

94. Synthesis, Base Pairing, and Structural Transitions of Oligodeoxyribonucleotides Containing 8-Aza-2'-deoxyguanosine

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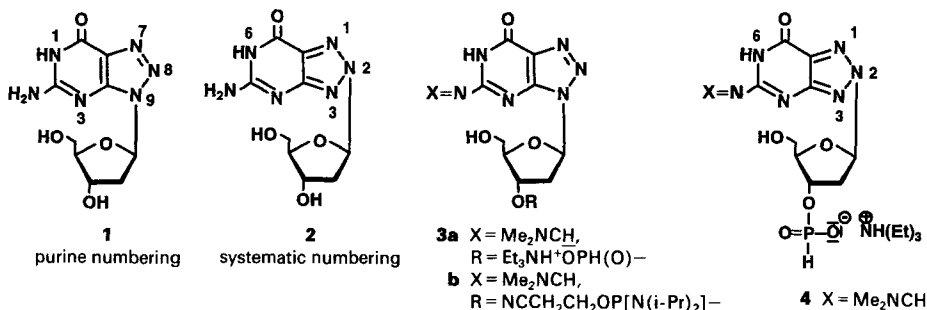
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The synthesis of oligonucleotides containing 8-aza-2'-deoxyguanosine (z^8G_d ; **1**) or its N^8 -regioisomer $z^8G_d^*$ (**2**) instead of 2'-deoxyguanosine (G_d) is described. For this purpose, the NH_2 group of **1** and **2** was protected with a (dimethylamino)methylidene residue ($\rightarrow$ **5**, **6**), a 4,4'-dimethoxytrityl group was introduced at 5'-OH ($\rightarrow$ **7**, **8**), and the phosphonates **3a** and **4** as well as the phosphoramidite **3b** were prepared. These building blocks were used in solid-phase oligonucleotide synthesis. The oligonucleotides were characterized by enzymatic hydrolysis and melting curves (T_m values). The thermodynamic data of the oligomers **12–15** indicate that duplexes were stabilized when **1** was replacing G_d . The aggregation of d(T-G-G-G-T) (**18**) was studied by *RP18* HPLC, gel electrophoresis and CD spectroscopy and compared with that of oligonucleotides containing an increasing number of z^8G_d residues instead of G_d . Similarly to $[d(C-G)]_3$ (**12a**), the hexamer d(C- z^8G -C- z^8G -C-G) (**14**) underwent salt-dependent B-Z transition.

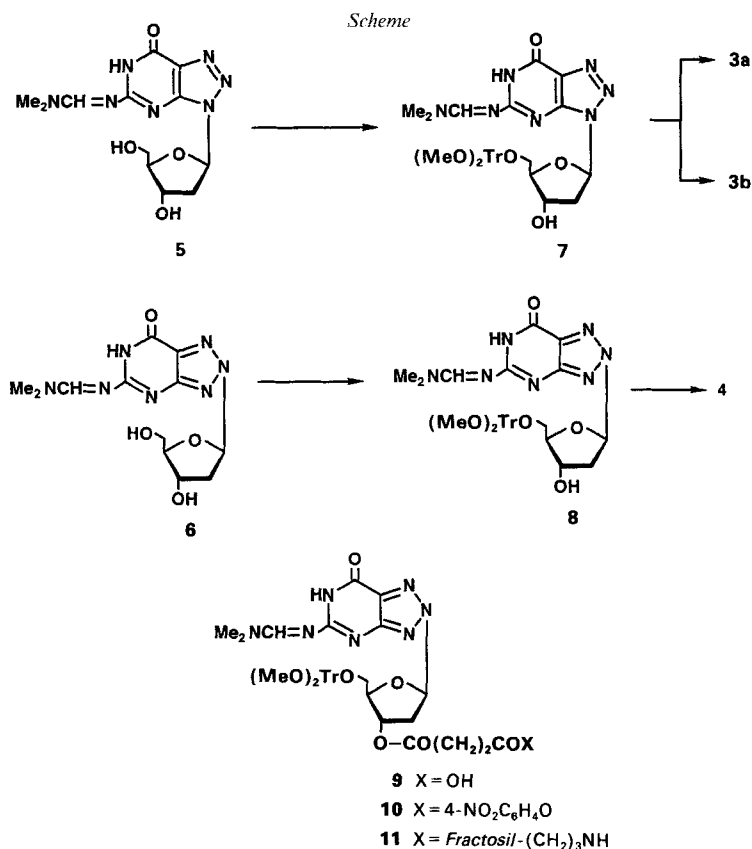
Introduction. – DNA can adopt various structures depending on the sequence or the environmental conditions [1]. Unusual duplexes are formed when modified bases are replacing the common DNA constituents [2]. In particular, the N-atoms located in the major or the minor groove are of decisive importance [3]. They can control the binding of enzymes or antibiotics. Special secondary structures such as the G-4 structure [4–6] or bended A_d tracts are influenced by the ring N-atom positions [7]. The N-atom pattern controls also the topology and strength of base pairs, thereby affecting duplex stability. It is interesting to mention that nucleosides with unusual N-glycosylic bond linkages such as N^7 -(2'-deoxyriboyl)purines [8] or N^2 -(2'-deoxyriboyl)pyrazolo[3,4-*d*]pyrimidines [9] are able to form duplex structures.

Recently, we investigated the glycosylation of 8-azaguanine and found that 8-aza-2'-deoxyguanosine (z^8G_d ; **1**) as well as the N^8 -regioisomer $z^8G_d^*$ (**2**) are formed as the main reaction products [10]. Both compounds are comparably stable and suitable for incorporation into oligonucleotides. In the following, we report on the synthesis of phosphonate and phosphoramidite building blocks **3a, b** and **4** derived from the nucleosides **1** or **2**. Furthermore, oligonucleotides are described containing an 8-azaguanine moiety with a sugar moiety attached to the N(8) or N(9) position of the base. Structural changes such as the B-Z transition and the aggregation of G_d -rich oligonucleotides are also investigated.

Results and Discussion. – *Building Blocks 3a, b and 4.* The N-glycosylic bond of 8-aza-2'-deoxyguanosine (z^8G_d ; **1**) was *ca.* 10 times more stable towards hydrolytic cleavage (0.1N HCl) than that of 2'-deoxyguanosine (G_d). Already the N^8 -isomer $z^8G_d^*$ (**2**) showed a *ca.* 5 times slower N–C(1') cleavage; the N^7 -isomer, however, was extremely



labile [10]. This is different to guanine 2'-deoxyribonucleosides: in this case, the N^7 -isomer was more stable than the N^9 -compound [11]. Accordingly, standard conditions can be used for the conversion of compounds **1** or **2** into the phosphonate or phosphoramidite building blocks **3a**, **b** or **4** used later in solid-phase synthesis. As NH_2 -protecting group, the (dimethylamino)methylidene residue was chosen for compounds **1** and **2** affording the amidines **5** and **6** as crystalline derivatives (*Scheme*). Their UV spectra were bathochromically shifted compared to the parent nucleosides **1** and **2**. The half-life values



of these compounds in 25% ammonia were determined UV-spectrophotometrically at 40°. Compound **5** showed a $t_{1/2}$ of 17 min and **6** a $t_{1/2}$ of 20 min. Under the same conditions, the (dimethylamino)methylidene derivative of 2'-deoxyguanosine had a half-life value of 19 min [12]. The corresponding amidines of related pyrazolo[3,4-*d*]pyrimidine 2'-deoxyribonucleosides were extremely labile [13].

Compounds **5** and **6** were converted into the 4,4'-dimethoxytrityl ((MeO)₂Tr) derivatives **7** and **8** which were isolated in more than 80% yield (*Scheme*). Reaction of **7** or **8** with PCl₃/*N*-methylmorpholine/1*H*-1,2,4-triazole [14] afforded the 3'-phosphonates which were isolated as the triethylammonium salts **3a** and **4**, respectively. The phosphoramidite **3b** was prepared from **7** and chloro(2-cyanoethyl)(diisopropylamino)phosphane. Compound **8** was also transformed into the *Fractosil*-linked derivative **11** by treatment with succinic anhydride (→ **9**), esterification with 4-nitrophenol (→ **10**), and reaction with aminopropyl-functionalized *Fractosil*.

Table 1 summarizes the ¹³C-NMR chemical shifts of the 8-azapurine nucleosides as well as of their protected derivatives. As reported earlier, only the bridgehead C-atoms could be assigned unambiguously in case of the compounds **1** and **2**. In case of the amidines **5** and **6**, the C(2) signal was additionally identified by gated-decoupled spectra. A small coupling (³*J* = 6.7 Hz) was found between C(2) and the CH of the (dimethylamino)methylidene residue. In case of the *N*⁸-derivatives **6** and **8**, the C(2) and C(6) signals showed very similar chemical shifts. Protection of OH–C(5') was established by a downfield shift (1.7–2.1 ppm) of the C(5') signal and an upfield shift (> 2 ppm) of the C(4') signal compared to those of the starting compounds.

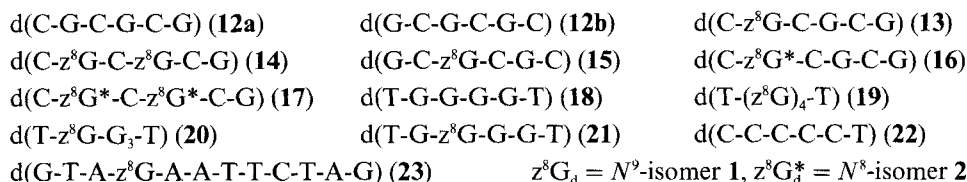
Table 1. ¹³C-NMR Chemical Shifts of 1,2,3-Triazolo[4,5-*d*]pyrimidine 2'-Deoxyribofuranosides in (D₆)DMSO at 25°

	C(7) ^a) ^c C(6) ^b)	C(5) ^b) ^c C(2) ^b)	C(3a) ^a) ^c C(4) ^b)	C(7a) ^a) ^c C(5) ^b)
1 [10]	155.7	155.7	151.5 ^d)	124.5
2 [10]	159.5	154.4	156.6	127.2
3a	156.5	159.8	150.0	126.6
4	158.8	159.1	157.6	129.1
5	156.5	160.1 ^e)	150.5	126.6
6	158.8	159.1 ^e)	157.5	128.9
7	156.5	160.0	150.5	126.6
8	158.7	159.0	157.6	129.0
9	158.8	159.2	157.5	129.0

	C(1')	C(2')	C(3')	C(4')	C(5')	Me	CH	(MeO) ₂ Tr
1 [10]	83.9	38.0	70.9	88.2	62.2	–	–	–
2 [10]	92.8	DMSO	70.7	88.6	62.2	–	–	–
3a	84.6	37.0	71.9	85.0	63.5	35.1	159.3	55.0
4	92.6	DMSO	71.7	85.0	63.6	34.8	158.8	55.0
5	84.3	DMSO	70.9	88.2	62.2	35.0	159.1	–
6	93.0	DMSO	70.7	88.7	62.2	34.8	158.8	–
7	83.9	DMSO	70.6	86.0	64.3	35.0	159.0	55.0
8	92.5	DMSO	70.1	86.1	63.9	34.8	158.8	55.0
9	92.7	DMSO	73.8	83.9	63.6	34.8	158.8	55.0

^a) Systematic numbering. ^b) Purine numbering. ^c) Tentative. ^d) [10]. ^e) From gated-decoupled spectra.

Oligonucleotides Containing 8-Aza-2'-deoxyguanosine (1) or the N⁸-Isomer 2. The phosphonates **3a** and **4** were used in automated solid-phase oligonucleotide synthesis using a protocol published recently [15]. Three types of oligonucleotides (see **12–23**) were prepared showing structural or functional characteristics of the parent purine-containing oligomers: *i*) oligonucleotide duplexes such as **12a** exhibiting the B-Z transition on increase of the NaCl concentration from 1 to 4M [16] [17]; *ii*) oligonucleotides such as **18** forming G-4 aggregates [18], and *iii*) the dodecamer d(G-T-A-z⁸G-A-A-T-T-C-T-A-G) (**23**) containing the recognition sequence of the endodeoxyribonuclease EcoRI.



The (MeO)₂Tr-protected oligonucleotides were removed from the support and purified by *RP-18* HPLC. After detritylation, the oligonucleotides were again submitted to *RP-18* HPLC, desalted, and lyophilized. The nucleoside content was determined by phosphodiester hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. It was observed that oligonucleotides containing **1** were significantly slower hydrolyzed than the unmodified compounds. Representative examples of the HPLC pattern obtained after enzymatic hydrolysis of compounds **14** and **16** are shown in *Fig. 1*.

It should be mentioned that oligonucleotides such as **23** are susceptible to regio-specific phosphodiester hydrolysis by restriction enzymes. The EcoRI endodeoxyribonuclease hydrolyzed the oligomer duplex of **23** into the fragments d(G-T-A-z⁸G) and d(pA-A-T-T-C-T-A-G), whereas a similar oligonucleotide containing 7-deazaguanosine

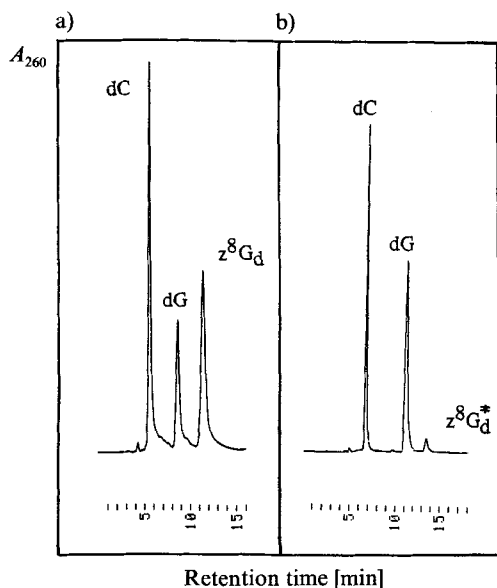


Fig. 1. HPLC Profiles of a) **14** and b) **16**, after enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. For conditions, see *Exper. Part*.

was completely resistant [19]. These data are in line with earlier observations by *Bodnar et al.* [20]. They polymerized the triphosphate of **1** on a template, thereby obtaining a z^8G_d -containing DNA. This fragment which contained various restriction sites was hydrolyzed by the restriction endonucleases *TaqI*, *AluI*, *RsaI*, and others.

To compare the stability of duplexes, the T_m values of the oligomers **12–17** were determined at 280 and 260 nm in aqueous 1M NaCl containing 60 mM sodium cacodylate. In all cases, cooperative transitions were observed from which T_m values were determined. The data of *Table 2* show that oligonucleotides containing z^8G_d (**1**) exhibit significant higher T_m values than the parent purine oligomers. This may be the result of the better proton-donor properties of $NH(3)$ as well as of the NH_2 group. On the other hand, if $z^8G_d^*$ (**2**) was replacing G_d , T_m was strongly reduced, indicating that this structural change cannot be accommodated by the *Watson-Crick* base pair.

Table 2. T_m Values and Thermodynamic Data of 8-Azaguanine-Containing Oligonucleotides

	Conc. [μ M]	T_m^a [$^{\circ}$ C]	$-\Delta H$ [kcal/mol]	$-\Delta S$ [cal/mol K]	Hypochromicity [%]
12a [d(C-G)] ₃	16	45	60.6	192	22
b [d(G-C)] ₃	14	45	60.0	190	29
13 d(C- z^8G -C-G-C-G)	14	49	67.0	206	23
14 d(C- z^8G -C- z^8G -C-G)	12	52	70.0	214	21
15 d(G-C- z^8G -C-G-C)	12	47	64.0	198	27
16 d(C- z^8G^* -C-G-C-G)	15	28	26	90	22
17 d(C- z^8G^* -C- z^8G^* -C-G)	14	29	24	84	16

^a) Measured in 1M NaCl containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate, pH 7.0 at 280 nm.

From the melting curves, the ΔH and ΔS values were calculated (see *Table 2*) using the two-state model for helix-coil transition [21]. It was of interest to determine a ΔH increment for the $z^8G_d \cdot C_d$ base pair within an oligonucleotide duplex. As the number of the modified oligonucleotides was limited, we used the ΔH values of the hexamers **13–15** and compared them with the unmodified [d(C-G)]₃ (**12a**) and [d(G-C)]₃ (**12b**). Using increments of *Breslauer et al.* [22] for the latter nucleosides, the value of a $z^8G_d \cdot C_d$ base pair was taken from the differences of ΔH values. An averaged enthalpy of *ca.* 15 kcal/mol was found for the $z^8G_d \cdot C_d$ base pair indicating a stabilization of 3–4.5 kcal/mol over the $G_d \cdot C_d$ base pair. However, this ΔH increment is only a rough value, as our determination neglects the nearest-neighbor influences.

Incorporation of 8-Aza-2'-deoxyguanosine (1) in d(T-G-G-G-T) (18) and Influence on the G-4 Structure. In a previous study, it was shown that the tetrameric structure of U-G-G-G-G-U was disaggregated when 7-deazaguanosine replaced guanosine [23]. Different forms of aggregates were detected by gel electrophoretic mobility changes of c^7G -containing oligonucleotides [23]. The tetraplex formation of G-rich RNA's was also discovered for DNA containing short runs of G_d [24] [25]. Guanine-rich tracts (telomers) were found at the end of chromosomes [25] [18]. *Sen* and *Gilbert* proposed a sodium-potassium switch which is realized by a structural transition from a tetraplex formed in the presence of Na^+ ions to a hairpin dimer which should be preferred in the presence of K^+ ions [25]. As a consequence, our interest was focussed on the oligo(deoxyribonucleotides) containing tracts of G_d in which G_d is replaced by z^8G_d (**1**). For these investigations,

d(T-G-G-G-G-T) (**18**) was chosen as parent having a similar sequence of bases as U-G-G-G-G-U.

Guanine is known to form tetrameric tracts. As proton acceptors, these tracts use N(7) and the 6-oxo group. The 2-NH₂ group and NH(3) are used as proton-donor sites [25] [26]. The self-pairing in these tracts follows the *Hoogsteen* mode and generates a parallel right-handed helix with all sugar residues in the 'anti'-conformation [27]. In telomers, the strands are folded back into hairpins with two antiparallel and two parallel strands and different 'syn'- and 'anti'-glycosylic bonds in the strands [26] [28]. Compared to purines, the 8-azapurines are comparably weak proton acceptors at the triazole N-atoms. This is indicated by the pK_a value of protonation which is below 1 for **1** compared to 2.5 [29] for 2'-deoxyguanosine. Moreover, the conformation at the N-glycosylic bond is 'high-anti' and different to that of purines [30]. From this point, the G-4 structure containing compound **1** should be less stable than that of 2'-deoxyguanosine.

We studied the influence on aggregation of a step-wise replacement of one or more G_d residues in d(T-G-G-G-G-T) (**18**) by z⁸G_d. To this end, the hexamers **12a** and **18–22** were first incubated overnight in 0.1M NaCl to equilibrate possible aggregates. The solutions were then applied to non-denaturing 21% polyacrylamide-gel electrophoresis (PAGE; see *Exper. Part*) [31]. After chromatography and staining with 'stains all', the single-stranded **22** was not detected (*Fig. 2*, lane 1). The oligomer d(T-G-G-G-G-T) (**18**) showed two bands (lane 4), a strong one with low mobility and a minor one migrating similarly to the duplex of [d(C-G)]₃ (**12a**; lane 6). The similar oligomer U-G-G-G-G-U showed only one slow-migrating band which was assigned to a tetramer [23]. Thus, the species of the slow-migrating band of **18** is expected to have a similar tetrameric structure, and that of the fast migrating zone can be assigned to a duplex (*cf.* duplex of **12a**). The same observation (two bands) was made with U-G-G-G-G-U when the G residue at position 3 was replaced by c⁷G [23]; it was explained by a partial destabilization of the tetrad induced by the incorporation of c⁷G which caused a loss of 2 H-bonds and thereby shifted the equilibrium towards the duplex. Apparently, the tetrad of **18** is less stable than that of U-G-G-G-G-U, thereby forming already two species (tetramer and dimer/monomer) under the conditions of PAGE.

In the case of a single G_d replacement by z⁸G_d (**1**) in position 2 of **18** (→ d(T-z⁸G-G-G-G-T) (**20**)), only the slow-migrating zone was observed on PAGE (*Fig. 2*, lane 2). The G_d replacement in position 3 (→ d(T-G-z⁸G-G-G-T) (**21**); lane 3) showed a light smearing of the slow migrating zone and the appearance of a slightly faster migrating sharp zone. The fully modified hexamer d[T-(z⁸G)₄-T] (**19**) showed only this new zone (lane 5). The single-stranded d(C₅-T) (**22**) was used for comparison (*Fig. 2*, lane 1; not stained with 'stains all', but visible under UV light), but it was more mobile than all other species. The disaggregation of d(T-(z⁸G)₄-T) (**19**) compared to **18** was also observed during *RP18* HPLC (*Fig. 3*). A slow-migrating aggregate (G-4 structure) was detected together with the disaggregated oligomer in the case of **18**. Such aggregates have been also reported for d(T-G-G-G-T) [32]. Contrary to this, only a fast-migrating zone was observed in the case of d(T-(z⁸G)₄-T) indicating that **19** does not form a tetrad, at least under these conditions.

It was shown by pK_a measurements that compound **1** is a weaker proton acceptor but a better proton donor than G_d (protonation: pK = 2.5 for G_d at N(7) [29], and pK < 1.0 for z⁸G_d; deprotonation: pK = 9.5 for G_d at N(3) and pK = 8.6 for z⁸G_d). These proper-

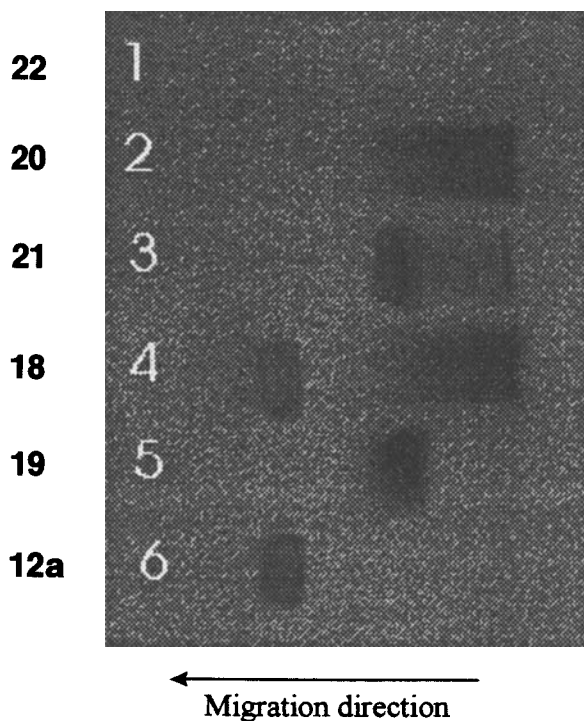


Fig. 2. Non-denaturing 21% polyacrylamide gel electrophoresis of the oligomers **12a** and **18–22** after incubation of $0.6 A_{260}$ units with 0.1 M NaCl . For conditions, see *Exper. Part*.

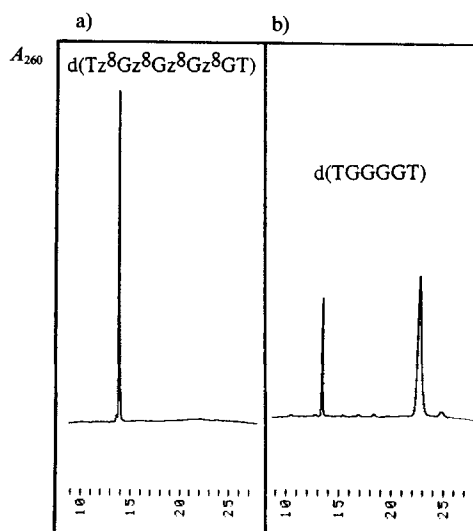


Fig. 3. HPLC Profiles of a) **19** and b) **18**. For conditions, see *Exper. Part*.

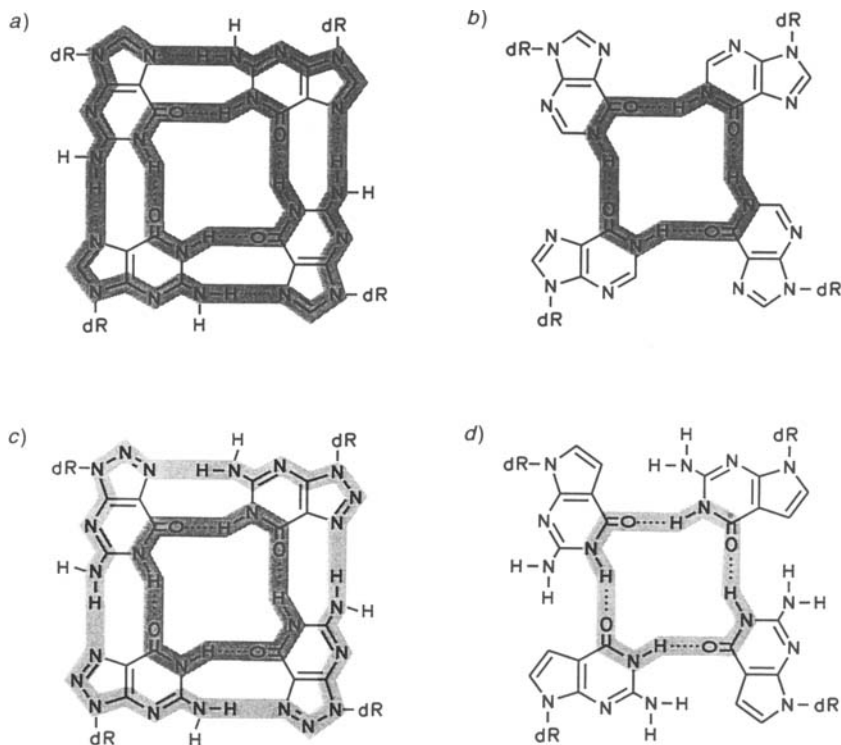


Fig. 4. Tetrameric structures a) proposed for $d(T-G-G-G-T)$ (**18**), b) of oligo(I_d) [32], and c) d) proposed for $d(T-z^8G-z^8G-z^8G-T)$ (**19**) and $d(T-c^7G-c^7G-c^7G-T)$, respectively. dR = 2'-Deoxyribsyl.

ties will stabilize the inner core of H-bonds ($NH \cdots O=C$) of the tetrad of **19** (Fig. 4c) compared to the parent tetrad of **18** (Fig. 4a) and may compensate the weaker H-bonding pattern within the outer core ($NH_2 \cdots N(7)$). This assumption is supported by the finding that tetrads were also formed by oligonucleotides containing 2'-deoxyinosine (I_d) [32]. Oligo(I_d)'s are less stable than G_d -rich oligomers, but they have the same structure [26] although they are devoid of an outer core of H-bonds (Fig. 4b). We already reported that c^7G -rich oligonucleotides did not form tetrads [23]. However, Fig. 4d suggests that tetrads can be expected even in this case. An explanation for their nonobservance is that in this tetrad, the outer core of H-bonds does not exist, and the inner core is destabilized due to the weaker H-bonding contributed by the six-membered ring of 7-deazaguanine [23]. Opposite to this, a stabilization of the inner H-bond core may occur in the case of a possible tetrad containing compound **1** (Fig. 4c). Consequently, the tetrad containing c^7G_d should be the most labile aggregate. However, according to the pK values and the conditions of electrophoresis, a partial deprotonation within the inner core may be also considered for **19**. The different conformation around the N-glycosylic bond of z^8G_d ('high-anti') [30] compared to G_d [30] seems to be of minor importance as 8-bromoguanosine which shows similar properties forms gel structures implying the formation

of a tetrad [33]. The inability to form a tetrad on the level of a homopolymer [34] may have simply steric reasons.

The CD spectrum of the unmodified d(T-G₄-T) (**18**; *Fig. 5a*) in 0.1M NaCl at pH 7.0 was very similar to that of d(T₄-G₄) or (3'-5')d(G₄-T₂)-(5'-3')d(T₂-G₄) which were assigned to parallel-stranded tetramers [35] [36]. Slightly different spectra were found for the hexamers **20** (*Fig. 5b*) and **21** (*Fig. 5c*) containing only one modified base residue. But compound **19** behaved differently showing an almost mirror-like CD spectrum (*Fig. 5d*). Such mirror-like spectra were already observed for G₄-rich sequences, e.g. d(G₄-T₄-G₄), in which two strands of the tetrad were in a parallel and the others in antiparallel orientation [34]. Another helical sense could also explain the mirror-like spectra. The CD spectrum of z⁸G₄ (**1**) showed opposite signs around 230 and 250 nm compared to G₄. Therefore, the change of the CD spectra of d[T-(z⁸G)₄-T] (**19**) may simply result from the properties of the monomeric nucleoside. The oligomers **20** and **21** showed the CD spectra of a tetrameric structure which was already observed for the tetrad of G₄. The maxima decreased with increasing temperature. Acidification to pH 1 changed the CD spectra of **18**, **20**, and **21** strongly. They became similar to the CD spectra obtained at high temperature which were also observed by *Sarma et al.* [37]. Detailed information on the particular structure of **19** can only be expected from additional physical data, preferably from NMR data or single-crystal X-ray analysis.

Replacement of 2'-Deoxyguanosine by 8-Aza-2'-deoxyguanosine (1) in [d(C-G)]₃ (12a) and Influence on the B-Z Transition. Spectroscopic studies on poly[d(G-C)] at different salt concentrations showed a conformational change of B-DNA to Z-DNA at high salt concentrations [38]. The zigzag (Z) nature, the 'syn'-orientation of the guanine residue, and the left-handed helical sense were established by a single-crystal X-ray analysis [39] [40]. The B-Z transition was already observed on short oligomers such as [d(C-G)]₃ (**12a**) [39] [40]. In a previous study, we replaced the G₄ residues of **12a** by 7-deaza-2'-deoxyguanosine or 8-aza-7-deaza-2'-deoxyguanosine [41] and recently also by 3-deaza-2'-deoxyguanosine (unpublished results). In all cases, a salt-induced B-Z helical change was not observed.

The salt-induced B-Z transition of poly(C-G) as well as of [d(C-G)]₃ can be detected by CD spectroscopy [16] [42]. Therefore, the CD spectra of the oligomers **12a**, **13**, **14**, **16**, and **17** were measured in 1 and 4M NaCl (*Fig. 6*). At 1M NaCl, each oligomer showed the CD spectra of a B-DNA. As expected, at 4M NaCl, the CD spectrum of [d(C-G)]₃ (**12a**) was different due to the transition into Z-DNA [16] [17]. Interestingly, the oligomers **13** and **14** modified at G₄ positions by z⁸G₄ (**1**) showed very similar transitions as the parent oligomer **12a** (*Fig. 6a* and *6b*) indicating that these oligomers are able to undergo B-Z transitions. With increasing temperature, the amplitudes of the CD spectra became smaller suggesting that the Z-form was destroyed. In **16** and **17**, G₄ residues were replaced by the N⁸-nucleoside **2**. When only one residue was altered as in the oligomer **16**, B-Z transition still occurred (*Fig. 6c*). However, if two G₄ residues were replaced as in the oligomer **17**, a completely different CD spectrum appeared, which was almost independent of the salt concentration (0.5–4M NaCl) (*Fig. 6d*).

From the Z-DNA structure it can be seen that the G₄·C₄ base pair occupies a position at the periphery of the molecule instead of the centre like in right-handed B-DNA. The N(7) and C(8) atoms of guanine are near the outer shell of the molecule, an arrangement in which N(7) can interact with counter ions. If the data obtained earlier from base-mod-

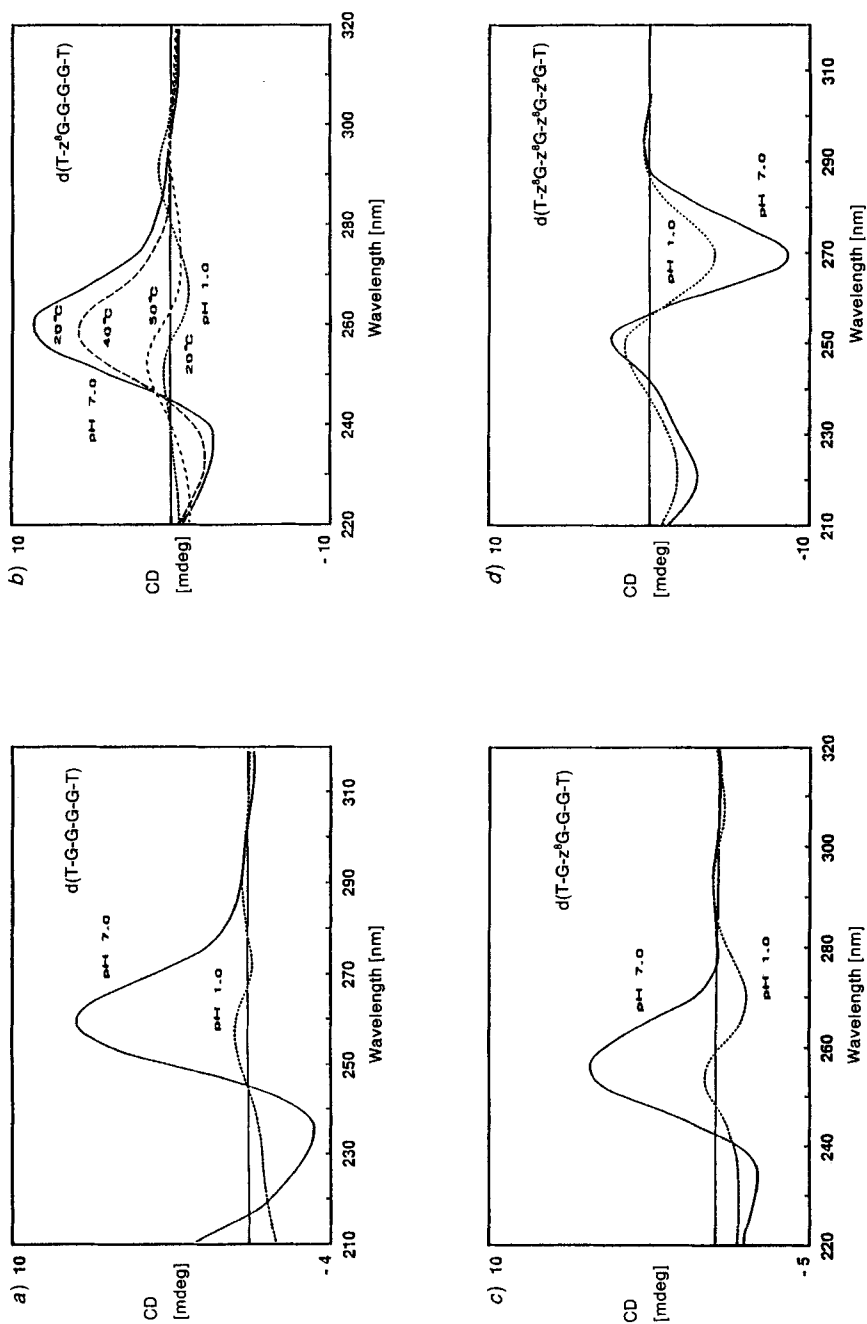


Fig. 5. CD Spectra of a) $d(T-G-G-G-G-T)$ (18), b) $d(T-z^8G-G-G-G-T)$ (20), c) $d(T-z^8G-z^8G-z^8G-z^8G-T)$ (21), and d) $d(T-z^8G-z^8G-z^8G-z^8G-T)$ (19). The solutions contained 0.5–0.6 A_{260} units of the oligonucleotide dissolved in 0.1M NaCl at pH 7.0 (20°). Temperature-dependent CD spectra at pH 7.0 and spectra at pH 1.0 as indicated.

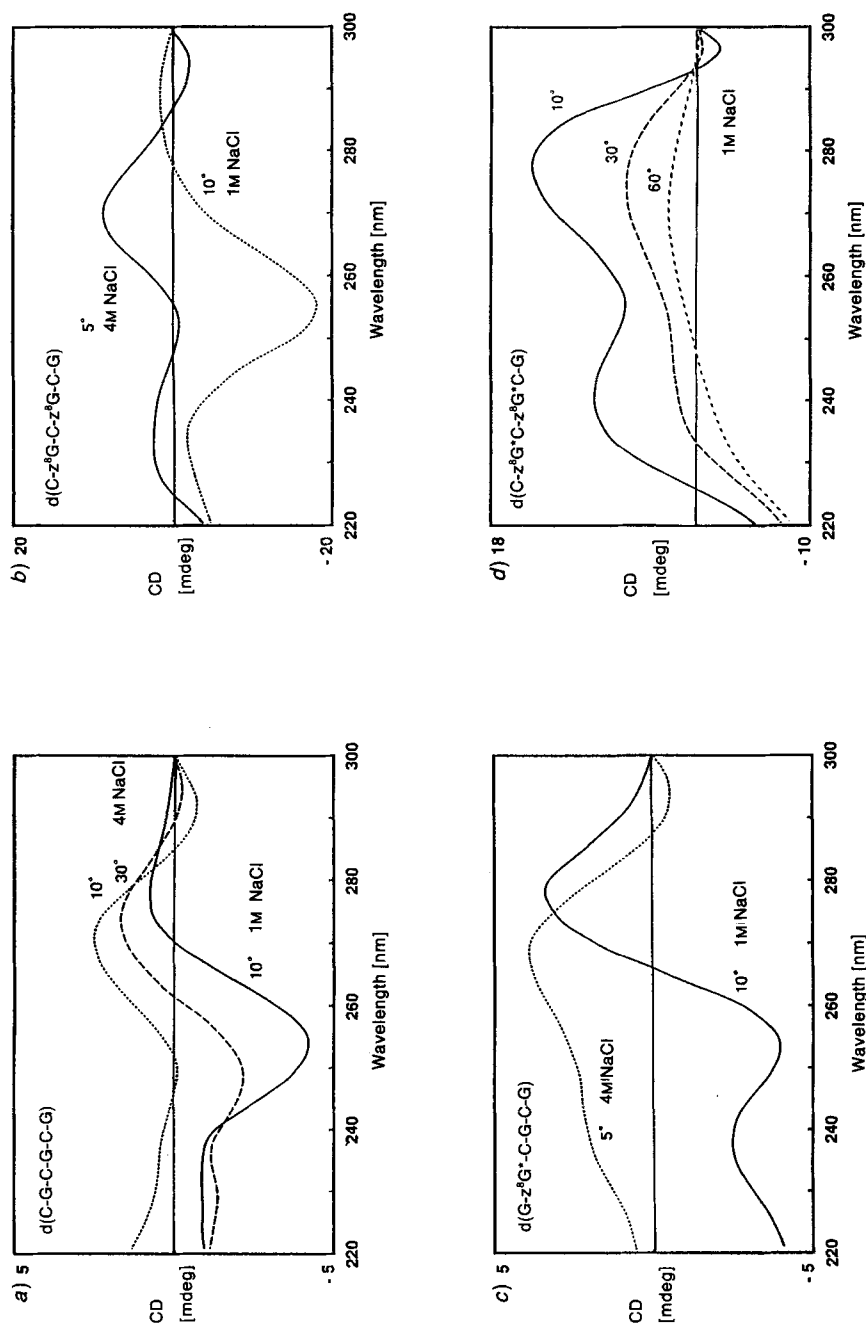


Fig. 6. CD Spectra of a) $d(C-G-C-G-C-G)$ (14), b) $d(C-z^8G-C-z^8G-C-G)$ (16), and c) $d(C-z^8G-C-z^8G-C-G)$ (17). Measured in 1M NaCl and 4M NaCl containing 60 mM Na-cacodylate, 100 mM $MgCl_2$ at pH 7.0. Temperatures as indicated. The oligomer concentration was 12–14 μM .

ified oligonucleotides [41] are compared to those discussed in this report, it can be seen that G_d derivatives lacking a N-atom in position 7 are not able to undergo the B-Z transition. The 'high-*anti*' conformation of 8-azanucleosides together with the presence of the purine N(7) leads probably to the ability of the hexamers **13** and **14** to undergo this transition. Apparently, the purine N(7) plays a crucial role during the transformation from B- to Z-DNA. This N-atom may interact with cations thereby inducing a positive charge onto the G_d residue. This is supported by the N⁷-methylation of the G_d residues within DNA which converts the molecule to Z-DNA already under low salt concentration conditions [43].

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Experimental Part

General. See [2]. The phosphonates of regular 2'-deoxynucleosides were purchased from Sigma, St. Louis, and CPG (30 μmol of immobilized protected 2'-deoxynucleoside/g of solid support) from Milligene, Eschborn, Germany. Oligonucleotide synthesis was carried out on a DNA synthesizer, model 380B, Applied Biosystems, Weiterstadt, Germany.

Enzymatic Hydrolysis of the Oligomers and Determination of the Hypochromicity. Hydrolysis was carried out as described [2] [19]. The mixture was analyzed on reversed-phase HPLC (RP-18, solvent system III; see below). Quantification of the material was made on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituents (ϵ_{260} : A_d 15400, C_d 7300, G_d 11700, T_d 8800, z⁸G_d 12000, and z⁸G_d⁺ 2200). Values obtained from the oligomers were in agreement with the calculated data. Hypochromicity values were determined by enzymatic digestion of 0.2 A₂₆₀ units of the corresponding oligonucleotides as described in [2].

HPLC Separation. See [2]. Gradients consisting of 0.1M (Et₃NH)OAc (pH 7.5)/MeCN 95:5 (A) and MeCN (B) were used. Gradient I, within 15 min 15–40% B in A; gradient II, within 20 min 0–20% B in A.

Melting Experiments. As described in [2] (linear temp. increase from 10 to 70°).

Electrophoresis. The oligomers **12a** and **18–22** (0.6 A₂₆₀) were evaporated in a Speed-Vac concentrator and then dissolved in 0.1M NaCl, containing 10 mM Tris-HCl (10 μl, pH 8.2), heated to 95° for 2 min, and incubated overnight at 5°. The samples were treated with 15% Ficoll/H₂O (5 μl); as reference, a loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 15% Ficoll) was taken. These samples were applied to anal. electrophoresis. Electrophoresis was carried out using a non-denaturing 21% polyacrylamid gel (ratio mono- vs. bis(acrylamid) 20:1; 15 × 30 × 0.1 cm) in Tris-borate EDTA buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.4) at 5° and 9 W until the bromophenol blue dye migrated 15 cm. The bands were visualized under UV or stained with 0.01% 'stains all' (= 4,5,4',5'-dibenzo-3,3'-diethyl-9-methylthiacarbocyanine bromide in formamide/H₂O) for photography (Fig. 2).

3-(2-Deoxy-β-D-erythro-pentofuranosyl)-5-{[(dimethylamino)methylidene]amino}-3,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (5). A soln. of **1** [10] (285 mg, 1.06 mmol) in anh. amine-free DMF (7 ml) was treated with *N,N*-dimethylformamide diethyl acetal (5 ml, 29.2 mmol) under stirring at r.t. Stirring was continued for 24 h. The soln. was evaporated and the residue co-evaporated with toluene. FC (silica gel, column 20 × 4 cm, 0.5 bar, CH₂Cl₂/MeOH 4:1) afforded **5** (315 mg, 92%) as colorless amorphous solid which crystallized from MeOH as colorless needles. M.p. 236°. TLC (CH₂Cl₂/MeOH 8:2): R_f 0.67. UV (MeOH): 301 (26500), 244 (15500). ¹H-NMR ((D₆)DMSO): 2.34 (m, H₂-C(2')); 2.90 (m, H₆-C(2')); 3.06, 3.19 (2s, 2 Me); 3.57 (m, 2 H-C(5')); 3.85 (m, H-C(4')); 4.48 (m, H-C(3')); 4.79 (br. s, OH-C(5')); 5.40 (br. s, OH-C(3')); 6.43 (t, J = 6.32, H-C(1')); 8.66 (s, CH). Anal. calc. for C₁₂H₁₇N₇O₄ (323.31): C 44.58, H 5.29, N 30.33; found: C 44.64, H 5.26, N 30.37.

2-[2-Deoxy- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -2,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (**6**). A soln. of **2** [10] (350 mg, 1.30 mmol) in anh. DMF (7 ml) and *N,N*-dimethylformamide diethyl acetal (5.5 ml, 32.1 mmol) was reacted and worked up as described for **5**. Colorless crystals (360 mg, 86%). M.p. 162°. TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.3. UV (MeOH): 290 (18900), 248 (13500). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 2.39 (*m*, $\text{H}_\alpha\text{-C}(2'')$); 2.74 (*m*, $\text{H}_\beta\text{-C}(2'')$); 3.04, 3.17 (2*s*, 2 Me); 3.33, 3.51 (*m*, 2 $\text{H-C}(5'')$); 3.88 (*m*, $\text{H-C}(4'')$); 4.46 (*m*, $\text{H-C}(3'')$); 4.73 (br. *s*, $\text{OH-C}(5'')$); 5.35 (br. *s*, $\text{OH-C}(3'')$); 6.34 (*t*, $J = 4.82$, $\text{H-C}(1'')$); 8.66 (*s*, CH); 11.54 (br. *s*, NH). Anal. calc. for $\text{C}_{12}\text{H}_{17}\text{N}_7\text{O}_4$ (323.31): C 44.58, H 5.29, N 30.33; found: C 44.72, H 5.39, N 30.38.

3-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -3,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (**7**). Compound **5** (170 mg, 0.53 mmol) was dried by repeated co-evaporation from anh. pyridine and then dissolved in pyridine (6 ml). The soln. was stirred at r.t. and 4,4'-dimethoxytriphenylmethyl chloride (263 mg, 0.7 mmol) added. After stirring for 3 h, the soln. was poured into 5% aq. NaHCO_3 soln. (40 ml) and extracted twice with CH_2Cl_2 (30 ml). The combined org. layers were dried (Na_2SO_4), filtered, and evaporated. The residue was submitted to FC (silica gel 60H, column 15×4 cm, 0.5 bar, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5): colorless foam (270 mg, 81%). TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5): R_f 0.3. UV (MeOH): 302 (24600), 235 (35000). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 2.40 (*m*, $\text{H}_\alpha\text{-C}(2'')$); 2.90 (*m*, $\text{H}_\beta\text{-C}(2'')$); 3.07, 3.22 (2*s*, Me); 3.37 (*m*, 2 $\text{H-C}(5'')$); 3.69 (*s*, 2 MeO); 3.97 (*m*, $\text{H-C}(4'')$); 4.57 (*m*, $\text{H-C}(3'')$); 6.48 (*m*, $\text{H-C}(1'')$); 6.70–7.39 (*m*, arom. H); 8.66 (*s*, CH); 12.03 (*s*, NH). Anal. calc. for $\text{C}_{33}\text{H}_{35}\text{N}_7\text{O}_6$ (625.69): C 63.35, H 5.64, N 15.67; found: C 63.42, H 5.72, N 15.71.

2-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -2,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one (**8**). Dimethoxytritylation and workup as described for **7** gave **8** (310 mg, 0.96 mmol). Colorless foam (480 mg, 80%). TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1): R_f 0.3. UV (MeOH): 237 (sh, 40200), 310 (19200). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 2.43 (*m*, $\text{H}_\alpha\text{-C}(2'')$); 2.80 (*m*, $\text{H}_\beta\text{-C}(2'')$); 2.99 (*m*, 2 $\text{H-C}(5'')$); 3.03, 3.17 (2*s*, 2 Me); 3.67 (*s*, 2 Me); 3.96 (*m*, $\text{H-C}(4'')$); 4.56 (*m*, $\text{H-C}(3'')$); 5.41 (*d*, $J = 5.2$, $\text{OH-C}(3'')$); 6.41 (*d*, $J = 5.0$, $\text{H-C}(1'')$); 6.73–7.39 (2*m*, arom. H); 8.68 (*s*, CH); 11.58 (*s*, NH). Anal. calc. for $\text{C}_{33}\text{H}_{35}\text{N}_7\text{O}_6$ (625.69): C 63.35, H 5.64, N 15.67; found: C 63.40, H 5.71, N 15.51.

3-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -3,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one 3'-(Triethylammonium Phosphonate) (**3a**). To a stirred soln. of PCl_3 (202 μl , 0.22 mmol) and *N*-methylmorpholine (2.7 ml, 2.24 mmol) in CH_2Cl_2 (10 ml) was added 1H-1,2,4-triazole (0.54 g, 7.56 mmol). The mixture was stirred for 30 min at r.t. After cooling to 0°, a soln. of **7** (220 mg, 0.35 mmol) pre-dried by evaporation with anh. MeCN in CH_2Cl_2 (5 ml) was added slowly. After stirring for 10 min at r.t., the mixture was poured into 1M aq. $(\text{Et}_3\text{NH})\text{HCO}_3$ (= TBK; pH 8.0, 30 ml), shaken, and separated. The aq. layer was extracted with CH_2Cl_2 , the combined org. extract dried (Na_2SO_4) and evaporated, and the colorless foam submitted to FC (silica gel 60H, column 4×15 cm, 0.5 bar, $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$ 88:10:2). The residue of the main zone was dissolved in CH_2Cl_2 (10 ml) and extracted several times with 0.1M TBK buffer (pH 8.0). The org. layer was dried (Na_2SO_4) and evaporated: colorless foam (235 mg, 85%). TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$ 88:10:2). R_f 0.3. UV (MeOH): 285 (sh, 14900), 303 (18400). $^1\text{H-NMR}$ ($(\text{D}_6)\text{DMSO}$): 1.13 (*m*, 3 MeCH_2); 2.56 (*m*, 2 $\text{H-C}(2'')$); 2.97 (*m*, 3 MeCH_2); 3.07, 3.23 (*s*, 2 Me, 2 $\text{H-C}(5'')$); 3.67 (*s*, 2 MeO); 4.09 (*m*, $\text{H-C}(4'')$); 5.22 (*m*, $\text{H-C}(3'')$); 6.53 (*m*, $J = 4.5$, $\text{H-C}(1'')$); 6.69–7.24 (*m*, arom. H); 5.45, 7.77 (*d*, $J = 585$, PH); 8.83 (*s*, CH); 11.68 (*s*, NH). $^{31}\text{P-NMR}$ ($(\text{D}_6)\text{DMSO}$): 1.16 ($^1J(\text{P,H}) = 585$, $^3J(\text{P,H-C}(3'')) = 8.90$). Anal. calc. for $\text{C}_{39}\text{H}_{51}\text{N}_8\text{O}_8\text{P}$ (790.87): C 59.23, H 6.50, N 14.17; found: C 59.33, H 6.79, N 13.95.

3-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -3,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one 3'-(2-Cyanoethyl *N,N*-Diisopropylphosphoramidite) (**3b**). To a soln. of **7** (50 mg, 0.08 mmol) in CH_2Cl_2 (1 ml), (*i*-Pr) $_2\text{EtN}$ (56 μl , 0.268 mmol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphane (115 μl , 0.51 mmol) were added. The soln. was stirred for 2 h at r.t. under Ar. After addition of 5% NaHCO_3 soln. (3 ml), the mixture was extracted twice with CH_2Cl_2 (30 ml), the extract dried (Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel 60H, column 7×2 cm, 0.5 bar, $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{Et}_3\text{N}$ 45:45:10): colorless foam (35 mg, 55%). TLC ($\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{Et}_3\text{N}$ 45:45:10): R_f 0.4. $^{31}\text{P-NMR}$ ($(\text{D}_6)\text{DMSO}$): 149.4, 148.9.

2-[2-Deoxy-5-O-(4,4'-dimethoxytrityl)- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -2,6-dihydro-7H-1,2,3-triazolo[4,5-d]pyrimidin-7-one 3'-(Triethylammonium Phosphonate) (**4**). As described for **3a**, **8** (190 mg, 0.30 mmol) gave **4** (170 mg, 72%). Colorless foam. TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Et}_3\text{N}$ 88:10:2): R_f

0.25. UV (MeOH): 300 (19400), 275 (22200), 235 (39800). $^1\text{H-NMR}$ ((D_6) DMSO): 1.11 (*m*, MeCH_2); 2.54 (*m*, 2 $\text{H-C}(2')$); 2.93 (*m*, MeCH_2 , 2 $\text{H-C}(5')$); 3.04, 3.17 (2*s*, 2 Me); 3.66, 3.68 (2*s*, 2 MeO); 4.11 (*m*, $\text{H-C}(4')$); 4.99 (*m*, $\text{H-C}(3')$); 6.42 (*d*, $J = 4.8$, $\text{H-C}(1')$); 6.72–7.23 (2*m*, arom. H); 5.38, 7.72 (*d*, $J = 586$, PH); 8.73 (*s*, CH); 11.61 (*s*, NH). $^{31}\text{P-NMR}$ ((D_6) DMSO): 1.14 ($^1J(\text{P,H}) = 585.7$, $^3J(\text{P,H-C}(3')) = 9.27$). Anal. calc. for $\text{C}_{39}\text{H}_{51}\text{N}_8\text{O}_8\text{P}$ (790.87): C 59.23, H 6.50, N 14.17; found: C 59.39, H 6.80, N 14.10.

2-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-3-*O*-succinyl- β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -2,6-dihydro-7H-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (**9**). To a soln. of **8** (110 mg, 0.18 mmol) in pyridine (5 ml) 4-(dimethylamino)pyridine (30 mg, 0.23 mmol) and succinic anhydride (90 mg, 0.88 mmol) were added. The mixture was stirred for 48 h at r.t., H_2O (2 ml) poured into the soln., and the resultant evaporated. Co-evaporation with toluene removed pyridine. The resulting oil was dissolved in CH_2Cl_2 and the soln. washed with 10% aq. citric acid and H_2O , dried (Na_2SO_4), and evaporated. The residue was dissolved in CH_2Cl_2 /pyridine 95:5 (2 ml) and added slowly to pentane/ Et_2O 1:1 (30 ml). The precipitate was filtered off: colorless powder (85 mg, 60%). TLC (silica gel, CH_2Cl_2 /MeOH 9:1): R_f 0.25. $^1\text{H-NMR}$ ((D_6) DMSO): 2.51 (*m*, 2 $\text{H-C}(2')$, 2 CH_2); 3.05, 3.18 (2*s*, 2 Me); 3.10 (*m*, 2 $\text{H-C}(5')$); 3.36 (*s*, 2 MeO); 4.21 (*m*, $\text{H-C}(4')$); 5.48 (*m*, $\text{H-C}(3')$); 6.46 (*m*, $J = 4.1$, $\text{H-C}(1')$); 6.75–7.28 (2*m*, arom. H); 8.71 (*s*, CH).

2-[2-Deoxy-5-*O*-(4,4'-dimethoxytrityl)-3-*O*- $\{3\text{-}[\text{N-}[\text{3-(Fractosyl)propyl}]\text{carbanoyl}\}\text{propanoyl}\}$ - β -D-erythro-pentofuranosyl]-5- $\{[(\text{dimethylamino})\text{methylidene}]\text{amino}\}$ -2,6-dihydro-7H-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (**11**). To a soln. of **9** (30 mg, 0.038 mmol) and 1,4-dioxane/pyridine 95:5 (1 ml), 4-nitrophenol (7 mg, 0.05 mmol) and *N,N*-dicyclohexylcarbodiimide (10 mg, 0.048 mmol) were added under stirring at r.t. After 2 h, the soln. was filtered and the supernatant containing 3'-*O*- $\{3\text{-}[(\text{nitrophenyloxy})\text{carbonyl}]\text{propanoyl}\}$ compound **10** was added to a suspension of Fractosil 200 (80 mg, 450 $\mu\text{mol/g}$; Merck) in DMF (1 ml). Et_3N (100 μl) was added and the mixture shaken for 4 h r.t. After addition of Ac_2O (20 μl), shaking was continued for another 30 min. The Fractosil derivative **11** 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 **11** with 0.1M toluene-4-sulfonic acid (5 ml) in MeCN. From the absorbance at 498 nm of the supernatant obtained after centrifugation, 64 μmol of linked 2/g of Fractosil were calculated ($A((\text{MeO})_2\text{Tr}) = 70000$).

Oligonucleotides 12–23. The oligonucleotides were synthesized on a 1- μmol scale with an automated DNA synthesizer (Applied Biosystems 380 B) employing phosphonate chemistry [15]. The oligomers were recovered from the synthesizer as 5'-*O*-(MeO)₂Tr derivatives. Deprotection of NH_2 groups was carried out by 25% $\text{NH}_3/\text{H}_2\text{O}$ at 60° overnight. The (MeO)₂Tr residues of the oligomers were removed by treatment with 80% aq. AcOH for 20 min at r.t. Purification was accomplished by HPLC (Table 3; RP-18 columns, gradient I for the (MeO)₂Tr derivatives and II for the detritylated oligomers). Desalting followed on a 4 $\times$ 25 mm cartridge (RP-18 silica gel) using H_2O for elution of the salt, while the oligomer was eluted with MeOH/ H_2O 3:2. The oligomers **12–22** were lyophilized on a Speed-Vac evaporator. The colorless residue was dissolved in H_2O (100 μl) and stored frozen at -25° . The average yield was around 9.0 A_{260} units.

Table 3. HPLC of Oligomers **12–21** and **23**

Oligomer	Retention time [min]		Gradient
d(C-G-C-G-C-G) (12a)	17.2	–	II
d(G-C-G-C-G-C) (12b)	17.2	(14.8)	II (I)
d(C-z ⁸ G-C-G-C-G) (13)	15.1	(12.5)	II (I)
d(C-z ⁸ G-C-z ⁸ G-C-G) (14)	15.8	(12.9)	II (I)
d(G-C-z ⁸ G-C-G-C) (15)	15.5	(12.5)	II (I)
d(C-z ⁸ G*-C-G-C-G) (16)	14.1	(11.9)	II (I)
d(C-z ⁸ G*-C-z ⁸ G*-C-G) (17)	14.7	(12.8)	II (I)
d(T-G-G-G-G-T) (18)	13.4, 22.5	(12.3)	II (I)
d(T-z ⁸ G-z ⁸ G-z ⁸ G-z ⁸ G-T) (19)	13.6	(12.4)	II (I)
d(T-z ⁸ G-G-G-G-T) (20)	13.4	(12.2)	II (I)
d(T-G-z ⁸ G-G-G-T) (21)	13.5	(12.1)	II (I)
d(G-T-A-z ⁸ G-A-A-T-T-C-T-A-G) (23)	15.0	(12.4)	II (I)

REFERENCES

- [1] W. Saenger, 'Principles of Nucleic Acid Structure', in 'Springer Advanced Texts in Chemistry', Ed. C. R. Cantor, Springer Verlag, New York–Berlin–Heidelberg–Tokyo, 1984.
- [2] F. Seela, T. Wenzel, *Helv. Chim. Acta* **1992**, *75*, 1111.
- [3] T. Grein, S. Lampe, K. Mersmann, H. Rosemeyer, H. Thomas, F. Seela, *Bioorg. Med. Lett.* **1994**, *4*, 971.
- [4] J. R. Fresco, J. Massoulié, *J. Am. Chem. Soc.* **1963**, *85*, 1352.
- [5] D. Thiele, W. Guschlbauer, *Biochimie* **1974**, *56*, 501.
- [6] M. Gellert, M. N. Lipsett, D. R. Davies, *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 2013.
- [7] F. Seela, T. Grein, *Nucleic Acids Res.* **1992**, *20*, 2297.
- [8] F. Seela, H. Winter, *Helv. Chim. Acta* **1994**, *77*, 597.
- [9] F. Seela, K. Kaiser, *Helv. Chim. Acta* **1988**, *71*, 1813.
- [10] F. Seela, S. Lampe, *Helv. Chim. Acta* **1993**, *76*, 2388.
- [11] F. Seela, P. Leonard, unpublished data.
- [12] J. Zemlicka, A. Holy, *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159.
- [13] K. Kaiser, Ph. D. Thesis, University of Osnabrück, 1989.
- [14] B. C. Froehler, P. G. Ng, M. D. Matteucci, *Nucleic Acids Res.* **1986**, *14*, 5399.
- [15] B. C. Froehler, in 'Methods in Molecular Biology', Ed. E. S. Agrawal, Humana Press, Totowa, N. J., 1993.
- [16] S. Uesugi, T. Shida, M. Ikehara, *Chem. Pharm. Bull.* **1981**, *29*, 3573.
- [17] H. Drew, T. Takano, S. Tanaka, K. Itakura, R. E. Dickerson, *Nature (London)* **1980**, *286*, 567.
- [18] J. Kim, C. Cheong, P. B. Moore, *Nature (London)* **1991**, *351*, 331.
- [19] F. Seela, H. Driller, *Nucleic Acids Res.* **1986**, *14*, 2319.
- [20] J. W. Bodnar, W. Zempsky, D. Warder, C. Bergson, D. C. Ward, *J. Biol. Chem.* **1983**, *258*, 15206.
- [21] J. Kehrnhahn, University of Osnabrück, Department of Physical Chemistry.
- [22] K. J. Breslauer, R. Frank, H. Blöcker, L. A. Marky, *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3746.
- [23] F. Seela, K. Mersmann, *Helv. Chim. Acta* **1993**, *76*, 1435.
- [24] D. Sen, W. Gilbert, *Nature (London)* **1988**, *334*, 364.
- [25] W. I. Sundquist, A. Klug, *Nature (London)* **1989**, *342*, 825.
- [26] W. Guschlbauer, J. F. Chantot, D. Thiele, *J. Mol. Struct. Dynamics* **1990**, *8*, 491.
- [27] I. G. Panyutin, O. I. Kovalsky, E. I. Budowsky, R. E. Dickerson, M. E. Rikhirev, A. A. Lipanov, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 867.
- [28] C. H. Kang, X. Zhang, R. Ratliff, R. Moyzis, A. Rich, *Nature (London)* **1992**, *356*, 126.
- [29] 'Handbook of Biochemistry and Molecular Biologie', 'Nucleic Acids Vol. I', Ed. G. D. Fasman, CRC Press, 1979.
- [30] S. Sprang, R. Scheller, D. Rohrer, M. Sundaralingam, *J. Am. Chem. Soc.* **1978**, *100*, 2867.
- [31] D. Grierson, in 'Gel Electrophoresis of Nucleic Acids – A Practical Approach', Eds. D. Rickwood and B. D. Hames, IRL Press, Oxford–Washington, DC, 1982, pp. 1–38.
- [32] B. L. Gaffney, C. Wang, R. A. Jones, *J. Am. Chem. Soc.* **1992**, *114*, 4047.
- [33] J. F. Chantot, W. Guschlbauer, *FEBS Lett.* **1969**, *4*, 173.
- [34] A. M. Michelson, C. Monny, A. M. Kapuler, *Biochim. Biophys. Acta* **1970**, *217*, 7.
- [35] M. Lu, Q. Guo, N. R. Kallenbach, *Biochemistry* **1993**, *32*, 598.
- [36] Q. Guo, M. Lu, L. A. Marky, N. R. Kallenbach, *Biochemistry* **1992**, *31*, 2451.
- [37] M. H. Sarma, J. Luo, K. Umamoto, R. Yuan, R. H. Sarma, *J. Biomol. Struct. Dyn.* **1992**, *9*, 1131.
- [38] F. M. Pohl, T. M. Jovin, *J. Mol. Biol.* **1972**, *67*, 375.
- [39] A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, A. Rich, *Nature (London)* **1979**, *282*, 680.
- [40] A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. van Boom, A. Rich, *Science* **1981**, *211*, 171.
- [41] F. Seela, H. Driller, *Nucleic Acids Res.* **1989**, *17*, 901.
- [42] D. DePrisco Albergo, D. H. Turner, *Biochemistry* **1981**, *20*, 1413.
- [43] A. Möller, A. Nordheim, S. R. Nichols, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4777.