.35876).

N-*{7-[5′*-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-*β-D-*ribofuranosyl]-7*H-*purin-2-yl}acetamide 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (β-D-**7**). To a soln. of β-D-**6** (86 mg, 140 µmol; co-evaporated twice with abs. THF (15 ml)) in abs. THF (10 ml), *Hünig*'s base (124 µl, 720 µmol, 5 eqiv.) and 2-cyanoethyl-diisopropylphosphoramidochloridite (64 µl, 290 µmol, *ca.* 2 equiv.) were added at 0°. The mixture was stirred for 15 min at 0° and then allowed to warm to r.t. After 1 h, more 2-cyanoethyl diisopropylphosphoramidochloridite (32 µl, 145 µmol, *ca.* 1 equiv.) was added, and the mixture was stirred for 2 more h. Then MeOH (2 ml) was added, the mixture evaporated, the crude product dissolved in AcOEt (20 ml), and the soln. washed with sat. $NaHCO_3$ soln. (2 × 20 ml). The aq. layers were re-extracted with AcOEt (20 ml) and the combined org. phases dried (Na_2SO_4) and evaporated. The residue was purified by FC (silica gel (15 g), CH_2Cl_2/MeOH 50:1) and the product then dissolved in CH_2Cl_2 (3 ml) and precipitated in cold hexane (100 ml): β-D-**7** (87 mg, 78%). Slightly beige foam. TLC (CH_2Cl_2/MeOH 10:1): R_f 0.42. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.09–1.19 (*m*, 2 Me_2CH); 2.46, 2.61 (2*t*, *J* = 6.2, CH_2CH_2CN); 2.56, 2.56 (2*s*, AcN); 2.66–2.73 (*m*, 2 H–C(2′)); 3.30–3.36 (*m*, 2 H–C(5′)); 3.54–3.77 (*m*, 2 Me_2CH, CH_2CH_2CN); 3.74, 3.75 (2*s*, 2 MeO); 4.26–4.35 (*m*, H–C(4′)); 4.69 (*m*, H–C(3′)); 6.26 (*m*, H–C(1′)); 6.75 (*m*, 4 arom. H); 7.18–7.33 (*m*, 9 arom. H); 7.28, 7.28 (2*s*, H–C(8)); 8.63 (br. *s*, NH); 8.92 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.2, 20.3, 20.4, 20.5 (4*t*, CH_2CH_2CN); 24.5, 24.5, 24.6, 24.6, 24.6, 24.7 (6*q*, Me_2CH); 25.1 (*q*, *Me*CON); 40.1, 40.1, 40.2 (3*t*, C(2′)); 43.3 (*dd*, *J*(C,P) = 12.4, Me_2CH); 43.4 (*dd*, *J*(C,P) = 12.4, Me_2CH); 55.2, 55.2 (2*q*, 2 MeO); 58.1 (*dt*, *J*(C,P) = 9.6, CH_2CH_2CN); 58.3 (*dt*, *J*(C,P) = 9.3,

CH_2CH_2CN); 63.1, 63.1 (2*t*, C(5′)); 73.4 (*dd*, *J*(C,P) = 16.5, C(3′)); 73.9 (*dd*, *J*(C,P) = 18.1, C(3′)); 86.0 (*dd*, *J*(C,P) = 6.9, C(4′)); 86.3 (*dd*, *J*(C,P) = 4.0, C(4′)); 86.3, 86.4 (2*d*, C(1′)); 86.7, 86.7 (2*s*, Ar_2C(Ph)); 113.2 (*d*, arom. C); 117.4, 117.6 (2*s*, CN); 121.0, 121.0 (2*s*, C(5)); 127.0, 127.0, 127.9, 128.0, 129.9, 130.0, 130.0 (7*d*, arom. C); 135.3, 135.3 (2*s*, arom. C); 142.3, 142.4 (2*d*, C(6)); 144.2 (*s*, arom. C); 146.1, 146.2 (2*s*, C(8)); 153.7, 153.8 (2*s*, C(2)); 158.6, 158.6 (2*s*, arom. C); 162.4 (*s*, C(4)); 171.6, 171.9 (2*s*, C=O). ^{31}P-NMR (81 MHz, $CDCl_3$): 148.6, 148.8.

N-*[6-Chloro-9-(3′,5′-di*-O-*acetyl-2′-deoxy-α,β*-D-*ribofuranosyl)-9*H-*purin-2-yl]acetamide* (*α/β*-D-**8**). To a suspension of **1** (1 g, 4.8 mmol) in abs. MeCN (20 ml), BSA (2.3 ml, 9.5 mmol, 2 equiv.) was added and the resulting soln. stirred at 60° for 1 h. After the addition of **2** (1.3 g, 5.6 mmol, 1.2 equiv.) and $Me_3SiOSO_2CF_3$ (1.7 ml, 9.5 mmol, 2 equiv.), the soln. was stirred at 60° for 3 h. The mixture was diluted in CH_2Cl_2 (200 ml) and washed with sat. NaCl (100 ml), sat. $NaHCO_3$ (100 ml), and sat. NaCl soln.(100 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue (1.6 g) purified by FC (silica gel (100 g), AcOEt/hexane 20 : 1): *α/β*-D-**8** (790 mg, 40%). TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.42. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.98, 2.05, 2.11, 2.13 (4*s*, 2 AcO); 2.51 (*s*, AcN); 2.58–2.66, 2.69–2.76, 2.87–2.98 (3*m*, 2 H–C(2′)); 4.21–4.28, 4.37–4.41 (2*m*, 2 H–C(5′)); 4.35, 4.62 (*m*, *dt*, *J* = 2.1, 4.3, H–C(4′)); 5.32, 5.44 (2*m*, H–C(3′)); 6.36, 6.47 (*dd*, *J* = 6.1, 7.4, *dd*, *J* = 2.0, 7.2, H–C(1′)); 8.05 (br., NH); 8.14, 8.25 (2*s*, H–C(8)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.7, 20.8 (2*q*, *Me*COO); 25.0, 25.1 (2*q*, *Me*CON); 37.3, 37.9 (2*t*, C(2′)); 63.5, 63.6 (2*t*, C(5′)); 74.1 (*d*, C(3′)); 82.7, 85.0 (2*d*, C(1′)); 84.2, 85.5 (2*d*, C(4′)); 128.3, 128.6 (2*s*, C(5)); 142.4, 142.7 (2*d*, C(8)); 143.9, 144.1 (2*s*, C(4)); 151.4 (*s*, C(6)); 151.9, 152.0 (2*s*, C(2)); 169.9, 170.2, 170.3 (3*s*, C=O).

N-*[9-(3′,5′-Di*-O-*acetyl-2′-deoxy-α/β*-D-*ribofuranosyl-9*H-*purin-2-yl]acetamide* (*α/β*-D-**9**). To a soln. of *α/β*-D-**8** (430 mg, 1.05 mmol) in MeOH (15 ml), Et_3N (175 µl, 1.25 mmol) and 10% Pd/C (525 mg, 500 mg/mmol) were added. The mixture was stirred under H_2 for 4 h and then filtered through *Celite*, the filtrate evaporated, and the residue (520 mg) purified by FC (silica gel (25 g), CH_2Cl_2/MeOH 20 : 1): *α/β*-D-**9** (286 mg, 73%). TLC (CH_2Cl_2/MeOH 10 : 1): R_f 0.55. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.97, 2.05, 2.10, 2.12 (4*s*, 2 AcO); 2.46, 2.52 (2*s*, AcN); 2.58–2.65, 2.70–2.79, 2.88–3.02 (3*m*, 2 H–C(2′)); 4.24–4.44, 4.6 (2*m*, 2 H–C(5′), H–C(4′)); 5.32, 5.44 (*dt*, *J* = 2.1, 7.7, *m*, H–C(3′)); 6.39, 6.51 (*dd*, *J* = 6.2, 7.7, *dd*, *J* = 2.2, 7.1, H–C(1′)); 8.11, 8.23 (2*s*, H–C(8)); 8.53 (br. *s*, NH); 8.94, 8.96 (2*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.8, 20.8, 20.9 (3*q*, *Me*COO); 25.1, 25.2 (2*q*, *Me*CN); 37.1, 37.8 (2*t*, C(2′)); 63.6, 63.6 (2*t*, C(5′)); 74.2, 74.3 (2*d*, C(3′)); 82.6, 84.0, (2*d*, C(1′)); 84.6, 85.0 (2*d*, C(4′)); 131.0, 131.3 (2*s*, C(5)); 142.5, 142.8 (2*d*, C(8)); 149.7, 149.9 (2*d*, C(6)); 151.6 (C(4)); 152.9, 153.0 (2*s*, C(2)); 170.0, 170.2, 170.4, 170.5 (4*s*, C=O). HR-LSI-MS: 378.14154 ($C_{16}H_{20}N_5O_6^+$, $[M+H]^+$; calc. 378.14136).

N-*[9-(2′-Deoxy-α,β*-D-*ribofuranosyl)-9*H-*purin-2-yl]acetamide* (*α/β*-D-**10**). As described for *α/β*-D-**5**, from *α/β*-D-**9** (244 mg, 0.65 mmol): *α/β*-D-**10** (189 mg, 99%), which was used without further purification. TLC (CH_2Cl_2/MeOH 5 : 1): R_f 0.54. ^{1}H-NMR (300 MHz, CD_3OD): 2.34 (*s*, AcN); 2.54, 2.89 (2*m*, 2 H–C(2′)); 3.70–3.85 (*m*, 2 H–C(5′)); 4.06, 4.41–4.68 (2*m*, H–C(3′), H–C(4′)); 6.59 (*m*, H–C(1′)); 8.59 (br. NH); 8.69, 8.73 (2*s*, H–C(8)); 8.94 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, CD_3OD): 25.0 (*q*, *Me*CON); 41.4, 41.5 (2*t*, C(2′)); 63.3, 63.6 (2*t*, C(5′)); 72.5, 73.0 (2*d*, C(3′)); 85.9, 86.6 (2*d*, C(1′)); 89.6, 90.7 (2*d*, C(4′)); 132.2 (*s*, C(5)); 146.3, 146.8 (2*d*, C(8)); 149.9, 150.0 (2*d*, C(6)); 153.1 (*s*, C(2)); 154.3 (C(4)); 172.5, 172.7 (2*s*, C=O).

N-*{9-[5′*-O-*(Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-α/β*-D-*ribofuranosyl}-9*H-*purin-2-yl}acetamide* (*α/β*-D-**11**). As described for *α/β*-D-**6**; from *α/β*-D-**10** (131 mg, 0.45 mmol), in abs. Py (15 ml), and $(MeO)_2$TrCl (195 mg, 0.58 mmol, 1.3 equiv.), after two FCs (CH_2Cl_2/MeOH 20 : 1): *α*-D-**11** (100 mg, 38%) and *β*-D-**11** (66 mg, 25%) as slightly yellow foams.

Data of α-D-**11**: TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.16. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.33 (*s*, AcN); 2.75, 3.05 (2*m*, 2 H–C(2′)); 3.21, 3.35 (2*m*, 2 H–C(5′)); 3.76 (*s*, 2 MeO); 4.49 (*m*, H–C(3′)); 4.54 (*m*, H–C(4′)); 4.90 (br., OH–C(3′)); 6.30 (*dd*, *J* = 2.8, 8.7, H–C(1′)); 6.81 (*d*, *J* = 8.9, 4 arom. H); 7.19–7.42 (*m*, 9 arom. H); 8.19 (*s*, H–C(8)); 8.66 (br. *s*, NH); 8.93 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.0 (*q*, *Me*CN); 40.3 (*t*, C(2′)); 55.2 (*q*, MeO); 64.3 (*t*, C(5′)); 73.1 (*d*, C(3′)); 86.3 (*d*, C(1′)); 88.0 (*d*, C(4′)); 113.2 (*d*, arom. C); 126.7, 127.9, 128.1, 130.0 (4*d*, arom. C); 132.0 (*s*, C(5)); 135.8, 135.9 (2*s*, arom. C); 144.7 (*s*, arom. C); 145.3 (*d*, C(8)); 149.8 (*d*, C(6)); 151.3 (*s*, C(2)); 152.2 (*s*, C(4)); 158.6 (*s*, arom. C). HR-LSI-MS 596.25092 ($C_{33}H_{34}NO_6^+$, $[M+H]^+$; calc. 596.25091).

Data of β-D-**11**: TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.14. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (*s*, *Me*CON); 2.64 (*m*, 2 H–C(2′)); 3.37 (*m*, 2 H–C(5′)); 3.71 (*s*, 2 MeO); 4.27 (*m*, H–C(4′)); 4.74 (*m*, H–C(3′)); 6.63 (*m*, H–C(1′)); 6.72–6.80 (*d*, *J* = 8.6, 4 arom. H); 7.11–7.27 (*m*, 7 arom. H); 7.34–7.36 (*d*, *J* = 7.4, 2 arom. H); 8.17 (*s*, H–C(8)); 9.10 (*s*, H–C(6)); 10.23 (br. *s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 25.0 (*q*, *Me*CON); 40.9 (*t*, C(2′)); 55.2 (*q*, MeO); 64.0 (*t*, C(5′)); 72.5 (*d*, C(3′)); 84.5 (*d*, C(1′)); 86.6 (*d*, C(4′)); 113.1 (*d*, arom. C); 126.9, 127.8, 128.1, 130.0 (4*d*, arom. C); 131.0 (*s*, C(5)); 135.6, 135.6 (2*s*, arom. C); 143.1 (*d*, C(8)); 144.5 (*s*, arom. C);

149.3 (*d*, C(6)); 151.8 (*s*, C(4)); 152.9 (*s*, C(2)); 158.5 (*s*, arom. C); 171.3 (*s*, C=O). HR-LSI-MS: 596.25067 ($C_{33}H_{34}N_5O_6^+$, $[M+H]^+$; calc. 596.25091).

N-*{9-[5′*-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-α*-D-*ribofuranosyl]-9*H-*purin-2-yl}acetamide 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (*α*-D-**12**). As described for *α*-D-**7**; from *α*-D-**11** (33 mg; 55.4 μmol) *Hünig*'s base (48 μl, 277 μmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (25 μl, 111 μmol). FC (silica gel (15 g), CH_2Cl_2 with 2% MeOH) and precipitation from hexane gave *α*-D-**12** (38 mg, 87%). White foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.35. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.00–1.26 (*m*, 2 Me_2CH); 2.39, 2.49 (2*t*, *J* = 2.1, CH_2CH_2CN); 2.51, 2.52 (2*s*, AcN); 2.54, 2.76 (2*m*, 2 H–C(2′)); 2.92 (*m*, 1 Me_2CH); 3.18–3.66 (*m*, 1 Me_2CH, 2 H–C(5′), CH_2CH_2CN); 3.77, 3.78 (2*s*, 2 MeO); 4.51, 4.59 (*m*, H–C(4′)); 4.64 (*m*, H–C(3′)); 6.58 (*m*, H–C(1′)); 6.81–6.85 (*dd*, *J* = 2.9, 9.0, 4 arom. H); 7.21–7.43 (*m*, 9 arom. H); 8.42 (br., NH); 8.47, 8.50 (2*s*, H–C(8)); 8.91 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.1, 20.2, 20.3 (3*t*, CH_2CH_2CN); 24.3, 24.4, 24.5, 24.6, 24.6 (5*q*, Me_2CH); 25.1 (*q*, *Me*CN); 40.0, 40.5 (2*t*, C(2′)); 43.2, 43.4 (2*d*, Me_2CH); 55.2 (*q*, MeO); 57.8, 58.0, 58.3 (*t*, ^3J(C,P) = 9.5, CH_2CH_2CN); 63.7 (*t*, C(5′)); 74.7, 75.0, 75.2 (3*d*, (C(3′)); 84.9, 84.9 (2*s*, Ar_2C(Ph)); 87.5, 87.6, 87.7 (3*d*, C(1′), C(4′)); 113.2 (*d*, arom. C); 117.3, 117.3 (2*s*, CN); 126.9, 127.9, 128.1, 130.0, 130.1 (5*d*, arom. C); 135.6, 135.7, 135.7 (3*s*, C(5), arom. C); 143.7, 143.9 (2*d*, C(8)); 144.5 (*s*, C(2)); 149.3, 149.3 (2*d*, C(6)); 152.8 (*s*, arom. C); 158.6 (*s*, C(4)). ^{31}P-NMR (81 MHz, $CDCl_3$): 148.4, 149.0.

N-*{9-[5′*-O-*[Bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-β*-D-*ribofuranosyl]-9*H-*purin-2-yl}acetamide 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (*β*-D-**12**). As described for *α*-D-**7**; from *β*-D-**11** (86 mg, 144 μmol), *Hünig*'s base (123 μmol, 720 μmol, 5 equiv.), and 2-cyanoethyl diisopropylphosphoramidochloridite (65 μl, 290 μmol, 2 equiv.). FC (silica gel (20 g), CH_2Cl_2/MeOH 50:1) and precipitation from hexane gave *β*-D-**12** (71 mg, 62%). White foam. TLC (CH_2Cl_2/MeOH 20:1): R_f 0.4. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.09–1.18 (*m*, 2 Me_2CH); 2.41 (*s*, AcN); 2.45, 2.62 (2*t*, *J* = 6.2, CH_2CH_2CN); 2.73, 2.84 (2*m*, 2 H–C(2′)); 3.35 (*m*, 2 H–C(5′)); 3.49–3.71 (*m*, 2 $Me_2CHCH_2CH_2CN$); 3.75 (*s*, 2 MeO); 4.26, 4.28 (2*m*, H–C(4′)); 4.70 (*m*, H–C(3′)); 6.41 (*t*, *J* = 6.5, H–C(1′)); 6.75 (*m*, 4 arom. H); 7.18–7.39 (*m*, 9 arom. H); 8.12, 8.14 (2*s*, H–C(8)); 8.51, 8.55 (2br. *s*, NH); 8.95 (*s*, H–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.1, 20.2, 20.2, 20.3, 20.3, 20.4 (6*t*, CH_2CH_2CN); 24.4, 24.4, 24.5, 24.5, 24.5, 24.6, 24.6 (7*q*, Me_2CH); 24.9, 24.9 (2*q*, *Me*CON); 39.6, 39.6 (2*t*, C(2′)); 42.8, 43.0, 43.2, 43.2, 43.3, 43.5 (6*d*, Me_2CH); 55.1, 55.1 (2*q*, MeO); 57.9 (*dt*, *J*(C,P) = 6.1, CH_2CH_2CN); 58.2 (*dt*, *J*(C,P) = 6.1, CH_2CH_2CN); 63.5, 63.5 (2*t*, C(5′)); 73.7 (*dd*, *J*(C,P) = 17.1, C(3′)); 74.2 (*dd*, *J*(C,P) = 17.7, C(3′)); 84.3, 84.4 (2*d*, C(1′)); 85.8 (*dd*, *J*(C,P) = 6.7, C(4′)); 86.0 (*dd*, *J*(C,P) = 3.7, C(4′)); 86.4 (*s*, Ar_2C(Ph)); 113.1 (*d*, arom. C); 117.3, 117.5 (2*s*, CN); 126.9, 126.9, 127.7, 127.8, 128.0, 128.1, 128.5, 129.1, 129.9, 130.0 (10*d*, arom. C); 131.2 (*s*, C(5)); 131.9, 131.9, 132.1 (3*d*, arom. C); 135.4, 135.5 (2*s*, arom. C); 143.1, C(8)); 144.4 (*s*, arom. C); 149.5, 149.6 (2*d*, C(6)); 151.7, 151.7 (2*s*, C(4)); 152.8 (*s*, C(2)); 158.5 (*s*, arom. C); 170.5 (*s*, C=O). ^{31}P-NMR (81 MHz, $CDCl_3$): 139.72 (br. *s*). HR-LSI-MS (2,2′,2″-nitrilotris[ethanol]): 796.35986 ($C_{42}H_{51}N_7O_7P^+$, $[M+H]^+$; calc. 796.35876).

Oligonucleotide Synthesis. Oligonucleotide synthesis was performed on a *Pharmacia Gene Assembler Special* connected to a *Compaq ProLinea 3/25-zs PC*. All syntheses were performed with the 1,3-μmol cycle and coupling times of 2 min for the purin-2-amine phosphoramidites. Solvents and solns. were prepared according to the manufacturer's protocol. Phosphoramidite (0.1M in MeCN) solns. were equal in concentration to those used for the synthesis of natural oligodeoxynucleotides. The activator 1*H*-tetrazole was replaced in all syntheses by 5-(benzylthio)-1*H*-tetrazole (0.25M in MeCN) [17]. Average coupling yields, monitored by the on-line trityl assay, were >98% for *α*-D- and *β*-D-**7**.

Deprotection and Purification of Oligonucleotides. Removal of the protecting groups and detachment from the solid support was effected in conc. NH_3 soln. (1–2 ml) at 55° for 18–20 h. The crude oligomers were purified by *MonoQ* ion-exchange HPLC, desalted over *Sep-Pak* (*Waters*), and their purity and composition confirmed by electrospray-MS analysis. *Table 2* contains synthetic and anal. data of the oligonucleotides described here. All natural DNA sequences used in this study were prepared according to standard phosphoramidite chemistry and purified by HPLC.

UV Melting Experiments. They were performed on a *Varian-Cary-3E* UV/VIS spectrometer equipped with a temp. controller unit and connected to a PC running the *Varian* WinUV software, temp. gradient 0.5°/min; data-point collection in intervals of *ca.* 1°; below 20°, the cell compartment was flushed with N_2 to avoid condensation of H_2O on the UV cells; the transition temperature T_m was determined as the maximum of the 1st derivative of the melting curve with the software package *Origin*™ V5.0.

Molecular Modeling. Molecular-modeling calculations were carried out with the Amber forcefield [18] as incorporated in the software package Insight II/Discover 3 V98.0 of *Molecular Simulations, Inc.* on an *SGI Octane* workstation. Only original Amber parameters and potentials were used. No explicit H_2O molecules and counterions were included. A distance-dependent permittivity of $\varepsilon = 4 \cdot r$ was used instead as a screening

Table 2. *Synthesis and Analytical Data of Oligonucleotides* **13**–**19**

Sequence	HPLC[a])		Isolated yield, *OD* (260 nm) ([%])	Electrospray-MS $[M-H]^-$	
	Conditions	t_R [min]		*m/z* (calc.)	*m/z* (found)
13 d(GCTA$^{\beta 7}$**ap**GTCGA)	22–55% *B* in 25 min	27.9	12 (10)	3052.3	3051.5
14 d(GCTA$^{\alpha 7}$**ap**GTCGA)	25–60% *B* in 25 min	16.5	51 (40)	3052.3	3051.4
15 d(GCTACGTCGA)	30–60% *B* in 25 min	28.1	57 (48)	3028.2	3027.4
16 d(TCGACATAGC)	25–70% *B* in 25 min	13.3	69 (56)	3012.2	3011.8
17 d(TCGACCTAGC)	25–70% *B* in 25 min	14.3	62 (54)	2988.2	2987.6
18 d(TCGACGTAGC)	25–55% *B* in 25 min	28.5	45 (38)	3028.2	3027.4
19 d(TCGACTTAGC)	25–70% *B* in 25 min	15.6	76 (65)	3003.2	3002.8

[a]) *Pharmacia MonoQ HR 10/10* ion-exchange column; *A:* 10 mM LiOH, pH 12; *B:* 10 mM LiOH, pH 12, 1M LiCl; flow 2.5 ml/min; detection at 260 nm.

function. 1–4 Nonbonded interactions were scaled by 0.5. No cut-offs were applied. Structures were built on the basis of the parameters of a B-DNA double helix [19]. They were first minimized until the energy gradient was below 0.05 kcal · mol^{-1} · Å^{-1}. Then, the structures were stepwise heated from 0 to 300 K over 20 ps (1 ps at 50 K, 1 ps at 100 K, 2 ps at 150 K, 2 ps at 200 K, 4 ps at 250 K, and 10 ps at 300 K) and submitted to 200 ps of unrestrained molecular dynamics at 300 K. The temp. was controlled by coupling to an external heat bath [20]. One-fs time steps were used in the numerical integration of the equation of motion. For the analysis, coordinates and energy terms were stored every 0.5 ps.

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