Synthesis and Pairing Properties of 3′-Deoxyribopyranose (4′ → 2′)-Oligonucleotides (‘*p*-DNA’)

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The preparation and the pairing properties of the new 3′-deoxyribopyranose (4′ → 2′)-oligonucleotide (= *p*-DNA) pairing system, based on 3′-deoxy-β-D-ribopyranose nucleosides is presented. D-Xylose was efficiently converted to the prefunctionalized 3-deoxyribopyranose derivative 4-*O*-[(*tert*-butyl)dimethylsilyl]-3-deoxy-D-ribopyranose 1,2-diacetate **8** (obtained as a 4:1 mixture of α- and β-D-anomers; *Scheme 1*). From this sugar building block, the corresponding, appropriately protected thymine, guanine, 5-methylcytosine, and purine-2,6-diamine nucleoside phosphoramidites **29**–**32** were prepared in a minimal number of steps (*Schemes 2–4*). These building blocks were assembled on a DNA synthesizer, and the corresponding *p*-DNA oligonucleotides were obtained in good yields after a one-step deprotection under standard conditions, followed by HPLC purification (*Scheme 5* and *Table 1*). Qualitatively, *p*-DNA shows the same pairing behavior as *p*-RNA, forming antiparallel, exclusively *Watson-Crick*-paired duplexes that are much stronger than corresponding DNA duplexes. Duplex stabilities within the three related (*i.e.*, based on ribopyranose nucleosides) oligonucleotide systems *p*-RNA, *p*-DNA, and 3′-*O*-Me-*p*-RNA were compared with each other (*Table 2*). Intrinsically, *p*-RNA forms the strongest duplexes, followed by *p*-DNA, and 3′-*O*-Me-*p*-RNA. However, by introducing the nucleobases purine-2,6-diamine (D) and 5-methylcytosine (M) instead of adenine and cytosine, a substantial increase in stability of corresponding *p*-DNA duplexes was observed.

1. Introduction. – In the context of an ongoing study about the chemical etiology of the natural nucleic acid’s structure, a novel pairing system, based on ribopyranose (4′ → 2′)-oligonucleotides (*p*-RNA) was introduced in 1993 by *Eschenmoser* and co-workers [1–6] (*Fig. 1*)[1]. The pairing properties of this constitutional isomer of RNA

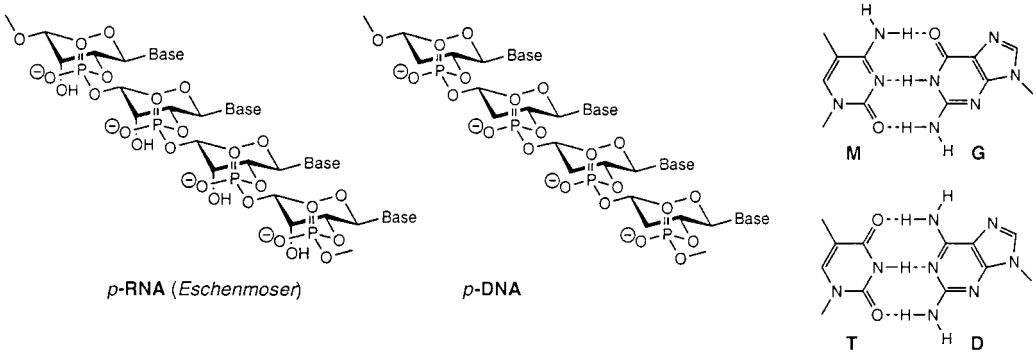

Fig. 1. *Structural representation of the* p-*RNA and the* p-*DNA backbone, shown in their idealized, linear conformations, and base pairs formed between the nucleobases 5-methylcytosine* (M) *and guanine* (G), *and between thymine* (T) *and purine-2,6-diamine* (D)

[1]) For a recent article by *Eschenmoser* and co-workers, which is related to this field, see [7].

differ substantially from those of the natural nucleic acids DNA and RNA: *p*-RNA duplexes are at the same time much stronger and formed more selectively than DNA or RNA duplexes. The almost linear structure of *p*-RNA duplexes and the strong inclination between the backbone and the base pairs leads to a strict antiparallel strand orientation and allows favorable purine-purine intrastrand stacking. Furthermore, no cross-pairing between *p*-RNA and DNA or RNA, respectively, is observed.

In the context of creating functionalized aptamers and ribozymes by chemical synthesis, we wanted to substitute RNA stems and RNA loops by another pairing system, ideally with the same properties as *p*-RNA [8]. Unfortunately, due to the harsh conditions required to remove the 3′-*O*-benzoyl protecting group present in each ribopyranose-nucleotide unit, the synthesis of *p*-RNA oligonucleotides is not compatible with the synthesis of RNA oligonucleotides[2]). Therefore, we developed the herein-reported synthesis of the analogous 3′-deoxyribopyranose (4′→2′)-oligonucleotide pairing system (= *p*-DNA), which is based on 3′-deoxy-β-D-ribopyranose nucleosides (*Fig. 1*)[3]).

Initial pairing studies with such oligonucleotides revealed that *p*-DNA sequences are forming weaker duplexes than corresponding *p*-RNA sequences. However, by replacing the natural nucleobases adenine and cytosine with the analogues purine-2,6-diamine (D) and 5-methylcytosine (M), respectively (*Fig. 1*), a substantial increase in pairing energy was achieved[4]); *p*-DNA duplexes forming G·M and D·T *Watson-Crick* base pairs have almost identical pairing properties as the corresponding G·C and A·T containing *p*-RNA duplexes (see below, *Table 2*).

In this report, the highly convergent synthesis of the four *p*-DNA phosphoramidites containing the nucleobases adenine, thymine, 5-methylcytosine, and purine-2,6-diamine (*Schemes 1–4*), the synthesis of oligonucleotides derived therefrom (*Scheme 5*, *Table 1*), and their pairing properties are reported (*Table 2*, *Fig. 2*).

2. Results. – 2.1. *Sugar Building Block*. In the context of this project, we evaluated different routes to the unnatural sugar 3-deoxyribopyranose. The obvious route included deoxygenation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose derivatives[5]), followed by selective cleavage of the 5,6-isopropylidene protecting group under acidic conditions, $NaIO_4$ cleavage of the resulting glycol, reduction of the resulting aldehyde with $NaBH_4$, and cleavage of the remaining 1,2-isopropylidene group. However, we were unable to prepare or to isolate pure pyranoside derivatives in

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[2]) Deprotection of *p*-RNA oligonucleotides involves hydrazine treatment (10% in H_2O, 4°, 20 h [1]), which causes very fast degradation of uracil-containing nucleosides.

[3]) In the same context, we also evaluated 3′-*O*-methyl-ribopyranose (4′-2′)-oligonucleotides (3′-*O*-Me-*p*-RNA) [9]. Duplexes derived from 3′-*O*-Me-*p*-RNA, however, exhibited much weaker pairing than the corresponding *p*-RNA duplexes and weaker pairing than the corresponding *p*-DNA duplexes (see below, *Table 2*).

[4]) For comparison, the thermodynamic parameters for duplex formation of the *p*-DNA duplexes $A_8 \cdot T_8$ and $D_8 \cdot T_8$ are shown in *Table 2* (see below).

[5]) In the literature, two different methods for this process are described, either reduction of its 3-(*S*-methyl dithiocarbonate) derivative with Bu_3SnH [10], or reduction of its 3-[(trifluoromethyl)sulfonyl] derivative with $(Bu_4N)BH_4$ in refluxing benzene [11]. We could improve the second method even further by performing the reaction in toluene at 100° (98% yield).

reasonable quantities [6]). Therefore, we decided to develop a new synthetic approach, leading not to 3-deoxyribose, but directly to a pyranoside derivative thereof. As in the earlier synthesis of L-ribonucleoside phosphoramidites (from D-glucose) [12], we furthermore aimed at the synthesis of a prefunctionalized sugar building block, containing two acyl groups at O–C(1) and O–C(2) (required for an unambiguous and efficient preparation of β-D-configurated nucleosides by nucleosidation under *Vorbrüggen* conditions [13]) and a trialkylsilyl group at O–C(4), serving as temporary substitute for the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) group.

In *Scheme 1*, the synthesis of the prefunctionalized sugar building block 4-*O*-[(*tert*-butyl)dimethyl)silyl]-3-deoxy-D-ribopyranose 1,2-diacetate (**8**) from benzyl α-D-xylopyranoside (**1**) [14] is presented. Within this sequence of simple reactions, only two purifications (filtrations on silica gel) were required, and the building block **8** was obtained in 50% total yield (0.5-mol scale). Thus, esterification of **1** with phenylboronic acid in refluxing toluene under removal of H_2O (*Dean-Stark* separator) gave the cyclic 2,4-phenylboronate derivative **2**, which was directly transformed into its 3-*O*-mesyl derivative **3** with $MeSO_2Cl/Et_3N$. After filtration of the by-product $Et_3N \cdot HCl$ and evaporation of toluene, **3** was treated with NaOMe in MeOH. Under these conditions, the cyclic phenylboronate was cleaved, and subsequently a 1:1 mixture of the two regioisomeric epoxides **4a** and **4b** was obtained by intramolecular substitution of the 3-mesyloxy group by O–C(2) or O–C(4). After extractive workup, the mixture **4a**/**4b** was submitted to hydride reduction with $LiAlH_4$ in THF. In this reaction, only one product, the 3-deoxyribopyranoside **5**, was obtained, indicating that attack of the hydride occurred in a stereoelectronically favored manner, exclusively at the C(3) position (in both epoxides **4a** and **4b**). The crude product **5** from this sequence of reactions was sufficiently pure (> 95% by ^{1}H-NMR; 90% yield based on **1**) to be used directly for the next step. Treatment of **5** with 1.2 equiv. of ^{t}bdms-Cl ((*tert*-butyl)chlorodimethylsilane) at $-78°$ in CH_2Cl_2 in the presence of $AgNO_3$ and ^{i}PrNEt resulted in the formation of the 4-*O*-silylated derivative **6** and the corresponding 2,4-di-*O*-silylated derivative[7]). The 4-*O*-silylated 3-deoxypyranoside **6** could be easily isolated by filtration over silica gel and was obtained in 68% yield (based on **1**; additionally 11% of the 2,4-di-*O*-silylated derivative was isolated). Removal of the benzyl protecting group of **6** by hydrogenolysis with H_2 and $Pd(OH)_2/C$ in EtOH gave the pyranose derivative **7**, which finally was converted into **8** by acetylation with Ac_2O in pyridine. After filtration through silica gel, the sugar building block **8** was obtained in pure form as a 4:1 mixture of the α- and β-D-anomers, and in 70% yield (based on **6**).

2.2. *Building Blocks for the Assembly of* p-*DNA Oligonucleotides.* In *Schemes 2* and *3*, the preparation of the four protected *p*-DNA nucleosides **12**, **15**, **21**, and **28** is shown. They served as precursors for the synthesis of the four phosphoramidite building blocks **29**–**32** and the four immobilized *p*-DNA nucleosides **37**–**40** (see below). After nucleoside formation with sugar building block **8** and appropriate nucleobase derivatives, followed by cleavage of the 4′-*O*-^{t}bdms protecting group, an unambiguous

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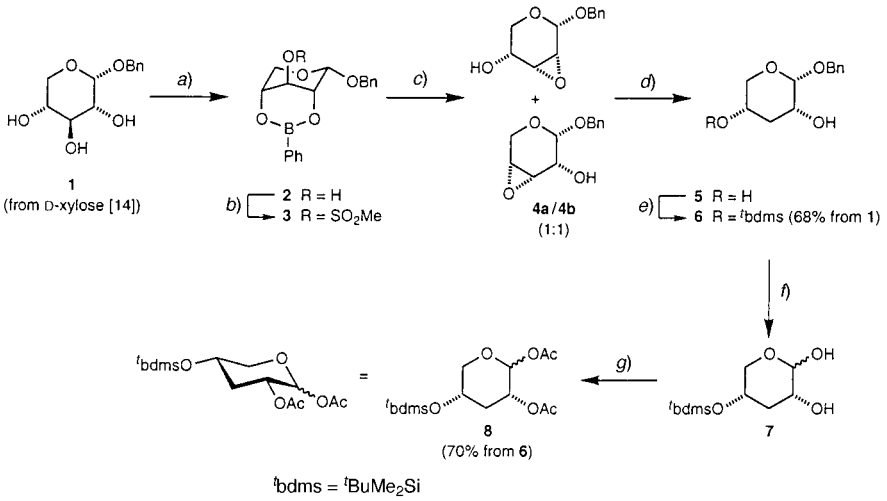
[6]) Glycoside formation with 3-deoxy-D-ribose and different alcohols (*Fischer* conditions, thermodynamic control) led always to 1:1 mixtures of the corresponding pyranosides and furanosides. Interestingly, 2-*O*-acylated 3-deoxyribose derivatives adopt almost exclusively the furanose forms.

[7]) The corresponding 2-*O*-silylated derivative was not detected.

Scheme 1



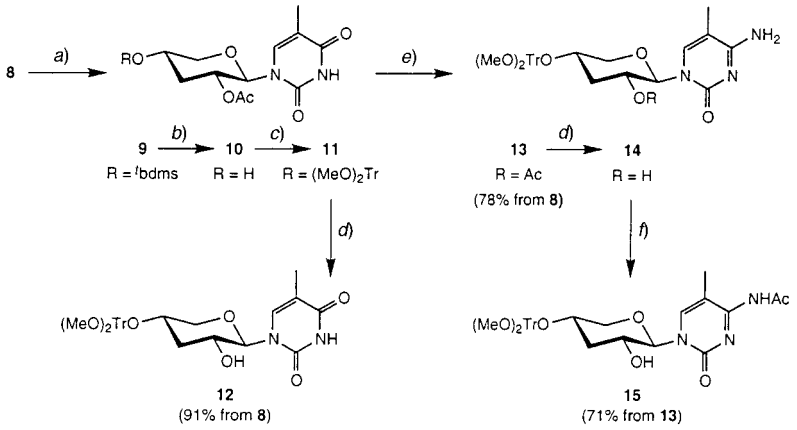
a) $PhB(OH)_2$, toluene, reflux (*Dean-Stark*). *b*) $MeSO_2Cl$, Et_3N, 4°. *c*) NaOMe, MeOH, 4° → 25°. *d*) $LiAlH_4$, THF, 4° → 25°. *e*) ^{t}bdms-Cl, $^i Pr_2NEt$, $AgNO_3$, CH_2Cl_2, −78° → 25°. *f*) H_2, $Pd(OH)_2$/C, EtOH, 25°. *g*) Ac_2O, pyridine, 25°.

introduction of the $(MeO)_2Tr$ group at the 4′-*O*-position of the nucleosides could be achieved. Only one nucleosidation reaction (with thymine) was required for the preparation of the two pyrimidine nucleosides **12** and **15**. The 5-methylcytosine derivative **13** was prepared from the thymine nucleoside intermediate **11** by a well-known base-transformation procedure according to [15].

Thus, under *Vorbrüggen* conditions [13], in MeCN as solvent and with Me_3SiOTf as *Lewis* acid, the sugar building block **8** reacted smoothly with *in situ* trimethylsilylated thymine, forming the thymine nucleoside **9** in quantitative yield. Removal of the ^{t}bdms protecting group with aqueous HCl in MeCN afforded nucleoside **10**. Dimethoxytritylation of this compound with $(MeO)_2TrCl$, $AgNO_3$, and collidine (=2,4,6-trimethylpyridine) in CH_2Cl_2 gave the 4′-*O*-$(MeO)_2Tr$-substituted nucleoside **11**. After removal of the 2′-*O*-acetyl group of **11** with NaOH in MeOH/THF/H_2O and subsequent filtration through silica gel, the thymine nucleoside **12** was isolated in 91% overall yield (based on **8**).

The 5-methylcytosine nucleoside **13** was prepared from the intermediate thymine nucleoside **11** by treatment with $(ClC_6H_4O)P(O)Cl_2$, triazole, and Et_3N in pyridine (→ formation of the 4-triazolide derivative), followed by NH_3 in dioxane and H_2O according to [15]. After silica-gel chromatography, **13** was isolated in 78% yield (based on **8**). Removal of the 2′-*O*-acetyl group with NaOMe in MeOH/THF/H_2O afforded nucleoside **14**, which was transformed into its N^4-acetyl derivative **15** by selective *N*-acetylation with Ac_2O in DMF. The 5-methylcytosine nucleoside **15** was isolated in 71% yield (based on **13**) after chromatography (silica gel).

Scheme 2



a) Thymine, *N,O*-bis(trimethylsilyl)acetamide, MeCN, 60°, then Me_3SiOTf, 40°. *b*) HCl, H_2O, MeCN. *c*) $(MeO)_2TrCl$, collidine, $AgNO_3$, CH_2Cl_2, 25°. *d*) NaOH, THF/MeOH/H_2O. *e*) 1. 4-chlorophenyl phosphorodichloridate, 1*H*-1,2,4-triazole, pyridine, Et_3N, 4° → 25°; 2. NH_3, H_2O, dioxane, 25°. *f*) Ac_2O, DMF, 25°.

To achieve regioselective nucleoside formation with **8**, the purine derivatives N^2-acetyl-O^6-(diphenylcarbamoyl)guanine (**16**) [16] and *N*-(6-chloro-9*H*-purin-2-yl)-2-methoxyacetamide (**23**), were employed for the synthesis of the guanine and the purine-2,6-diamine nucleosides **21** and **28**, respectively (*Scheme 3*)[8]). The purine derivative **23** was obtained in 57% yield from 6-chloro-9*H*-purin-2-amine (**22**) upon treatment with $(MeOCH_2CO)_2O$ in *N,N*-dimethylacetamide at 120°, followed by aqueous workup and crystallization.

Nucleosidation of sugar **8** with the *in situ O*-trimethylsilylated guanine derivative **16** under reported conditions (developed for the nucleosidation of ribofuranose derivatives [16]) afforded a very complex product mixture. However, by changing the solvent (benzene instead of toluene), the reaction temperature (45° instead of 80°), and the *Lewis* acid (Et_3SiOTf instead of Me_3SiOTf), the reaction proceeded much more cleanly, and the nucleoside **17** was formed as major product. Without purification, the 4′-*O*-^{t}bdms group of **17** was cleaved with Et_4NF/AcOH in MeCN, and the nucleoside **18** was isolated in 55% yield after chromatography (silica gel). Under our standard conditions, with $(MeO)_2TrCl$, $AgNO_3$, and collidine in CH_2Cl_2, **18** was converted to the corresponding 4′-*O*-$(MeO)_2Tr$ derivative **19**. At this stage, we wanted to remove the diphenylcarbamoyl protecting group[9]). Treatment of **19** with N^1,N^1,N^3,N^3-tetramethylguanidinium 2-pyridine-*syn*-carbaldoximate (= pyridine-2-carboxaldehyde

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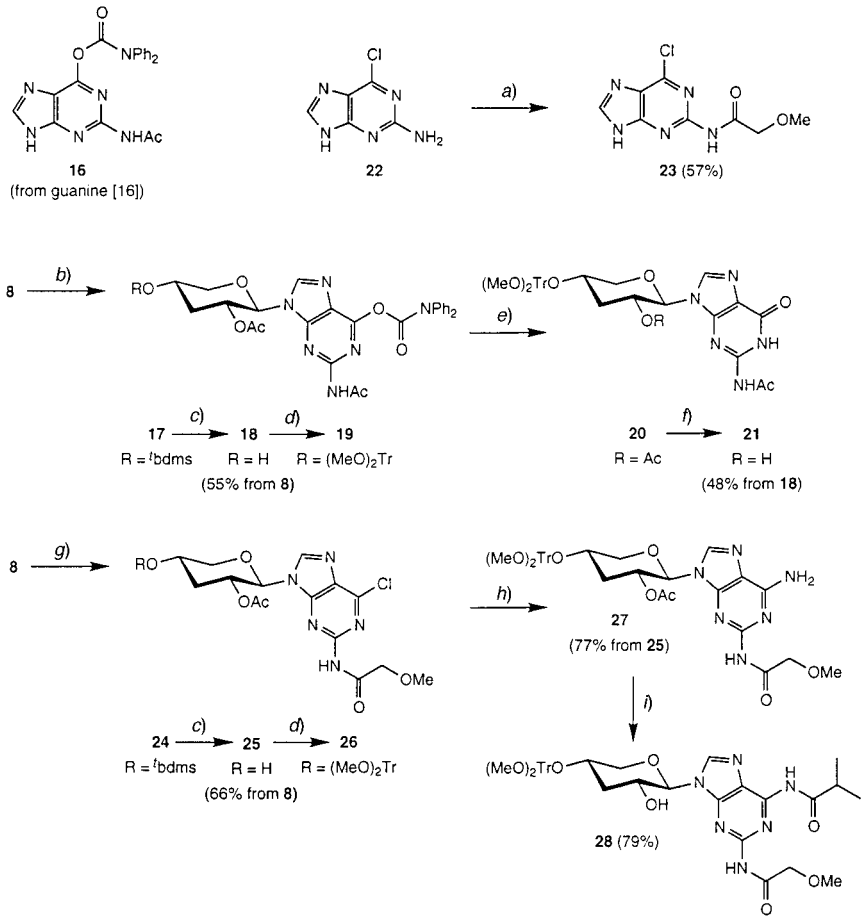
[8]) Nucleosidation with N^2-acylated guanine derivatives and with N^2,N^6-diacylated purinediamine derivatives leads always to mixtures of the corresponding N^9- and N^7-connected nucleosides. In contrast, nucleoside formation with O^6-(diphenylcarbamoyl)-protected guanine derivatives [16] and 6-chloro-substituted purin-2-amine derivatives [17] leads almost exclusively to the corresponding N^9-connected nucleosides.

[9]) We earlier had found that under our preferred oligonucleotide deprotection conditions ($MeNH_2$ in H_2O/EtOH [18]), O^6-(diphenylcarbamoyl)-protected guanines are partially converted into N^6-methylpurine-2,6-diamine nucleosides, suggesting attack of $MeNH_2$ at C(6).

Scheme 3



a) $(MeOCH_2CO)_2O$, *N,N*-dimethylacetamide, 120°, then H_2O crystallization. *b*) 1. **16**, *N,O*-bis(trimethylsilyl)acetamide, $(CH_2Cl)_2$, 60°; 2. C_6H_6, Et_3SiOTf, 45°. *c*) $Et_4NF \cdot 2\,H_2O$, AcOH, MeCN, 25°. *d*) $(MeO)_2TrCl$, collidine, $AgNO_3$, CH_2Cl_2, 25°. *e*) $NaNO_2$, DMSO, 75°. *f*) NaOH, THF, MeOH, H_2O. *g*) 1. **23**, *N,O*-bis(trimethylsilyl)acetamide, MeCN, 60°; 2. Et_3SiOTf, 45°. *h*) 1. NaN_3, pyridine, 65°; 2. PPh_3, 25°; 3. $Et_3N \cdot$ AcOH, MeOH/THF, 65°. *i*) 1. Isobutyryl chloride, pyridine, CH_2Cl_2, 25°; 2. NaOH, THF/MeOH/H_2O.

[*c*(*z*)]-oximate) in dioxane/H_2O according to [19] resulted in a complex reaction mixture. However, we found that the protecting group was cleanly removed upon treatment of **19** with $NaNO_2$ in DMSO at 75°. Under these neutral conditions, all other (acid- and base-sensitive) protecting groups remained intact[10]). Without purification, the N^2-acetylguanine nucleoside **20** was 2′-*O*-deacetylated under standard conditions

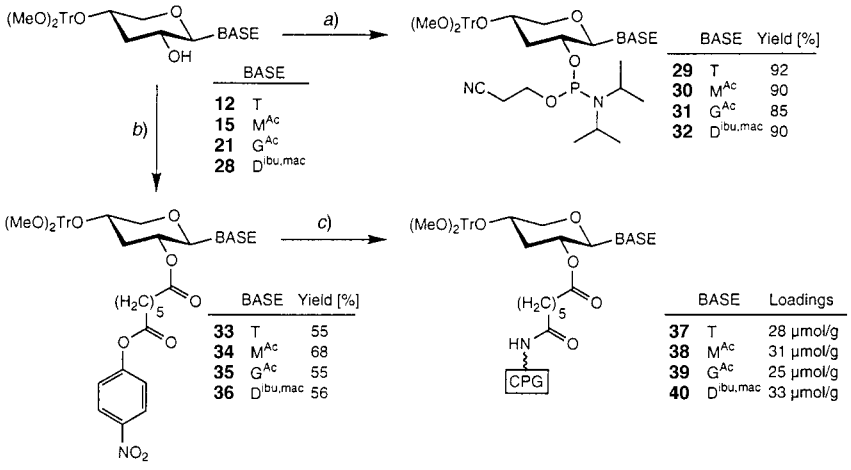
[10]) Under similar conditions (50° instead of 75°), a clean conversion of N^2-acyl-6-chloropurin-2-amine nucleosides to the corresponding N^2-acylguanines occurs.

with NaOH in MeOH/H_2O/THF, and the guanine nucleoside **21** was isolated in a moderate yield of 48% after chromatography (silica gel).

Nucleosidation of **8** with the *in situ* trimethylsilylated nucleobase derivative **23** was carried out under the above-described conditions, at 45° with Et_3SiOTf as *Lewis* acid, but in the solvent MeCN. The crude product **24** was again desilylated with Et_4NF/AcOH in MeCN, and the resulting nucleoside **25** was isolated by chromatography in a good yield of 66%. Introduction of the 4′-*O*-$(MeO)_2Tr$ group with $(MeO)_2TrCl$, $AgNO_3$, and collidine in CH_2Cl_2 led to the 6-chloropurine-2-amine nucleoside **26**. Without purification, this intermediate was converted to the corresponding partially protected purine-2,6-diamine nucleoside **27** under *Staudinger* conditions, by first forming the 6-azido derivative with NaN_3 in pyridine, and then treating this intermediate with PPh_3, followed by $Et_3N \cdot AcOH$ in MeOH/H_2O/THF. Compound **27** was isolated in a good yield of 77% after chromatography (silica gel). The N^6-isobutyrylated, 2′-*O*-deacetylated derivative **28** was obtained by treating **27** first with isobutyryl chloride in pyridine/CH_2Cl_2 and then with NaOH in MeOH/THF/H_2O. After purification by chromatography (silica gel), the protected purine-2,6-diamine nucleoside **28** was isolated in a yield of 79%.

Under standard conditions, with 2-cyanoethyldiisopropylphosphoramidochloridite/$^iPr_2NEt$ in CH_2Cl_2, the four protected 3′-deoxyribopyranose nucleosides **12**, **15**, **21**, and **28** were converted into their corresponding phosphoramidite building blocks **29**–**32** and isolated in good yields by silica gel chromatography (*Scheme 4*). The solid supports were synthesized by first preparing the 2′-(4′-nitrophenyl heptanedioates) **33**–**36** from the nucleosides **12**, **15**, **21**, and **28**, and then immobilizing these activated esters on aminoalkyl-functionalized controlled-pore glass (CPG). To prevent additional N^2-acetylation of the purinediamine nucleoside, the final capping of the solid support **40**

Scheme 4



a) 2-Cyanoethyl diisopropylphosphoramidochloridite, $^iPr_2NEt$, CH_2Cl_2, 25°. *b*) Bis(4-nitrophenyl) heptanedioate, pyridine, DMAP (*N*,*N*-dimethylpyridin-4-amine), 25°. *c*) 1. Long-chain-alkylamino CPG, $^iPr_2NEt$, DMF, 25°; 2. Ac_2O, pyridine, 25° for **37**–**39**; $(MeOCH_2CO)_2O$, pyridine, 25° for **40**.

was carried out with $(MeOCH_2CO)_2O$ instead of Ac_2O[11]). Typical loadings of 25–33 μmol/g were obtained (*Scheme 4*).

Prior to the synthesis of *p*-DNA oligonucleotides, we established the conditions required for the removal of the nucleobase protecting groups under our preferred conditions with $MeNH_2$ (10M in H_2O/EtOH 1:1, 25°), developed for the synthesis of RNA sequences from 2′-*O*-tom-protected ribonucleoside phosphoramidites (tom = [(triisopropylsilyl)oxy]methyl) [18]. By UV measurements and reversed-phase HPLC chromatography, we determined $t_{1/2}$ values of 2 min for the N^4-acetyl-5-methylcytosine nucleoside **15**, 4 min for the N^2-acetylguanine nucleoside **21**, and 3 min for the N^6-isobutyryl-N^2-(methoxyacetyl)-protected purine-2,6-diamine nucleoside **28**, respectively[12]).

2.3. p-*DNA Oligonucleotides.* The synthesis of *p*-DNA oligonucleotides from the phosphoramidite building blocks **29**–**32** and the solid supports **37**–**40** was carried out on 1.0- and 10-μmol scales with a *Pharmacia Gene Assembler* by essentially the protocol developed for the synthesis of RNA oligonucleotides from 2′-*O*-tom-protected phosphoramidites [16] (*Scheme 5*). To prevent N^6-acetylation of the purinediamine nucleosides during each capping step, it was carried out with $(MeOCH_2CO)_2O$ instead of Ac_2O. The coupling step was performed with 12 equiv. (1.0-μmol scale) or 3.5 equiv. (10-μmol scale) of *p*-DNA phosphoramidites and in the presence of 5-(benzylthio)-1*H*-tetrazole as activator. The coupling times were adjusted to 5 min (1.0-μmol scale) or 9 min (10-μmol scale). The individual coupling yields obtained under these conditions were >98% (detritylation assay) and could not be increased further by using more equiv. of phosphoramidites or longer reaction times.

The removal of the base- and phosphate-protecting groups and the cleavage from the solid support were carried out by treatment with 10M $MeNH_2$ in H_2O/EtOH 1:1 for 3 h at 25° (*Scheme 5*); the supernatants were evaporated and the oligonucleotides subsequently purified by anion-exchange HPLC and characterized by MALDI-TOF-MS according to [21] (*Table 1*).

The *p*-DNA sequences shown in *Table 1* were isolated in acceptable yields and at purity >98% (HPLC analysis). The self-complementary sequence $[pd^{3'}(MGDDTTMG)]_2$[13]), containing all four nucleosides, was prepared on a larger scale (15 mg yield after purification) and subsequently subjected to a detailed NMR analysis (performed by *Jaun* and *Ebert* [22]), which demonstrated the correct constitution of this new oligonucleotide pairing system[14]).

2.4. *Pairing Properties of* p-*DNA Oligonucleotides.* In *Fig. 2*, the transition curves (determined by temperature-dependent UV spectroscopy) and CD spectra of some *p*-DNA single strands and duplexes, carried out at a single-strand concentration of 10 μM,

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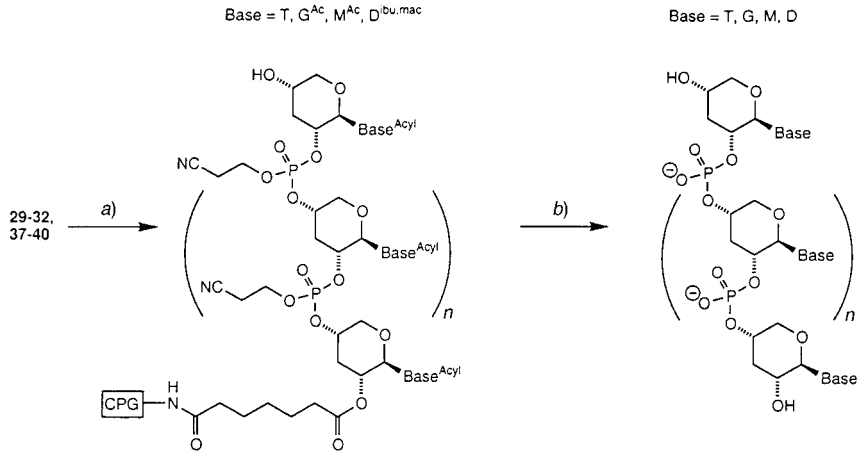
11) Under standard acylation conditions, N^2-acylated purin-2-amines are (at least partially) reacting to the corresponding N^2-diacylated compounds, resulting in a scrambling of acyl-protecting groups [20].

12) First, we evaluated the deprotection of the corresponding N^2,N^6-diacetyl-substituted purine-2,6-diamine nucleoside. We found that the N^6-acetyl group was removed very rapidly ($t_{1/2} < 2$ min) and the N^2-acetyl group very slowly ($t_{1/2} \approx 30$ min). Therefore, we chose the more stable isobutyryl group for N^6-protection and the more labile methoxyacetyl group for N^2-protection, respectively.

13) The sequence descriptor '$pd^{3'}$' stands for 'pyranosyl, 3′-deoxy' (in analogy to the descriptor '*p*r', which is used for *p*-RNA sequences).

14) The structure of this duplex has not yet been completely determined [22].

Scheme 5



a) Assembly of *p*-DNA sequences on a DNA synthesizer: detritylation with 4% dichloroacetic acid in $ClCH_2CH_2Cl$ (1.0-μmol scale: 2 min; 10-μmol scale: 4 min); coupling with 0.1M phosphoramidite in MeCN, promoted by 0.25M 5-(benzylthio)-1*H*-tetrazole in MeCN (1.0-μmol scale: 0.12 ml + 0.36 ml, 5 min; 10-μmol scale: 0.36 ml + 0.60 ml, 9 min); capping with a 1:1 mixture of $(MeOCH_2CO)_2O$/2,6-lutidine (=2,6-dimethylpyridine)/THF 1:1:8 and 16% (*v*/*v*) 1-methyl-1-*H*-imidazole/THF (1.0-μmol scale: 1 min; 10-μmol scale: 3 min); oxidation with I_2/H_2O/pyridine/THF 3:2:20:75 (1.0-μmol scale: 0.7 min; 10-μmol scale: 2.5 min). *b*) Deprotection of *p*-DNA oligonucleotides: 10M $MeNH_2$ in H_2O/EtOH 1:1, 25°, 3 h.

Table 1. *Preparation and Characterization of* p-*DNA Sequences* (*p* = pyrano)

$pd^{3'}$(4′-sequence-2′)[13]	Scale [μmol]	Isolated yield[a])		MS[c]) [*m*/*z*]	
		a.u. (260 nm)[b])	mg [%]	calc.[d])	found
$pd^{3'}(TTTTTTTT) = pd^{3'}(T)_8$	1	41	1.5 (50)	2371.6	2371.1
$pd^{3'}(DDDDDDDD) = pd^{3'}(D)_8$	1	30	1.0 (35)	2563.8	2563.8
$pd^{3'}(MMMMMM) = pd^{3'}(M)_6$	1	25	1.0 (45)	1757.3	1757.2
$pd^{3'}(GGGGGG) = pd^{3'}(G)_6$	1	27	0.8 (45)	1913.3	1913.0
$pd^{3'}$(MGDDTTMG)	1	37	1.2 (50)	2467.7	2468.1
	10	450	15.0 (55)	2467.7	2467.8
$pd^{3'}(DMDMDM) = pd^{3'}(DM)_3$	1	25	0.8 (50)	1832.4	1832.4
$pd^{3'}(GTGTGT) = pd^{3'}(GT)_3$	1	28	0.8 (50)	1838.3	1838.4

[a]) Yield after purification by anion-exchange chromatography; according to analytical anion-exchange HPLC, the purity was >98%. [b]) a.u. = absorption unit. [c]) MALDI-TOF-MS: measured in 2,4-dihydroxyacetophenone (ammonium citrate) according to [21]. [d]) For fragment $[M - H]^-$.

in 0.15M NaCl and at pH 7.4, are presented. The completely reversible, sigmoidal transition curves obtained from 1:1 mixtures of the complementary[15]) *p*-DNA sequences $pd^{3'}(D)_8 \cdot pd^{3'}(T)_8$ (*Fig. 2*,*d*) and $pd^{3'}(G)_6 \cdot pd^{3'}(M)_6$ (*Fig. 2*,*e*), and the self-complementary sequence $[pd^{3'}(MGDDTTMG)]_2$ (*Fig. 2*,*c*) clearly indicate a reversible and cooperative duplex formation. The transition temperatures (T_m values) were 36°, 63°, and 54°, respectively, and the hyperchromicity values ranged between 10–

[15]) Complementary according to the *Watson-Crick* pairing rules.

25%. In contrast, no indication for any cooperative interaction of the four *p*-DNA single strands $pd^{3'}(D)_8$, $pd^{3'}(T)_8$ (*Fig. 2,a*), $pd^{3'}(G)_6$, and $pd^{3'}(M)_6$ (*Fig. 2,b*), respectively, could be detected by temperature-dependent UV spectroscopy. In summary,

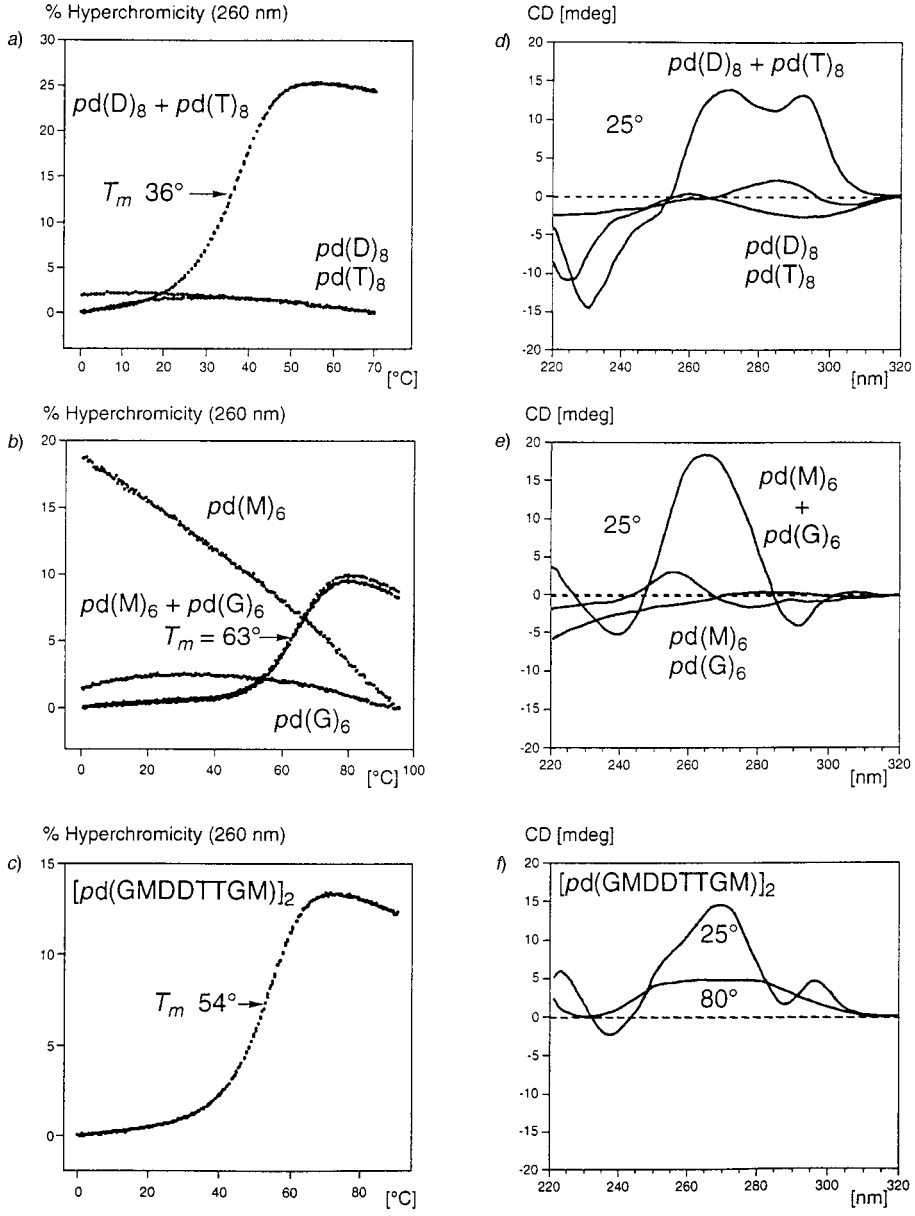


Fig. 2. a)–c) *Transition curves* (heating and cooling curves) *and* d)–f) *CD spectra of* p-*DNA duplexes and single strands.* All measurements were carried out at an oligonucleotide single-strand concentration of 10 μM, in 0.15M NaCl at a pH value of 7.0 (0.01M *Tris*·HCl buffer). $pd = pd^{3'}$.

these results show that the pairing rules of *p*-DNA oligonucleotides are the same as of *p*-RNA oligonucleotides, both forming exclusively antiparallel paired duplexes according to the *Watson-Crick* pairing rules.

The CD spectra obtained from 1:1 mixtures of complementary *p*-DNA sequences, *i.e.*, of $pd^{3'}(D)_8 \cdot pd^{3'}(T)_8$ (*Fig. 2,d*) and $pd^{3'}(G)_6 \cdot pd^{3'}(M)_6$ (*Fig. 2,e*), differ substantially from the CD spectra of the corresponding single strands; furthermore, they are very closely related to the CD spectra obtained from the similar *p*-RNA duplexes $pr(A)_8 \cdot pr(U)_8$ [1] and $pr(G)_8 \cdot pr(C)_8$ [2], respectively.

In *Table 2*, the pairing properties of *p*-DNA oligonucleotide duplexes are summarized and shown in comparison with the properties of *p*-RNA, 3′-*O*-Me-*p*-RNA[16], and DNA duplexes. Additionally, we determined the pairing properties of the *p*-DNA duplex $pd^{3'}(A)_8 \cdot pd^{3'}(T)_8$ for comparison, and the influence of the concentration and the type of salt on the pairing behavior of the *p*-DNA duplex $pd^{3'}(DM)_3 \cdot pd^{3'}(GT)_3$.

Table 2. *Pairing Properties of* p-*DNA Duplexes in Comparison with* p-*RNA, 3′*-O-*Me*-p-*RNA and DNA Duplexes.* T_m values were determined by temperature-dependent UV spectroscopy and thermodynamic data of duplex formation from concentration dependence of T_m values according to *Marky* and *Breslauer* [23]. All measurements were carried out at pH 7.0 (0.01M *Tris*·HCl) and in 0.15M NaCl (when not stated otherwise).

Duplex base sequences	Oligonucleotide backbone	T_m (10 μM)	ΔG (25°) [kcal/mol]	ΔH [kcal/mol]	$T\Delta S$ (25°) [kcal/mol]
-R R R R R R R R	*p*-DNA (R = D)	36°	−9.6	−41.0	−31.4
T T T T T T T T-	*p*-DNA (R = A)[a])	26°	−7.4	−39.7	−32.3
	p-RNA (R = D)[b])	50°			
	p-RNA (R = A)[c])	40°	−10.5	−62.2	−51.7
	3′-*O*-Me-*p*-DNA (R = A)[a])	22°	−7.5	−45.0	−37.5
	DNA (R = A)[d])	< 10°			
-G G G G G G	*p*-DNA (Y = M)	63°	−13.6	−48.4	−35.2
Y Y Y Y Y Y-	*p*-RNA (Y = C)[b])	61°	−11.3	−54.3	−40.8
	3′-*O*-Me *p*-RNA (Y = C)[a])	49°	−10.9	−44.3	−33.4
	DNA (Y = C)[b])	22°[e])	−7.1	−61.3	−54.2
-Y G R R T T Y G	*p*-DNA (R = D, Y = M)	54°	−11.4	−52.1	−11.4
G Y T T R R G Y-	*p*-RNA (R = A, Y = C)[b])	60°	−12.6	−54.9	−12.6
	3′-*O*-Me *p*-RNA (R = A, Y = C)[a])	41°	−10.1	−61.5	−10.1
	DNA (R = A, Y = C)[b])	32°	−8.2	−54.4	−46.2
-D M D M D M	*p*-DNA	35° (0.15M NaCl)	−9.0	−42.3	−33.3
T G T G T G-		36° (1.0M NaCl)			
		36° (1.0M $MgCl_2$)			

[a]) Unpublished work from our group (see [9]). [b]) Data taken from [2]. [c]) Data taken from [6]. [d]) Synthesized according to standard procedures. [e]) Extrapolated from thermodynamic data.

The duplex stabilities of the four pairing systems *p*-DNA, *p*-RNA, 3′-*O*-Me-*p*-RNA, and DNA differ substantially from each other. All three unnatural, pyranose-based oligonucleotides form stronger duplexes than DNA, but to a different extent. The ΔG values of duplex formation (and the T_m values), obtained for the duplexes with the

[16]) The synthesis of this oligonucleotide pairing system is not yet published (see [9]).

identical base composition $A_8 \cdot T_8$, show that *p*-RNA is clearly the strongest pairing system among these three, followed by *p*-DNA and 3′-*O*-Me-*p*-RNA. By replacing adenine with the nucleobase purine-2,6-diamine (X=D), a substantial increase in duplex stability of *p*-RNA and *p*-DNA duplexes resulted ($\Delta T_m = +10^\circ$). The stability of the *p*-DNA duplex $pd^{3'}(DM)_3 \cdot pd^{3'}(GT)_3$ showed only a very moderate dependence on the type and the concentration of added salt. An increase in T_m of $+1^\circ$ was observed upon changing the NaCl concentration from 0.15M to 1.0M, and no difference in T_m was determined by replacing 1.0M NaCl by 1.0M $MgCl_2$.

3. Discussion. – By employing a prefunctionalized sugar building block and base-transformation reactions, we were able to develop a short and highly convergent synthesis of *p*-DNA phosphoramidites. Their assembly to oligonucleotides and their deprotection was carried out under standard conditions, which are fully compatible with RNA (and DNA) synthesis. Meanwhile, we have prepared a number of *p*-DNA/RNA hybrids and could demonstrate that *p*-DNA duplexes are good substitutes for hairpin loops within aptamers and ribozymes [8]. Not surprisingly, the qualitative pairing properties of *p*-DNA duplexes are very similar to the properties of *p*-RNA duplexes: both form much stronger duplexes than the natural oligonucleotide systems RNA and DNA, and both are more selective pairing systems than RNA and DNA.

In the context of finding *p*-RNA-related pairing systems that are compatible with RNA synthesis and deprotection, we have prepared and evaluated 3′-*O*-Me-*p*-RNA and *p*-DNA oligonucleotides. Among these three structurally closely related oligonucleotide systems, *p*-RNA intrinsically forms the strongest duplexes, followed by *p*-DNA and 3′-*O*-Me-*p*-RNA (*Table 2*)[17]). The differences in duplex stability among them are rather large and, so far, difficult to comprehend, mainly due to the lack of structural data. Constitutionally, the three pairing systems differ only in the type of 3′-substituents within each nucleotide unit. The three substituents (OH, H, and MeO within *p*-RNA, *p*-DNA, and 3′-*O*-Me-*p*-RNA, resp.) are, however, sterically and electronically different and can, in principle, induce significantly different backbone conformations, *e.g.*, by steric interactions with other sugar substituents or by altering the hydration shell around the backbone. With one *p*-RNA duplex, a detailed NMR-based structural analysis has been carried out, revealing its quasi-linear overall structure [4]. Recently, a similar analysis has been carried out with a similar *p*-DNA duplex, but its structure could not yet be determined completely [22]. However, significant differences among the backbone angles β and ε were found for *p*-RNA and *p*-DNA; the value for angle β is 145° and 160°, and the value for angle ε is -85° and -60° in *p*-RNA and *p*-DNA, respectively. For 3′-*O*-Me-*p*-RNA, no structural analysis has been carried out so far.

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Experimental Part

General. Reagents and solvents from *Fluka*, unless otherwise stated; 5-(benzylthio)-1*H*-tetrazole was synthesized according to [23]. Workup implies partition of the reaction mixture between CH_2Cl_2 and sat. aq.

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[17]) This trend is very different from the situation among the three analogous pairing systems based on furanose nucleotides, where 2′-*O*-Me-RNA forms the strongest duplexes, followed by RNA and DNA.

$NaHCO_3$ soln., followed by drying the org. layer ($MgSO_4$), and evaporation. TLC: precoated silica gel plates from *Merck*, stained by dipping into a soln. of anisaldehyde (10 ml), conc. H_2SO_4 soln. (10 ml), and AcOH (2 ml) in EtOH (180 ml) and subsequent heating with a heat gun. CC (column chromatography): silica gel *60* (230–400 mesh) from *Fluka* or Al_2O_3 from *Woelm* (act. III was obtained according to the manufacturer's instruction). Anion-exchange HPLC (prep.): *Mono Q HR 5/5* (*Pharmacia*), flow 1 ml/min; eluent *A:* 10 mM sodium phosphate in H_2O, pH 11.5; eluent *B:* 10 mM sodium phosphate/1M NaCl in H_2O, pH 11.5; detection at 260 nm, elution at 25°. Anion-exchange HPLC (anal.): *DNAPAC PA-100* (*Dionex*), flow 0.75 ml/min; eluent *A:* 2 mM *Tris*·HCl (pH 7.4), 10 mM $NaClO_4$, 6M urea; eluent *B:* 2 mM *Tris*·HCl (pH 7.4), 0.55M $NaClO_4$, 6M urea; detection at 260 nm, elution at 80°. Optical rotation ($[\alpha]_D^{25}$): *c* (g/100 ml) as indicated in parentheses. UV Spectra: λ_{max} in nm, ε in dm^3/mol/cm in parentheses; all measurements at 25°. NMR: chemical shift δ in ppm and coupling constants *J* in Hz. MS: FAB in the positive mode, 3-nitrobenzyl alcohol as matrix; ESI in the presence of 10^{-5} M NH_3·AcOH; HR-MALDI in the positive mode, 2,5-dihydroxybenzoic acid as matrix; in *m/z* (rel. intensity in %).

Oligonucleotide Synthesis and Deprotection. The oligonucleotides were assembled on a *Pharmacia Gene Assembler Plus* under the conditions described in *Scheme 5*. HPLC-Grade MeCN was dried by refluxing over CaH_2 (24 h). Prior to the assembly, the phosphoramidite solns. (0.1M in MeCN), the 5-(benzylthio)-1*H*-tetrazole soln. (0.25M in MeCN) and the MeCN were stored over 4-Å molecular sieves for 14 h. All syntheses were carried out in the 'trityl-off' mode. After the assembly, the solid supports were removed from the cartridges and treated with 1.5 ml (1-μmol scale) or 7 ml (10-μmol scale) of 12M $MeNH_2$ in H_2O/8M $MeNH_2$ in EtOH (1:1 mixture). By centrifugation, the supernatant solns. were separated from the solid supports and evaporated and the residues purified by anion-exchange HPLC. The final desalting was carried out according to *Pitsch* [12].

Thermal Denaturation Studies. Absorbance *vs.* temperature profiles were recorded in fused quartz cuvettes at 260 nm with a *Cary Bio-1* spectrophotometer equipped with a *Peltier* temperature controlling device. The samples were prepared from stock solns. of the oligonucleotide, 1M *Tris*·HCl buffer (pH 7.0), and 3M NaCl and subsequently degassed. A layer of silicon oil was placed on the surface of the soln. Prior to the measurements, each sample was briefly heated to 80°. The curves were obtained with both a cooling and a heating ramp of 0.3°/min. The transition temperatures ($= T_m$ values) were obtained after differentiation of the melting curves and analyzed according to [24].

*Benzyl 4-*O*-[(*tert*-Butyl)dimethylsilyl]-3-deoxy-α-*D*-ribopyranoside* (**6**). A soln. of benzyl α-D-xylopyranoside (**1**; 10.0 g, 41.5 mmol; prepared according to [14]) and $PhB(OH)_2$ (5.3 g, 43.75 mmol) in toluene (160 ml) was heated to reflux for 2 h (→ **2**), then cooled to 0°, and treated with Et_3N (7.5 ml, 54 mmol) and $MeSO_2Cl$ (4.2 ml, 54 mmol). After warming to 25° and stirring for 1 h, the solid (Et_3N·HCl) was filtered off and the filtrate evaporated. The residue **3** was dissolved in MeOH (160 ml) and the soln. cooled to 4°, slowly treated with NaOMe (6.72 g, 124.5 mmol), warmed to 25°, and stirred for 1 h. After workup, the crude product **4a**/**4b** was dissolved in THF (160 ml) and the soln. cooled to 4°, treated with $LiAlH_4$ (3.15 g, 83.0 mmol), stirred for 30 min at 25°, cooled to 0°, and carefully treated with AcOEt (300 ml) and then with 2M NaOH (500 ml). Phase separation and extraction gave crude **5** (8.5 g) as a solid, which was dissolved in CH_2Cl_2 (200 ml) and $^iPr_2NEt$ (13.5 ml, 79 mmol). This soln. was cooled to −78°, treated in turn with ^{t}bdms-Cl (6.25 g, 41.5 mmol) and $AgNO_3$ (8.0 g, 47.4 mmol), and allowed to warm up to 25° overnight. Filtration, workup, and CC (silica gel (100 g), AcOEt/hexane 2:98 → 10:90) gave **6** (9.5 g, 68%). Colorless viscous liquid. TLC (hexane/AcOEt 1:1): R_f 0.90. $[\alpha]_D^{25} = +108$ ($c = 0.89$, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$): 0.077, 0.085 (2*s*, Me_2Si); 0.85–0.92 (*m*, ^{t}Bu); 1.73 (*dd*, $J = 10.6$, 11.5, H−C(3)); 2.07–2.14 (*m*, H′−C(3), OH); 3.44–3.53 (*m*, H−C(4), H−C(5)); 3.63–3.70 (*m*, H−C(2)); 3.72–3.82 (*m*, H′−C(5)); 4.53, 4.81 (2*d*, $J = 11.6$, $PhCH_2O$); 4.75 (*d*, $J = 3.7$, H−C(1)); 7.26–7.40 (*m*, 5 arom. H). ^{13}C-NMR (75 MHz, $CDCl_3$): 17.3 (*s*, Me_3C); 25.0 (*q*, Me); 37.1 (*t*, C(3)); 63.5 (*t*, $PhCH_2O$); 68.6 (*t*, C(5)); 65.2, 66.6 (2*d*, C(2), C(4)); 95.8 (*d*, C(1)); 127.3, 127.4, 127.7 (3*d*, arom. C); 136.8 (*s*, arom. C). ESI-MS: 339.4 (14, $[M+H]^+$), 361 (18, $[M+Na]^+$), 356 (100).

*4-*O*-[(*tert*-Butyl)dimethylsilyl]-3-deoxy-α/β-*D*-ribopyranose 1,2-Diacetate* (**8**). A soln. of **6** (24.0 g, 71 mmol) in EtOH (200 ml) was treated with $Pd(OH)_2$/C (4.4 g; *Aldrich*) and subjected to 6 bar H_2 for 48 h at 25°. After filtration over *Celite*, evaporation, and co-evaporation with benzene, the residue **7** was dissolved in pyridine (250 ml), treated with Ac_2O (16.7 ml, 178 mmol), and stirred overnight at 25°. MeOH (10 ml) was added, the soln. evaporated, and the residue co-evaporated with toluene and absorbed on SiO_2 (20 g). CC (silica gel (60 g), hexane → hexane/AcOEt 8:2) gave **8** (16.0 g, 70%), α-/β-D 4:1 (by ^{1}H-NMR). Colorless, viscous liquid. TLC (hexane/AcOEt 4:1): R_f 0.65. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.06–0.08 (*m*, Me_2Si); 0.86–0.92 (*m*, ^{t}Bu); 1.62–1.72 (*m*, 0.8 H, H−C(3)(α)); 1.86 (*q*, $J = 11.7$, 0.2 H, H−C(3)(β)); 2.03, 2.06, 2.10, 2.16 (4*s*, 12 H, Ac); 2.28–2.38 (*m*, 1 H, H′−C(3)(α+β)); 3.37–3.92 (*m*, 3 H, H−C(4)(α+β), H−C(5)(α+β), H′−C(5)(α+β)); 4.78 (*ddd*, $J = 5.0$, 6.9, 9.7, 0.8 H, H−C(2)(α)); 4.91 (*ddd*, $J = 3.4$, 5.0, 12.4, 0.2 H, H−C(2)(β)); 5.65 (*d*, $J =$

6.9, 0.8 H, H–C(1)(α)); 6.10 (*d*, *J* = 3.1, 0.2 H, H–C(1)(β)). ^{13}C-NMR (75 MHz, $CDCl_3$): 18.0 (*s*, Me_3C); 20.9, 21.0 (2*q*, Me_2Si); 25.7 (*q*, *Me*$_3$C); 33.6, 35.9 (2*q*, *Me*CO); 64.7, 65.2, 67.3, 67.9 (4*d*, C(2), C(4)); 65.3, 69.8 (2*t*, C(3), C(5)); 88.3, 93.2 (2*d*, C(1)); 169.6, 169.6, 170.2, 170.4 (4*s*, Me*C*O). ESI-MS: 350.4 (100, $[M+NH_4]^+$), 682 (5, $[2M+Na]^+$).

1-[3′-Deoxy-4′-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl]thymine 2′-Acetate* (**11**). A suspension of **8** (5.60 g, 17.3 mmol) and thymine (2.40 g, 19.1 mmol) in MeCN (65 ml) was heated to 60°, treated with *N,O*-bis(trimethylsilyl)acetamide (9.3 ml, 38.6 mmol) and stirred for 45 min at 60°. After adjusting the temp. to 40°, the clear soln. was treated with Me_3SiOTf (9.4 ml, 52.0 mmol), stirred for 15 min at 40°, and poured into a mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (500 ml). The residue **9** obtained after workup was dissolved in MeCN (100 ml) and treated with conc. aq. HCl soln. (5 ml) for 10 min at 25°. After workup, co-evaporation with benzene, and drying *in vacuo*, the crude product **10** was dissolved in CH_2Cl_2 (70 ml), treated with $(MeO)_2TrCl$ (6.5 g, 19.1 mmol), *sym*-collidine (=2,4,6-trimethylpyridine; 4.6 ml, 34.7 mmol), and $AgNO_3$ (3.24 g, 19.1 mmol), and stirred 20 min at 25°. After filtration over *Celite*, evaporation, and removal of collidine by destillation *in vacuo*, the residue **11** (10.4 g) was used further without purification. A small amount of **11** was purified by CC for characterization. TLC (AcOEt): R_f 0.5. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.83 (*q*, *J* = 11.5, H–C(3′)); 1.85 (*d*, *J* = 0.9, Me–C(5)); 1.97 (*s*, Ac); 2.14–2.18 (*m*, H′–C(3′)); 3.00 (*ddd*, *J* = 1.9, 4.7, 10.6, H–C(5′)); 3.21 (*t*, *J* = 10.6, H′–C(5′)); 3.69–3.77 (*sept.*, *J* = 4.8, H–C(4′)); 3.796, 3.800 (2*s*, 2 MeO); 4.66–4.75 (*m*, H–C(2′)); 5.48 (*d*, *J* = 9.4, H–C(1′)); 6.82–6.86 (*m*, 4 arom. H); 6.95 (*d*, *J* = 1.1, H–C(6)); 7.25–7.48 (*m*, 9 arom. H); 8.30 (*s*, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 12.4 (*q*, *Me*–C(5)); 20.8 (*q*, *Me*CO); 36.6 (*t*, C(3′)); 55.3 (*q*, MeO); 66.3, 67.1 (2*d*, C(2′), C(4′)); 71.1 (*t*, C(5′)); 82.1 (*d*, C(1′)); 86.8 (*s*, arom. C); 111.4 (*d*, C(5)); 113.3, 127.1, 128.0, 128.3, 130.0, 130.4, 130.1 (7*d*, arom. C); 135.1 (*d*, C(6)); 136.2, 136.4, 145.2 (3*s*, arom. C); 150.4 (*s*, C(2)); 158.8 (*s*, arom. C); 163.1 (*s*, C(4)); 169.7 (*s*, Me*C*O). HR-MALDI-MS: 609.224 (22, $[M+Na]^+$, $C_{33}H_{34}N_2NaO_8^+$; calc. 609.222), 303.138 (100).

1-[3′-Deoxy-4′-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl]thymine* (**12**). A soln. of crude **11** (10.4 g, *ca.* 17.3 mmol) in THF/MeOH 5:4 (450 ml) was cooled to 4°, treated with 2M NaOH (50 ml, 100 mmol) for 5 min and then with AcOH (6 g, 100 mmol). After usual workup, the crude product was adsorbed on SiO_2 (20 g), subjected to CC (silica gel (30 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 8:92): **12** (8.6 g, 91% based on **8**). Off-white foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.60. $[\alpha]_D^{25°} = +34$ (*c* = 0.83, $CHCl_3$). UV (MeOH): 265 (11100), 255 (10200), 235 (20600). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.76 (*d*, *J* = 1.1, Me–C(5)); 1.86 (*q*, *J* = 11.6, H–C(3′)); 2.32–2.35 (*m*, H′–C(3′)); 2.92 (*m*, H–C(5′)); 3.20 (*t*, *J* = 10.4, H′–C(5′)); 3.38–3.45 (br. *m*, H–C(2′)); 3.66–3.73 (*m*, H–C(4′)); 3.758, 3.766 (2*s*, 2 MeO); 4.30 (br. *s*, OH); 5.36 (*d*, *J* = 9.0, H–C(1′)); 6.80–6.84 (*m*, 4 arom. H); 6.95 (*d*, *J* = 1.1, H–C(6)); 7.18–7.51 (*m*, 9 arom. H); 10.2 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$, 100 MHz): 12.3 (*q*, *Me*–C(5)); 39.7 (*t*, C(3′)); 55.2 (*q*, MeO); 66.9, 67.6 (2*d*, C(2′), C(4′)); 70.9 (*t*, C(5′)); 85.0 (*d*, C(1′)); 86.6 (*s*, arom. C); 113.2, 127.0, 127.9, 128.1, 130.1, 130.2 (6*d*, arom. C); 135.6 (*d*, C(6)); 136.5, 136.7, 145.5 (3*s*, arom. C); 151.5 (*s*, C(2)); 158.6, 158.7 (2*s*, arom. C); 164.0 (*s*, C(4)). HR-MALDI-MS: 567.209 (4, $[M+Na]^+$, $C_{31}H_{32}N_2NaO_7^+$; calc. 567.211), 303.138 (100).

1-[3′-Deoxy-4′-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl]-5-methylcytosine 2′-Acetate* (**13**). A soln. of crude **11** (4.2 g, *ca.* 7.0 mmol) in pyridine (20 ml) and Et_3N (13.6 ml, 98 mmol) was cooled to 4°, treated with 1*H*-1,2,4-triazole (5.8 g, 83.7 mmol) and 4-chlorophenyl phosphorodichloridate (2.3 ml, 14.1 mmol), allowed to warm to 25°, and stirred 48 h at 25°. The mixture was diluted with dioxane (30 ml), treated with 25% aq. NH_3 soln. (20 ml), and stirred 1 h at 25°. After evaporation of the dioxane and usual workup, the residue was adsorbed on SiO_2 (6 g). Purification by CC (silica gel (12 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 8:92) gave **13** (3.2 g, 78%). Yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.30. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.88, 1.91 (2*s*, Ac, Me–C(5)); 1.86–1.94 (*m*, H–C(3′)); 2.14–2.18 (*m*, H′–C(3′)); 2.98 (*dd*, *J* = 3.4, 10.8, H–C(5′)); 3.23 (*t*, *J* = 10.8, H′–C(5′)); 3.69–3.76 (*sept.*, *J* = 5.3, H–C(4′)); 3.786, 3.789 (2*s*, 2 MeO); 4.66–4.75 (*m*, H–C(2′)); 5.70 (*d*, *J* = 9.3, H–C(1′)); 6.82–6.86 (*m*, 4 arom. H); 7.02 (*s*, H–C(6)); 7.20–7.48 (*m*, 9 arom. H); 8.17 (*s*, NH_2). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.2, (*q*, *Me*–C(5)); 20.8 (*q*, *Me*CO); 36.7 (*t*, C(3′)); 55.3 (*q*, MeO); 66.5, 67.2 (2*d*, C(2′), C(4′)); 71.7 (*t*, C(5′)); 82.1 (*d*, C(1′)); 86.7 (*s*, arom. C); 102.9 (*d*, C(5)); 113.2, 126.7, 127.1, 127.9, 128.0, 128.4, 130.4, 130.1 (8*d*, arom. C); 136.3, 136.5 (2*s*, arom. C); 137.9 (*d*, C(6)); 145.3 (*s*, arom. C); 156.3 (*s*, C(2)); 158.7 (*s*, arom. C); 165.5 (*s*, C(4)); 170.0 (*s*, Me*C*O). HR-MALDI-MS: 608.235 (25, $[M+Na]^+$, $C_{33}H_{35}N_3NaO_7^+$; calc. 608.238), 303.133 (100).

N^4-*Acetyl-1-[3′-deoxy-4′*-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl]-5-methylcytosine* (**15**). A soln. of **13** (2.65 g, 4.5 mmol) in THF/MeOH 5:4 (90 ml) was cooled to 4°, treated with 2M NaOH (10 ml, 20 mmol), stirred for 10 min at 4°, treated with AcOH (1.2 g, 20 mmol), concentrated to 20 ml, and worked up. The residue was dissolved in DMF (20 ml), treated with Ac_2O (425 µl, 4.5 mmol), and stirred at 25° overnight. After extraction and evaporation, DMF was removed by destillation *in vacuo*. The crude product was adsorbed on SiO_2 (5 g).

Purification by CC (silica gel (12 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 6:94) gave **15** (1.88 g, 71%). Solid foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.20. $[\alpha]_D^{25°} = +71$ ($c = 0.80$, $CHCl_3$). UV (MeOH): 305 (6600), 290 (5500), 282 (6300), 269 (5800), 236 (26000). ^{1}H-NMR (400 MHz, (D_6)DMSO): 1.55 (q, $J = 11.5$, H–C(3′)); 1.84–1.89 (m, Me–C(5), H′–C(3′)); 2.22 (br. s, Ac); 2.96 (dd, $J = 2.9$, 9.8, H–C(5′)); 3.10 (t, $J = 10.6$, H′–C(5′)); 3.51–3.59 (m, H–C(2′)); 3.60–3.68 (m, H–C(4′)); 3.743, 3.747 (2s, 2 MeO); 5.17 (d, $J = 6.1$, H–C(1′)); 5.27 (br. d, $J = 8.2$, OH); 6.91–6.94 (m, 4 arom. H); 7.22–7.45 (m, 9 arom. H); 7.75 (s, H–C(6)); 9.75 (br. s, NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 13.2 (q, *Me*–C(5)); 24.8 (q, *Me*CO); 55.0 (q, MeO); 64.9, 66.4 (2d, C(2′), C(4′)); 69.9 (t, C(5′)); 85.5 (d, C(1′)); 85.8 (s, arom. C); 105.7 (d, C(5)); 113.2, 126.7, 127.6, 127.8, 128.2, 129.6, 129.7 (7d, arom. C); 136.1, 136.3 (2s, arom. C); 143.3 (d, C(6)); 145.5 (s, arom. C); 154.6 (s, C(2)); 158.2 (s, arom. C); 162.3 (s, C(4)); 170.5 (s, Me*C*O). HR-MALDI-MS: 608.236 (1, $[M + Na]^+$, $C_{33}H_{35}N_3NaO_7^+$; calc. 608.238), 303.137 (100).

N^2-*Acetyl-9-(3′-deoxy-β-*D*-ribopyranosyl)*-O^6-*(diphenylcarbamoyl)guanine 2′-Acetate* (**18**). A soln. of **8** (9.8 g, 30.4 mmol) and *N*^{2}-acetyl-*O*^{6}-(diphenylcarbamoyl)guanine (**16**; 14.7 g, 39.5 mmol; prepared according to [16]) in $(CH_2Cl)_2$ (105 ml) was heated to 60° and treated with *N,O*-bis(trimethylsilyl)acetamide (19.3 ml, 79 mmol) for 45 min at 60°. The solvent was evaporated and the residue redissolved in benzene (105 ml). The mixture was heated to 45°, treated with Et_3SiOTf (11.7 ml, 51.6 mmol), stirred 90 min at 45°, poured into a mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (500 ml), filtered, and worked up. The residue **17** was dissolved in MeCN (150 ml) containing $Et_4NF \cdot 2\,H_2O$ (27.8 g, 150 mmol) and AcOH (2.0 ml, 35 mmol), and stirred 20 min at 25°. After workup, the crude product was adsorbed on SiO_2 (30 g) and purified by CC (silica gel (75 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 8:92): **18** (9.13 g, 55%). Orange foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.65. $[\alpha]_D^{25°} = +9$ ($c = 0.71$, $CHCl_3$). UV (MeOH): 277 (12400), 267 (11500), 254 (sh, 16400), 225 (31800). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.77 (q, $J = 10.9$, H–C(3′)); 1.86 (s, AcO); 2.35 (br. s, OH); 2.57 (s, *Ac*NH); 2.66–2.71 (m, H′–C(3′)); 3.47 (t, $J = 9.7$, H–C(5′)); 4.06–4.14 (m, H–C(4′), H′–C(5′)); 5.43 (ddd, $J = 4.7$, 9.0, 10.9, H–C(2′)); 5.55 (d, $J = 9.0$, H–C(1′)); 7.23–7.44 (m, 10 arom. H); 8.03 (s, H–C(8)); 8.09 (s, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 20.7, 25.2 (2q, *Me*COO, *Me*CONH); 37.8 (t, C(3′)); 64.1, 67.8 (2d, C(2′), C(4′)); 72.1 (t, C(5′)); 83.0 (d, C(1′)); 120.9 (s, C(5)); 128.5, 129.5 (2d, arom. C); 141.9 (s, arom. C); 142.3 (d, C(8)); 150.6 (s, Ph_2NCOO); 152.6 (s, C(2)); 155.3 (s, C(4)); 156.5 (s, C(6)); 169.7, 171.3 (2s, Me*C*ONH, Me*C*OO). HR-MALDI-MS: 569.173 (100, $[M + Na]^+$, $C_{27}H_{26}N_6NaO_7^+$; calc. 569.176).

N^2-*Acetyl-9-[3′-deoxy-4′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]guanine* (**21**). A soln. of **18** (4.05 g, 7.4 mmol) in CH_2Cl_2 (30 ml) was treated with *sym*-collidine (2.0 ml, 15 mmol), $AgNO_3$ (1.39 g, 8.2 mmol), and $(MeO)_2TrCl$ (2.77 g, 8.2 mmol), stirred 30 min at 25°, filtered over *Celite*, and worked up. The residue **19** was dissolved in a soln. of $NaNO_2$ (4.14 g, 60 mmol) in DMSO (60 ml) and kept 4 h at 75°. After workup, *sym*-collidine and DMSO were distilled off *in vacuo*, and the crude product **20** was dissolved in THF/MeOH 5:4 (135 ml). The soln. was cooled to 4°, treated 8 min with 2M NaOH (15 ml), and then AcOH (1.7 ml, 30 mmol) was added. The mixture was concentrated to 40 ml, worked up and adsorbed on SiO_2 (12 g). CC (silica gel (24 g), CH_2Cl_2 (+2% Et_3N) $\rightarrow$ MeOH/CH_2Cl_2 7:93 (+2% Et_3N)) and subsequent extraction (aq. $NaHCO_3$ soln./CH_2Cl_2) gave **21** (2.17 g, 48%).Yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.50. $[\alpha]_D^{25°} = -4$ ($c = 0.83$, $CHCl_3$). UV (MeOH): 280 (sh, 12700), 274 (13100), 270 (12900), 256 (sh, 17200), 236 (25900). ^{1}H-NMR (400 MHz, (D_6)DMSO): 1.56 (q, $J = 11.2$, H–C(3′)); 1.94–1.99 (m, H′–C(3′)); 2.17 (s, *Me*COO); 2.98 (dd, $J =$ 2.6, 10.8, H–C(5′)); 3.11 (t, $J = 10.7$, H′–C(5′)); 3.68 (*sept.*, $J = 4.8$, H–C(4′)); 3.745, 3.750 (2s, 2 MeO); 3.96 (br. s, H–C(2′)); 5.00 (d, $J = 9.1$, H–C(1′)); 5.32 (br. s, OH); 6.92–6.95 (m, 4 arom. H); 7.21–7.47 (m, 9 arom. H); 8.05 (s, H–C(8)); 11.9 (br. s, 2 NH). ^{13}C-NMR (100 MHz, (D_6)DMSO): 23.7 (q, *Me*CO); 45.6 (t, C(3′)); 55.0 (q, MeO); 65.0, 66.4 (2d, C(2′), C(4′)); 69.82 (t, C(5′)); 84.9 (d, C(1′)); 85.9 (s, arom. C); 113.3 (d, arom. C); 119.9 (s, C(5)); 126.77, 127.57, 127.88, 129.66, 129.70 (5d, arom. C); 136.10, 136.29 (2s, arom. C); 138.1 (d, C(8)); 145.5 (s, arom. C); 147.7, 148.9, 154.8 (3s, C(2), C(4), C(6)); 158.2 (s, arom. C); 173.4 (s, Me*C*O). HR-MALDI-MS: 634.230 (1, $[M + Na]^+$, $C_{33}H_{33}N_5NaO_7^+$; calc. 634.228), 303.138 (100).

N-*(6-Chloro-9*H*-purin-2-yl)-2-methoxyacetamide* (**23**). A suspension of 6-chloro-9*H*-purin-2-amine (**22**; 15 g, 88 mmol) in a soln. of $(MeOCH_2CO)_2O$ (28.7 g, 177 mmol) in *N,N*-dimethylacetamide (120 ml) was stirred for 20 min at 120°, cooled to 25°, and treated with H_2O (50 ml). After stirring for 10 min, H_2O was evaporated, the suspension filtered, and the residue washed with toluene (30 ml). The residue was recrystallized by suspending it in EtOH (120 ml), heating for 5 min to reflux temp. and slow cooling. Isolation of the colorless crystals by filtration gave **23** (12.2 g, 57%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.40. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.19 (s, CH_2); 8.48 (s, H–C(8)); 9.52 (s, H–N(9)); 13.7 (br. s, NH–C(2))[18]).

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[18]) MeO Signal covered by H_2O signal.

*6-Chloro-9-(3′-deoxy-β-*D*-ribopyranosyl)-*N^2*-(methoxyacetyl)-9*H*-purin-2-amine 2′-Acetate* (**25**). A soln. of **8** (7.7 g, 23.5 mmol) and **23** (6.34 g, 26.2 mmol) in MeCN (100 ml) was heated to 60° and treated for 20 min with *N,O*-bis(trimethylsilyl)acetamide (12.8 ml, 52.5 mmol) at 60°. After adjusting the temp. to 45°, the clear soln. was treated with Et_3SiOTf (9.2 ml, 40.7 mmol), stirred for 50 min at 45°, and then poured into a mixture of sat. aq. $NaHCO_3$ soln./AcOEt 1:1 (500 ml). The residue **24** obtained after workup was dissolved in MeCN (180 ml) containing $Et_4NF \cdot 2\,H_2O$ (33.3 g, 180 mmol) and AcOH (5.1 ml, 90 mmol), and stirred for 3.5 h at 25°. Workup, adsorption on SiO_2 (25 g), and CC (silica gel (110 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 5:95) gave **25** (6.33 g, 66%). Yellow foam. TLC (AcOEt): R_f 0.10. $[\alpha]_D^{25°} = +21$ ($c = 0.88$, $CHCl_3$). UV (MeOH): 285 (7200), 268 (5100), 256 (7400), 240 (5500), 227 (17400). ^{1}H-NMR (400 MHz, $CDCl_3$): 1.83 (*s*, Ac); 1.88 (*q*, *J* = 11.5, H–C(3′)); 2.68–2.73 (*m*, H′–C(3′)); 3.40 (br. *s*); 3.54 (*s*, MeO); 3.58 (*q*, *J* = 10.5, H–C(5′)); 4.14 (*s*, MeOCH_2); 4.11–4.20 (*m*, H–C(4′), H′–C(5′)); 5.39 (*ddd*, *J* = 4.8, 9.1, 11.5, H–C(2′)); 5.80 (*d*, *J* = 9.1, H–C(1′)); 8.19 (*s*, H–C(8)); 9.16 (*s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.6 (*q*, MeO); 37.8 (*t*, C(3′)); 59.4 (*q*, *Me*CO); 64.1, 68.1 (2*d*, C(2′), C(4′)); 72.05, 72.21 (2*t*, C(5′), MeO*C*H_2); 82.5 (*d*, C(1′)); 128.5 (*s*, C(5)); 142.9 (*d*, C(8)); 151.4, 152.8 (2*s*, C(2), C(4)); 167.6 (*s*, MeOCH_2*C*O); 169.5 (*s*, C(6)). HR-MALDI-MS: 422.084 (100, $[M + Na]^+$, $C_{15}H_{18}ClN_5NaO_6^+$; calc. 422.085).

*9-[3′-Deoxy-4′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]-*N^2*-(methoxyacetyl)-9*H*-purine-2,6-diamine 2′-Acetate* (**27**). A soln. of **25** (6.27 g, 15.7 mmol), $(MeO)_2$TrCl (5.84 g, 17.2 mmol), and *sym*-collidine (4.2 ml, 31.4 mmol) in CH_2Cl_2 (55 ml) was treated with $AgNO_3$ (2.66 g, 15.7 mmol) and stirred for 30 min at 25°. After filtration over *Celite*, evaporation, and co-evaporation with toluene, the residue **26** was dissolved in pyridine (70 ml). The mixture was treated with NaN_3 (2.04 g, 31.4 mmol) for 3 h at 65°, cooled to 25°, treated with PPh_3 (6.2 g, 23.5 mmol), and stirred for 45 min at 25°. After workup (1. $NaHCO_3$ soln. 2. 10% citric acid), evaporation, and co-evaporation with toluene, the residue was dissolved in MeOH/THF/1M $Et_3N \cdot$ AcOH 5:4:2 (220 ml) and stirred at 65° overnight, concentrated to 60 ml, worked up, and adsorbed on SiO_2 (30 g). CC (silica gel (100 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 5:95) gave **27** (8.29 g, 77%). Yellow foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.80. $[\alpha]_D^{25°} = +17$ ($c = 1.06$, $CHCl_3$). UV (MeOH): 271 (17600), 266 (sh, 17300), 251 (12700), 225 (42700). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.80 (*s*, Ac); 1.89 (*q*, *J* = 11.8, H–C(3′)); 2.30–2.33 (*m*, H′–C(3′)); 3.02 (*ddd*, *J* = 1.8, 4.9, 11.3, H–C(5′)); 3.27 (*t*, *J* = 11.1, H′–C(5′)); 3.45 (*s*, *Me*OCH$_2$CO); 3.784, 3.789 (2*s*, 2 *Me*OC$_6$H$_4$); 3.87 (*sept.*, *J* = 5.1, H–C(4′)); 4.39 (br. *s*, MeOCH_2CO); 5.21 (br. *m*, H–C(2′)); 5.37 (br. *d*, *J* = 7.9, H–C(1′)); 6.10 (br. *s*, NH_2); 6.83–6.87 (*m*, 4 arom. H); 7.21–7.56 (*m*, 9 arom. H); 7.71 (*s*, H–C(8)); 9.1 (br. *s*, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 20.6 (*q*, *Me*CO); 36.8 (*t*, C(3′)); 55.185, 55.189 (2*q*, *Me*OC$_6$H$_4$); 59.2 (*q*, *Me*OCH$_2$CO); 66.2, 67.7 (2*d*, C(2′), C(4′)); 71.0 (*t*, C(5′)); 73.0 (br. *t*, MeOCH_2CO); 82.5 (br. *d*, C(1′)); 86.8 (*s*, arom. C); 113.31, 113.36 (2*d*, arom. C); 116.5 (*s*, C(5)); 127.0, 128.40, 128.50, 132.01, 132.09 (5*d*, arom. C); 136.11, 136.36 (2*s*, arom. C); 137.9 (*d*, C(8)); 145.2 (*d*, arom. C); 150.2, 152.6, 156.4 (3*s*, C(2), C(4), C(6)); 158.75, 158.77 (2*s*, arom. C); 169.2 (*s*, Me*C*O). HR-MALDI-MS: 705.265 (1, $[M + Na]^+$, $C_{36}H_{38}N_6NaO_9^+$; calc. 705.265), 303.138 (100).

*9-[3′-Deoxy-4′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]-*N^6*-isobutyryl-*N^2*-(methoxyacetyl)-9*H*-purine-2,6-diamine* (**28**). A soln. of **27** (8.18 g, 12.0 mmol), DMAP (73 mg, 0.6 mmol), and isobutyryl chloride (1.40 g, 13.2 mmol) in CH_2Cl_2/pyridine 5:1 (50 ml) was stirred for 1 h at 25°. After workup, the residue was dissolved in THF/EtOH 5:4 (144 ml), cooled to 4°, treated with 2M NaOH (16 ml), and after 5 min treated with AcOH (2 ml). The mixture was concentrated to 30 ml, worked up, and adsorbed on SiO_2 (15 g). CC (silica gel (60 g), $CH_2Cl_2 \rightarrow$ MeOH/CH_2Cl_2 5:95) and crystallization (MeOH/CH_2Cl_2 2:3, 40 ml) gave **28** (6.7 g, 79%). White powder. TLC (CH_2Cl_2/MeOH 19:1): R_f 0.35. $[\alpha]_D^{25°} = +54$ ($c = 0.85$, $CHCl_3$). UV (MeOH): 284 (13900), 270 (11000), 233 (47900). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.21, 1.25 (2*d*, *J* = 6.8, *Me*$_2$CH); 2.06 (*q*, *J* = 12.0, H–C(3′)); 2.53–2.56 (*m*, H′–C(3′)); 2.66 (*sept.*, *J* = 6.9, Me$_2$C*H*); 2.87 (*dd*, *J* = 3.2, 11.3, H–C(5′)); 3.23 (*t*, *J* = 10.7, H′–C(5′)); 3.58 (*s*, *Me*OCH$_2$); 3.783, 3.791 (2*s*, 2 *Me*OC$_6$H$_4$); 3.81–3.88 (*m*, H–C(4′)); 3.92–3.98 (*m*, H–C(2′)); 4.02, 4.22 (2*d*, *J* = 15.5, MeOCH_2CO); 5.38 (*d*, *J* = 8.8, H–C(1′)); 6.16 (*d*, *J* = 5.5, OH); 6.84–6.87 (*m*, 4 arom. H); 7.21–7.54 (*m*, 9 arom. H); 7.76 (*s*, H–C(8)); 8.91, 9.58 (2*s*, 2 NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 18.92, 19.44 (2*q*, *Me*$_2$CH); 36.6 (*d*, Me$_2$*C*H); 39.1 (*t*, C(3′)); 55.2 (*q*, *Me*OC$_6$H$_4$); 59.7 (*q*, *Me*OCH$_2$CO); 66.9, 68.5 (2*d*, C(2′), C(4′)); 70.5 (*t*, C(5′)); 72.7 (*t*, MeOCH_2CO); 85.8 (*d*, C(1′)); 86.6 (*s*, arom. C); 113.27, 113.31 (2*d*, arom. C); 117.6 (*s*, C(5)); 127.0, 127.94, 128.18, 130.11, 130.22, 136.59, 136.86 (5*d*, arom. C); 136.59, 136.86 (2*s*, arom. C); 140.5 (*d*, C(8)); 145.6 (*s*, arom. C); 148.3, 151.70, 151.91 (3*s*, C(2), C(4), C(6)); 158.69, 158.71 (2*s*, arom. C); 168.6 (br. *s*, MeOCH$_2$*C*O); 175.4 (*s*, Me$_2$C*H*C*O*). HR-MALDI-MS: 733.295 (2, $[M + Na]^+$, $C_{38}H_{42}N_6NaO_8^+$; calc. 733.296), 303.139 (100).

*1-[3′-Deoxy-4′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]thymine 2′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**29**). A soln. of **12** (2.0 g, 3.65 mmol) in CH_2Cl_2 (15 ml) was treated in turn with ^{i}Pr$_2$NEt (1.56 ml, 9.11 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (1.14 g, 4.8 mmol). After stirring

for 4 h at 25°, the mixture was subjected to CC (silica gel (35 g), hexane/AcOEt 4:1 → 2:3 (+2% Et_3N)): **29** (2.5 g, 92%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:4): R_f 0.80, 0.75. UV (MeCN): 263 (12400), 257 (12200), 236 (23200). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.01, 1.08, 1.10, 1.11 (4*d*, *J* = 6.8, 2 Me_2CH); 1.820, 1.832 (2*q*, *J* = 11.4, H–C(3′)); 1.86, 1.88 (2*d*, *J* = 1.2, Me–C(5)); 2.16–2.19, 2.24–2.26 (2*m*, H–C(5′)); 2.43–2.58 (*m*, OCH_2CH_2); 2.97, 3.00 (2*ddd*, *J* = 2.1, 5.0, 11.0, H–C(5′)); 3.20 (*t*, *J* = 10.7, H′–C(5′)); 3.43–3.75 (*m*, OCH_2CH_2CN, H–C(2′), H–C(4′)); 3.788, 3.789, 3.793, 3.796 (4*s*, 2 MeO); 5.41 (*d*, *J* = 8.9, H–C(1′)); 6.82–6.87 (*m*, 4 arom. H); 6.97, 6.98 (2*d*, *J* = 1.2, H–C(6)); 7.20–7.50 (*m*, 9 arom. H); 8.59 (br. *s*, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 12.33, 12.38 (2*q*, *Me*–C(5)); 22.33, 22.39 (2*t*, OCH_2CH_2CN); 24.27, 24.34, 24.42, 24.47, 24.51, 24.55, 24.61 (7*q*, Me_2CH); 39.17, 39.39 (2*t*, C(3′)); 55.22, 55.25 (2*q*, MeO); 57.5, 58.1 (2*t*, *J*(C,P) = 18, OCH_2CH_2CN); 66.44, 66.51 (2*d*, C(4′)); 68.2, 69.1 (2*d*, *J*(C,P) = 12, 15, C(2′)); 70.97, 71.04 (2*t*, C(5′)); 83.9 (*s*, C(1′)); 86.51, 86.61 (2*s*, Ar_2CPh); 110.85, 110.97 (2*s*, C(5)); 113.3 (*d*, arom. C); 117.58, 117.67 (2*s*, CN); 126.97, 127.02, 127.93, 128.02, 128.07, 130.07, 130.15, 130.21 (8*d*, arom. C); 135.4, 135.7 (2*d*, C(6)); 136.39, 136.47, 136.55, 136.61, 145.40, 145.49 (6*s*, arom. C); 150.46, 150.60 (2*s*, C(2)); 162.6 (*s*, arom. C); 163.30, 163.43 (2*s*, C(4)). ^{31}P-NMR: 149.62; 148.08. HR-MALDI-MS: 767.318 (1, $[M+Na]^+$, $C_{40}H_{49}N_4NaO_8P^+$; calc. 767.319), 303.139 (100).

N^4-*Acetyl-1-[3′-deoxy-4′*-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl)]-5-methylcytosine 2′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**30**). As described for **29**, with **15** (1.85 g, 3.16 mmol), CH_2Cl_2 (12 ml), ^{i}Pr$_2$NEt (1.08 ml, 6.33 mmol), and ^{i}Pr$_2$NPCl(OCH$_2$CH$_2$CN); (900 mg, 3.8 mmol). CC (silica gel (40 g), hexane/AcOEt 4:1 → 3:7 (+2% Et_3N)) afforded **30** (2.2 g, 90%). Yellow foam (1:1 mixture of diastereoisomers). TLC (hexane/AcOEt 3:7): R_f 0.30, 0.50. UV (MeCN): 309 (10400), 268 (5300), 236 (25800). ^{1}H-NMR (500 MHz, (D_6)DMSO): 0.84, 1.018, 1.027, 1.050 (4*d*, *J* = 6.7, 2 Me_2CH); 1.687, 1.698 (2*q*, *J* = 11.5, H–C(3′)); 1.868, 1.962 (2 br. *s*, Me–C(5)); 1.95–2.02 (*m*, H′–C(3′)); 2.60, 2.745, 2.754 (3*t*, OCH_2CH_2CN); 2.94, 2.99 (2 br. *d*, *J* = 8.0, H–C(5′)); 3.20, 3.24 (2*t*, *J* = 10.5, H′–C(5′)); 3.34–3.62 (*m*, OCH_2CH_2CN, 2 Me_2CH); 3.70–3.75 (*m*, H–C(4′)); 3.742, 3.747, 3.749 (3*s*, 2 MeO); 3.80–3.90, 3.91–4.00 (2*m*, H–C(2′)); 5.48 (br. *s*, H–C(1′)); 6.89–6.94 (*m*, 4 arom. H); 7.22–7.46 (*m*, 9 arom. H); 7.83 (br. *s*, H–C(6)); 9.80 (br. *s*, NH). ^{13}C-NMR (125 MHz, (D_6)DMSO): 13.2 (*q*, *Me*–C(5)); 19.52, 19.69 (2*t*, *J*(C,P) = 7, OCH_2CH_2CN); 23.80, 23.96, 24.04, 24.26 (4*t*, *J*(C,P) = 7, Me_2CH); 39.0 (*t*, C(3′)); 42.36, 42.42 (2*d*, *J*(C,P) = 12, Me_2CH); 54.96, 54.99 (2*q*, MeO); 57.77, 58.27 (2*t*, *J*(C,P) = 18, OCH_2CH_2CN); 66.06, 66.15 (2*d*, C(4′)); 67.7, 68.5 (2*t*, C(2′)); 69.73, 69.82 (2*t*, C(5′)); 84.1 (*d*, C(1′)); 85.68, 85.88 (2*s*, arom. C); 105.8 (*s*, C(5)); 113.23, 113.25 (2*d*, arom. C); 118.60, 118.83 (2*s*, CN); 127.51, 127.56, 127.82, 127.85, 126.74 (5*d*, arom. C); 136.03, 136.09, 136.13, 136.20 (4*s*, arom. C); 142.9, 143.2 (2*d*, C(6)); 145.42, 145.44 (2*s*, arom. C); 154.3 (*s*, C(2)); 158.2 (*s*, arom. C); 162.3 (*s*, C(4)); 170.6 (*s*, Me*CO*). ^{31}P-NMR: 149.66; 148.20. FAB-MS: 786.4 (5, $[M+H]^+$), 303.1 (100).

N^2-*Acetyl-9-[3′-deoxy-4′*-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosl]guanine 2′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**31**). As described for **29**, with **21** (2.30 g, 3.75 mmol), CH_2Cl_2 (15 ml), ^{i}Pr$_2$NEt (1.6 ml, 9.4 mmol), and ^{i}Pr$_2$NPCl(OCH$_2$CH$_2$CN) (1160 mg, 4.9 mmol). CC (Al_2O_3 (100 g), hexane/AcOEt 4:1 → AcOEt, then CH_2Cl_2 → MeOH/CH_2Cl_2 10:90) afforded **31** (2.6 g, 85%). White foam (1:1 mixture of diastereoisomers). TLC (AcOEt): R_f 0.30, 0.35. UV (MeCN): 280 (sh, 10400), 275 (10600), 268 (9800), 259 (sh, 14000), 237 (22200). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.76, 0.98, 1.02, 1.03 (4*d*, *J* = 6.8, 2 Me_2CH); 1.77, 1.79 (2*q*, *J* = 11.4, H–C(3′)); 2.19–2.24 (*m*, H′–C(3′)); 2.24, 2.26 (2*s*, Ac); 2.31–2.45, 2.57–2.60 (2*m*, OCH_2CH_2CN); 3.12–3.37 (*m*, H–C(4′), H–C(5′), H′–C(5′), 2 Me_2CH); 3.51–3.76, 3.81–3.88 (2*m*, OCH_2CH_2CN); 3.781, 3.784, 3.786, 3.790 (4*s*, 2 MeO); 4.02–4.06, 4.22–4.56 (2*m*, H–C(2′)); 5.06, 5.13 (2*d*, *J* = 8.9, H–C(1′)); 6.82–6.87 (*m*, 4 arom. H); 7.20–7.51 (*m*, 9 arom. H); 7.67, 7.71 (2*s*, H–C(8)); 8.89, 9.05 (2 br. *s*, NH–C(2)); 11.95 (br. *s*, H–N(1)). ^{13}C-NMR (125 MHz, $CDCl_3$): 20.05, 20.47 (2*t*, *J*(C,P) = 7, OCH_2CH_2CN); 24.03, 24.10, 24.21, 24.37, 24.41, 24.46, 24.54 (7*q*, Me_2CH); 39.51, 39.57 (2*t*, C(3′)); 42.91, 43.01 (2*d*, *J*(C,P) = 2.4, Me_2CH); 55.22, 55.26 (2*q*, MeO); 57.2, 57.8 (2*t*, *J*(C,P) = 20, 18, OCH_2CH_2CN); 66.38 (*d*, *J*(C,P) = 11, C(4′)); 69.10, 69.16 (2*d*, *J*(C,P) = 15, C(2′)); 71.08 (*t*, *J*(C,P) = 5, C(5′)); 84.71, 86.67 (2*d*, *J*(C,P) = 5, C(1′)); 113.3 (*s*, arom. C); 117.82, 118.01 (2*s*, CN); 121.1, 121.5 (2*s*, C(5)); 126.69, 127.05, 127.94, 127.96, 128.00, 128.04, 130.09, 130.16, 130.18, 130.22 (10*d*, arom. C); 136.34, 136.47, 136.48, 136.55 (4*s*, arom. C); 137.6, 138.0 (2*d*, C(8)); 145.44, 145.54 (2*s*, arom. C); 146.98, 147.26, 148.43, 148.59, 155.59 (5*s*, C(2), C(4), C(6)); 158.72, 158.76 (2*s*, arom. C); 171.80, 171.85 (2*s*, Me*CO*). ^{31}P-NMR: 148.90; 148.63. HR-MALDI-MS: 834.335 (3, $[M+Na]^+$, $C_{42}H_{50}N_7NaO_8P^+$; calc. 834.336), 303.139 (100).

9-[3′-Deoxy-4′-O-*(4,4′-dimethoxytrityl)-β*-D-*ribopyranosyl]*-N^2-*(methoxyacetyl)*-N^6-*isobutyryl-9*H-*purine-2,6-diamine 2′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**32**). As described for **29**, with **28** (1.30 g, 1.83 mmol), CH_2Cl_2 (7.5 ml), ^{i}Pr$_2$NEt (630 µl, 3.66 mmol), and ^{i}Pr$_2$NPCl(OCH$_2$CH$_2$CN) (520 mg, 2.2 mmol). CC (silica gel (30 g), hexane/AcOEt 4:1 → 3:7 (+2% Et_3N)) afforded **32** (1.45 g, 90%). White foam (1:1 mixture of diastereoisomers). TLC (hexane/AcOEt 1:4): R_f 0.20, 0.25. UV (MeCN): 284 (16300), 270 (12900),

234 (49700). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.63, 0.96, 1.01, 1.02 (4*d*, *J* = 6.8, 2 *Me*$_2$CHN); 1.25–1.28 (*m*, *Me*$_2$CHCO); 1.88, 1.90 (2*q*, *J* = 9.7, H–C(3′)); 2.23–2.28 (*m*, 0.5 H, H′–C(3′)); 2.309, 2.315 (2*t*, *J* = 6.2, 1 H, OCH$_2$C*H*$_2$CN); 2.40 (br. *s*, 0.5 H, H′–C(3′)); 2.549, 2.553, 2.746, 2.752 (4*t*, *J* = 6.0, 1 H, OCH$_2$C*H*$_2$CN); 3.08–3.35 (*m*, H–C(5′), H′–C(5′), OC*H*$_2$CH$_2$N, 2 Me$_2$C*H*N); 3.500, 3.503 (2*s*, *Me*OCH$_2$CO); 3.51–3.58, 3.63–3.69 (2*m*, Me$_2$C*H*CO); 3.758, 3.789, 3.791, 3.797 (4*s*, 2 *Me*OC$_6$H$_4$); 3.85, 3.88 (2 *sept.*, *J* = 5.3, H–C(4′)); 4.03–4.15 (*m*, H–C(2′)); 4.17–4.23 (*m*, MeOC*H*$_2$CO); 5.37, 5.39 (2*d*, *J* = 8.9, H–C(1′)); 6.83–6.87 (*m*, 4 arom. H); 7.05–7.52 (*m*, 9 arom. H); 7.95, 8.00 (2*s*, H–C(8)); 8.10, 8.69 (2 br. *s*, NH); 8.94, 9.00 (2*s*, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 19.14, 19.16, 19.20 (3*q*, *Me*$_2$CHCO); 19.95, 20.10, 20.30 (3*t*, *J*(C,P) = 7, OCH$_2$*C*H$_2$CN); 23.83, 23.90, 24.32, 24.34, 24.37, 24.40, 24.43, 24.49 (8*q*, *Me*$_2$CHN); 35.67, 35.85 (2*d*, Me$_2$*C*HCO); 39.48, 39.51 (2*t*, C(3′)); 42.95, 43.05 (2*d*, *J*(C,P) = 12, Me$_2$*C*HN); 55.209, 55.245, 55.252 (3*q*, *Me*OC$_6$H$_4$); 57.6, 57.8, 58.1 (3*t*, *J*(C,P) = 5, 18, 19, O*C*H$_2$CH$_2$CN); 59.4 (*q*, *Me*OCH$_2$CO); 66.37, 66.42 (*d*, C(4′)); 69.4, 70.3 (2*t*, *J*(C,P) = 15, C(2′)); 71.09, 71.41 (2*t*, C(5′)); 72.5 (*t*, MeO*C*H$_2$CO); 84.13, 84.37 (2*d*, C(1′)); 86.16, 86.64 (2*s*, arom. C); 113.3 (*d*, arom. C); 117.5 (*s*, CN); 118.6, 118.8 (2*s*, C(5)); 126.99, 127.03, 127.95, 128.03, 128.08, 130.09, 130.17, 130.2 (8*d*, arom. C); 136.37, 136.45, 136.53, 136.60 (4*s*, arom. C); 140.42, 140.68 (*d*, C(8)); 145.43, 145.52 (2*s*, arom. C); 149.49, 149.58, 151.72, 151.75, 152.56, 152.93 (6*s*, C(2), C(4), C(6)); 158.7 (br. *s*, MeOCH$_2$*C*O); 176.35, 176.64 (2*s*, Me$_2$CH*C*O). ^{31}P-NMR: 149.45; 148.63. FAB-MS: 911.5 (15, $[M+H]^+$), 303.1 (100).

1-[3′-Deoxy-4′-O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]thymine 2′-(4-Nitrophenyl Heptanedioate)* (**33**). A soln. of **12** (200 mg, 0.37 mmol) and ^{i}Pr$_2$NEt (63 µl, 0.37 mmol) in pyridine (5.5 ml) was treated with bis(4-nitrophenyl) heptanedioate (1180 mg, 2.94 mmol) and DMAP (45 mg, 0.37 mmol). After stirring for 14 h at 25°, the mixture was worked up (CH_2Cl_2/10% citric acid in H_2O) and subjected to CC (silica gel (5 g), hexane/AcOEt 8:2 → 2:8): **33** (165 mg, 55%). TLC (hexane/AcOEt 3:7): R_f 0.70. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.28–1.36 (*m*, CH$_2$); 1.44–1.65 (*m*, CH$_2$); 1.67–1.81 (*m*, H–C(3′), 1 CH$_2$); 1.85 (*d*, *J* = 2.0, *Me*–C(5)); 2.10–2.18 (*m*, H′–C(3′)); 2.25, 2.38 (2*t*, *J* = 7.3, 2 CH$_2$); 3.03 (*ddd*, *J* = 2.1, 5.0, 11.1, H–C(5′)); 3.21 (*t*, *J* = 10.7, H′–C(5′)); 3.75 (*sept.*, *J* = 4.7, H–C(4′)); 3.793, 3.795 (2*s*, 2 MeO); 4.71 (*ddd*, *J* = 4.9, 9.3, 11.6, H–C(2′)); 5.49 (*d*, *J* = 9.3, H–C(1′)); 6.65–6.87 (*m*, 4 arom. H); 6.99 (*d*, *J* = 1.2, H–C(6)); 7.20–7.44 (*m*, 9 arom. H); 7.45–7.47 (*m*, 2 arom. H); 8.23–8.25 (*m*, 2 arom. H); 9.08 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 12.4 (*q*, *Me*–C(5)); 24.20, 24.32, 28.22, 33.75, 33.94 (4*t*, CH$_2$); 36.6 (*t*, C(3′)); 55.3 (*q*, MeO); 67.1, 66.25 (2*d*, C(2′), C(4′)); 71.1 (*t*, C(5′)); 82.2 (*d*, C(1′)); 86.8 (*s*, Ar$_2$*C*Ph); 111.3 (*s*, C(5)); 113.35, 113.38, 115.7, 122.5, 125.2, 127.1, 128.0, 130.0 (8*d*, arom. C); 135.4 (*d*, C(6)); 136.18, 136.38, 145.2 (3*s*, arom. C); 150.5 (*s*, C(2)); 155.5 (*s*, arom. C); 163.7 (*s*, C(4)); 171.1, 172.1 (2*s*, C(O)). HR-MALDI-MS: 830.297 (85, $[M+Na]^+$, $C_{44}H_{45}N_3NaO_{12}^+$; calc. 830.290), 814.300 (100).

N^4*-Acetyl-1-[3′-deoxy-4′*-O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]-5-methylcytosine 2′-(4-Nitrophenyl Heptanedioate)* (**34**). As described for **33**, with **15** (179 mg, 0.31 mmol), ^{i}Pr$_2$NEt (157 µl, 0.92 mmol), pyridine (4.5 ml), bis(4-nitrophenyl) heptanedioate (860 mg, 2.14 mmol), and DMAP (37 mg, 0.31 mmol). CC (silica gel (4 g), hexane/AcOEt 8:2 → 1:9): **34** (180 mg, 68%). TLC (AcOEt): R_f 0.80. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.28–1.34 (*m*, CH$_2$); 1.51–1.58 (*m*, CH$_2$); 1.67–1.76 (*m*, CH$_2$); 1.82–1.90 (*m*, H–C(3′)); 1.91 (*d*, *J* = 1.0, Me–C(5)); 2.09–2.18 (*m*, H–C(3′)); 2.24 (*t*, *J* = 7.4, 1 CH$_2$); 2.38 (br. *s*, MeCO); 2.56 (*t*, *J* = 7.5, 1 CH$_2$); 3.03 (*ddd*, *J* = 2.1, 5.0, 11.3, H–C(5′)); 3.23 (*t*, *J* = 10.7, H′–C(5′)); 3.76 (*sept.*, *J* = 5.2, H–C(4′)); 3.796, 3.797 (2*s*, 2 MeO); 4.69 (*ddd*, *J* = 4.9, 9.2, 11.5, H–C(2′)); 5.60 (*d*, *J* = 9.2, H–C(1′)); 6.83–6.86 (*m*, 4 arom. H); 7.16 (*s*, H–C(6)); 7.23–7.38 (*m*, 9 arom. H); 7.45–7.47 (*m*, 2 arom. H); 8.24–8.28 (*m*, 2 arom. H). ^{13}C-NMR (125 MHz, $CDCl_3$): 13.5 (*q*, *Me*–C(5)); 24.2, 24.3, 28.2, 33.79, 33.91 (5*t*, CH$_2$); 36.6 (*t*, C(3′)); 55.2 (*q*, MeO); 66.3, 67.8 (2*d*, C(2′), C(4′)); 71.1 (*t*, C(5′)); 82.7 (*d*, C(1′)); 86.8 (*s*, Ar$_2$*C*Ph); 102.0 (*s*, C(5)); 113.34, 113.37, 115.7, 122.44, 122.47, 125.19, 125.20, 127.1, 128.0, 130.02, 130.08 (11*d*, arom. C); 136.18, 136.38 (2*s*, arom. C); 138 (br. *s*, C(6)); 145.22, 145.28 (2*s*, arom. C); 151.1, 159.4 (2 br. *s*, (C(2), C(4)); 155.5, 158.80, 158.81 (3*s*, arom. C); 171.05, 171.16, 172.5 (3*s*, C=O). FAB-MS: 849.4 (4, $[M+H]^+$), 303.1 (100).

N^2*-Acetyl-9-[3′-deoxy-4′*-O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]guanine 2′-(4-Nitrophenyl Heptanedioate)* (**35**). As described for **33**, with **21** (210 mg, 0.34 mmol), ^{i}Pr$_2$NEt (236 µl, 1.40 mmol), pyridine (5.5 ml), bis(4-nitrophenyl) heptanedioate (1100 mg, 2.75 mmol), and DMAP (42 mg, 0.34 mmol). CC (silica gel (4 g), hexane/AcOEt 8:2 → AcOEt): **36** (165 mg, 55%). TLC (AcOEt): R_f 0.75. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.10–1.08 (*m*, CH$_2$); 1.34–1.46 (*m*, CH$_2$); 1.66–1.83 (*m*, H–C(3′), 1 CH$_2$); 2.02–2.18 (*m*, CH$_2$); 2.20–2.27 (*m*, H′–C(3′)); 2.24 (*s*, MeCO); 2.52 (*t*, *J* = 7.4, 1 CH$_2$); 3.06 (*ddd*, *J* = 1.8, 5.0, 11.4, H–C(5′)); 3.23 (*t*, *J* = 10.7, H′–C(5′)); 3.78, 3.79 (2*s*, 2 MeO); 3.87 (*sept.*, *J* = 5.1, H–C(4′)); 5.14–5.22 (*m*, H–C(2′)); 5.20 (*s*, H–C(1′)); 6.83–6.86 (*m*, 4 arom. H); 7.20–7.40 (*m*, 9 arom. H); 7.46–7.48 (*m*, 2 arom. H); 7.66 (*s*, H–C(8)); 8.22–8.27 (*m*, 2 arom. H); 9.17 (br. *s*, NH). ^{13}C-NMR (100 MHz, $CDCl_3$): 24.07 (*t*, CH$_2$); 24.29 (*Me*CO); 24.35, 27.7, 33.59, 33.77 (4*t*, CH$_2$); 36.9 (*t*, C(3′)); 55.3 (*q*, MeO); 66.1, 67.5 (2*d*, C(2′), C(4′)); 71.1 (*t*, C(5′)); 82.9 (*t*, C(1′)); 86.9 (*s*, arom. C); 113.40, 113.43 (2*d*, arom. C); 121.3 (*s*, C(5)); 122.4, 122.5, 125.2, 127.1,

127.9, 128.0, 130.0 (7*d*, arom. C); 136.1, 136.3 (2*s*, arom. C); 137.3 (*d*, C(8)); 145.3 (*s*, arom. C); 147.6, 148.0 (2*s*, C(2), C(4)); 158.9 (*s*, arom. C); 171.1, 171.6, 171.7 (3*s*, C=O). HR-MALDI-MS: 897.305 (77, $[M+Na]^+$, $C_{46}H_{46}N_6NaO_{12}^+$; calc. 897.307), 881.311 (100).

*9-[3′-Deoxy-4′-*O*-(4,4′-dimethoxytrityl)-β-*D*-ribopyranosyl]-*N^6*-isobutyryl-2-(methoxyacetyl)-9*H*-purine-2,6-diamine 2′-(4-Nitrophenyl Heptanedioate)* (**36**). As described for **33**, with **28** (148 mg, 0.22 mmol), ^{i}Pr$_2$NEt (150 µl, 0.88 mmol), pyridine (3.2 ml), bis(4-nitrophenyl) heptanedioate (700 mg, 1.74 mmol), and DMAP (26 mg, 0.22 mmol). CC (silica gel (3.5 g), hexane/AcOEt 8:2 → 1:9): **36** (120 mg, 56%). TLC (hexane/AcOEt 3:7): R_f 0.60. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.05–1.13 (*m*, CH_2); 1.24 (*d*, *J* = 6.8, *Me*$_2$CH); 1.34 (*sept.*, *J* = 7.1, CH_2); 1.51–1.59 (*m*, CH_2); 1.92 (*q*, *J* = 11.5, H–C(3′)); 1.99–2.13 (*m*, CH_2); 2.22–2.25 (*m*, H′–C(3′)); 2.47 (*t*, *J* = 7.5, CH_2); 3.08 (*ddd*, *J* = 2.0, 5.0, 11.3, H–C(5′)); 3.22 (br. *s*, Me$_2$C*H*); 3.32 (*t*, *J* = 10.1, H′–C(3′)); 3.499 (*s*, *Me*OCH$_2$CO); 3.791, 3.795 (2*s*, 2 MeO); 3.88 (*sept.*, *J* = 5.0, H–C(4′)); 4.13 (*s*, MeOC*H*$_2$CO); 5.13 (*ddd*, *J* = 4.8, 9.4, 11.5, H–C(2′)); 5.53 (*d*, *J* = 9.4, H–C(1′)); 6.84–6.87 (*m*, 4 arom. H); 7.22–7.32 (*m*, 5 arom. H); 7.36–7.40 (*m*, 2 arom. H); 7.46–7.49 (*m*, 2 arom. H); 7.91 (*s*, H–C(8)); 8.23–8.26 (*m*, 2 arom. H); 8.60, 8.94 (2 br. *s*, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 19.13, 19.17 (2*q*, *Me*$_2$CH); 24.14, 24.39, 27.97, 33.56, 33.84 (5*t*, CH_2); 36.0 (*d*, Me$_2$CH); 36.8 (*t*, C(3′)); 55.2 (*q*, *Me*OC$_6$H$_4$); 59.4 (*q*, *Me*OCH$_2$CO); 66.2, 68.0 (2*d*, C(2′), C(4′)); 71.1, 72.5 (2*t*, C(5′), MeOCH$_2$CO); 82.1 (*d*, C(1′)); 86.9 (*s*, arom. C); 113.38, 113.41 (2*d*, arom. C); 118.6 (*s*, C(5)); 122.47, 125.18, 127.1, 128.0, 130.1 (5*d*, arom. C); 136.2, 136.4 (2*s*, arom. C); 140.0 (*d*, C(8)); 145.3 (*s*, arom. C); 149.6, 151.9, 152.6 (3*s*, C(2), C(4), C(6)); 155.4, 158.8 (2*s*, arom. C); 167.7 (br. *s*, MeOCH$_2$*C*O); 170.9, 171.8 (2*s*, C=O); 176.1 (*s*, Me$_2$CH*C*O). FAB-MS 974.6 (5, $[M+H]^+$), 303.1 (100).

Preparation of Solid Supports. To a soln. of the active esters **33**–**36** (0.12 mmol) in DMF (4 ml), long-chain-alkylamino CPG (500-Å pore size; 1 g) was added, and then ^{i}Pr$_2$NEt (0.8 ml). The mixtures were shaken for 20 h at 25°. After filtration, the solids were washed with DMF and CH_2Cl_2, dried, suspended in pyridine (5 ml) and Ac_2O (3 ml) for **37**–**39** (pyridine (5 ml) and MeOCH$_2$CO)$_2$O (3 ml) for **40**), and shaken for 2 h at 25°. After filtration, the solids were washed with DMF and CH_2Cl_2 and dried under high vacuum. The following loadings were obtained: T-solid support **37**, 28 µmol/g; M-solid support **38**, 31 µmol/g; G-solid support **39**, 25 µmol/g; D-solid support **40**, 33 µmol/g.

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