5-Aza-7-deaza-2′-deoxyguanosine: Oligonucleotide Duplexes with Novel Base Pairs, Parallel Chain Orientation and Protonation Sites in the Core of a Double Helix

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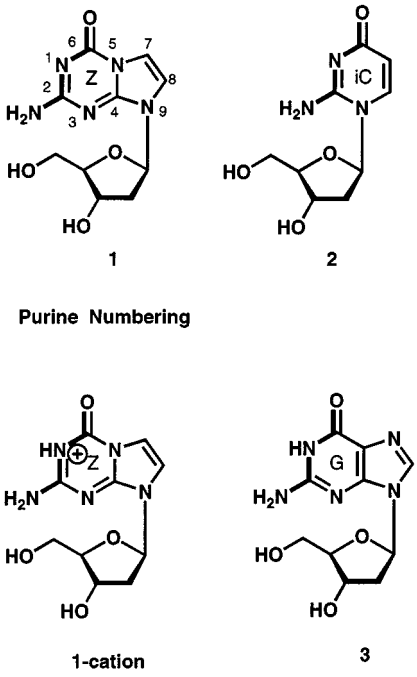
Oligonucleotides containing 5-aza-7-deaza-2′-deoxyguanosine (**1**) were synthesized. Solid-phase synthesis was performed with the phosphonate **15** or the phosphoramidite **5**. The amino-unprotected phosphonate **4** was also employed. Hybridization studies of oligonucleotides containing **1** resulted in new base pairs leading to duplexes with parallel (ps) or antiparallel (aps) chain orientation. Among those with parallel chains a stable "purine-purine" base pair was observed between 5-aza-7-deazaguanine and guanine or 7-deazaguanine. Antiparallel stranded duplexes are formed when 5-aza-7-deazaguanine pairs with cytosine. This base pair has only two hydrogen bonds under neutral conditions but is stabilized by a third one in acidic medium. A new base pair is also detected between the base of **1** and isoguanine (neutral medium).

Base-modified 2′-deoxyribonucleosides having nitrogen patterns different from those of the naturally occurring DNA constituents are of significant importance in DNA research and biotechnology.[1] Due to their ability to restrict the base pairing to certain motifs[2] or to form entirely new base pairs[3] novel DNA structures are accessible. Modified nucleosides are also valuable to probe the complex formation of DNA with ligands or proteins and to study other biochemical interactions.

In the past a large number of base-modified purine 2′-deoxyribonucleosides have been synthesized and incorporated into oligonucleotides.[4] Among them, the 7-deazapurines play an important role.[5−9] The 5-aza-7-deazapurine nucleosides are related to the latter. The 5-aza-7-deaza-2′-deoxyisoguanosine is the only compound which has already been incorporated into oligonucleotides.[10] The related 5-aza-7-deaza-2′-deoxyguanosine (**1**, dZ)[11] which is the subject of this study, is a structural analogue of 2′-deoxyguanosine (**3**). Although the base, 5-aza-7-deazaguanine, has very similar spatial requirements as guanine, N-7 as a proton acceptor site is absent. On one hand, N-1 is now a proton acceptor and no longer a proton donor as in the parent guanine. On the other hand, the *s*-triazine ring of **1** represents the same Watson−Crick-type site as 2′-deoxyisocytidine (**2**), and, therefore, it becomes similar to **3** when it is protonated at N-1 (**1**-cation) (Scheme 1).

According to the changes of the donor-acceptor pattern, various new base pairs are conceivable and novel nucleic acid structures can be formed. This study describes building blocks of 5-aza-7-deaza-2′-deoxyguanosine (**1**) for solid-phase oligonucleotide synthesis (**4**, **5**) (Scheme 2) and investigations concerning the pairing modes of the neutral and the protonated 5-aza-7-deazaguanine base with cytosine, guanine and related bases.



Scheme 1

Building Blocks

5-Aza-7-deaza-2′-deoxyguanosine (**1**) was prepared according to Rosemeyer and Seela[11] starting with compound **7**, and was then converted into oligonucleotide building blocks after precedent protection of the functional groups.[12,13] Due to the different nitrogen pattern of 5-aza-7-deazaguanine compared to guanine an altered reactivity of the 2-amino function of **1** was anticipated. The protect-

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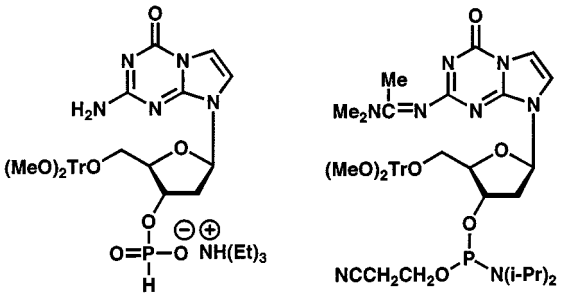


4 5

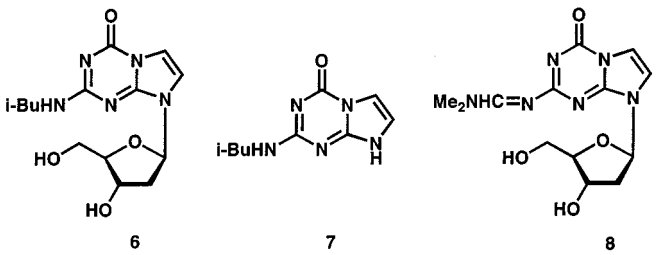
Scheme 2

ing groups (see compounds shown in Table 1) (Schemes 3 and 4) were studied. Only the acetamidine **12** was found to be suitable. However, even this compound is not stable and forms the methoxy compound **13** during the workup procedure.

Table 1. Half-life values of deprotection of 2-deoxyribofuranosyl derivatives of 8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one in 25% aq. NH_3 solution at 40°C

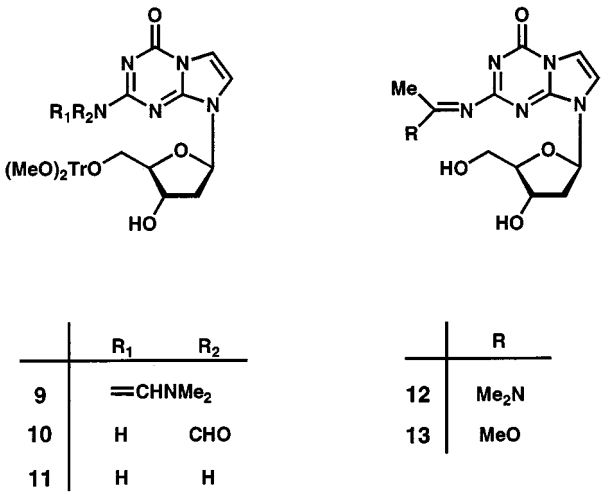
Compound	λ [nm][a]	τ [min]	Compound	λ [nm][a]	τ [min]
6	295	< 1	**10**	298	< 1
7	295	> 500	**12**	300	43
8	300	3	**13**	300	4

[a] Wavelength at which the kinetics was determined.



6 7 8

Scheme 3



	R_1	R_2
9	=CHNMe$_2$	
10	H	CHO
11	H	H

	R
12	Me$_2$N
13	MeO

Scheme 4

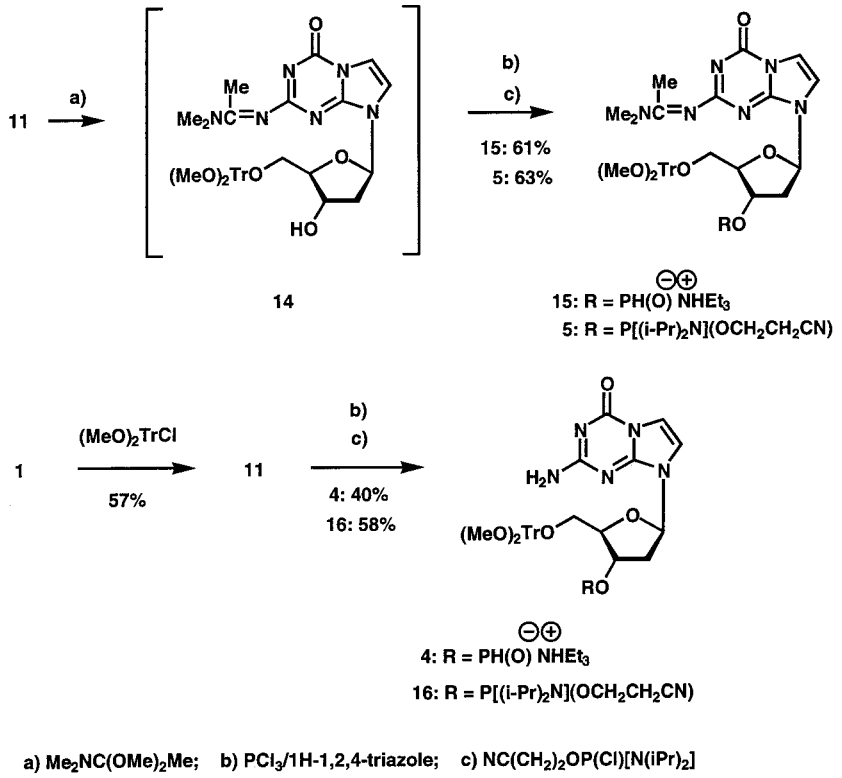
In order to circumvent this problem the following procedure was employed: (i) reaction of **1** with bis(4-methoxyphenyl)(phenyl)methyl chloride under formation of compound **11**. (ii) Subsequent reaction of **11** with *N*,*N*-dimethylacetamide dimethyl acetal[14] without isolation of the reaction product **14**. (iii) Further reaction of compound **14** to either the phosphonate **15** or the phosphoramidite **5** using standard conditions.[15,16]

Although the protection of the amino group was necessary in the case of the phosphoramidite coupling, it was unnecessary to introduce a protecting group when the protocol of phosphonate chemistry was employed.[17−19] For this purpose compound **11** was directly converted into the phosphonate **4** using a mixture of PCl_3/*N*-methylmorpholine/1,2,4-triazole. Similarly, the phosphitylation of **11** with $NC(CH_2)_2OP(Cl)[N(iPr)_2]$ furnished the base-unprotected phosphoramidite **16** (Scheme 5). During oligonucleotide synthesis it turned out that both fully protected building blocks − the phosphonate **15** and the phosphoramidite **5** − as well as the amino-unprotected phosphonate **4** but not the unprotected phosphoramidite **16** gave excellent coupling yields in oligonucleotide synthesis.

Properties of the Monomers

The compounds described above were characterized by ^{1}H-, ^{31}P- and ^{13}C-NMR spectra as well as by elemental analyses (see Experimental Section). Table 2 summarizes the ^{13}C-NMR data of the 5-aza-7-deazapurine nucleosides as well as of their protected derivatives. The signal assignment of the starting material **1** has already been performed.[11] However, in the case of the base only C-7, C-8 and the bridge-head C atoms could be assigned unambiguously whereas the assignment of C-2 and C-6 remained tentative. In the case of the amidine **8**, the C-2 signal can be identified by gated-decoupled ^{13}C-NMR spectra. A small coupling (6.8 Hz) between C-2 and the CH of the (dimethylamino)methylidene group is indicative for this position (Table 3).

Previous studies have shown that compound **1** is protonated on the *s*-triazine moiety exhibiting a pK_{BH+} value of 3.7.[11] Now, the site of protonation has been established using ^{13}C-NMR spectroscopy. Spectra were measured in D_2O within a pD range of 1−14. A significant downfield shift of the C-2 and C-6 resonances ($\Delta\delta$ = 8.4 ppm, both) was observed when changing the pD from 5 to 1 while all other chemical shifts remain almost constant (Table 2). These experiments prove that N-1 is the protonation site. This is different from 2′-deoxyguanosine (pK_{BH+} = 3.5)[20] which is protonated at N-7. Only one transition in the UV spectra of **1** at acidic pH was observed. The UV spectra of **1** in anhydrous dioxane has a maximum at 266 nm, whereas in water the maximum is shifted to 258 nm (spectra not shown). This phenomenon is not observed in the case of 7-deaza-2′-deoxyguanosine. It implies that different tautomers are formed in solvents of different polarity − a finding which has already been proposed earlier based on ^{13}C-



Scheme 5

Table 2. ^{13}C-NMR-chemical shifts of 2-deoxyribofuranosyl derivatives of 8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one[a]

[b] / [c]	C-2 / C-2	C-4 / C-6	C-6 / C-7	C-7 / C-8	C-8a / C-4				
1	150.1	165.4	108.4	114.2	150.2				
1[d]	144.6	156.7	110.3	118.0	149.0				
1[e]	149.5	161.6	109.4	116.4	149.6				
1[f]	153.0	165.1	109.0	115.4	150.1				
1[g]	153.8	166.0	109.8	116.2	150.9				
1[h]	154.7	166.9	110.7	117.0	151.8				
1[i]	153.4	165.5	109.3	115.7	150.5				
1[j]	152.3	164.4	108.1	114.6	149.3				
6	150.1	169.3	108.3	115.4	149.8				
8	149.9	165.4	108.2	114.0	150.3				
9	150.0	169.4	108.3	115.3	150.1				
10	148.9	161.0	108.9	116.3	149.5				
12	149.8	169.0	108.2	115.5	150.5				
13	164.1	169.2	108.6	116.3	150.6				
11	149.2	160.1	108.8	116.1	150.0				
4	149.9	165.3	108.4	113.7	150.4				
15	149.9	162.0	108.1	115.1	150.2				
	C-1′	C-2′	C-3′	C-4′	C-5′	C=N(O)	N−CH$_3$	CH$_3$	O−CH$_3$
1	87.7	38.8	70.6	82.8	61.5				
1[d]	87.9	39.1	71.1	85.1	61.8				
1[e]	87.4	38.4	71.0	84.4	61.6				
1[f]	87.2	38.6	71.0	84.0	61.6				
1[g]	88.0	39.5	71.9	84.9	62.5				
1[h]	88.9	40.3	72.7	85.7	63.3				
1[i]	87.6	38.9	71.3	84.4	62.0				
1[j]	86.8	38.2	70.2	83.1	61.0				
6	83.1	n.d.	70.4	87.8	61.4			19.0	
8	82.6	38.6	70.1	85.6	63.9	159.7	34.6		
9	85.7	40.6	70.1	82.9	63.9	159.6	34.7		
10	85.5	n.d.	70.0	83.7	63.9	161.0			
12	83.3	n.d.	70.5	87.7	61.5	161.9	[k]	17.0	
13	83.6	n.d.	70.3	88.0	61.3	164.1		17.0	53.7
11	87.9	n.d.	70.4	83.4	61.4				55.0
4	82.7	37.7	72.3	85.6	63.7				55.0
15	83.1	37.2	72.1	85.6	63.6	158.0	16.0	8.5	

[a] Measured in [D$_6$]DMSO at 303 K. – [b] Systematic numbering. – [c] Purine numbering. – [d] pH = 1.0. – [e] pH = 3.8. – [f] pH = 5.0. – [g] pH = 6.3. – [h] pH = 9.0. – [i] pH = 12.0. – [j] pH = 14.0. – [k] Superimposed by DMSO.

NMR spectroscopy of compound **1** in D_2O and [D_6]DMSO.[11]

The concept of preorganization – brought forth by Cram in the analysis of small organic guest-host complexes[21] – was later discussed and applied to nucleic acids by Eschenmoser et al.[22] as well as by Kool.[23] This concept can be used to design a modification which rigidifies a ligand, e.g. an oligonucleotide single strand prior to binding to its complement so that it more resembles the bound conformation. This would increase the binding strength. On the other hand the importance of stereoelectronic effects of a nucleobase on the sugar puckering of the nucleoside and therewith on the preorganization of a corresponding oligonucleotide secondary structure has been discussed.[24][25] The results described above prompted us to examine the sugar conformation of compound **1**. This was performed on the basis of seven vicinal $^1H,^1H$-coupling constants determined in D_2O. In particular, the sugar puckering [$^{3'}T_{2'}$ (N) $\rightleftharpoons$ $_{3'}T^{2'}$ (S)] and the conformation about the C(4′)–C(5′) [$\gamma^{(g)} \rightleftharpoons \gamma^{t} \rightleftharpoons \gamma^{-(g)}$] bond were determined applying the PSEUROT (6.2) program of Altona and co-workers[26] as well as the method of Westhof et al.[27] Using the [H,H] coupling constants 1′,2′, 1′,2′′, 2′,3′, 2′′,3′, and 3′,4′ the N/S conformer populations were determined to be 37% N and 63% S. Compared to dG (29% N, 71% S) the sugar conformation is slightly shifted towards the N conformation. The conformation at the C(4′)–C(5′) bond of **1** [$\gamma^{+(g)}$ 48%, γ^{t} 33%, $\gamma^{-(g)}$ 19%] is similar to that of dG [$\gamma^{+(g)}$ 53%, γ^{t} 30%, $\gamma^{-(g)}$ 17%]. This means that 5-aza-7-deazapurine exerts a slightly stronger electron-withdrawing influence on the 2′-deoxyribose moiety than guanine thereby biasing the N/S equilibrium of the sugar towards that of a ribonucleoside. Although there are significant changes of the sugar moiety of base-modified nucleosides which will affect the preorganization of the sugar-phosphate backbone, there is no simple correlation between stereoelectronic effects within a base-modified nucleoside and the stability of an oligonucleotide duplex.[28]

Table 3. *J*(C,H) values [Hz] of the nucleosides **1** and **8**[a]

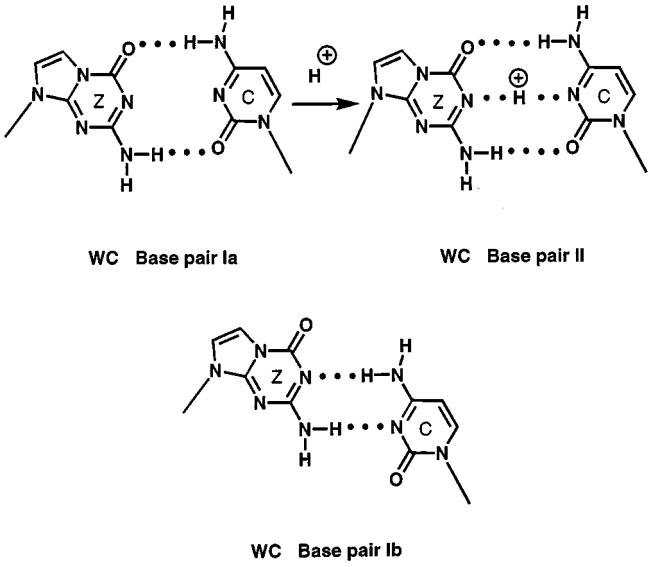
J(C,H)	**1**	**8**
CH, CH	–	179.4
C(2), CH=N	–	6.8
C(6), H-C(6)	205.2	205.4
C(6), H-C(7)	10.7	10.9
C(7), H-C(7)	200.7[b]	200.8
C(7,) H-C(1′)	4.5[b]	4.2
C(1′), H-C(1′)	167.7	165.9
C(3′), H-C(3′)	150.6	148.3
C(4′), H-C(4′)	147.6	148.0
C(5′), H-C(5′)	140.5	140.3

[a] Measured in [D_6]DMSO at 303 K. – [b] See ref.[11]

Oligonucleotides with 5-Aza-7-deazaguanine Opposite to Cytosine

An interesting feature of the 5-aza-7-deazaguanine is its ability to act as hydrogen-bond donor at N-1 (analogous to guanine) in the protonated form.[29,30] Appropriate base pairing is possible with other bases providing a proton to N-1 of the 5-aza-7-deazaguanine moiety. Stable, tridentate dZ–dC Watson–Crick (WC) base pairs are therefore expected in acidic solution (Scheme 6, WC base pair II) whereas under neutral conditions (Scheme 6, WC base pair Ia or Ib) a lower stability is anticipated. Such a stabilization of a base pair in acidic medium has been already studied for triplexes formed between cytosine and a guanine–cytosine base pair.[31]

In order to realize such a duplex structures, oligodeoxythymidylates containing one central dG or dZ residue (**17** and **19**) were synthesized and hybridized with oligodeoxyadenylates. The latter possesses a central dC or 5-methyl-2′-deoxycytidine residue (m^5C_d) opposite to dZ or dG when hybridized with the oligothymidylates (Table 4). The T_m values of the oligonucleotides were measured as a function of the pH by using buffer solutions (10 mM sodium cacodylate, 10 mM $MgCl_2$, and 100 mM NaCl) which had been adjusted to pH = 5.0, 6.0, 7.0, 8.5, and 9.5 by adding either 0.1 N HCl or 0.1 N NaOH. In all cases cooperative melting profiles were observed. The T_m values of the resulting duplexes are summarized in Table 4. From the data it is apparent that protonation – either at dZ (pK = 3.7), dC (pK = 4.3) or m^5C_d (pK = 4.5), respectively – stabilizes the duplex



Scheme 6

Table 4. T_m values and thermodynamic data of 5′-d(TTTTT**X**TTTTTT) · 5′-d(AAAAAA**Y**AAAAA) at pH = 7.0, 5.0 and 9.5[a]

	X · Y	pH	T_m[b] [°C]	Postulated base pair involving Z
17 · 18	G · C	7.0	40	
		5.0	39	
		9.5	39	
19 · 18	Z · C	7.0	27	base pair Ia or Ib, Scheme 6
		5.0	35	base pair II, Scheme 6
		9.5	22	base pair Ia or Ib, Scheme 6
19 · 20	Z · m^5C	7.0	28	base pair Ia or Ib, Scheme 6
		5.0	37	base pair II, Scheme 6
		9.5	27	base pair Ia or Ib, Scheme 6
19 · 21	Z · G	7.0	30	wobble base pair III, Scheme 7
		5.0	28	wobble base pair III, Scheme 7
		9.5	24	wobble base pair III, Scheme 7

[a] Oligomer concentration 10 μM of single strands. – [b] Measured in 0.1 M NaCl containing 10 mM $MgCl_2$ and 10 mM sodium cacodylate.

containing a dZ−dC (ΔT_m = +8°C) or a dZ−m^5C_d base pair (ΔT_m = +9°C). The situation is different, however, in the case of **19 · 21.** Here, a dG−dZ base pair (Scheme 7, Pur · Pur wobble pair III) with an antiparallel strand orientation is conceivable even under neutral conditions which contains two hydrogen bonds. This would explain the still relatively high T_m value of **19 · 21** at pH = 7 (30°C). Upon protonation as well as upon deprotonation a destabilization of the duplex is observed (Table 4, ΔT_m = 2−6°C) which is due to the fact that in both cases the number of hydrogen bonds is reduced. The observed T_m decrease of the duplex **19 · 18** between pH = 7.0 and 9.5 might be the result of the relatively lower stability of the dZ−dC base pair compared to those of dZ−m^5C_d or dG−dC. As a consequence, the dT deprotonation (pH ≈ 9.5) might occur earlier when it is close to a dZ−dC base pair.

Using the helix axis as a dyad axis, rotation of one strand of an antiparallel duplex by 180° results in a parallel arrangement. Doing this, the conformation of the sugar−phosphate backbone must not be necessarily changed in order to get a stable right-handed duplex. The oligonucleotides depicted in Table 4 might adopt such structures with parallel as well as antiparallel strand orientation.[32] In order to circumvent this problem another oligonucleotide sequence was chosen (see oligonucleotides of Table 5) which can only form aps duplex structures. In this case the modified duplexes contain either two or four dZ−dC base pairs. In all modified duplexes a stabilization occurred under acidic but not under neutral conditions.

From the structural point of view the protonated duplexes **24 · 23**, **22 · 25** and **24 · 25** are of particular interest. They represent double helices with a spine of positive charges in the core of the double helix. The additional hydrogen bonds lead to duplex stabilization while the positive charges might reduce base stacking. Therefore, it is not surprising that the modified and protonated duplex of Table 5 do not reach the stability of the parent duplex (**22 · 23**). To our best knowledge these protonated double helices are the first ones formed by purine−pyrimidine base pairs of the Watson−Crick type. Related structures of the Hoogsteen type are long known from triplexes when a dG−dC duplex binds a third strand containing dG.

In conclusion, it was demonstrated that 5-aza-7-deazaguanine forms a stable base pair with cytidine in acidic solutions under antiparallel chain orientation. In this case a proton is inserted between N-1 of 5-aza-7-deazaguanine and N-3 of cytosine forming an additional hydrogen bond.

Table 5. T_m values and thermodynamic data of oligonucleotide duplexes

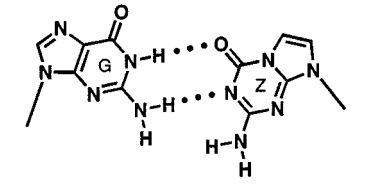
5′-d (T	A	X	X	T	C	A	A	T	A	C	T)
3′-d (A	T	C	C	A	Y	T	T	A	T	Y	A)

XXY...Y		pH	T_m[a] [°C]	Postulated base pair involving dZ
GGG...G	**22 · 23**	5	46	
		7	45	
ZZG...G	**24 · 23**	5	34	Z−C: base pair II, Scheme 7
		7	23	Z−C: base pair Ia or Ib, Scheme 7
GGZ...Z	**22 · 25**	5	37	C−Z: base pair II, Scheme 7
		7	23	C−Z: base pair Ia or Ib, Scheme 7
ZZZ...Z	**24 · 25**	5	25	Z−C and C−Z: all base pair II, Scheme 7
		7	<10	Z−C and C−Z: all base pair Ia or Ib Scheme 7

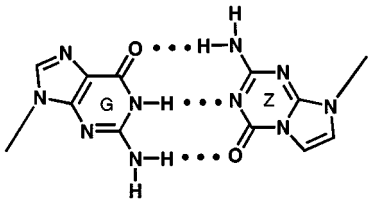
[a] Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate at 7 μmol single-strand concentration.

Oligonucleotides with 5-Aza-7-deazaguanine Opposite to Guanine or Isoguanine

According to the fact that 5-aza-7-deazaguanine has the same Watson−Crick recognition site as isocytosine (Scheme 1) and isocytosine forms parallel duplex structures with guanine it was anticipated that also 5-aza-7-deazaguanine can form parallel duplexes with guanine (Scheme 7, Pur · Pur base pair IV). In order to realize such a structure , the oligonucleotide d($T_4Z_4T_4$) (**30**) was synthesized along with other oligomers, namely d($A_4G_4A_4$) (**27**), d($T_4G_4T_4$) (**26**) and d($A_4C_4A_4$) (**31**). These oligonucleotides exhibit only linear increases of the absorbance upon heating. Therefore, self-pairing can be ruled out under these conditions. Moreover, in contrast to d($T_4G_4T_4$) (**26**),[27] the oligomer d($T_4Z_4T_4$) (**30**) is not able to form a quartet structure. When the oligomer **26** is hybridized with **27** a T_m value of only 13°C is obtained (Table 6). Obviously, this duplex is mainly held together by eight dA−dT base pairs. This duplex becomes strongly stabilized (ΔT_m = +10°C) when only one of the dG residues is replaced by a dZ moiety (duplexes **28 · 27** and **29 · 27**), independently of the position of substitution. The replacement of all four dG residues by dZ (**30 · 27**) increases the T_m value to 55°C (ΔT_m =



Pur•Pur Wobble Pair III (aps)



Pur•Pur Base Pair IV (ps)

Scheme 7

+42°C). As this duplex is even more stable than the duplex **26 · 31** (T_m = 45°C) containing 4 dG−dC and 8 dA−dT base pairs, it is concluded that also **30 · 27** represents a fully matched hybrid with 12 base pairs (8 × 2 + 4 × 3 H bonds) and with a parallel strand orientation (Scheme 7, Pur · Pur base pair IV). At this moment also for the oligonucleotides **28 · 27** and **29 · 27** a parallel strand orientation can be tentatively postulated notwithstanding the fact that also antiparallel dZ−dG base pairs are principally conceivable even under neutral conditions (Scheme 7, Pur · Pur wobble pair III). On the other hand, substitution of one dG base by dZ within the antiparallel duplex d($T_4G_4T_4$) · d($A_4C_4A_4$) (**26 · 31**) causes a destabilization by about 6°C under neutral conditions d($T_4ZG_3T_4$) · d($A_4C_4A_4$) (**28 · 31**) with retention of the chain orientation (Table 6) (3 versus 2 hydrogen bonds, Scheme 6, WC base pair Ia or Ib).

The formation of dG−dZ base pairs can also be demonstrated in the case of the duplex **30 · 26** compared to **26 · 32**. Both have dT tails at the 3′ and the 5′ termini which normally do not form hydrogen bonds with each other. From the T_m values of these duplexes (Table 6) it is evident that the four dG−dZ base pairs in the ps duplex **30 · 26** (Scheme 7, Pur · Pur base pair IV) are more stable than the G−C base pairs within the aps duplex **26 · 32**. Obviously, dZ dictates the parallel strand orientation with three hydrogen bonds within each of the four dG−dZ base pairs.

The base pair formed between dZ and dG under neutral conditions can be either a purine−purine analogue base pair with three (Scheme 7, Pur · Pur base pair IV) or a base pair with two hydrogen bonds (Scheme 7, Pur · Pur wobble pair III) − the first with parallel, the second with antiparallel orientation of the strands. Moreover, under acidic conditions where dZ and/or dG are protonated on the base moiety three different Hoogsteen type dG−dZ base pairs with either parallel or antiparallel strand orientation can be formulated (Scheme 8).

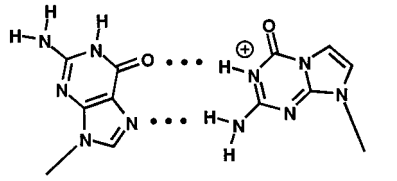
The strand orientation of oligonucleotide duplexes can be deduced from hybridization studies performed on oligonucleotides containing tracts of identical bases either at their 3′- or at the 5′-termini.[33,34] For such studies the oligomers **33** and **34** as well as the homo-dZ oligomer **35** were synthesized, and their duplex formation was studied. In the case of parallel chain orientation it is expected that neither the oligomer **33** nor **34** form duplexes or at best duplexes of low stability in the case of partial hybridization. A stable duplex should be formed when **33** and **34** are hybridized. It was observed that self-pairing of **33** as well as **34** occurs but that the duplexes exhibit relatively low T_m values (Table 7).

The duplexes **33 · 33** and **34 · 34** can exhibit parallel or antiparallel chain orientation by self-pairing (Scheme 9). For the self-paired oligomer **33** the antiparallel structure A

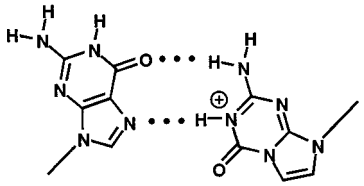
Table 6. T_m values and thermodynamic data of oligonucleotide duplexes[a]

		T_m[b] [°C]	H [%]	Postulated base pair involving dZ
5′-d(T_4GGGGT_4) 3′-d(A_4GGGGA_4)	**26 · 27**	13	5	
5′-d(T4**Z**GGGT4)	**28 · 27**	23	13	Z−G: base pair IV, Scheme 8
5′-d(T_4G**Z**GGT_4) 5′-d(A_4GGGGA_4)	**29 · 27**	23	10	Z−G: base pair IV, Scheme 8
5′-d(T_4Z Z Z ZT_4)	**30 · 27**	55	14	Z−G: base pair IV, Scheme 8
5′-d(T_4GGGGT_4) 3′-d(A_4CCCCA_4)	**26 · 31**	45	14	
5′-d(T_4GGGGT_4)	**28 · 31**	39	13	Z−C: base pair Ia or Ib, Scheme 7
5′-d(T_4 ZZZZ T_4) 5′-d(T_4GGGGT_4)	**30 · 26**	25	8	Z−G: base pair IV, Scheme 8
5′-d(T_4GGGGT_4) 3′-d(T_4CCCCT_4)	**26 · 32**	15	15	

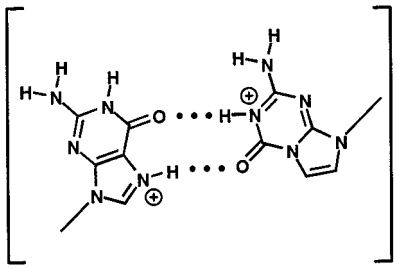
[a] Oligomer concentration 10 μM of single strands. − [b] Measured in 1 M NaCl containing 10 mM $MgCl_2$ and 10 mM sodium cacodylate, pH = 7.0.



Base pair V (aps)



Base pair VI (ps)



Base pair VII (ps)

Scheme 8

(base pair mode III, Scheme 9) is the most likely one (bold face) as it contains the highest number of hydrogen bonds (12 H bonds). The T_m value is 27°C (Table 7); upon protonation (pH = 4) its stability is decreased to 22°C because in this case the dZ−dG base pair is disturbed. The CD spectrum of **33 · 33** is shown in Figure 1a and exhibits the typical pattern of a B-type helix. Even upon protonation (pH = 4) the shape of the CD spectrum is maintained from which it can be deduced that the B-DNA structure with aps Watson−Crick-type base pairing and blunt ends is conserved.

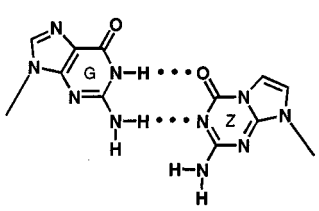
The T_m value of the self-paired duplex **34 · 34** amounts to only 20°C which is 7°C lower than that of **33 · 33** (Table 7)**.** This may be traced back either to a different secondary structure, namely a parallel B-DNA with Watson−Crick-type base pairing and sticky ends (Scheme 9: structure E, mode IV) containing only nine hydrogen bonds or to the inverted base stacking. Upon protonation (pH = 4) the T_m value of this self-paired duplex is significantly enhanced to 32°C (ΔT_m = 12°C). Moreover, the CD spectrum of **34 · 34** is drastically changed (Figure 1b). In this case a reorientation of the duplex from the ps structure E to an aps structure with Hoogsteen-type base pairing (Scheme 8, mode V) might take place.

A mixture of the oligomers **33** and **34** gives rise to a much more stable duplex compared to the self-paired ones with a T_m value of 50°C (Table 7). The concentration dependence of the T_m values indicates duplex melting (data not shown). According to these findings the duplex **33 · 34** must adopt a parallel strand orientation following the base pair mode IV (Scheme 9, structure H, 18 H bonds, blunt ends). This structure is conservative upon protonation which is demon-

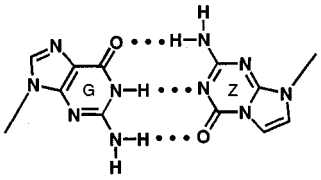
Table 7. T_m values and thermodynamic data of self-complementary oligonucleotides[a]

		T_m[b] [°C]	pH	H [%]	Postulated base pair involving dZ
5′-d(Z-Z-Z-G-G-G)	**33**	27	7.0	18	Z−G: wobble pair III, Scheme 9
5′-d(Z-Z-Z-G-G-G)	**33**	27	5.0	19	Z−G: wobble pair III, Scheme 9
5′-d(Z-Z-Z-G-G-G)	**33**	22	4.0	28	Z−G: wobble pair III, Scheme 9
5′-d(G-G-G-Z-Z-Z)	**34**	20	7.0	12	G−Z: base pair IV, Scheme 8
5′-d(G-G-G-Z-Z-Z)	**34**	32	5.0	9	G−Z: base pair V, Scheme 8
5′-d(G-G-G-Z-Z-Z)	**34**	32	4.0	8	G−Z: base pair V, Scheme 8
5′-d(Z-Z-Z-G-G-G) 5′-d(G-G-G-Z-Z-Z)	**33 · 34**	50	7.0	18	Z−G, G−Z: base pair IV, Scheme 9
5′-d(Z-Z-Z-G-G-G) 5′-d(G-G-G-Z-Z-Z)	**33 · 34**	52	5.0	10	Z−G, G−Z: base pair IV, Scheme 9
5′-d(Z-Z-Z-G-G-G) 5′-d(G-G-G-Z-Z-Z)	**33 · 34**	48	4.0	14	Z−G, G−Z: base pair IV, Scheme 9
5′-d$(Z)_6$	**35**	[c]			
5′-d$(Z)_6$ 5′-d$(c^7G)_6$	**35 · 36**	41	7.0	14	Z−c^7G: base pair IV, Scheme 9
5′-d$(c^7G)_6$	**36**	[c]			
5′-d(iGiGiG-C-C-C)[d] 5′-d(C-C-C-iGiGiG)	**37 · 38**	47	7.0	–	–
5′-d(G-G-G-Z-Z-Z)	**34 · 37**	41	7.0	20	Z−iG: base pair IX, Scheme 10

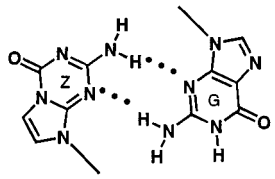
[a] Oligomer concentration 10 μM of single strands. – [b] Measured in 1 M NaCl containing 100 mM $MgCl_2$ and 60 mM sodium cacodylate, pH = 7.0. – [c] Linear increase of absorbance at 260 nm. – [d] Ref.[28]



Pur•Pur Wobble Pair III (aps)



Pur•Pur Base Pair IV (ps)



Pur•Pur Base Pair VIII (ps)

A 5'-d(Z Z Z G G G)
(G G G Z Z Z)d-5'
aps-33 • 33

B 5'-d(Z Z Z G G G)
5'-d(Z Z Z G G G)
ps-33 • 33

C 5'-d(Z Z Z G G G)
5'-d(Z Z Z G G G)
ps-33 • 33

D 5'-d(G G G Z Z Z)
(Z Z Z G G G)d-5'
aps-34 • 34

E **5'-d(G G G Z Z Z)**
5'-d(G G G Z Z Z)
ps-34 • 34

F 5'-d(G G G Z Z Z)
5'-d(G G G Z Z Z)
ps-34 • 34

G 5'-d(Z Z Z G G G)
(Z Z Z G G G)d-5'
aps-33 • 34

H **5'-d(Z Z Z G G G)**
5'-d(G G G Z Z Z)
ps-33 • 34

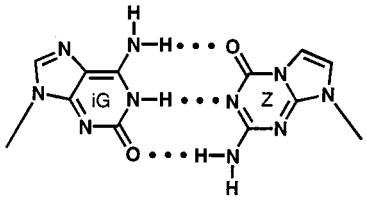
I 5'-d(Z Z Z G G G)
5'-d(G G G Z Z Z)
ps-33 • 34

Scheme 9

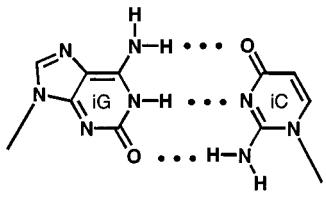
strated by pH-dependent CD spectra (Figure 1c). The T_m value is only slightly reduced (Table 7) when changing the pH from 7 to 4 which is consistent with our structural assumption.

The parallel orientation of oligonucleotide duplexes containing dG−dZ base pairs is supported by experiments performed on the homooligonucleotides 5′-d(Z-Z-Z-Z-Z-Z) (**35**) and 5′-d(c^7G-c^7G-c^7G-c^7G-c^7G-c^7G) (**36**). Both oligonucleotides contain 7-deazapurine bases (7-deazaguanine and 5-aza-7-deazaguanine), excluding Hoogsteen-type base pairing. The T_m value of **35** · **36** (Table 7) was determined UV-spectrophotometrically as well as by temperature-dependent CD spectra. Both T_m values were found to be 41°C. According to this T_m value the duplex must follow the Watson−Crick-type base pair IV with parallel chain orientation (analogous structure H, Scheme 9); only here, a T_m value which is in the range of that of the duplex **33** · **34** (50°C). The difference (ΔT_m = 9°C) can be attributed to the altered sequence as well as to the presence of c^7G_d instead of dG.

As isocytosine and 5-aza-7-deazaguanine have the same donor-acceptor pattern (Scheme 1), an antiparallel duplex structure should also be formed in the case of an oligonucleotide built up from 5-aza-7-deazaguanine-isoguanine base pairs (mode IX**,** Scheme 10). Similarly, isoguanine pairs with isocytidine with aps chain orientation (mode X, Scheme 10).[34,35] In order to prove this hypothesis the oligonucleotides 5′-d(G_3-Z_3) (**34**) and 5′-d(isoG_3-C_3) (**37**) were hybridized. Also in this case a stable aps duplex with a T_m value of 41°C was formed (Table 7). The corresponding ps duplex of **37** · **38** showed a T_m value of 47°C.



Base pair IX (aps)



Base pair X (aps)

Scheme 10

The experiments performed with oligonucleotides containing dZ opposite to G_d show that fully matched duplexes

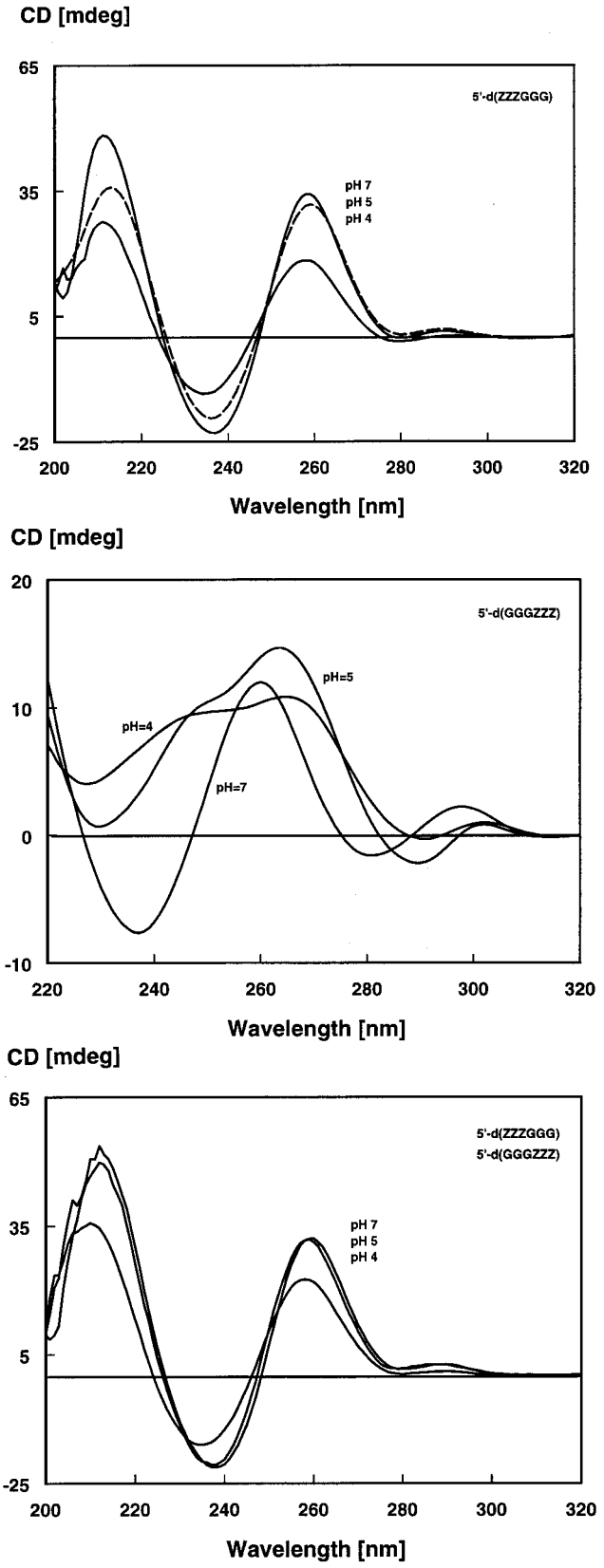


Figure 1. pH-dependent CD spectra a) of self-complementary oligomer d(ZZZGGG) (**33**), b) of self-complementary oligomer d(GGGZZZ) (**34**) and c) of duplex d(ZZZGGG) − d(GGGZZZ) (**33** · **34**), measured at 0°C in 60 mM sodium cacodylate, 1 M NaCl, and 100 mM $MgCl_2$ at pH = 4.0, 5.0 and 7.0; the oligomer concentration was 10 μM of single-strands

with parallel chain orientation (Pur · Pur base pair IV) are formed whereas those containing dZ opposite to $isoG_d$ (base pair IX) form duplexes with antiparallel chains.

In general it was demonstrated that various new base pairs can be constructed when 5-aza-7-deazaguanine replaces guanine within oligonucleotide duplexes. Depending on the complementary base parallel as well as antiparallel hybrids are accessible. In particular, "purine−purine" base pairs (dG−dZ) (base pair IV)[36] are remarkably stable when the chain orientation is parallel.

Experimental Section

General: Solvent systems for flash chromatography (FC) and thin-layer chromatography (TLC): CH_2Cl_2/MeOH, 95:5 (A); CH_2Cl_2/MeOH, 9:1 (B); CH_2Cl_2/MeOH, 8:2 (C); CH_2Cl_2/MeOH, 75:25 (D); CH_2Cl_2/MeOH/Et_3N, 95:3:2 (E); CH_2Cl_2/MeOH/Et_3N, 88:10:2 (F); CH_2Cl_2/MeOH/Et_3N, 78:20:2 (G); CH_2Cl_2/EtOAc/Et_3N, 45:45:10 (H); CH_3CN/H_2O, 9:1 (I). − Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). Oligonucleotide synthesis was carried out with a DNA synthesizer, model 381 A (Applied Biosystems, Weiterstadt, Germany), on a 1-μmol scale. The enzymatic hydrolysis of the oligomers was carried out as described.[37] The mixture was analyzed on reversed-phase HPLC (RP-18, solvent system III). Quantification of the resulting nucleosides was made on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituents (λ_{260}: dA 15400, dC 7300, dG 11700, dT 8800, dZ 11500). − UV: Hitachi U 3200 UV-VIS spectrophotometer. − NMR: Bruker AMX-500 (500.15 MHz and 125.4 MHz for ^1H and ^{13}C, respectively). For ^1H, [D_6]DMSO as solvent δ_H = 2.50; for ^{13}C NMR, [D_6]DMSO δ_C = 39.5. Bruker AC-250 (101.1 MHz for ^{31}P, relative to 85% H_3PO_4 as external standard). All spectra were measured at 30°C. − MALDI-TOF spectra were recorded by Mrs. Julia Gross, Westfälische Wilhelms Universität Münster, Institut für Medizinische Physik und Biophysik (Head: Prof. Dr. F. Hillenkamp) with a home-built apparatus.

pK_a Values: The pK_a values were determined UV-spectrophotometrically at 20°C in Teorell−Stenhagen buffer.[38]

HPLC Separation: HPLC was carried out according to Seela et al.[39] The solvent gradients consisting of 0.1 M (Et_3NH)OAc, pH = 7.0/MeCN 95:5 (A) and MeCN (B) were used in the following order: gradient I, 3 min 15% B in A, 7 min 15−40% B in A, 10 min 40% B in A, 5 min 40−15% B in A; flow rate 1 mL/min; gradient II, 20 min 0−20% B in A, flow rate 1 mL/min; gradient III, 20 min 100% A, flow rate 0.6 mL/min.

T_m Experiments: Melting curves were measured with a Cary-1/3 UV/VIS spectrophotometer (Varian, Australia). The temperature of the solution was increased linearly with time at a rate of 60°C/h using a Lauda PM-350 programmer and a Lauda RCS 6 bath equipped with an R 22 unit (MWG Lauda, FRG). The actual temperature was measured in the reference cell with a Pt-100 resistor. The T_m values were calculated using the software package provided by Dr. H. Apel, Varian, (Darmstadt, Germany).

8-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-(isobutyrylamino)-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (6): Compound **1**[11] (50 mg, 0.19 mmol) was dried by co-evaporation with pyridine (3 × 5 mL). The residue was suspended in pyridine (5 mL), and Me_3SiCl (0.48 mL, 3.75 mmol) was added at room temp. After 15 min of stirring, the

solution was treated with isobutyric anhydride (0.62 mL, 3.78 mmol) and maintained at room temp. for 3 h. The mixture was cooled (ice bath) and water (1 mL) was added. Then, 25% aq. ammonia solution (1 mL) was added and stirring was continued for 15 min. The solution was concentrated to give an oil. FC (silica gel, column 20 × 1.8 cm, 100 mL of CH_2Cl_2, F) furnished a main zone which gave **6** (21 mg, 33%) as a colorless, amorphous solid. TLC (silica gel, B): R_f = 0.35. − UV (MeOH): λ_{max} (lg ε) = 260, 295 nm (4.09, 4.16). − ^{1}H NMR ([D$_6$]DMSO): δ = 7.72 (m, 1 H, 7-H), 7.63 (m, 1 H, 6-H), ("t", *J* = 6.4 Hz, 1′-H), 5.32 (m, 1 H, 3′-OH), 5.00 (m, 1 H, 5′-OH), 4.41 (m, 1 H, 3′-H), 3.84 (m, 1 H, 4′-H), 3.63 (m, 2 H, 5′-H), 2.90 (m, 1 H, 2′-H$_\alpha$), 2.27 (m, 1 H, 2′-H$_\beta$), 1.27 [s, 1 H, C*H*(CH$_3$)$_2$], 1.0 [d, *J* = 6.7 Hz, 6 H, C(C*H*$_3$)$_2$].

8-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-{[(dimethylamino)methylidene]amino}-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (8): To a suspension of compound **1** (60 mg, 0.22 mmol) in MeOH (3 mL) *N*,*N*-dimethylformamide diethyl acetal (0.16 mL, 1.07 mmol) was added under stirring at room temp. The stirring was continued overnight. After concentration, the residue was submitted to FC (silica gel, column 15 × 1.8 cm, C) yielding **8** as a colorless amorphous solid which crystallized from MeOH in colorless needles (47 mg, 66%), m.p. 178°C; TLC (silica gel, C): R_f = 0.45. − UV(MeOH): λ_{max} (lg ε) = 259, 298 nm (4.16, 3.67). − ^{1}H NMR ([D$_6$]DMSO): δ = 8.72 [s, 1 H, C*H*N(CH$_3$)$_2$], 7.60 (s, 1 H, 7-H), 7.48 (s, 1 H, 6-H), 6.32 (s, br., 1 H, 1′-H), 5.30 (s, 1 H, 3′-OH), 5.00 (s, br., 1 H, 5′-OH), 4.35 (s, br., 1 H, 3′-H), 3.84 (s, 1 H, 4′-H), 3.56 (s, br., 2 H, 5′-H), 3.16 and 3.03 [2 s, 6 H, N(C*H*$_3$)$_2$], 2.50 (m, 1 H, 2′-H$_\alpha$), 2.24 (m, 1 H, 2′-H$_\beta$). − $C_{13}H_{18}N_6O_4$ (322.33): calcd. C 48.44, H 5.63, N 26.07; found C 48.37, H 5.60, N 25.96.

Reaction of 8 with Bis(4-methoxyphenyl)(phenyl)methyl Chloride: Compound **8** (205 mg, 0.63 mmol) was dried by repeated co-evaporation with absolute pyridine and then dissolved in absolute pyridine (3 mL). Then bis(4-methoxyphenyl)(phenyl)methyl chloride (235 mg, 0.70 mmol) (Ar) was added and the solution was stirred for 3.5 h at ambient temp. After addition of 5% aq. $NaHCO_3$ (40 mL), the solution was extracted with CH_2Cl_2 (25 mL, twice). The combined organic extracts were dried (Na_2SO_4), filtered, concentrated, and the residue was separated by FC into two zones (silica gel, column 22 × 1.8 cm, 100 mL of CH_2Cl_2, F).

8-{5-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-β-D-*erythro*-pentofuranosyl}-2-{[(dimethylamino)methylidene]amino}-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (9): From the slow migrating zone compound **9** (155 mg, 39%) was obtained as colorless amorphous solid; TLC (silica gel, C): R_f = 0.3. − ^{1}H NMR ([D$_6$]DMSO): δ = 8.73 [s, 1 H, C*H*N(CH$_3$)$_2$], 7.46 (d, *J* = 2.6 Hz, 1 H, 7-H), 7.40 (d, *J* = 2.7 Hz, 1 H, 6-H), 7.37−7.23 (m, aromatic H), 6.85 (m, aromatic H), 6.34 ("t", *J* = 6.7 Hz, 1 H, 1′-H), 5.38 (m, 1 H, 3′-OH), 4.37 (s, br., 1 H, 3′-H), 3.95 (m, 4′-H), 3.74 (2 s, 6 H, 2 OC*H*$_3$), 3.51 (m, 1 H, 5′-H$_\alpha$), 3.17 [m, 4 H, 2′-H$_\alpha$ and N(C*H*$_3$)], 3.05 [s, 3 H, N(C*H*$_3$)], 2.32 (m, 1 H, 2′-H$_\beta$).

8-{5-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-β-D-*erythro*-pentofuranosyl}-2-(formylamino)-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (10): From the faster migrating zone compound **10** (23 mg, 6%) was obtained as a colorless foam; TLC (silica gel, E): R_f = 0.4. − ^{1}H NMR ([D$_6$]DMSO): δ = 10.8 (s, br., 1 H, N*H*), 9.34 (s, OC*H*), 7.61 (d, *J* = 2.5 Hz, 1 H, 7-H), 7.53 (d, *J* = 2.5 Hz, 1 H, 6-H), 7.36−7.19 (m, aromatic H), 7.86−7.82 (m, aromatic H), 6.30 ("t", *J* = 6.8 Hz, 1 H, 1′-H), 5.39 (m, 1 H, 3′-OH), 4.39 (m, 1 H, 3′-H), 3.96 (m, 1 H, 4′-H), 3.73 (2 s, 6 H, 2 OC*H*$_3$), 3.18 (m, 2 H, 5′-H), 2.63−2.59 (m, 1 H, 2′-H$_\alpha$), 2.35−2.31 (m, 1 H, 2′-H$_\beta$).

Reaction of Compound 1 with *N*,*N*-Dimethylacetamide Dimethyl Acetal: Compound **1** (197 mg, 0.74 mmol) was suspended in DMF (3 mL). After 10 min, *N*,*N*-dimethylacetamide dimethyl acetal (0.7 mL 4.2 mmol) was added under stirring. The reaction was continued at room temp. for 3 d. After evaporation of the solvent, the residue was submitted to FC (column: 15 × 1.8 cm, silica gel). Flash chromatography with solvent C furnished two zones.

8-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-[(dimethylamino)ethylidene]amino)]-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (12): From the slow migrating zone compound **12** (116 mg, 47%) was obtained as colorless amorphous solid; TLC (silica gel, C): R_f = 0.3. − ^{1}H NMR ([D$_6$]DMSO): δ = 7.61 (d, *J* = 2.8 Hz, 1 H, 7-H), 7.49 (d, *J* = 2.7 Hz, 1 H, 6-H), 6.25 ("t", *J* = 6.7 Hz, 1 H, 1′-H), 5.33 (m, 1 H, 3′-OH), 5.01 ("t", *J* = 5.3 Hz, 1 H, 5′-OH); 4.34 (s, br., 1 H, 3′-H), 3.83 (m, 1 H, 4′-H), 3.55 (m, 1 H, 5′-H$_\alpha$), 3.54 (m, 1 H, 5′-H$_\beta$), 3.07−3.01 [m, 6 H, N(C*H*$_3$)$_2$], 2.46−2.40 (m, 1 H, 2′-H$_\alpha$), 2.27−2.20 (m, 1 H, 2′-H$_\beta$), 2.14 [s, 3 H, C(C*H*$_3$)].

8-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-{[(methoxy)ethylidene]amino)}-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (13): The faster migrating zone furnished compound **13** (26 mg, 11%) as colorless amorphous solid; TLC (silica gel, C): R_f = 0.3. − ^{1}H NMR ([D$_6$]DMSO): δ = 7.76 (m, 1 H, 7-H), 7.63 (s, br., 1 H, 6-H), 6.29 ("t", *J* = 6.3 Hz, 1 H, 1′-H), 5.36 (m, 1 H, 3′-OH), 5.06 ("t", *J* = 4.8 Hz, 1 H, 5′-OH), 4.35 (s, br., 1 H, 3′-H), 3.85 (m, 1 H, 4′-H), 3.71 (s, 3 H, OC*H*$_3$), 3.60−3.53 (m, 2 H, 5-H$_2$), 2.48−2.43 (m, 1 H, 2′-H$_\alpha$), 2.28−2.25 (m, 1 H, 2′-H$_\beta$), 1.99 (s, 3 H, C*H*$_3$).

2-Amino-8-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-β-D-*erythro*-pentofuranosyl}-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (11): Compound **1** (197 mg, 0.74 mmol) was dried by repeated co-evaporation from absolute pyridine and then dissolved in absolute pyridine (2 mL). Thereupon, bis(4-methoxyphenyl)(phenyl)methyl chloride (325 mg, 0.96 mmol) was introduced under Ar. The solution was stirred for 3.5 h at ambient temp., 5% aq. $NaHCO_3$ (40 mL) was added and the solution was extracted with CH_2Cl_2 (25 mL, twice). The combined organic extracts were dried (Na_2SO_4), filtered, concentrated, and the residue was applied to FC (silica gel, column 22 × 1.8 cm, 100 mL of CH_2Cl_2, F). Concentration of the main zone afforded **11** (240 mg, 57%) as colorless, amorphous solid; TLC (silica gel, B): R_f = 0.35. − ^{1}H NMR ([D$_6$]DMSO): δ = 7.37−7.34 (m, 3 H, 7-H, 6-H and aromatic H), 7.30−7.22 (m, aromatic H), 6.95 (s, br., 2 H, *NH*$_2$), 6.87−6.84 (m, aromatic H), 6.21 ("t", *J* = 6.5 Hz, 1 H, 1′-H), 5.35 (s, 1 H, 3′-OH), 4.36 (m, 1 H, 3′-H), 3.92 (m, 1 H, 4′-H), 3.74 (s, 6 H, OC*H*$_3$); 3.15 (m, 2 H, 5′-H), 2.50 (m, 1 H, 2′-H$_\alpha$), 2.28 (m, 1 H, 2′-H$_\beta$). − $C_{31}H_{31}N_5O_6$ (569.62): calcd. C 65.37, H 5.49, N 12.29; found C 65.32, H 5.55, N 12.37.

2-Amino-8-{5-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-β-D-*erythro*-pentofuranosyl-3′-(phosphonato)]-8*H*-imidazo[1,2-*a*][1,3,5]-triazin-4-one (4): To a solution of PCl_3 (132 µl, 1.55 mmol) and *N*-methylmorpholine (1.7 mL, 15.2 mmol) in absolute CH_2Cl_2 (14 mL) was added 1*H*-1,2,4-triazole (345 mg, 5.0 mmol). After stirring for 20 min at room temp. and cooling to 0°C, a solution of compound **11** (230 mg, 0.4 mmol) in CH_2Cl_2 (9 mL) was added slowly. Stirring was continued for 30 min at 0°C, then the mixture was poured into 1 M (Et_3NH)HCO_3 (TBK, pH = 8.0, 17 mL), shaken and separated. The aqueous phase was extracted with CH_2Cl_2 (12 mL, three times) and the combined organic extracts were dried (Na_2SO_4) and concentrated. FC (silica gel, column 15 × 1.8 cm, B (100 mL); C (100 mL); then D) furnished a main zone which was concentrated. The residue was dissolved in CH_2Cl_2 (20 mL), extracted with aq. 0.1 M TBK (10 × 15 mL), dried with Na_2SO_4 and concentrated. Compound **4** (119 mg, 40%) was obtained as yellow-

ish foam; TLC (silica gel, C): R_f = 0.45. − ^{1}H NMR ([D$_6$]DMSO): δ = 7.36−7.17 (m, 11.5 H, 7-H, 6-H, P*H*, aromatic H), 6.98 (s, br., 2 H, NH_2), 6.85 (m, 4 H, aromatic H), 6.20 (m, 1 H, 1′-H), 6.04 (s, 1/2 H, P*H*), 4.71 (s, br., 1 H, 3′-H), 4.07 (s, 1 H, 4′-H), 3.73 (m, 3 H, OCH_3); 3.15 (m, 3 H, 5′-H and CH_2), 2.94 (m, 1 H, CH_2), 2.40 (m, 1 H, 2′-H$_a$), 1.02 (m, 6 H, CH_3). − ^{31}P NMR ([D$_6$]DMSO): δ = 2.01 [^1J(P,H) = 581.68 Hz; ^3J(P,H) = 8.27]. − $C_{37}H_{47}N_6O_8P$ (734.79): calcd. C 60.48, H 6.45, N 11.44; found C 60.60, H 6.63, N 11.18.

Phosphoramidite 16: To a solution of **11** (100 mg, 0.18 mmol) and (*i*Pr)$_2$EtN (91 μL, 0.50 mmol) in anhydrous CH_2Cl_2 (2.3 mL), chloro(2-cyanoethoxy)(diisopropylamino)phosphane (115 μL, 0.50 mmol) was added within 2 min at room temp. After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (11 mL), cooled to 0°C and quenched by adding a 5% aq. $NaHCO_3$ solution (11 mL). Then, the aqueous layer was extracted with CH_2Cl_2 (3 × 6 mL), the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was applied to FC (silica gel, column 8 × 2 cm, CH_2Cl_2/EtOAc/Et$_3$N, 69:30:1). Colorless foam of **16** (81 mg, 58%); TLC (silica gel, CH_2Cl_2/EtOAc/Et$_3$N, 69:30:1): R_f = 0.27. − ^{31}P NMR ($CDCl_3$): δ = 149.71, 149.64.

8-{5-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-2-deoxy-β-D-*erythro*-pentofuranosyl}-2-{[(dimethylamino)ethylidene]amino}-3′-(phosphonato)-8*H*-imidazo[1,2-*a*][1,3,5]triazin-4-one (15): 184 mg (0.32 mmol) of **11** was suspended in 6 mL of MeOH. After 10 min, *N,N*-dimethylacetamide diethyl acetal (0.56 mL, 3.90 mmol) was added under stirring. The reaction was continued at 50°C overnight. After concentration, the residue was co-evaporated with MeCN and dissolved in absolute CH_2Cl_2 (11.5 mL). The resulting mixture was added within 10 min to a previously prepared solution of PCl_3 (105 μL, 1.24 mmol), 1,2,4-triazole (276 mg, 4.0 mmol) and *N*-methylmorpholine (1.38 mL, 12.16 mmol) in absolute CH_2Cl_2 (11.5 mL, 0°C). After stirring for 20 min, the reaction mixture was hydrolyzed by addition of TBK buffer (1 M, pH = 7.5, 14 mL). The aqueous phase was extracted three times with CH_2Cl_2 (9 mL each) and the combined organic extracts were dried (Na_2SO_4). After evaporation of the solvent, the residue was submitted to FC (column: 15 × 1.8 cm, solvents: B (100 mL); C (100 mL); then D). The main zone was concentrated, the residue dissolved in CH_2Cl_2 (20 mL) and extracted three times with TBK buffer (0.1 M). After drying (Na_2SO_4) and concentration, compound **15** (160 mg, 61%) was obtained as a yellowish foam; TLC (silica gel, C): R_f = 0.45. − UV (MeOH): λ_{max} (lg ε) = 237, 255, 275 nm (4.35, 4.22, 4.10). − ^{1}H NMR ([D$_6$]DMSO): δ = 7.44 (s, 1 H, 6-H), 7.35−7.21 (m, 10.5 H, 7-H, 1/2 P*H*, aromatic H), 6.84 (m, 4 H, aromatic H), 6.23 (t, *J* = 6 Hz, 1 H, 1′-H), 6.03 (s, 1/2 P*H*); 4.72 (s, br., 1 H, 3′-H), 4.09 (s, 1 H, 4′-H), 3.73 (m, OCH_3), 3.17 (s, 2 H, 5′-H), 3.03 (s, br., 2 H, MeCH_2), 2.95 [m, 6 H, N(CH_3)$_2$], 2.13 (s, CH_3), 1.14 (m, 10 H, MeCH_2). − ^{31}P NMR ([D$_6$]DMSO): 2.27 [^1J(P,H) = 291.94 Hz; ^3J(P,H) = 9.01). − $C_{41}H_{54}N_7O_8P$·3/5HCl (825.78): calcd. C 59.66, H 6.59, N 11.87; found C 59.58, H 6.51, N 11.45.

Phosphoramidite 5: Compound **11** (136 mg, 0.24 mmol) was suspended in MeOH (1 mL). After 10 min, *N,N*-dimethylacetamide diethyl acetal (0.35 mL, 2.43 mmol) was added under stirring. The reaction was continued at 50°C overnight. After concentration, the residue was co-evaporated with MeCN, dissolved in absolute CH_2Cl_2 (9 mL) followed by addition of (*i*Pr)$_2$EtN (140 μL, 0.32 mmol). To the resulting mixture chloro(2-cyanoethoxy)(diisopropylamino)phosphane (177 μL, 0.77 mmol) was added within 2 min at room temp. After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (36 mL), cooled to 0°C, and quenched by adding a 5% aqueous $NaHCO_3$ solution (9 mL). Then the aqueous layer was extracted with CH_2Cl_2 (3 × 24 mL), the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was applied to flash chromatography (silica gel column 8 × 2 cm, CH_2Cl_2/MeOH/ Et$_3$N, 96:3:1); colorless foam (126 mg, 63%); TLC (silica gel, CH_2Cl_2/MeOH/Et$_3$N, 96:3:1): R_f = 0.35. − ^{31}P NMR ($CDCl_3$): δ = 149.45, 149.38.

Solid-Phase Synthesis of the Oligonucleotides 17−38: The synthesis of the oligonucleotides **17, 19, 30, 28** and **26** was performed using 3′′-phosphonates of [(MeO)$_2$Tr]ib^{2}G$_d$, [(MeO)$_2$Tr]ib^{2}c^{7}G$_d$, [(MeO)$_2$Tr]Bz^{4}C$_d$, [(MeO)$_2$Tr]A$_d$, and [(MeO)$_2$Tr]T$_d$, as well as compounds **4** and **5**. The synthesis followed the regular protocol of the DNA synthesizer for 3′-phosphonates.[25] The synthesis of the oligomers **18, 20, 21, 22−25, 27, 26, 31, 33−38** was carried out on a 1-μmol scale in a automated DNA synthesizer (Applied Biosystems, ABI 392−08) using the regular phosphoramidite protocol.[19] The phosphoramidite **5** as well as those of the regular DNA constituents were employed. The oligonucleotides were recovered from the synthesizer as the 5′-[bis(4-methoxyphenyl)(phenyl)methyl]ated derivatives. They were isolated, deprotected, and purified on oligonucleotide cartridges.[40] Their purity was checked by reverse-phase HPLC. The nucleoside composition of the oligomers was determined from reverse-phase HPLC profiles, obtained after hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. MALDI-TOF mass spectra have been measured for several cases (Table 8). The oligomers were lyophilized in a Speed-Vac evaporator to yield a colorless foam which was dissolved in H_2O (100 μL) and stored frozen at −18°C.

Table 8. Molecular weights determined by MALDI-TOF mass spectra of oligonucleotides

	Yield [A_{260} units]	Nucleoside composition		M^+ (calcd.)	M^+ (found)
d(T$_5$ZT$_6$)	22	dT:dZ	11.3:1.0	3612	3612
d(G$_3$Z$_3$)	21	dG:dZ	1.1:1.0	1913	1910
d(Z$_3$G$_3$)	20	dG:dZ	1.0:1.1	1923	1915
d(T$_4$ Z$_4$T$_4$)	18	dT:dZ	2.1:1.0	3685	3687
d(T$_4$ GZGGT$_4$)	18	dT:dG:dZ	8.1:3.1:1.0	3685	3686
d(T$_4$ ZG$_4$T$_4$)	21	dT:dG:dZ	8.2:3.9:1.0	4017	4020
d(Z)$_6$	16	−		1913	1913
d(TAGGTCAATACT)	24	[a]		3644	3643
d(AGTATTGACCTA)	23	[a]		3644	3651
d(TAZZTCAATACT)	17	[a]		3644	3645
d(AZTATTZACCTA)	18	[a]		3644	3641

[a] Not determined.

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[1] J. A. Montgomery in *Nucleosides, Nucleotides and Their Biological Applications* (Eds.: J. L. Rideout, D. W. Henry, L. M. Beacham III) Academic Press, New York, **1983**, pp. 19−46.
[2] F. Seela, H. Driller, *Nucleic Acids Res.* **1985**, *13*, 911−926.
[3] C. Y. Switzer, S. E. Moroney, S. A. Benner, *Biochemistry* **1993**, *32*, 10489−10496.
[4] G. R. Revankar, R. K. Robins, "The Synthesis of Heterocyclic Analogues of Purine Nucleosides and Nucleotides" in *Chemistry of Nucleosides and Nucleotides, 2* (Eds.: L. B. Townsend) Plenum Press, New York, London, **1991**, pp. 161−398.
[5] [5a] F. Seela, A. Kehne, *Biochemistry* **1987**, *26*, 2232−2238. − [5b] F. Seela, K. Kaiser, *Helv. Chim. Acta* **1988**, *71*, 1813−1823.
[6] [6a] S. Mizusawa, S. Nishimura, F. Seela, *Nucleic Acids Res.* **1986**, *14*, 1319−1324. − [6b] F. Seela, S. Menkhoff, *Liebigs Ann. Chem.* **1985**, 1360−1366. − [6c] F. Seela, H. Driller, U. Liman, *Liebigs Ann. Chem.* **1985**, 312−320.
[7] F. Seela, A. Röling, *Nucleic Acids Res.* **1992, 20,** 55−61.
[8] F. Seela, H. Steker, *Helv. Chim. Acta* **1986**, *69*, 1602−1613.
[9] [9a] N. Ramzaeva, F. Seela, *Helv. Chim. Acta* **1996**, 79, 1549−1558. − [9b] F. Seela, H. Thomas, *Helv. Chim. Acta* **1995**, *78*, 94−108. − [9c] F. Seela, M. Zulauf, *Chem. Eur. J.* **1998**, *4*, 1781−1790.
[10] J. J. Voegel, S. A. Benner, *Helv. Chim. Acta* **1996**, *79*, 1881−1898.
[11] H. Rosemeyer, F. Seela, *J. Org. Chem.* **1987**, *52*, 5136−5143.
[12] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.
[13] A. Holy, J. Zemlicka, *Collect. Czech. Chem. Commun.* **1969**, *34*, 2449−2458.
[14] L. J. McBride, R. Kierzek, S. L. Beaucage, M. H. Caruthers, *J. Am. Chem. Soc.* **1986**, *108*, 2040−2048.
[15] S. L. Beaucage "Protocols for Oligonucleotides and Analogs" in *Methods in Molecular Biology* (Ed.: E. S. Agrawal), Humana Press, Totowa, New Jersey, **1994**, vol. 20, p. 33.
[16] B. C. Froehler, *Protocols for Oligonucleotides and Analogs* in *Methods in Molecular Biology* (Ed.: E. S. Agrawal), Humana Press, Totowa, New Jersey, **1994**, vol. 20, p. 63.
[17] T. Wada, Y. Sato, F. Honda, S. Kawahara, M. Sekine, *J. Am. Chem. Soc.* **1997**, *119*, 12710−12721.
[18] P. P. Kung, R. A. Jones, *Tetrahedron Lett.* **1992**, *33*, 5869−5872.
[19] S. M. Gryasnov, R. L. Letsinger, *J. Am. Chem. Soc.* **1991**, *113*, 5876−5877.
[20] R. P. Panzica, R. J. Rousseau, R. K. Robins, L. B. Townsend, *J. Am. Chem. Soc.* **1972**, *94*, 4708−4714.
[21] D. J. Cram, *Chemtech* **1987**, *17*, 120.
[22] A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992**, *75*, 218.
[23] E. T. Kool, *Chem. Rev.* **1997**, *97*, 1473−1487.
[24] C. Thibaudeau, J. Plavec, K. A. Watanabe, J. Chattopadhyaya, *J. Chem. Soc., Chem. Commun.* **1994**, 537−540.
[25] H. Rosemeyer, F. Seela, *J. Chem. Soc., Perkin Trans. 2* **1997**, 2341−2345.
[26] J. van Wijk, C. Altona, *PSEUROT 6.2 − A program for the conformational analysis of five-membered rings*, University of Leiden, July **1993**.
[27] E. Westhof, O. Röder, I. Croneiss, H.-D. Lüdemann, *Z. Naturforsch., C* **1975**, *30*, 131.
[28] F. Seela, I. Münster, U. Löchner, H. Rosemeyer, *Helv. Chim. Acta* **1998**, *81*, 1139−1155.
[29] F. Seela, A. Melenewski, C. Wei, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2173−2176.
[30] F. Seela, Y. Chen, A. Melenewski, H. Rosemeyer, C. Wei, *Acta Biochim. Pol.* **1996**, *43*, 45−52.
[31] 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**.
[32] K. Rippe, N. B. Ramsing, R. Klement, T. M. Jovin, *J. Biomol. Struct. Dyn.* **1990**, *7*, 1199−1209.
[33] F. Seela, C. Wei, A. Melenewski, *Nucleic. Acids Res.* **1996**, *24*, 4940−4945.
[34] F. Seela, C. Wei, *Helv. Chim. Acta* **1997**, *80*, 73−85.
[35] Y. Tor, P. B. Dervan, *J. Am. Chem. Soc.* **1993**, *115*, 4461−4467.
[36] K. Groebke, J. Hunziker, W. Fraser, L. Peng, U. Diederichsen, K. Zimmermann, A. Holzner, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1998**, *81*, 375−474.
[37] F. Seela, T. Wenzel, *Helv. Chim. Acta* **1992**, *75*, 1111−1122.
[38] T. Teorell, E. Stenhagen, *Biochem. Z.* **1953**, *299*, 416−419.
[39] F. Seela, K. Kaiser, U. Bindig, *Helv. Chim. Acta* **1989**, *72*, 868−881.
[40] Applied Biosystems, Oligonucleotide Purification Cartridges.

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