122. Xylose-DNA Containing the Four Natural Bases

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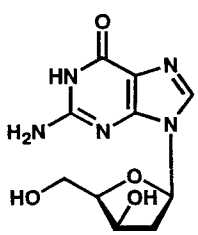
Oligonucleotides containing (2′-deoxy-β-D-xylofuranosyl)guanine have been prepared. For this purpose 2-aminoadenosine (**5**) was synthesized and converted to 2′-deoxy-β-D-xyloguanosine (**1**). The related 2′-deoxy-β-D-xyloisoguanosine (**3**) and 2′-deoxy-β-D-xyloxanthosine (**4**) were also synthesized. Compound **1** was converted to the phosphonate and phosphoramidite building blocks **10** and **11**, respectively. The oligodeoxynucleotide (5′-3′)d(xG-xT-xA-xG-xA-xA-xT-xT-xC-xT-xA-xC-T) (**18**) formed a duplex with the same T_m as the parent (5′-3′)-(G-T-A-G-A-A-T-T-C-T-A-C) (**19**), but with an inverted CD spectrum.

Xylose-DNA is one of the four conceivable isomeric forms of DNA resulting from a configurational variation at one (C(3′)) of the two chiral centers (C(3′) and C(4′)) of its sugar-phosphate backbone. Recently, our laboratory reported on the synthesis of oligonucleotides built-up from (2′-deoxyxylosyl)thymine, 2′-deoxyxyloadenosine, and 2′-deoxyxylocytidine [1–4]. Oligomers containing these nucleotides exhibit generally a mirror-image-like secondary structure compared to their 2′-deoxyribo counterparts and possess a higher stability towards exonucleases. Comparison of the *van't Hoff* thermodynamic constants and T_m values of a series of self-complementary double-stranded oligo(2′-deoxy-β-D-xylonucleotides) with the corresponding data of the parent oligo(2′-deoxyribonucleotides) demonstrated that duplex formation of the first is generally enthalpically unfavorable but entropically favored [5]. This manuscript reports on the synthesis of 2′-deoxy-β-D-xyloguanosine (xG_d, **1**) and related nucleosides, such as **3** and **4**. Compound **1** is converted into its phosphonate as well as its phosphoramidite building blocks. The first is employed in solid-phase synthesis of oligonucleotides, one of them containing all four natural bases. The oligomers are studied with regard to duplex stability and compared with their non-modified counterparts.



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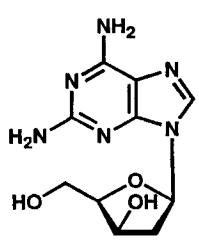

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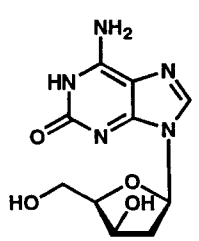

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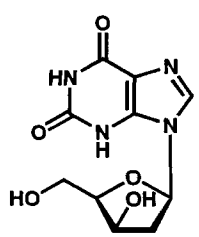

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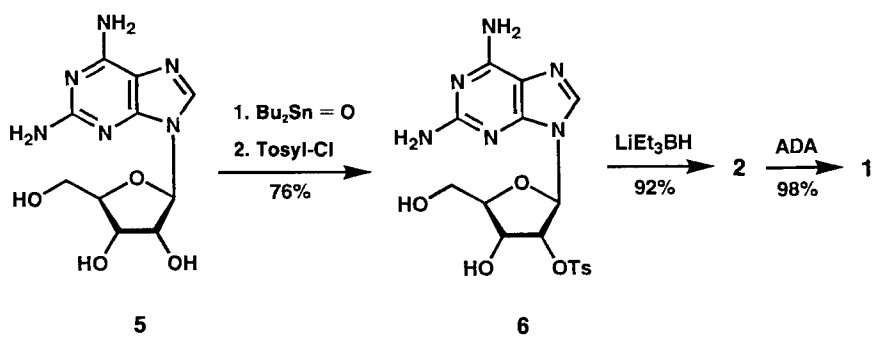
Results and Discussion. – *Monomers.* The synthesis of 2′-deoxy-β-D-xyloguanosine (**1**) was first described by *Robins* and coworkers [6]. It was performed by condensation of 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-β-D-xylofuranose with N_2-acetylguanine followed by deacetylation and subsequent deoxygenation of the 2′-OH group. More convenient seemed to us the route developed by *Herdewijn et al.* [7] starting with a regioselective tosylation of guanosine at its 2′-OH group followed by reduction with $LiEt_3BH$. Purification of the products, however, turned out to be tedious due to the aggregation of guanosine derivatives. Therefore, it seemed to be logical to generate the amino/oxo substituent pattern of the guanine base at the end of the synthesis and to perform the transformation of the ribose to the 2′-deoxyxylose moiety on the stage of the 2,6-diaminopurine nucleoside.

For this purpose, 2-aminoadenosine (**5**) was converted to the 2′-*O*-tosylate **6** *via* its 2′,3′-*O*-(dibutylstannylene) derivative according to *Wagner et al.* [8]. Subsequent reduction of **6** with $LiEt_3BH$ [9] afforded the 2-amino-9-(2-deoxy-β-D-xylofuranosyl)adenine (**2**). It was converted into 2′-deoxy-β-D-xyloguanosine (**1**) with adenosine deaminase (ADA; calf-intestine mucosa). On a preparative scale, 1 g of **2** was converted quantitatively into xG_d overnight using 0.1 mg of enzyme. The *Michaelis-Menten* constants of the adenosine-deaminase-catalyzed reaction of **2** (K_m, v_{max}) were determined and compared with those of xA_d and dA (*Table 1*) [10]. As can be seen, both 2′-deoxy-β-D-xylonucleosides are deaminated at a significantly lower rate compared to dA. This results is in line with the deamination of β-D-xyloadenosine which was slower than that of adenosine [2].

Scheme 1



5 **6**

Table 1. Michaelis-Menten *Constants of Adenosine-Deaminase-Catalyzed Reaction on Nucleosides*

	K_m [μM]	v_{max} [mM min^{-1} mg^{-1}]
2	142	10.0
xA_d	238	28.2
dA	22	186
A	35	200
2-NH_2A	34	50.0

Deamination of **2** with $NaNO_2$/AcOH [11] afforded 2′-deoxy-β-D-xyloisoguanosine (**3**) which shows unpleasant aggregation properties in aqueous solution. Desalting of **3** by silica-gel chromatography led to a material which was much easier to handle. Qualitative experiments (UV monitoring) showed that 2′-deoxy-β-D-xyloisoguanosine (**3**) can be slowly deaminated by the action of adenosine deaminase. On preparative scale, 0.1 g of **3** furnished 2′-deoxy-β-D-xyloxanthosine (**4**) within one week using 3 mg of enzyme (UV monitoring). Compound **4** was characterized by ^{1}H- and ^{13}C-NMR spectra (*Table 2*) but it appeared that it was difficult to handle due to aggregation.

Table 2. *^{13}C-NMR Data of Nucleosides and Derivatives*[a])

	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
dG	153.8	151.0	116.5	156.7	135.4	82.7	DMSO	70.6	87.7	61.6
1	153.6	150.6	116.3	156.8	136.1	81.7	DMSO	69.0	85.1	59.8
2	156.2	150.7	113.3	159.9	136.7	81.8	DMSO	69.2	84.7	59.9
3[a])	157.3	152.4	110.3	151.5	140.7	84.1	40.0	70.3	84.7	60.5
3[b])	156.4	152.6	109.5	152.6	138.4	82.4	40.0	69.3	84.8	59.9
4	158.9	154.3	115.6	145.4	136.1	83.3	40.2	69.3	84.7	59.9
5	156.4	151.5	113.7	160.1	136.6	87.3	73.3	70.8	85.7	61.8
6	156.2	150.1	113.8	159.6	136.3	87.1	78.8	70.0	84.2	61.8
7	148.1	148.1	120.3	154.7	136.3	79.7	DMSO	72.4	83.4	61.4
8	148.2	148.2	119.9	154.9	138.2	82.4	40.5	68.9	85.7	59.8
9	148.3	148.1	120.1	155.0	138.0	83.0	DMSO	69.3	84.1	63.3
10	148.5	148.1	120.1	154.9	138.1	82.4	DMSO	70.1	82.6	62.8
12	148.4	148.2	120.4	154.9	136.9	81.3	DMSO	72.3	82.8	61.4

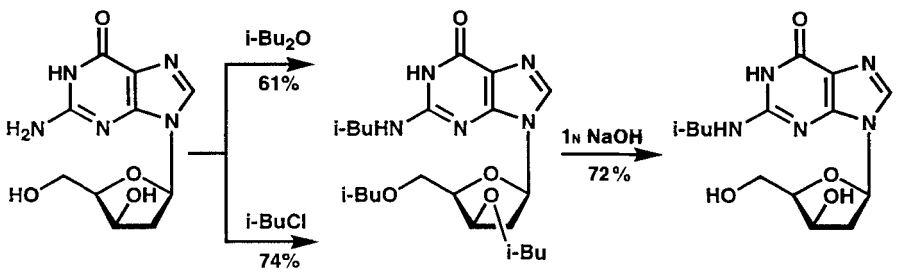
	C=O	Me_2CH	Me_2CH	CH_2
7	180.1	158.2	55.0	–
8	180.2	158.1	55.0	–
9	180.1	158.1	55.0	–
10	180.2	158.1	55.0	–
12	180.2	158.1	55.0	28.7

[a]) Measured in (D_6)DMSO. [b]) Measured in D_2O; 303 K.

All 2′-deoxyxylonucleosides show ^3J(H–C(1′), H_β–C(2′)) coupling constants of 2.0–2.5 Hz. Applying the PSEUROT program [12] for the evaluation of conformational parameters of the glyconic ring from vicinal ^{1}H,^{1}H-couplings, N-type ($^3{}'T_{2'}$) sugar puckering ($P_N > 95\%$) was determined. This is in line with ^{1}H-NOE measurements performed typically on compounds **1** and **2**. Irradiation of H–C(1′) gives only NOE's on H_α–C(2′) and H–C(4′) but not on H–C(3′) despite the fact that it is also positioned on the α-face of the glyconic moiety. This has to be interpreted by an N-type sugar puckering of the 2′-deoxyxylonucleoside [1] [2] – an observation which is in contrast to 2′-deoxyribonucleosides. The latter exhibit S-type sugar puckering ($^3{}'T_{2'}$) [13]; thus the sugar conformation of 2′-deoxy-β-D-xylonucleosides mimic that of β-D-ribonucleosides.

Next, compound **1** was converted into a building block for oligonucleotide synthesis. For this purpose, it was blocked with isobutyryl residues furnishing the perisobutyrylated **7** [14]. Selective deacetylation of the sugar protecting groups (1N aqueous NaOH, 0°) afforded derivative **8**. Its structure was established by ^{1}H- and ^{13}C-NMR spectra as well as elemental

Scheme 2



i-Bu = $Me_2CHC(O)$

analysis. The assignment of the ^{13}C resonances was made on the basis of gated-decoupled and $^1H,^{13}C$-correlation spectra. The half-life value (τ) of the isobutyryl protecting group was determined UV-spectrophotometrically in 25% aqueous NH_3 solution at 300 nm (40°). The half-life of 126 min corresponds to that of ibG_d (τ 112 min; ib = isobutyryl). Subsequent 5′-dimethoxytritylation of **8** [15] yielded compound **9** which was then reacted with PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole [16] to give 3′-phosphonate **10** as triethylammonium salt, after flash chromatography and extraction with aqueous $(Et_3NH)HCO_3$.

Alternatively, **9** was reacted with chloro(2-cyanoethoxy)(diisopropylamino)phosphane [17] to give phosphoramidite **11**. Succinylation of **9** in the presence of 4-(dimethylamino)pyridine gave acid **12** which was subsequently activated to its 4-nitrophenyl ester and then coupled to amino-functionalized *Fractosil* (→ **13**). The nucleoside content in **13** was determined to be 73 μmol of **1**/g *Fractosil* [1] [2].

Synthesis of Oligonucleotides and Duplex Stability. The oligonucleotides **14**–**19** were prepared applying a slightly altered protocol of phosphonate synthesis which was already published [18] [19]. The composition of the oligomers was determined by tandem hydrolysis with snake-venom phosphodiesterase (SVPDE) and alkaline phosphatase (AP) followed by reversed-phase HPLC (*RP-18*). Oligomer **16** was synthesized to test the efficiency of the *Fractosil*-bound xG_d (**13**).

d(G-G-C-A-xG-A-A-G-A-C) **14**

d(G-G-C-A-G-A-A-G-A-C) **15**

d(C-C-G-T-C-T-T-C-T-xG) **16**

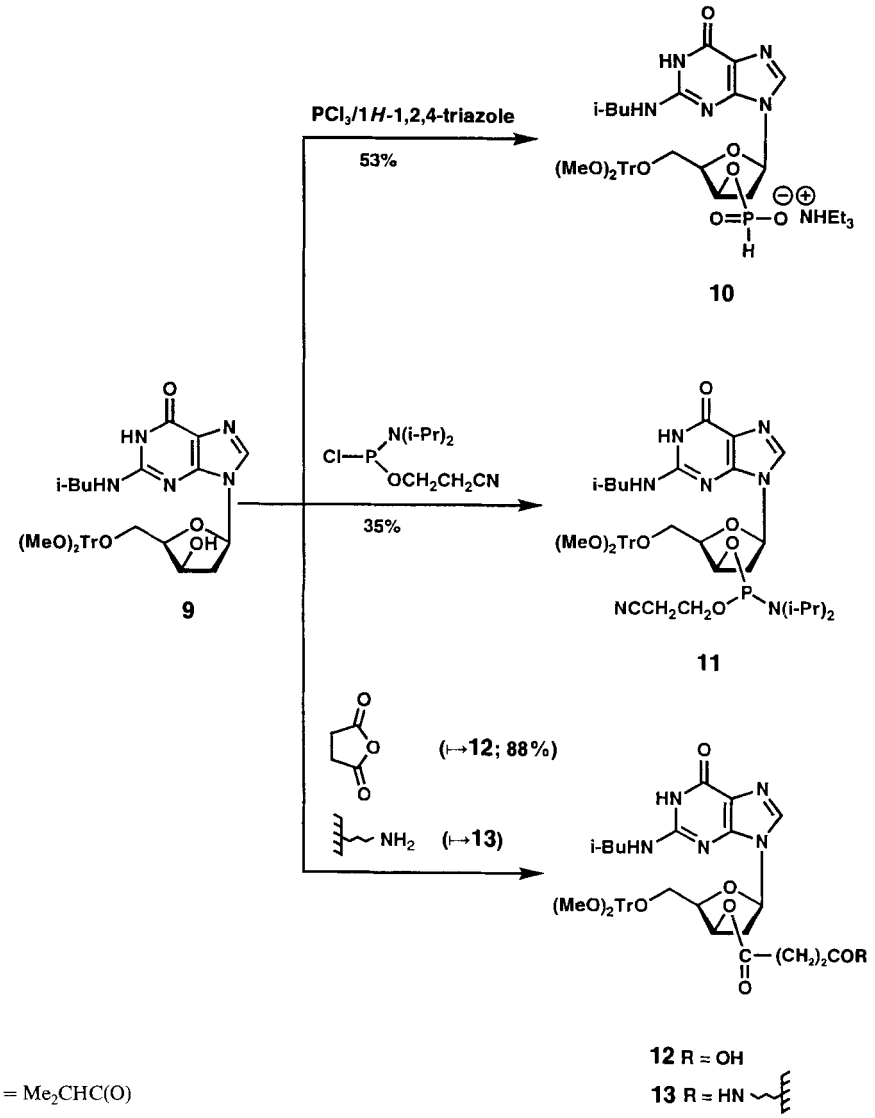
d(C-C-G-T-C-T-T-C-T-G) **17**

d(xG-xT-xA-xG-xA-xA-xT-xT-xC-xT-xA-xC-T) **18**

d(G-T-A-G-A-A-T-T-C-T-A-C) **19**

In order to prove the base-pairing properties of the oligomers **14** and **15**, they were hybridized with the complementary strand **17**. The resulting duplexes with antiparallel strand orientation contain a two-base overhang on both termini. A parallel strand orientation could be ruled out, because it was shown earlier by us that **15**·**17** could be ligated by T4 DNA ligase after enzymatic phosphorylation of the single strands by

Scheme 3



i-Bu = $Me_2CHC(O)$

polynucleotide kinase [20]. In case of a parallel strand orientation, the resulting blunt-ended duplexes would not have been a substrate for T4 DNA ligase [21].

From sigmoidal melting curves, complex formation can be deduced in both cases (**14·17**, **15·17**), although the stability of the modified **14·17** is significantly lower compared to **15·17** (**14·17**: T_m 31°; **15·17**: T_m 43°). From concentration-dependent T_m measurements (*Fig. 1*), the thermodynamics of duplex formation of **14·17** and **15·17** were calculated according to [22] (*Table 3*). The modified duplex is entropically more

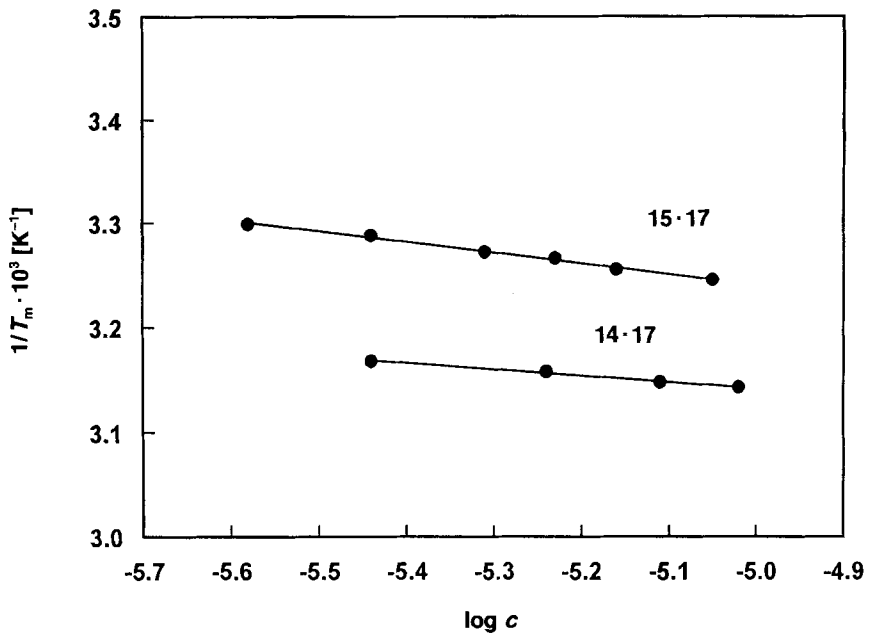


Fig. 1. $1/T_m$ vs. *log* c *of* **14·17** *and* **15·17**. Measured at 260 nm in 60 mM Na-cacodylate, 1M NaCl, and 100 mM $MgCl_2$ at pH 7.0.

Table 3. *Thermodynamic Data of Oligonucleotide-Duplex Formation*[a])

	T_m [°C]	$H_{therm.}$ [%]	ΔH [kcal/mol]	ΔS [cal/K mol]
14·17	31	14	−42.1	−159
15·17	43	16	−71.4	−208
18[b])	45	10	−62.0	−169
19[b])	46	30	−90.0	−257

[a]) 60 mM Na-cacodylate (pH 7), 1M NaCl, 100 mM $MgCl_2$. [b]) 2.8 μM of single strands.

favorable than **15·17** but enthalpically less favorable: the ΔH value of the modified duplex **14·17** ($\Delta H = -42.1$ kcal/mol) implies that xG_d does not form a base pair with dC within such an oligomer. Moreover, the difference in ΔH of **14·17** and **15·17** (29 kcal/mol) implies that also the neighboring dA·dT base pairs are abolished. In this case, only five base pairs in **14·17** would still exist. Despite the probable loop formation in case of **14·17**, both duplexes exhibit almost identical CD spectra (data not shown) which are typical for B-DNA. Interestingly, the modified oligonucleotide **14** bearing one central xG_d residue is hydrolyzed by SVPDE (0.1M *Tris*-HCl buffer, pH 8.3) at a higher rate (τ 3.9 min) compared to **15** (τ 7.4 min). As both thermal hypochromicity values were almost identical (**14**: 21%; **15**: 23%), a complete hydrolysis of both oligomers is considered. This is in contrast to oligonucleotide duplexes in which the replacement of regular nucleotides by 2′-deoxyxylonucleotides generally leads to a decrease of the hydrolysis rate [1] [2].

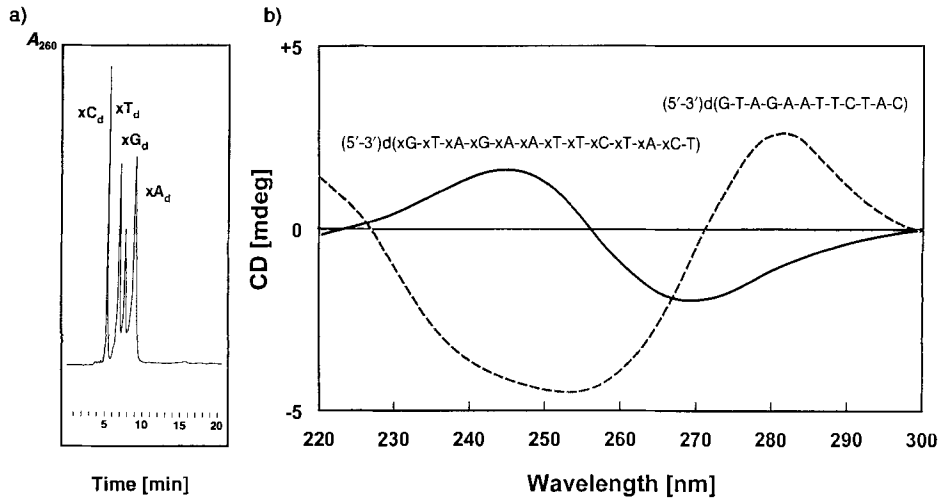


Fig. 2. a) *HPLC Profile of (5'-3')d(xG-xT-xA-xG-xA-xA-xT-xT-xC-xT-xA-xC-T)* (**18**) *after enzymatic tandem hydrolysis in 0.1* M Tris·*HCl buffer* (pH 8.3) *with snake-venom phosphodiesterase followed by alkaline phosphatase.* For details, see *Exper. Part.* b) *CD Spectra of the oligonucleotides* **18** *and* **19**. Oligomer conc., 2.8 μM in 60 mM Na-cacodylate (pH 7), 1M NaCl, and 100 mM $MgCl_2$ at 25°.

The oligo(2'-deoxy-β-D-xylonucleotide) **18** represents the first oligomer in which all internucleotide linkages are configurationally altered and which contains all four natural bases. Like the other oligomers described in this work, it was prepared by standard phosphonate chemistry. Enzymatic hydrolysis by SVPDE (17 h) and AP (5 h) was complete after 17 + 5 h and gave the expected pattern on reversed-phase HPLC (*RP-18*; *Fig. 2, a*). The oligo(2'-deoxyxylonucleotide) **18** exhibits an almost mirror-image-like CD spectrum compared to the unmodified **19** (*Fig. 2, b*). Temperature-dependent UV and CD measurements (258 nm) of **18** afforded a T_m value of 45° (*Fig. 3, a* and *b*). Surprisingly, this is identical to that of the regular counterpart **19** [1].

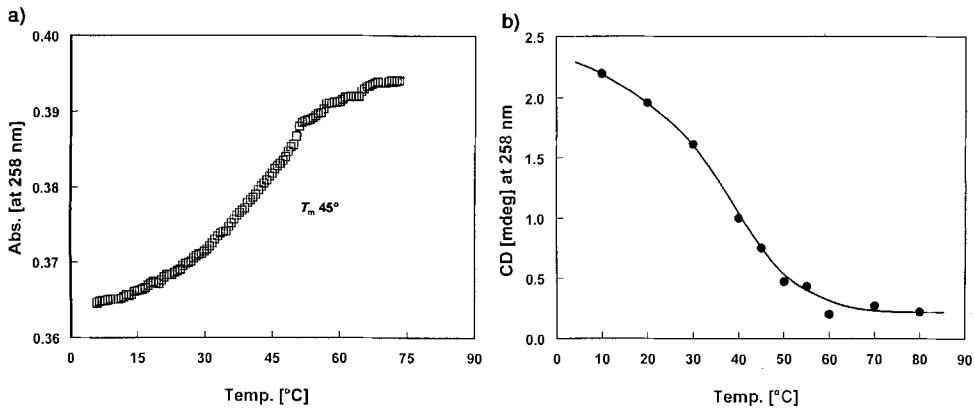


Fig. 3. a) *Melting profile of oligomer* **18**. Measured at 258 nm in 60 mM Na-cacodylate (pH 7), 1M NaCl, and 100 mM $MgCl_2$. b) *Temperature-dependent ellipticity* (CD in mdeg) *of the* B_{2u} *transition of* **18**. Buffer and oligomer concentration as in *Fig. 1*.

Evaluation of the thermodynamics of duplex formation of both oligonucleotides according to a two-state model, however, revealed a significant difference (*Table 3*). Compound **18** shows an enthalpy of duplex formation $\Delta H = -62$ kcal/mol which is 30–40% lower than that of the parent d(G-T-A-G-A-A-T-T-C-T-A-C) (**19**; $\Delta H = -90$ kcal/mol) [1]. On the other hand, duplex formation is entropically favored in the case of **18**. This result is in line with that obtained for a series of oligonucleotides containing xA_d and xT_d residues [5].

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Experimental Part

General. See [1] [2]. Flash chromatography (FC) and TLC: solvent systems: AcOEt/MeOH 8:2 (*A*), AcOEt/MeOH 85:15 (*B*), i-PrOH/aq. NH_3 soln./H_2O 3:1:1 (*C*), $CHCl_3$/MeOH 9:1 (*D*), CH_2Cl_2/MeOH 8:2 (*E*), CH_2Cl_2/MeOH 95:5 (*F*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*G*), CH_2Cl_2/MeOH/Et_3N 78:20:2 (*H*), CH_2Cl_2/AcOEt/Et_3N 80:18:2 (*I*), MeCN/H_2O 9:1 (*K*), i-PrOH/H_2O 1:9 (*L*), CH_2Cl_2/MeOH 1:1 (*M*). HPLC Separation: see [1]; solvents: *A*, 5% MeCN in 0.1M (Et_3NH)OAc buffer (pH 7); *B*, 100% MeCN; *C*, 100% H_2O; *D*, MeOH/H_2O 3:2; *E*, 1.5% MeCN in 0.1M (Et_3NH)OAc buffer (pH 7); gradients: *I*, 3 min 15% *B* in *A*, and 17 min 15–40% *B* in *A*; *II*, 20 min 0–20% *B* in *A*; *III*, 15 min *C* and 10 min *D*; *IV*, 20 min *A*; *V*, 20 min *E*; *VI*, 30 min 0–20% *B* in *A*. UV: λ_{max} (ε) in nm.

Enzymatic Hydrolysis and Melting Experiments of the Oligomers **14–19** see [1] [2]. Determination of the *Michaelis-Menten* constants according to [2].

*2,6-Diamino-9-([2′-O-(*p*-tosyl)sulfonyl]-β-*D*-ribofuranosyl)purine* (**6**) [6]. Compound **5** (2.8 g, 10 mmol) and dibutyltin oxide (2.5 mmol, 10 mmol) were suspended in MeOH (250 ml) and refluxed for 3 h. After cooling to r.t. and addition of Et_3N (21 ml), *p*-toluenesulfonyl chloride was added, and stirring was continued for another 20 min. Thereupon, the soln. was concentrated to 50 ml and the residue dissolved with H_2O (200 ml). After washing with Et_2O (2 × 100 ml), the aq. phase was evaporated to *ca.* 100 ml. After storing at 8° overnight, crude **6** precipitated partially. FC (column 20 × 3 cm, *A*) afforded pure **6** (0.9 g, 21%) as colorless crystals. Concentration of the mother liquor to 1/3 of its volume and FC (column 25 × 6 cm, *B*) afforded a second crop of **6** (2.4 g). Total yield: 3.3 g (76%). M.p. 134° ([6]: 136°). TLC (silica gel, *B*): R_f 0.45. ^{1}H-NMR ((D$_6$)DMSO): 7.70 (*s*, H–C(8)); 7.42, 7.06 (2*d*, arom. H); 6.83 (br. *s*, NH_2); 5.91 (*d*, J = 7.5, H–C(1′)); 5.67 (br. *s*, NH_2); 5.41 (*dd*, J(H–C(2′), H–C(3′)) = 5.0, H–C(2′)); 4.29 (*m*, H–C(3′)); 4.01 (*m*, H–C(4′)); 3.55 (*m*, 2 H–C(5′)); 2.29 (*s*, Me).

*2,6-Diamino-9-(2′-deoxy-β-*D*-threo-pentofuranosyl)purine* (**2**) [9]. Compound **6** (2 g, 4.6 mmol) was reduced with $LiEt_3BH$ in dry DMSO as described in [9]. After ion-exchange chromatography on *Dowex 1* × *2* (OH^- form), **2** (1.13 g, 92%) was isolated as colorless crystals. M.p. 175–176° ([9]: m.p. 175–176°). ^{1}H-NMR ((D$_6$)DMSO): 7.94, (*s*, H–C(8)); 6.74 (*s*, NH_2); 6.06 (*m*, H–C(1′), OH–C(3′)); 5.76 (*s*, NH_2); 4.64 (*s*, OH–C(5′)); 4.30 (*s*, H–C(3′)); 3.84 (*s*, H–C(4′)); 3.66 (*m*, 2 H–C(5′)); 2.73 (*m*, H_α–C(2′)); 2.30 (*m*, H_β–C(2′)). All anal. data were identical in all respects with those reported in [9].

*9-(2′-Deoxy-β-*D*-threo-pentofuranosyl)guanine* (**1**) [6]. Compound **2** (1.0 g, 3.6 mmol) was dissolved in H_2O (200 ml) under warming, then cooled to 30°, and adenosine deaminase (EC 3.5.4.4, calf intestine mucosa; 50 μg) was added. The mixture was stirred overnight at r.t. and evaporated. Repeated co-evaporation from acetone and crystallization from H_2O gave colorless crystals of **1** (980 mg, 98%). M.p. 250° ([6]: m.p. 300°). TLC (silica gel, *C*): R_f 0.6. ^{1}H-NMR ((D$_6$)DMSO): 10.65 (br. *s*, NH); 7.95 (*s*, H–C(8)); 6.49 (br. *s*, NH_2); 6.05 (*d*, J = 7.7, H–C(1′)); 5.43 (br. *s*, OH–C(3′)); 4.67 (br. *s*, OH–C(5′)); 4.33 (*m*, H–C(3′)); 3.88 (*m*, H–C(4′)); 3.71 (*m*, 2 H–C(5′)); 2.68 (*m*, H_α–C(2′)); 2.17 (*d*, J = –14.4, H_β–C(2′)).

*9-[2′-Deoxy-3′,5′-bis-*O*-(2-methylpropanoyl)-β-*D*-threo-pentofuranosyl]-*N^2*-(2-methylpropanoyl)guanine* (**7**). *Method A*: Compound **1** (500 mg, 1.87 mmol) was dried by repeated co-evaporation from abs. pyridine. After addition of pyridine/isobutyric anhydride 1:1 (40 ml), the mixture was refluxed for 3 h. After evaporation, the residue was submitted to FC (column 20 × 5 cm, *D*). The main zone was evaporated. Crystallization from MeOH/H_2O 1:1 afforded **7** (514 mg, 61%). Colorless plates. M.p. 91°.

Method B: Compound **1** (500 mg, 1.87 mmol) was dried by repeated co-evaporation from abs. pyridine and then dissolved in abs. pyridine under slight warming. After cooling to 0°, isobutyryl chloride (1.95 ml, 18.5 mmol) was added (Ar). The turbid mixture was stirred for 1 h and then poured into a soln. of $NaHCO_3$ (2.5 g) in H_2O (40 ml). After concentration to 20 ml, the soln. was stored at 8° overnight. The precipitated material was filtered off and washed with Et_2O (20 ml): colorless **7** (620 mg, 74%). TLC (silica gel, *E*): R_f 0.4. UV (MeOH): 260 (16700), 280 (12600). ^{1}H-NMR ((D_6)DMSO): 12.10, 11.70 (br., NH); 8.09 (*s*, H–C(8)); 6.20 (*d*, J = 6, H–C(1′)); 5.48 (*m*, H–C(3′)); 4.42 (*m*, H–C(4′)); 4.28 (*m*, 2 H–C(5′)); 2.90 (*m*, H_α–C(2′)); 2.73 (*m*, H_β–C(2′)); 1.05 (6 Me). Anal. calc. for $C_{22}H_{31}N_5O_7$ (477.51): C 55.34, H 6.54, N 14.67; found: C 55.18, H 6.57, N 14.73.

*9-[2′-Deoxy-β-*D-threo-*pentofuranosyl]-*N^2*-(2-methylpropanoyl)guanine* (**8**). Compound **7** (1.2 g, 2.51 mmol) was dissolved in MeOH (40 ml) and cooled to 0°. Thereupon, 1N aq. NaOH was added until the pH raised to 12. After 50 min, the reaction was quenched by addition of ion-exchange resin (*Dowex W* × *8*; pyridinium form), and the resin was filtered off. After washing with MeOH (300 ml), the combined solns. were evaporated, and the residue was crystallized from H_2O: colorless needles (560 mg, 72%). M.p. 250°. TLC (silica gel, *F*): R_f 0.42. UV (MeOH): 260 (16100), 281 (11900). ^{1}H-NMR ((D_6)DMSO): 12.05 (br. *s*, NH); 11.70 (br. *s*, NH); 8.20 (*s*, H–C(8)); 6.14 (*d*, J = 7.8, H–C(1′)); 5.30 (*s*, OH–C(3′)); 4.68 (*s*, OH–C(5′)); 4.36 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′)); 3.72 (*m*, 2 H–C(5′)); 2.77 (*m*, H_α–C(2′)); 2.28 (*d*, J = –14.5, H_β–C(2′)); 1.13 (*d*, J = 6.8, 2 Me). Anal. calc. for $C_{14}H_{19}N_5O_5$ (337.33): C 49.85, H 5.68, N 20.76; found: C 49.98, H 5.81, N 20.75.

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-threo-*pentofuranosyl]-*N^2*-(2-methylpropanoyl)guanine* (**9**). Compound **8** (300 mg, 0.89 mmol) was dried by repeated co-evaporation from abs. pyridine and then dissolved in abs. pyridine (5 ml). After addition of 4-(dimethylamino)pyridine (16 mg, 0.13 mmol), 4,4′-dimethoxytrityl chloride (150 mg, 0.4 mmol) was added (Ar), and the soln. was stirred for 4 h at r.t. After addition of 5% aq. $NaHCO_3$ soln. (45 ml), the soln. was extracted twice with CH_2Cl_2. The combined org. extracts were dried (Na_2SO_4), filtered, and evaporated. FC (column 20 × 5 cm, *G*) and evaporation of the main zone afforded a colorless, amorphous solid (423 mg, 74%). TLC (silica gel, *G*): R_f 0.4. UV (MeOH): 236 (22600), 251 (18500), 276 (13300). ^{1}H-NMR ((D_6)DMSO): 12.09 (br. *s*, NH); 11.77 (br. *s*, NH); 8.07 (*s*, H–C(8)); 6.22 (*m*, H–C(1′)); 5.30 (*s*, OH–C(3′)); 4.34 (*m*, H–C(3′)); 4.21 (*m*, H–C(4′)); 3.71 (*s*, MeO); 3.20 (*m*, 2 H–C(5′)); 2.74 (*m*, H_α–C(2′)); 2.30 (*d*, J = –14.5, H_β–C(2′)); 1.12 (2 Me). Anal. calc. for $C_{35}H_{37}N_5O_7$ (639.71): C 65.72, H 5.83, N 10.95; found: C 65.61, H 5.98, N 10.91.

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-threo-*pentofuranosyl]-*N^2*-(2-methylpropanoyl)guanine 3′-(Triethylammonium Phosphonate)* (**10**). To a soln. of PCl_3 (135 µl, 1.55 mmol) and *N*-methylmorpholine (1.7 ml, 15.2 mmol) in abs. CH_2Cl_2 (12 ml), 1*H*-1,2,4-triazole (358 mg, 5 mmol) was added at r.t. After stirring for 30 min and cooling to 0°, a soln. of **9** (200 mg, 0.31 mmol) in CH_2Cl_2 (10 ml) was added within 10 min. After stirring for 20 min, the mixture was hydrolyzed by addition of 1M TBK buffer ($(Et_3NH)HCO_3$, pH 7.7; 17 ml). The aq. phase was extracted with CH_2Cl_2 (3 × 15 ml), the combined org. extract dried (Na_2SO_4) and evaporated, and the residue submitted to FC (column 20 × 3 cm, CH_2Cl_2 (1 l), then *G* (500 ml) and *H*). The main zone was evaporated and the residue dissolved in CH_2Cl_2 (20 ml) and extracted six times with 0.1M TBK buffer. Drying (Na_2SO_4) and evaporation gave a pale yellow foam (132 mg, 53%). TLC (silica gel, *G*): R_f 0.43. UV (MeOH): 237 (22400), 255 (16700), 275 (12600). ^{1}H-NMR ((D_6)DMSO): 12.06 (br. *s*, NH); 11.97 (br. *s*, NH); 8.15 (*s*, H–C(8)); 7.67 (*d*, J = 588, PH); 7.33–6.78 (*m*, 13 arom. H); 6.17 (*m*, H–C(1′)); 4.80 (*m*, H–C(3′)); 4.24 (*m*, H–C(4′)); 3.68 (*m*, MeO); 3.35 (*m*, 2 H–C(5′)); 3.36 (*m*, H_α–C(2′)); 3.20 (*d*, J = –14.5, H_β–C(2′)); 1.02 (2 Me). ^{31}P-NMR ((D_6)DMSO): 1.2 (^1J(P,H) = 583.3, ^3J(P,H) = 8.2). Anal. calc. for $C_{41}H_{53}N_6O_9P$ (804.92): C 61.18, H 6.64, N 10.44; found: C 61.27, H 6.76, N 10.26.

*9-{2′-Deoxy-3′-*O*-[(diisopropylamino)(2-cyanoethoxy)phosphino]-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D-threo-*pentofuranosyl}-*N^2*-(2-methylpropanoyl)guanine* (**11**). To a soln. of **9** (100 mg, 0.16 mmol) in abs. CH_2Cl_2 (5 ml), (i-Pr)$_2$EtN (40 µl, 0.23 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (83 µl) were added, and the soln. was stirred for 2 h under Ar at r.t. After addition of 10% aq. $NaHCO_3$ soln. (5 ml), the mixture was extracted with CH_2Cl_2, the combined org. phase dried (Na_2SO_4) and evaporated, and the residue purified by prep. TLC (*I*). The main zones were pooled and eluted with *I*. Evaporation afforded a colorless oil (45 mg, 35%). TLC (silica gel, *I*): R_f 0.30, 0.23. ^{31}P-NMR ($CDCl_3$): 151.4, 147.8.

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-3′-*O*-succinyl-β-*D-threo-*pentofuranosyl]-*N^2*-(2-methylpropanoyl)guanine* (**12**). To a soln. of **9** (180 mg, 0.28 mmol) in abs. pyridine (6 ml), 4-(dimethylamino)pyridine (45 mg, 0.34 mmol) and succinic anhydride (144 mg, 1.44 mmol) were added. After stirring for 48 h at 40°, the reaction was quenched by addition of H_2O (3 ml). Pyridine was removed by repeated co-evaporation from toluene. Thereupon, the residue was dissolved in CH_2Cl_2, the soln. washed with aq. citric acid soln. and H_2O, dried (Na_2SO_4), and evaporated, the residue dissolved in CH_2Cl_2/pyridine 95:5 (2 ml), and the crude product precipitated by addition of pentane/Et_2O 1:1 (50 ml) at 0°. The crude material was submitted to FC (column 6 × 10 cm, *K*).

Evaporation of the main zone afforded an amorphous solid (185 mg, 88%). TLC (silica gel, *K*): R_f 0.7. ^{1}H-NMR ((D$_6$)DMSO): 12.10 (br. *s*, NH); 11.80 (br. *s*, NH); 7.90 (*s*, H–C(8)); 7.36–6.81 (*m*, 13 arom. H); 6.27 (*m*, H–C(1′)); 5.46 (*m*, H–C(3′)); 4.40 (*m*, H–C(4′)); 3.71 (2 MeO); 3.28 (*m*, 2 H–C(5′)); 2.90 (*m*, H$_\alpha$–C(2′)); 2.75 (*m*, H$_\beta$–C(2′)); 2.28 (2 CH$_2$); 1.10 (2 Me). Anal. calc. for $C_{39}H_{41}N_4O_{11}$ (741.77): C 63.15, H 5.57, N 9.47; found: C 62.84, H 5.72, N 9.39.

*6-Amino-9-(2′-deoxy-β-*D*-*threo*-pentofuranosyl)-1,9-dihydro-2*H*-purin-2-one* (= *2′-Deoxy-β-*D*-xyloisoguanosine*; **3**). Compound **2** (300 mg, 1.08 mmol) was dissolved in H_2O (35 ml), and $NaNO_2$ (350 mg) was added. After warming the soln. to 50°, AcOH (0.45 ml) was added and stirring continued for 5 min, before 25% of aq. NH_3 soln. was added until the pH had reached 8. Thereupon, the mixture was diluted with H_2O (250 ml) and submitted to ion-exchange chromatography (*Serdolit AD-4*, column 4 × 6 cm). After washing with H_2O (500 ml), **3** was eluted with solvent *L*. Subsequent FC (column 6 × 6 cm, *M*) gave an amorphous solid (180 mg, 60%). TLC (silica gel, *C*): R_f 0.7. UV (H_2O): 247 (9000), 292 (10000). ^{1}H-NMR ((D$_6$)DMSO): 8.2 (br. *s*, NH_2); 8.11 (*s*, H–C(8)); 6.16 (*m*, *J* = 7.8, H–C(1′)); 4.43 (*m*, H–C(3′)); 3.98 (*m*, H–C(4′)); 3.83 (*m*, 2 H–C(5′)); 2.87 (*m*, H$_\alpha$–C(2′)); 2.37 (*d*, *J* = −14.6, H$_\beta$–C(2′)). Anal. calc. for $C_{10}H_{13}N_5O_4$ (267.2): C 44.94, H 4.90, N 26.20; found: C 44.87, H 4.96, N 26.04.

*9-(2′-Deoxy-β-*D*-*threo*-pentofuranosyl)-3,9-dihydro-2*H*-purin-2,4(1*H*)-dione* (= *2′-Deoxy-β-*D*-xyloxanthosine*; **4**). Compound **3** (100 mg, 0.36 mmol) was dissolved in H_2O (10 ml), and adenosine deaminase (calf intestine mucosa, EC 3.5.4.4; 0.1 mg) was added. After stirring for 7 days at 37°, the solvent was evaporated. Colorless oil (100 mg, 100%). ^{1}H-NMR ((D$_6$)DMSO): 7.81 (*s*, H–C(8)); 6.06 (*d*, *J* = 7.5, H–C(1′)); 4.36 (*m*, H–C(3′)); 3.89 (*m*, H–C(4′)); 3.73 (*m*, 2 H–C(5′)); 2.78 (*m*, H$_\alpha$–C(2′)); 2.25 (*d*, *J* = −14.5, H$_\beta$–C(2″)).

*9-(2′-Deoxy-β-*D*-*threo*-pentofuranosyl)guanine 3′-[3-(*N*-'Fractosil'-carbamoyl)propanoate]* (**13**). To a soln. of **12** (100 mg, 0.16 mmol) in 1,4-dioxane/pyridine 95:5 (2 ml), 4-nitrophenol (40 mg, 0.28 mmol) and *N*,*N*-dicyclohexylcarbodiimide (60 mg, 0.28 mmol) were added under stirring at r.t. After 2 h, dicyclohexylurea was removed by filtration. To the filtrate, *Fractosil 200* (200 mg, 450 μequiv. NH_2/g) and DMF (1 ml) were added. After addition of Et_3N (200 μl), the suspension was shaken for 4 h at r.t. Then Ac_2O (60 μl) was added, and shaking was continued for another 30 min. The *Fractosil* derivative **13** was filtered off, washed with DMF, EtOH, and Et_2O, and dried *in vacuo*. The amount of silica gel bound nucleoside was determined by treatment of **13** (5 mg) with 0.1 M TsOH (10 ml) in MeCN. From the absorbance at 498 nm of the supernatant, 73 μmol of linked nucleoside/g *Fractosil* was calculated (ε((MeO)$_2$Tr) = 70000).

Solid-Phase Synthesis of the Oligonucleotides **14**–**19**. The synthesis of the oligonucleotides was accomplished on a 1-μmol scale using a protocol already published [1] [2]. Results in *Table 4*.

Table 4. *Synthesis of Oligonucleotides* **14**–**19**

	t_R [min][a])	Yield [A_{260} units]	$H_{therm.}$ [%]
14	12.6	15.0	15
15	13.2	18.3	19
16	12.7	7.5	5
17	10.8	10.4	7
18	12.3	2.0	10

[a]) Gradient *II*, flow rate 1 ml/min; 4 × 250 mm *RP-18 Lichrosorb*.

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