83. 1-(2′-Deoxy-β-D-xylofuranosyl)cytosine: Base Pairing of Oligonucleotides with a Configurationally Altered Sugar-Phosphate Backbone

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Solid-phase synthesis of the oligo(2′-deoxynucleotides) **19** and **20** containing 2′-deoxy-β-D-xylocytidine (**4**) is described. For this purpose, 1-(2-deoxy-β-D-*threo*-pentofuranosyl)cytosine (= 1-(2-deoxy-β-D-xylofuranosyl)-cytosine; **4**) was protected at its 4-NH_2 group with a benzoyl (→ **5**) or an isobutyryl (→ **8**) residue, and a dimethoxytrityl group was introduced at 5′-OH (→ **7**, **10**; *Scheme 2*). Compounds **7** and **10** were converted into the 3′-phosphonates **11a,b**. While **19** could be hybridized with **21** and **22** under formation of duplexes with a two-nucleotide overhang on both termini (**19**·**21**: T_m 29°; **19**·**22**: T_m 22°), the decamer **20** bearing four xC_d residues could no longer be hybridized with one of the opposite strands. Moreover, the oligonucleotides d[$(xC)_8$–C] (**13**), d[$(xC)_4$–C] (**14**), d[C–$(xC)_4$–C] (**15**), and d[C–$(xC)_3$–C] (**16**) were synthesized. While **13** exhibits an almost inverted CD spectrum compared to $d(C_9)$ (**17**), the other oligonucleotides show CD spectra typical for regular right-handed single helices. At pH 5, d[$(xC)_8$–C] forms a stable hemi-protonated duplex which exhibits a T_m of 60° (d[$(CH^+)_9$]·$d(C_9)$: T_m 36°). The thermodynamic parameters of duplex formation of (**13**H^+·**13**) and (**17**H^+·**17**) were calculated from their melting profiles and were found to be identical in ΔH but differ in ΔS (**13**H^+·**13**: ΔS = −287 cal/K mol; **17**H^+·**17**: ΔS = −172 cal/K mol).

The torsion angles around the bonds of the sugar-phosphate DNA backbone are of decisive importance for the secondary structure of DNA as well as for base-base recognition [1]. Recently, we reported on the synthesis of homooligonucleotides containing 2′-deoxyxylothymidine (xT_d) or 2′-deoxyxyloadenosine (xA_d) [2] [3]. The oligomers exhibit reversed *Cotton* effects in their CD spectra and could be hybridized with oligo(2′-deoxyribonucleotides). Already the dimer d(xTpxT) [4] exhibits an inverted CD spectrum compared to TpT which upon heating becomes similar to that of monomeric xT_d. This clearly demonstrates that this CD spectrum is due to a particular secondary structure, most likely with a left-handed helical sense. Moreover, mixed oligo(2′-deoxynucleotides) in which only some of the regular nucleotide units are replaced by their 2′-deoxyxylo counterparts were synthesized and their structural properties determined by temperature- and concentration-dependent UV and CD spectroscopy.

Besides their structural peculiarities, oligonucleotides either built-up of or containing 2′-deoxyxylonucleotides exhibit other interesting properties: generally, such modified oligomers are cleaved by snake-venom phosphodiesterase (oligonucleotide-5′-nucleotidohydrolase) completely, but at a significantly reduced hydrolysis rate. On the other hand, they are resistant towards calf-spleen phosphodiesterase (oligonucleotide-3′-nucleotidohydrolase). This offers the possibility of protecting antisense oligonucleotides against their catabolic deactivation by cellular nucleases.

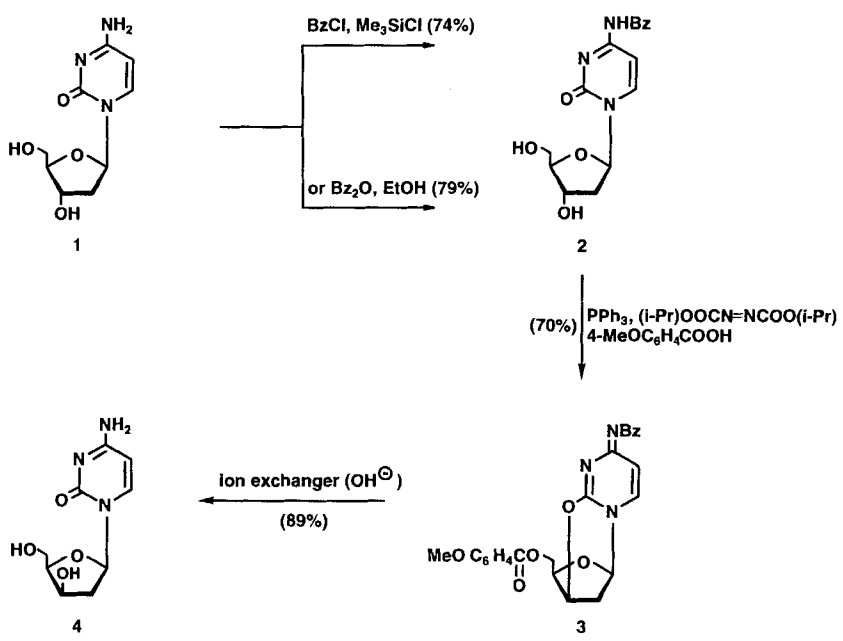
Xylose is a product of the formose reaction (see [5] and lit. cit. therein) so that it might be possible that this sugar was formed during prebiotic chemical evolution. Also nucleosides and nucleotides containing this glyconic moiety might have been formed. However,

due to a neighboring group (OH–C(3′)) participation in these 3′,4′-*threo*-configurated xylose derivatives, their chemical phosphorylations give exclusively 3′,5′-cyclic phosphates instead of 5′-monophosphates. As a result, 5′-triphosphates are very unstable. Consequently, such molecules are not suitable for the chemical storage of energy so that xylo-DNA could not be formed [6].

In the following, solid-phase synthesis of oligonucleotides containing 2′-deoxy-β-D-xylocytidine (xC_d, **4**) using xC_d phosphonates as monomeric building blocks is described [7]. Base-pairing properties as well as the stability of oligonucleotides containing 2′-deoxyxylocytidine are discussed.

Results and Discussion. – *Oligo(2′-deoxyxylonucleotides).* The 2′-deoxy-β-D-xylocytidine [8] (**4**, xC_d) was synthesized starting from 2′-deoxycytidine [9] (**1**) (*Scheme 1*). After benzoylation of **1** [10–12], the resulting **2** was submitted to a *Mitsunobu* reaction [13] yielding the 2,3′-anhydro derivative **3**. The latter was treated with *Dowex-1* × *2* ion-exchange resin (OH^- form) [14] to give **4** in 89% yield.

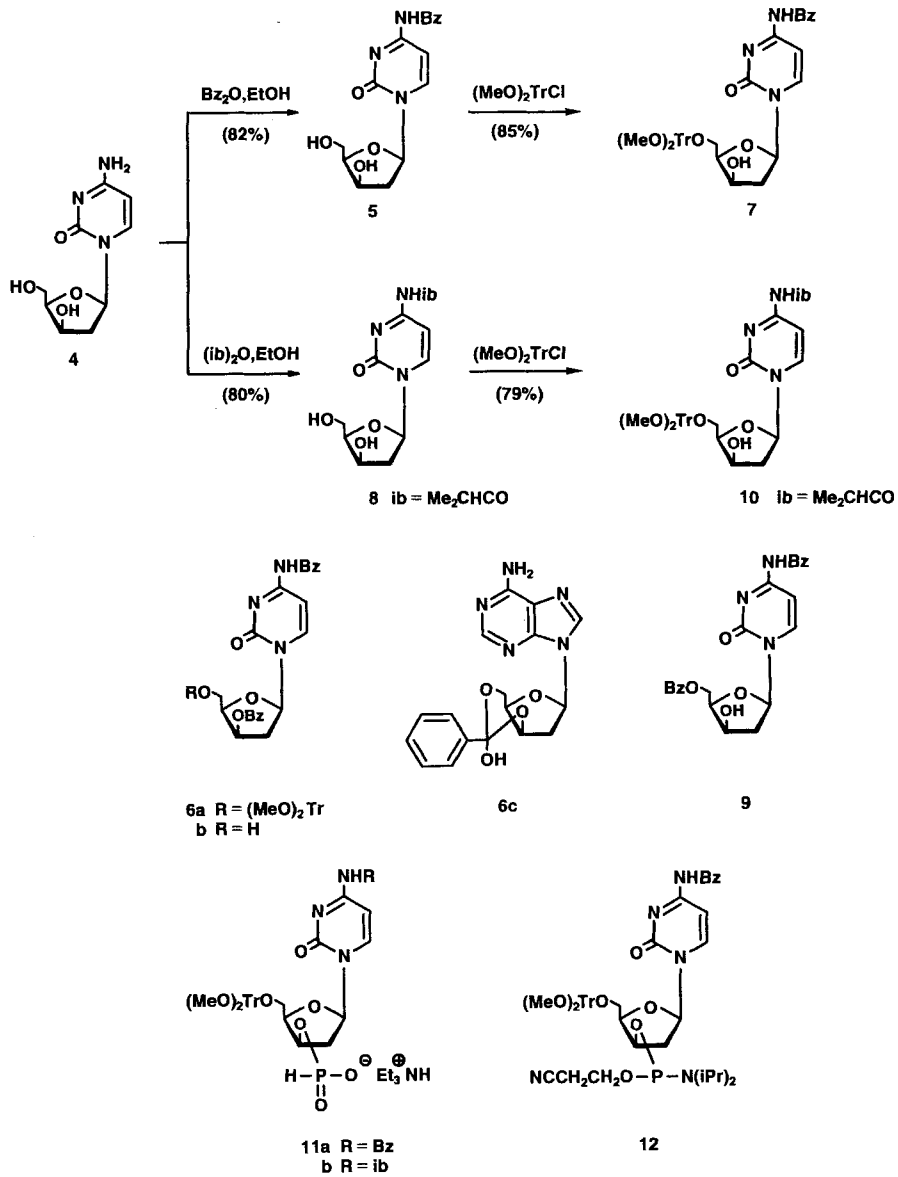
Scheme 1



Analysis of vicinal $^1H,^1H$-coupling constants of the sugar moiety (^3J(H–C(1′), H_β–C(2′)) ⩽ 1 Hz) using the PSEUROT program according to *Haasnoot et al.* [15] demonstrated a significantly preferred N-type sugar puckering ($^3T_{2'}$) of the 2′-deoxyxylose ring of **4** (N-conformer population ⩾ 95%). This is in line with findings on other 2′-deoxy-β-D-xylonucleosides as well as on d(xTpxT). The rigid, cyclic structure of **3** results in significantly altered ^{13}C-NMR resonances when compared to **2**. The 3′,4′-*threo*-configurated 2′-deoxyxylonucleoside **4** exhibits high-field-shifted glyconic resonances; the C(1′) and C(4′) signals coincide which is different from other 2′-deoxyxylonucleosides [2] [3].

Protection of the amino group of **4** with BzCl was performed employing the protocol of transient silyl protection of the sugar OH groups. TLC Monitoring (silica gel, CH_2Cl_2/MeOH 9:1) of the silylation in the absence of BzCl showed that silylation of the 3′-OH in case of **4** was incomplete compared to **1**. This explains the formation of the N^4,3′-dibenzoylated **6b**, besides the desired **5** (*Scheme 2*), when the reaction was carried out in the

Scheme 2



presence of BzCl. The by-product **6b** was isolated from the mixture and compared with an authentic sample which was prepared from **7** *via* **6a** (see *Exper. Part*).

A similar reaction was observed during the benzoylation of 2′-deoxyxyloadenosine (xA_d). In this case, the ortho ester **6c** was isolated which is apparently formed from a 3′-benzoylated intermediate similar to **6b**. The formation of **6c** which was isolated and characterized by ^{1}H- and ^{13}C-NMR spectra[1]) as well as elemental analysis [16] can be explained by two mechanisms: *i*) the trimethylsilyloxy residue is expelled by attack of the carbonyl O-atom forming an acyloxonium intermediate which, upon hydrolysis, gives the product or *ii*) the 5′-*O*-silyl group is hydrolyzed upon addition of H_2O, and the 5′-OH attacks the carbonyl group. It is still unclear why in this case the exocyclic NH_2 group is not benzylated. Both reactions, however, are enforced by neighboring-group participation. In view of these results, we repeated the benzoylation of xA_d and found that the ortho ester **6c** always formed but that its amount depended on the reaction and workup conditions. Therefore, the formamidine or another non-acyl group is recommended for protection of xA_d.

For these reasons, protection of xC_d (**4**) was performed by acylation according to the conditions of *Otter* and *Fox* [11] using benzoic or isobutyric anhydride in absolute EtOH: compounds **5** and **8** were obtained in 82 and 80% yield, respectively (*Scheme 2*). In the former case, due to the reactivity of the 5′-OH group, trace amounts of the N^4,5′-dibenzoylated compound **9** were isolated and characterized by ^{1}H- and ^{13}C-NMR spectra (*Table 1*[2])). Subsequently, the dimethoxytrityl group ($(MeO)_2Tr$) was introduced under standard conditions [2] [3] in **5** and **8**, as 5′-*O*-protecting group, giving **7** and **10**, respectively. The ^{13}C-NMR resonances of C(1′) and C(4′) which coincide for xC_d (**4**; *Table 1*) are well separated in case of the acylated derivatives **7** and **10**. Proton-coupled ^{13}C-NMR as well as 2D ^{1}H,^{13}C-NMR spectra, however, reveal that the order of resonances is changed compared to compounds **1**–**3**. This is a general phenomenon for all xC_d derivatives and may be due to an altered sugar puckering compared to the 3′,4′-*erythro*-configurated compounds.

To prove the applicability of the acyl protecting groups in the solid-phase oligonucleotide synthesis, the half-life values ($t_{1/2}$) of compounds **5** and **8** in 25% aqueous NH_3 solution (40°) were measured UV-spectrophotometrically at the wavelength indicated in *Table 2*. As can be seen, both, the benzoyl as well as the isobutyryl group, are suitable for solid-phase oligonucleotide synthesis. Both compounds were reacted with PCl_3/1*H*-1,2,4-triazole/*N*-methylmorpholine to afford the phosphonates **11a,b** which could directly be employed in solid-phase oligonucleotide synthesis [17]. The structure of the phosphonates **11a,b** was established by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra (*Table 1* and *Exper. Part*).

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[1]) **6c**: ^{1}H-NMR ($(CD_3)_2SO$; δ in ppm, J in Hz): 8.28 (*s*, H–C(8)); 8.06 (*s*, H–C(2)); 7.85 (*d*, J = 7.5, 2 H, Bz); 7.55 (*t*, J = 7.0, 1 H, Bz); 7.41 (*t*, J = 7.5, 2 H, Bz); 7.23 (br. *s*, NH_2); 6.22 (*dd*, J(H–C(1′),H_α–C(2′)) = 8.5, J(H–C(1′),H_β–C(2′)) = 2.0, H–C(1′)); 4.45 (CH_2(5′)); 4.38 (*m*, H–C(4′)); 4.19 (*m*, H–C(3′)); 2.76 (*m*, H_α–C(2′)); 2.25 (*m*, H_β–C(2′)). ^{13}C-NMR ($(CD_3)_2SO$; δ in ppm): 165.7 (Bz); 156.2 (C(6)); 152.3 (C(2)); 148.6 (C(4)); 140.0 (C(8)); 133.4, 129.6, 129.3, 128.8 (Bz); 119.1 (C(5)); 82.9 (C(1′)); 81.7 (C(4′)); 69.7 (C(3′)); 64.3 (C(5′)); 40.5 (C(2′)). Anal. calc. for $C_{17}H_{17}N_5O_4$: C 57.46, H 4.82, N 19.71; found: C 57.63, H 4.97, N 19.62.

[2]) N^4-Benzoyl-1-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)cytosine (**9**): ^{1}H-NMR ($(CD_3)_2SO$; δ in ppm, J in Hz): 11.16 (br. *s*, NH); 8.36 (*d*, J = 7.3, H–C(6)); 8.00–7.35 (*m*, 10 arom. H, H–C(5)); 6.08 (*d*, J = 7.0, H–C(1′)); 5.40 (br. *s*, OH–C(3′)); 4.68 (*m*, H–C(3′), H–C(4′)); 4.38 (*m*, CH_2(5′)); 2.64 (*m*, H_α–C(2′)); 2.08 (*m*, H_β–C(2′)).

Table 1. *^{13}C-NMR Chemical Shifts of Nucleosides*[a])

	C(2)	C(4)	C(5)	C(6)	COO	CON	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	MeO
1[a])	155.2	165.6	94.1	141.1	–	–	85.0	40.4	70.5	87.2	61.5	–
1[b])	157.3	166.4	96.4	141.8	–	–	86.4	39.6	70.8	86.9	61.6	–
2	154.4	163.0	96.2	145.0	–	167.6	86.3	41.0	70.1	88.0	61.0	–
3	163.4	162.4	105.9	140.0	165.1	177.3	87.4	32.8	78.1	82.2	62.0	55.5
4[a])	155.5	165.7	93.5	142.1	–	–	85.0	40.7	68.9	85.0	59.7	–
4[b])	157.8	166.6	95.7	142.6	–	–	85.2	41.0	69.9	86.9	60.3	–
5	154.5	162.8	95.4	145.6	–	167.3	86.4	41.5	68.7	86.3	59.5	–
8	154.5	162.4	94.6	145.6	–	177.6	86.3	41.4	68.7	86.1	59.5	–
6b	154.5	163.1	95.9	144.9	164.8	167.4	86.7	41.5	72.9	84.6	58.9	–
9	154.6	163.0	95.7	145.8	165.8	167.4	87.0	41.6	69.2	83.0	63.8	–
7	154.5	162.9	95.0	145.1	–	167.2	87.0	41.5	69.1	84.7	62.7	55.0
10	154.6	162.5	94.4	145.2	–	177.8	87.0	41.5	69.1	84.7	62.8	55.1
11a	154.4	162.4	94.2	144.7	–	177.7	86.6	DMSO	71.5[c])	83.8[d])	62.5	55.0
b	154.5	163.0	95.1	144.9	–	167.4	86.9	DMSO	71.6[c])	84.0[d])	62.6	55.1

[a]) Measured in $(CD_3)_2SO$ at 303 K; not all resonances of protecting groups are given.
[b]) Measured in D_2O.
[c]) $J(P,C) = 6.8$ Hz.
[d]) $J(P,C) = 3.4$ Hz.

Table 2. *Half-life Values of Base-Catalyzed Hydrolysis of Actylated Nucleosides*[a])

	$t_{1/2}$ [min]	Wavelength [nm]
2	18.6	302
5	19.1	302
ib^{4}C$_d$	2.2	298
8	2.5	298

[a]) 25% aqueous NH_3 solution; 40°.

Alternatively, compound **7** was reacted with chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphane to give the phosphoramidite **12** [18]. TLC Monitoring revealed significant side reactions, and compound **12** could, therefore, be isolated in only 17% yield.

In the course of the synthesis of oligonucleotides containing xT$_d$ and xA$_d$, it appeared that solid-phase synthesis with 2′-deoxyxylo 2-cyanoethyl phosphoramidites required ten-times longer coupling times than the corresponding 2′-deoxyribo phosphoramidites. In addition, phosphoramidites of 2′-deoxyxylonucleosides were accessible with difficulty and, as seen above in case of xC$_d$, the yields were very low. To circumvent this problem, phosphonate chemistry was chosen for solid-phase oligonucleotide syntheses containing 2′-deoxyxylonucleosides. In these cases, pivaloyl chloride was used as condensation reagent, and oxidation, which was carried out on the oligomeric level, was completed by repeated reaction (5 times) with a 1:1 mixture (*v*/*v*) of 0.2M I_2 in THF and *N*-methylmorpholine/H_2O/THF 1:1:8, followed by a 1:1 mixture (*v*/*v*) of 0.2M I_2 in THF and Et_3N/H_2O/THF 1:1:8 [19]. The oligonucleotides were removed from the support by the action of conc. aqueous NH_3 solution (60°) and then purified as 5′-*O*-$(MeO)_2$Tr derivatives by

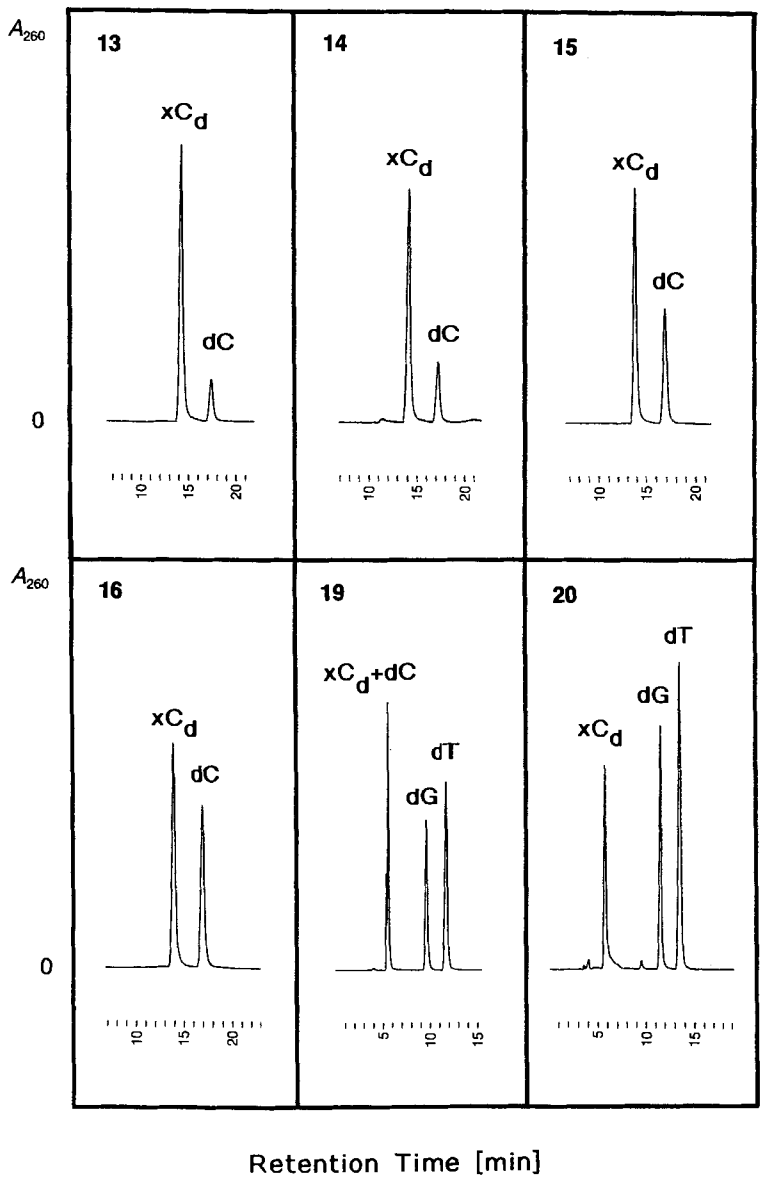

Fig. 1. *HPLC Profiles after enzymatic tandem hydrolysis in 0.1* M Tris-*HCl buffer* (pH 8.3) *of the oligomers* **13–16**, **19**, *and* **20** *with snake-venom phosphodiesterase followed by alkaline phosphatase*. Conditions, see [2], 2.5 μM of single strands, each; **13–16**: gradient *III*, flow rate 0.6 ml/min; **19** and **20**: gradient *IV*, flow rate 0.6 ml/min.

reversed-phase HPLC. In case of oligomers carrying a 2′-deoxyxylonucleoside at the 5′-terminus, detritylation was difficult and needed a 1-h treatment with AcOH (80% aqueous solution, 1 h, followed by neutralization with Et_3N). The detritylated oligomers were again submitted to reversed-phase HPLC, desalted, and lyophilized. Thus, the oligomers **13**–**23** were synthesized (for **21** and **23**, see [20]; for **22**, see [21]) and their composition determined by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase followed by HPLC (*Fig. 1*). Also the mixed oligomers **19** and **20** were synthesized using phosphonate **11a**, together with those of the corresponding regular 2′-deoxynucleosides employing the protocol of standard phosphonate chemistry.

d[$(xC)_8$–C] (**13**)
d[$(xC)_4$–C] (**14**)
d[C–$(xC)_4$–C] (**15**)
d[C–$(xC)_3$–C] (**16**)
d(C_9) (**17**)
d(C_5) (**18**)

d(C-C-G-T-C-T-T-xC-T-G) (**19**)
d(xC-xC-G-T-xC-T-T-xC-T-G) (**20**)
d(G-G-C-A-G-A-A-G-A-C) (**21**) [20]
d(G-G-C-A-xG-A-A-G-A-C) (**22**) [21]
d(C-C-G-T-C-T-T-C-T-G) (**23**) [20]

Duplex Formation of Oligonucleotides Containing 2′-Deoxyxylocytidine. Compound **19** was hybridized with equimolar amounts of either the cognate strand **21** or with the oligomer **22** bearing a xG_d unit to be opposite to xC_d [21]. Both duplexes exhibit a two-nucleotide overhang on both termini. *Fig. 2b* displays the melting profiles of the

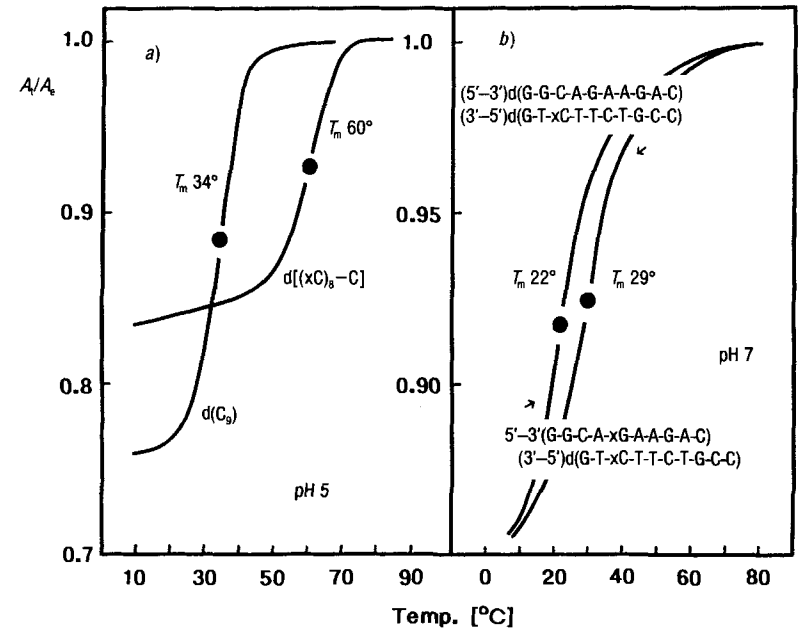

Fig. 2. *Normalized melting profiles of oligonucleotides. a*) Na-phosphate buffer (10 mM, pH 5, 10 mM NaCl, 1 mM EDTA); *b*) 60 mM Na-cacodylate (pH 7, 100 mM $MgCl_2$, 1M NaCl). Oligomer concentration 3.6 μM.

duplexes **19·21** and **19·22**. The double-modified duplex **19·22** bearing one $xC_d \cdot xG_d$ core shows a significantly lower T_m value compared to the corresponding unmodified parent duplex **23·21** ($\Delta T_m = -19°$) [20], while a single modification such as in **19·21** causes only a reduction in T_m of −12° compared to **23·21**. If all dC residues of the parent sequence **23** were replaced by xC_d (see **20**), no complex formation with the complementary strand **21** could be detected.

Fitting of the truncated melting curves to a two-state model and calculation of the *van't Hoff* enthalpy and entropy values of duplex formation shows that the ΔH values of **19·21** and **19·22** are almost identical but significantly lower than the ΔH value of the unmodified duplex **23·21** (*Table 3*). The ΔH value of the latter (−71.8 kcal/mol) is significantly lower than a calculated value (−46 kcal/mol) using published increments which were determined by *Breslauer et al.* [22] for aqueous solutions containing 1M NaCl. The nucleation process is associated with a $\Delta H = 0$ because H-bonding, the only major interaction in nucleation, has negligible enthalpy in H_2O [23]. This finding points to aggregate formation because of the dangling ends. The ΔH values for the modified duplexes **19·21** and **19·22** are significantly lower than that of the parent sequence (*Table 3*). This can be interpreted by formation of an internal bulge loop which consists of the modified core as well as its flanking $dA \cdot dT$ base pairs.

Table 3. *Thermodynamic Parameters of Oligonucleotide Melting*[a])

Complex	T_m [°C]	h [%]	ΔH [kcal/mol]	ΔS [cal/K mol]
13H^+·**13**	60	17	−90.7	−171.6
17H^+·**17**	34	24	−88.4	−287.3
19·21	29	14	−55.0	−182.7
19·22	22	16	−56.2	−192.1
23·21	40	11	−71.8	−205.0

[a]) Oligomer conc., 8 μM.

It was shown that homomeric dodecamers containing xT_d or xA_d exhibit reversed *Cotton* effects in the CD spectra compared to their regular counterparts. The same phenomenon was already observed for the dimer d(xTpxT). Upon heating to 90°, its CD spectrum becomes similar to that of xT_d indicating that the spectrum of d(xTpxT) is foremost the consequence of its particular secondary structure which is assumed to be that of a left-handed helix [20]. To substantiate this assumption, we applied the conformational analysis of *Eschenmoser* and *Dobler* [1] for evaluation of the most preferred structure of a DNA on oligo(2′-deoxyxylonucleotides). The critical structural parameter is the endocyclic torsion angle δ which, in a ribose ring, is always greater than 60°, as a consequence of the angle strain which induces flattening of the ring. Arranging the backbone of an oligo(2′-deoxyxylonucleotide) under consideration of the conformational analysis of *Eschenmoser* and *Dobler*, the exocyclic torsion angle δ is estimated to be in the range of 20–40° assuming an S-type sugar puckering of the furanose ring (N-type sugar puckering is implausible due to steric repulsion between the heterocyclic base and the 3′-phosphate residue, both placed on the β-site of the glyconic ring). Indeed, a computer modeling study on d(xTpxT) [4] gave a δ value of 38° confirming the proposed structure of a left-handed helix. Another alteration concerns the exocyclic torsion angle γ

(O(5′)–C(5′)–C(4′)–C(3′)) which for regular A- and B-DNA is around 60°. In case of an oligo(2′-deoxyxylonucleotide), such a value would lead to a 1,5-repulsion between O(5′) and O(3′), the extent of which depends on the amplitude of sugar puckering (ν_{max}; the higher the degree of puckering, the lower the 1,5-repulsion). Therefore, it seems reasonable to change γ from +60° to ±180°.

The CD spectra of d[(xC)$_8$–C] (**13**) and d(C$_9$) (**17**) [24] are shown in *Fig. 3* and exhibit a generally inverted shape. The axis of symmetry is tilted by 15°. Increase of the tempera-

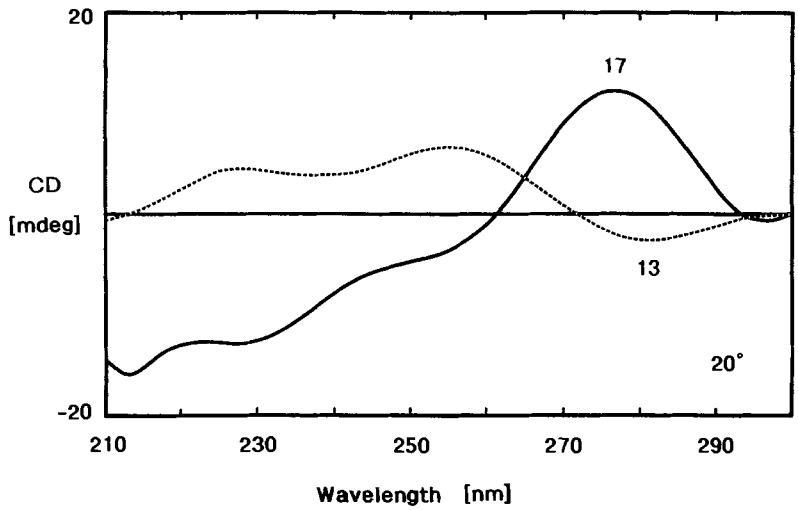

Fig. 3. *CD Spectra of d[(xC)$_8$–C]* (**13**) *and d(C$_9$)* (**17**) *in 60 mM Na-cacodylate* (pH 7, 100 mM $MgCl_2$, 1M NaCl). Oligomer concentration 3.6 μM.

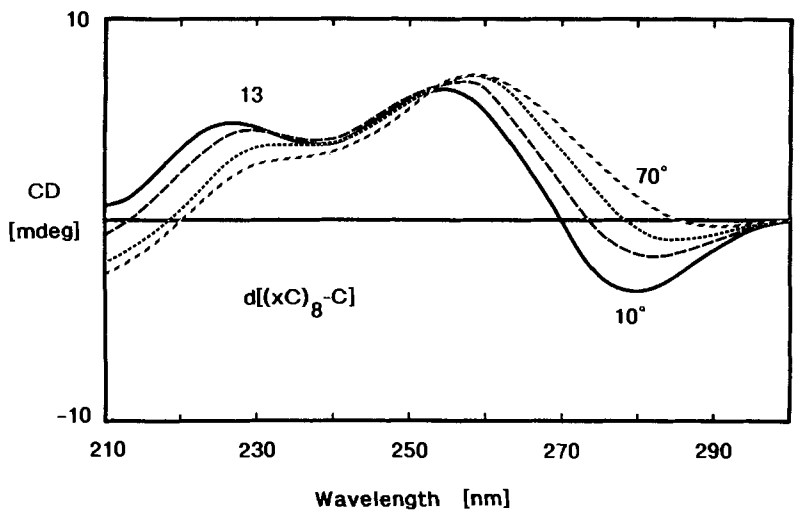

Fig. 4. *Temperature-dependent CD spectra of d[(xC)$_8$–C]* (**13**). Conditions: see *Fig. 3*.

ture from 10 to 70° changes the CD spectrum in such a way that the negative *Cotton* effect at 280 nm disappears completely (*Fig. 4*). Interestingly, shorter oligomers than **13**, *e.g.* d[(xC)$_4$–C] (**14**), exhibit a CD spectrum which shows the characteristics of a right-handed B-DNA. The same phenomenon is observed for the oligomers **15** and **16** (*Fig. 5*), both containing an excess of configurationally altered oligonucleotide linkages. This indicates that the helicity is inverted at a particular oligomer chain length.

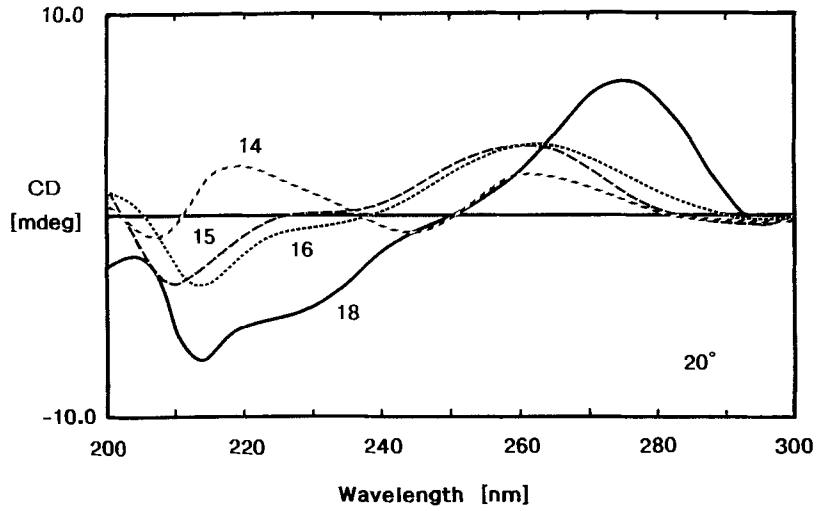

Fig. 5. *CD Spectra of the oligonucleotides* **14**–**16** *and* **18**. Conditions: see *Fig. 3*.

It has been a dogma that oligo(dC) forms a hemi-protonated duplex with parallel strand orientation at pH 5 $\{d[(CH^+)_n]\cdot d(C_n)\}$ [25] [26]. The stability of these base pairs is higher than that of dG·dC. A recent NMR [27] study at high oligomer concentration suggests now a different structure which is no longer dimeric but tetrameric. The dC·dCH$^+$ base pairs of two parallel strands are intercalated between the base pairs of a second duplex in an antiparallel arrangement. Similarly to the hemi-protonated complex of d(C$_9$) (**17**) that of d[(xC)$_8$–C] (**13**) exhibits cooperative melting at pH 5 (*Fig. 2a*). However, the T_m value is much higher than that of the complex of **17** ($\Delta T_m = +26°$; *Table 3*) while its hypochromicity is lower ($h = 17\%$). This implies stronger base pairing but weaker stacking interactions in case of d[(xC)$_8$–C] compared to d(C$_9$) ($h = 24\%$).

Fitting of the melting curves to a two-state model and calculation of the *van't Hoff* enthalpy and entropy values of complex formation shows that the exothermic heat values are almost identical in case of **17** and **13**, but that the entropy values differ significantly (d(C$_9$); $\Delta S = -287$ cal/K mol; d[(xC)$_8$–C]: $\Delta S = -171.6$ cal/K mol). This means that complex formation in case of d[(xC)$_8$–C] is entropically more favorable. Similar results were recently found for oligo(2′-deoxyxylonucleotides) which are completely built up from xT$_d$ and xA$_d$ [28]. The addition of one base pair to a double helix involves the restriction of 12 rotors (10 around the bonds of the sugar-phosphate backbones plus 2 around the glycosylic bonds) [29]. These increments of ΔS for extension of a double helix

balance against the formation of two base-pair stacks and the formation of 2 dA·dT or three dG·dC H-bonds. Moreover, the formation of double-helical structures of short duplexes from random-coil single strands involves the loss of rotational and translational free energy going along with a biomolecular association. These demanding geometrical restraints imply that already the preferred conformation of a single strand is of importance with respect to a preorganization towards a corresponding duplex. At pH 5, oligo(2′-deoxyxylocytidine) seems to be preorganized to a higher degree towards a duplex (or tetraplex) structure compared to regular oligo(dC) – probably as a consequence of the configurational change at C(3′) which causes an almost unflexible glyconic ring (N-type conformer population $\geq 95\%$) [30].

Another reason of such differences in the thermodynamics of duplex formation might be brought about by the different exposure to solvent of the heterocyclic nucleobases upon denaturation. In a usual native B-DNA double helix, the aromatic bases are burried between the two sugar-phosphate backbone strands and not as easily accessible for solvent molecules as in the denaturated state. The ΔS values measured imply that upon complex formation of $d[(xCH^+)_8{-}CH^+]\cdot d[(xC)_8{-}C]$, more H_2O molecules are set free than in the case of $d[(CH^+)_9]\cdot d(C_9)$.

Studies regarding the base pairing properties of xC_d with 2′-deoxyxyloguanosine as well as a detailed NMR spectroscopic analysis of xylo-DNA are under current investigation.

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Experimental Part

General Methods. See [2] [3]. Flash chromatography (FC): silica gel *60 H*, with CH_2Cl_2/MeOH 9:1 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 8:2 (*C*), CH_2Cl_2/MeOH 7:3 (*D*), CH_2Cl_2/Et_3N 98:2 (*E*), CH_2Cl_2/MeOH/Et_3N 93:5:2 (*F*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*G*), CH_2Cl_2/acetone 6:1 (*H*), CH_2Cl_2/acetone 55:45 (*I*), and CH_2Cl_2/AcOEt/cyclohexane 1:1:1 +5% Et_3N (*J*). HPLC: 4 × 250 and 4 × 25 mm (7 μm) or 10 × 250 mm *RP-18-LiChrosorb* columns (*Merck*); *Merck-Hitachi* HPLC apparatus with one pump (model *655 A-12*), connected with a proportioning valve, a variable-wavelength monitor (model *655 A*), and a controller (model *L-5000*), connected with an integrator (model *D*-2000); 0.1M (Et_3NH)OAc (pH 7)/MeCN 95:5 (*A*), 0.1M (Et_3NH)OAc (pH 7) (*B*), MeCN (*C*), H_2O (*D*), and MeOH/H_2O 3:2 (*E*) were used in the following order: gradient *I*, 3 min 15% *C* in *A* and 17 min 15–40% *C* in *A*; gradient *II*; 20 min 0–20% *C* in *A*; system *III*, 20 min *A*; system *IV*, 20 min *B*. UV: λ_{max} in nm, ε in M^{-1} cm^{-1}. NMR Spectra (^1H, ^{13}C, ^{31}P): *Bruker-AMX-500* (500 MHz) spectrometer; δ in ppm downfield from internal $SiMe_4$ (^{31}P, 85% H_3PO_4).

Melting Experiments. Cary 1/3 UV spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller at 260 and 280 nm, simultaneously. The actual temp. was measured in the reference cell with a Pt-100 resistor. The UV melting curves were measured between 10 and 80° with two data points per deg. Melting hypochromicity values were calculated from the initial and final absorbance as described by *Rosemeyer* and *Seela* [2]. The evaluation of the melting curves was performed on an *AT 286* personal computer using a software package (2hDNA) provided by Dr. *H. Apel* (*Varian*, Darmstadt, Germany). T_m Values were obtained *i*) graphically from the individual melting profiles, *ii*) from differential melting curves, and *iii*) by dividing ΔH of duplex formation by ΔS. The latter were calculated from the melting curves applying a program package developed by *J. Kehrhahn* (Physikalische Chemie, University of Osnabrück). The enzymatic hydrolysis of the oligonucleotides and determination of hypochromicity values were performed according to *Rosemeyer* and *Seela* [2].

2,3′-Anhydro-N^4*-benzoyl-1-[2-deoxy-5-*O*-(4-methoxybenzoyl)-β-*D*-threo-pentofuranosyl]cytosine* (**3**). To a suspension of **2** (3.31 g, 10 mmol) and PPh_3 (4.0 g, 15 mmol) in dimethylformamide (20 ml), a soln. of 4-methoxybenzoic acid (2.28 g, 15 mmol) and diisopropyl azodicarboxylate (3 ml, 15 mmol) in dimethylformamide (10 ml)

was added within 10 min at r.t. under Ar. After stirring for 20 min, a 2nd portion of 4-methoxybenzoic acid (2.28 g, 15 mmol) and diisopropyl azodicarboxylate (3 ml, 15 mmol) was added and stirring continued for 10 min. The mixture was poured into cold Et_2O (250 ml) and stored at 4° overnight. The precipitate was filtered off, washed with cold Et_2O, and dried. Crystallization from EtOH afforded colorless plates (3.14 g, 70%). M.p. 187–189°. TLC (silica gel, *C*): R_f 0.4. UV (MeOH): 254 (30400), 318 (17200). ^{1}H-NMR ($(CD_3)_2SO$): 7.68 (*d*, *J* = 7.3, H–C(6)); 7.01–7.58 (*m*, 9 arom. H); 6.52 (*d*, *J* = 7.3, H–C(5)); 6.00 (*d*, H–C(1′)); 5.47 (*m*, H–C(3′)); 4.58 (*m*, H–C(4′)); 4.41 (*m*, CH_2(5′)); 3.76 (*s*, MeO); 2.59–2.71 (*m*, CH_2(2′)). Anal. calc. for $C_{24}H_{23}N_3O_6$: C 64.44, H 4.73, N 9.39; found: C 64.49, H 4.71, N 9.31.

*1-(2-Deoxy-β-*D*-*threo*-pentofuranosyl)cytosine* (**4**). A soln. of **3** (2.0 g, 4.48 mmol) in EtOH/H_2O 1:1 (250 ml) was heated to 50°. Ion-exchange resin (*Dowex 1 × 2*, 100–200 mesh, OH^- form), suspended in H_2O (300 ml), was added and the mixture stirred at 50° for 20 h. Then the resin was filtered off and washed with H_2O, the combined filtrate evaporated, the oily residue dissolved in MeOH (20 ml), AcOEt (20 ml) added, and the soln. evaporated: colorless foam (903 mg, 89%). TLC (silica gel, *D*): R_f 0.34. UV (MeOH): 271 (8000). ^{1}H-NMR ($(CD_3)_2SO$): 7.82 (*d*, *J* = 7.5, H–C(6)); 7.04 (br., NH_2); 5.99 (*d*, H–C(1′)); 5.71 (*d*, *J* = 7.5, H–C(5)); 5.15 (*s*, OH–C(3′)); 4.68 (br., OH–C(5′)); 4.21 (*m*, H–C(3′)); 3.81 (*m*, H–C(4′)); 3.69 (*m*, CH_2(5′)); 1.80 (*d*, *J* = −14.5, H_β–C(2′)).

*N⁴-Benzoyl-1-(2-deoxy-β-*D*-*threo*-pentofuranosyl)cytosine* (**5**). *Method A*: Compound **4** (850 mg, 3.74 mmol) was dried by repeated co-evaporation with abs. pyridine and then dissolved in abs. pyridine (10 ml). Me_3SiCl (4 ml, 31.5 mmol) was added and the mixture stirred for 100 min at r.t. under Ar. Then a 2nd portion of Me_3SiCl (2 ml, 15.8 mmol) was introduced and stirring continued for another 40 min. After addition of benzoyl chloride (2.6 ml, 22.4 mmol), the mixture was stirred for 4.5 h, then cooled to 0°, and 25% aq. NH_3 soln. (20 ml) was added. After 2 h, the solvent was evaporated and the residue partitioned between H_2O (50 ml) and AcOEt (10 ml). From both layers, crude **5** (930 mg) could be obtained upon concentration. This material was submitted to FC (column 6 × 10 cm, *A*): colorless crystals (650 mg, 52%). M.p. 177° (H_2O).

Method B: A soln. of **4** (454 mg, 2 mmol) in abs. EtOH (45 ml) was heated under reflux, and benzoic anhydride (452 mg, 2.0 mmol) was added. Within the next 3 h, 3 further portions of benzoic anhydride (452 mg, 2.0 mmol, each) were added. After stirring for another 5 h, the soln. was evaporated and the residue triturated with Et_2O. The solid precipitate was filtered off and submitted to FC (column 2 × 10 cm, *A*): amorphous solid (540 mg, 82%). TLC (silica gel, *A*): R_f 0.4. UV (MeOH): 302 (10500), 258 (22200). ^{1}H-NMR ($(CD_3)_2SO$): 11.19 (br., NH); 8.27 (*d*, *J* = 7.5, H–C(6)); 8.01–7.47 (*m*, 5 arom. H); 7.32 (*d*, *J* = 7.5, H–C(5)); 5.99 (*d*, H–C(1′)); 5.09 (br., OH–C(3′)); 4.76 (br. OH–C(5′)); 4.24 (*m*, H–C(3′)); 3.98 (*m*, H–C(4′)); 3.78 (*m*, CH_2(5′)); 2.00 (*d*, *J* = −14.3, H_β–C(2′)). Anal. calc. for $C_{16}H_{17}N_3O_5$: C 58.01, H 5.17, N 12.68; found: C 58.14, H 5.29, N 12.71.

*N⁴-Benzoyl-1-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*threo*-pentofuranosyl]cytosine* (**7**). Compound **5** (1.07 g, 3.23 mmol) was dried by repeated co-evaporation with abs. pyridine and dissolved in dry pyridine (15 ml). Then 4,4′-dimethoxytriphenylmethyl chloride (1.64 g, 4.85 mmol) was added and the soln. stirred for 5 h under Ar at r.t. The mixture was poured into 5% aq. $NaHCO_3$ soln. (60 ml) and extracted twice with CH_2Cl_2 (50 ml, each). The combined org. layers were dried (Na_2SO_4), filtered, and evaporated. FC (column 6 × 10 cm, *I*) gave a colorless foam (1.735 g, 85%). TLC (silica gel, *I*): R_f 0.36. UV (MeOH): 301 (14500), 256 (28800), 236 (37000). ^{1}H-NMR ($(CD_3)_2SO$): 11.20 (br., NH); 7.99 (*m*, H–C(6), 2 arom. H); 7.64–7.20 (*m*, 13 arom. H); 6.90 (*m*, H–C(5), 3 arom. H); 6.04 (*d*, H–C(1′)); 5.06 (br., OH–C(3′)); 4.20 (br., H–C(3′), H–C(4′)); 3.74 (*s*, 2 MeO); 3.37 (*m*, CH_2(5′)); 2.00 (*d*, *J* = −14.5, H_β–C(2′)). Anal. calc. for $C_{37}H_{35}N_3O_7$: C 70.13, H 5.57, N 6.63; found: C 70.15, H 5.64, N 6.46.

*N⁴-Benzoyl-1-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-*D*-*threo*-pentofuranosyl]cytosine 3′-(Triethylammonium Phosphonate)* (**11a**). To a soln. of PCl_3 (500 µl, 5.68 mmol) and *N*-methylmorpholine (6.25 ml, 56.8 mmol) in CH_2Cl_2 (59 ml), 1*H*-1,2,4-triazole (1.31 g, 19 mmol) was added and the mixture stirred for 40 min at r.t. After cooling to 0°, a soln. of **7** (600 mg, 0.95 mmol) in CH_2Cl_2 (21 ml) was added dropwise within 20 min. The mixture was stirred for 40 min at r.t., poured into 1M aq. $(Et_3NH)HCO_3$ (TBK, 60 ml, pH 7.8), and shaken. Then the aq. layer was extracted twice with CH_2Cl_2 (30 ml, each), the combined org. extract dried (Na_2SO_4) and evaporated, and the resulting colorless foam submitted to FC (column 6 × 15 cm, 600 ml of *E*, then 300 ml of *F*, then *G*). The residue of the main zone was dissolved in CH_2Cl_2 (30 ml) and extracted twice with 1M aq. $(Et_3NH)HCO_3$ (pH 7.8, 15 ml, each), the org. layer dried (Na_2SO_4) and evaporated: colorless foam (433 mg, 57%). TLC (silica gel, *G*): R_f 0.6. UV (MeOH): 304 (11200), 256 (24000), 236 (31600). ^{1}H-NMR ($(CD_3)_2SO$): 11.24 (br., NH); 7.98–6.88 (*m*, H–C(5), H–C(6), 18 arom. H); 6.05 (*d*, H–C(1′)); 5.14 (*s*, PH); 4.59 (*m*, H–C(3′)); 4.29 (*m*, H–C(4′)); 3.74 (*s*, MeO); 3.38 (*m*, CH_2(5′)); 2.91 (*q*, 3 MeCH_2); 2.23 (*m*, *J* = −14.5, H_β–C(2′)); 1.06 (*t*, 3 *Me*CH_2). ^{31}P-NMR ($(CD_3)_2SO$): 0.52 (^1J(P,H) = 587, ^3J(P,H–C(3′)) = 8.8). Anal. calc. for $C_{43}H_{51}N_4O_9P$: C 64.65, H 6.43, N 7.01; found: C 64.43, H 6.55, N 7.00.

*N^4-Benzoyl-1-[2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-threo-pentofuranosyl]cytosin 3'-[(2-Cyanoethyl) N,N-Diisopropylphosphoramidite]* (**12**). To a soln. of **7** (250 mg, 0.39 mmol) in CH_2Cl_2 (5 ml), *N*-ethyldiisopropylamine (100 µl, 0.57 mmol), and chloro(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphane (226 µl), were added at r.t. under Ar. After stirring for 30 min, the reaction was quenched by addition of 5% aq. $NaHCO_3$ soln. (10 ml). The mixture was extracted with CH_2Cl_2 (2 × 5 ml) and the org. layer dried (Na_2SO_4) and evaporated. FC (column 3 × 6 cm, *J*) gave a colorless oil (56 mg, 17%). TLC (silica gel, *I*): R_f 0.76, 0.82. ^{31}P-NMR ($(CD_3)_2SO$): 150.5, 146.4.

*1-(2-Deoxy-β-*D*-threo-pentofuranosyl)-4-(2-methylpropanoyl)cytosine* (**8**). A soln. of **4** (600 mg, 2.64 mmol) in abs. EtOH (60 ml) was heated under reflux in the presence of isobutyric anhydride (440 µl, 2.64 mmol), 4 additional portions of the anhydride (440 µl, each) being added after each h. After a total reaction time of 5 h, the mixture was evaporated and the residue submitted to FC (column 4 × 10 cm, *A*): colorless solid (630 mg, 80%). TLC (silica gel, *A*): R_f 0.6. UV (MeOH): 298 (6600), 247 (13100). ^{1}H-NMR ($(CD_3)_2SO$): 10.76 (br., NH); 8.19 (*d*, *J* = 7.5, H–C(6)); 7.21 (*d*, *J* = 7.5, H–C(5)); 5.95 (*d*, H–C(1')); 5.03 (br., OH–C(3')); 4.73 (br., OH–C(5')); 4.21 (*m*, H–C(3')); 3.95 (*m*, H–C(4')); 3.74 (*m*, CH_2(5')); 2.70 (*m*, H_α–C(2')); 1.96 (*d*, *J* = −14.4, H_β–C(2')); 1.09 (*d*, 2 Me). Anal. calc. for $C_{13}H_{19}N_3O_5$: C 52.52, H 6.44, N 14.13; found: C 52.48, H 6.52, N 14.01.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-threo-pentofuranosyl]-4-(2-methylpropanoyl)cytosine* (**10**). Compound **8** (1.0 g, 3.36 mmol) was reacted with 4,4'-dimethoxytrityl chloride (1.6 g, 4.72 mmol) and worked up as described for **7**. FC (column 6 × 15 cm, *I*) afforded a colorless foam (1.5 g, 79%). TLC (silica gel, *I*): R_f 0.3. UV (MeOH): 298 (8300), 285 (7600), 237 (28700). ^{1}H-NMR ($(CD_3)_2SO$): 10.79 (br., NH); 7.92 (*d*, *J* = 7.5, H–C(6)); 7.45–7.22 (*m*, 9 arom. H); 7.10 (*d*, *J* = 7.5, H–C(5)); 6.90–6.67 (*m*, 4 arom. H); 6.00 (*d*, H–C(1')); 5.01 (*s*, OH–C(3')); 4.18 (*m*, H–C(3'), H–C(4')); 3.73 (*s*, 2 MeO); 3.36 (*m*, CH_2(5')); 2.70 (*m*, H_α–C(2')); 1.95 (*d*, *J* = −14.9, H_β–C(2')); 1.05 (*m*, 2 Me). Anal. calc. for $C_{34}H_{37}N_3O_7$: C 68.10, H 6.22, N 7.01; found: C 68.00, H 6.29, N 6.98.

*1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-threo-pentofuranosyl]-4-(2-methylpropanoyl)cytosine 3'-(Triethylammonium Phosphonate)* (**11b**). As described for **7**, **10** (600 mg, 1.0 mmol) was converted into **11b**: 428 mg (56%). Colorless foam. TLC (silica gel, *G*): R_f 0.5. UV (MeOH): 298 (8300), 285 (7600), 237 (28700). ^{1}H-NMR ($(CD_3)_2SO$): 10.85 (br., NH); 7.89 (*d*, *J* = 7.5, H–C(6)); 7.48 (*d*, *J* = 589, PH); 7.45–7.23 (*m*, 9 arom. H); 7.12 (*d*, *J* = 7.4, H–C(5)); 6.88 (*m*, arom. H); 6.01 (*d*, H–C(1')); 5.13 (*d*, *J* = 589, PH); 4.60 (*m*, H–C(3')); 4.29 (*m*, H–C(4')); 3.73 (*s*, 2 MeO); 3.32 (*m*, CH_2(5')); 2.85 (*q*, 2 CH_2); 2.62 (*m*, H_α–C(2')); 2.20 (*m*, *J* = −14.5, H_β–C(2')); 1.04 (*t*, 5 Me). ^{31}P-NMR ($(CD_3)_2SO$): 0.48 (^1J(P,H) = 589, ^3J(P,H–C(3')) = 8.8). Anal. calc. for $C_{40}H_{52}N_4O_9P$: C 62.90, H 6.86, N 7.34; found: C 63.06, H 7.05, N 7.39.

*N^4-Benzoyl-1-[3-O-benzoyl-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-threo-pentofuranosyl]cytosine* (**6a**). To a soln. of **7** (600 mg, 0.95 mmol) in abs. MeCN, benzoyl cyanide (372 mg, 2.84 mmol) and Et_3N (400 µl, 2.84 mmol) were added. After stirring for 6 h at r.t., the mixture was evaporated and the residue submitted to FC (column 6 × 20 cm, *H*): colorless foam (582 mg, 83%). TLC (silica gel, *H*): R_f 0.2. UV (MeOH): 304 (8300), 258 (18200), 234 (31800). ^{1}H-NMR ($(CD_3)_2SO$): 11.17 (br., NH); 8.12 (*d*, *J* = 7.5, H–C(6)); 7.97 (*d*, 2 arom. H); 7.68–7.18 (*m*, H–C(5), 17 arom. H); 6.78 (*m*, 4 arom. H); 6.07 (*d*, H–C(1')); 5.67 (*m*, H–C(3')); 4.58 (*m*, H–C(4')); 3.70 (*s*, 2 MeO); 3.47 (*m*, CH_2(5')); 2.92 (*m*, H_α–C(2')); 2.35 (*d*, *J* = −15.4, H_β–C(2')). Anal. calc. for $C_{44}H_{39}N_3O_8$: C 71.63, H 5.33, N 5.69; found: C 71.85, H 5.51, N 5.69.

*N^4-Benzoyl-1-[3-O-benzoyl-2-deoxy-β-*D*-threo-pentofuranosyl]cytosine* (**6b**). To AcOH (98%; 15 ml) preheated to 50°, **6a** (500 mg, 0.68 mmol) was added and the soln. stirred for 1 h at r.t. H_2O (15 ml) was added and the mixture evaporated, the oily residue triturated with MeOH (15 ml), the mixture again evaporated, and the residue submitted to FC (column 4 × 10 cm, *H*): colorless solid (277 mg, 94%). TLC (silica gel, *B*): R_f 0.4. UV (MeOH): 304 (10200), 259 (21700), 232 (22800). ^{1}H-NMR ($(CD_3)_2SO$): 11.15 (br., NH); 8.47 (*d*, *J* = 7.5, H–C(6)); 7.97, 7.87 (*d*, 2 H arom. H); 7.63–7.41 (*m*, H–C(5), 6 arom. H); 6.07 (*d*, H–C(1')); 5.59 (*m*, H–C(3')); 5.02 (*t*, OH–C(3')); 4.38 (*m*, H–C(4')); 3.90 (*m*, CH_2(5')); 2.92 (*m*, H_α–C(2')); 2.31 (*d*, *J* = −15.3, H_β–C(2')). Anal. calc. for $C_{23}H_{21}N_3O_6$: C 63.44, H 4.86, N 9.65; found: C 63.33, H 4.97, N 9.49.

Solid-Phase Synthesis of the Oligomers **13**–**20**. The synthesis of the oligonucleotides was carried out on a 1-µmol scale using the 3'-phosphonates of $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^4C_d$, and $[(MeO)_2Tr]T_d$ as well as compounds **11a**, **b**. The syntheses and deprotection of the oligonucleotides followed a slightly modified protocol of the DNA synthesizer for 3'-phosphonates [7]. In case of the modified oligomers **15** and **16**, the $(MeO)_2$Tr-group hydrolysis took 1 h. Yields, hypochromicities, and retention times of **13**–**20** in *Table 4*.

Table 4. *Yields, Hypochromicities, and Retention Times of the Oligomers* **13–20**

Oligomer	Yield		Hypochromicity [%]	Retention time[a]) [min]
	A_{260}	Units [%]		
d[$(xC)_8$–C] (**13**)	6.3	9.6	2.9	12.0; 10.3
d[$(xC)_4$–C] (**14**)	0.6	1.6	2.1	12.6; 7.3
d[C–$(xC)_4$–C] (**15**)	2.6	5.9	1.8	11.7; 8.1
d[C–$(xC)_3$–C] (**16**)	1.6	4.4	1.6	11.6; 7.7
$d(C_9)$ (**17**)	9.8	15	11	11.6; 12.9
$d(C_5)$ (**18**)	13	36	7.2	12.1; 11.6
d(C-C-G-T-C-T-T-xC-T-G) (**19**)	13	17	8.3	12.1; 13.1
d(xC-xC-G-T-xC-T-T-xC-T-G) (**20**)	13	15	5.4	11.8; 12.7

[a]) The 1st values (gradient *I*) refer to the $(MeO)_2Tr$ derivatives, the 2nd values to the deprotected oligomers (gradient *II*); flow rate, 3.5 ml/min column: 10 × 250 mm.

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