The C^8-(2′-Deoxy-β-D-ribofuranoside) of 7-Deazaguanine: Synthesis and Base Pairing of Oligonucleotides with Unusually Linked Nucleobases

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The 7-deazaguanine (2-aminopyrrolo[2,3-*d*]pyrimidin-4-one) C^8-(2′-deoxy-β-D-ribofuranoside) (**6b**), which possesses an unusual glycosylation site, was synthesized and incorporated in oligonucleotides. The oligonucleotides were prepared by solid-phase synthesis using phosphoramidite chemistry and were hybridized to form duplex DNA. Compound **6b** is able to form base pairs with 2′-deoxy-5-methylisocytidine (m^5isoC_d) in oligonucleotide duplexes with antiparallel chain orientation and with dC in parallel duplex DNA. Thus, the C^8-nucleoside **6b** shows a similar base recognition as 2′-deoxyisoguanosine but not as 2′-deoxyguanosine. This indicates that the nucleic acid recognition not only depends on the donor−acceptor pattern of the nucleobase but is influenced by the glycosylation site. Base pairs of compound **6b** formed with canonical and modified nucleosides are proposed.

Introduction

Chargaff's observation that the sum of purines in DNA equals that of pyrimidines[1] together with the X-ray data of Astbury,[2] Franklin,[3] and Wilkins[4] resulted in the model of the right-handed DNA double helix that was proposed by Watson and Crick.[5] The principle of DNA base pair complementarity—guanine pairs with cytosine and adenine with thymine—is of utmost importance to the formation of a stable DNA helix. As the bidentate dA−dT base pair is isomorphous to the tridentate dG−dC pair, it is obvious that the shape of the base pairs and thus of the nucleobases is of central importance for the DNA structure. Consequently, modified purine and pyrimidine bases showing the same or a similar shape as the canonical DNA constituents and displaying an identical donor acceptor pattern of the Watson−Crick recognition site were incorporated in DNA and have been found to fit well in nucleic acids.[6] Some of them are well accepted by DNA polymerases[7] in the form of their triphosphates and lead to duplex structures even more stable than their natural counterparts.[8]

Among the number of base-modified nucleosides the pyrrolo[3,4-*d*]pyrimidine nucleosides are ideal mimics of the purine compounds. The nucleobases have an almost identical shape as the purines and the Watson−Crick recognition site is not altered. Thus, they are well accommodated in a DNA duplex and are effective substrates for various DNA polymerases in the form of their triphosphates. As a result, pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) nucleosides, e.g., compounds **1a**,**b** (Scheme 1) have found a widespread application in nucleic acid chemistry and molecular biology.[9]

Apart from the 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) N^9-nucleosides the 9-deazapurine (pyrrolo[3,2-*d*]-pyrimidine) *C*-nucleosides such as compounds **2a**,**b** have also been found attention, and compound **2b** was incorporated in oligonucleotides (purine numbering is used throughout the Results and Discussion).[10] It has also been shown that pyrrolo[3,2-*d*]pyrimidine nucleosides (**3b**), glycosylated at the unusual 7-position can form stable duplex structures.[11] Other N^7-linked nucleosides such as ^7A_d and ^7G_d were already incorporated in oligonucleotides, and their base pairing properties have been investigated.[12]

Only a few nucleosides and oligonucleotides exist having the sugar moiety attached to the 8-position. The pyrazolo[3,4-*d*]pyrimidine nucleoside **4b**[13] and the pyrazolo[4,5-*d*]pyrimidine N^8-nucleoside (P2, **5**)[14] are among

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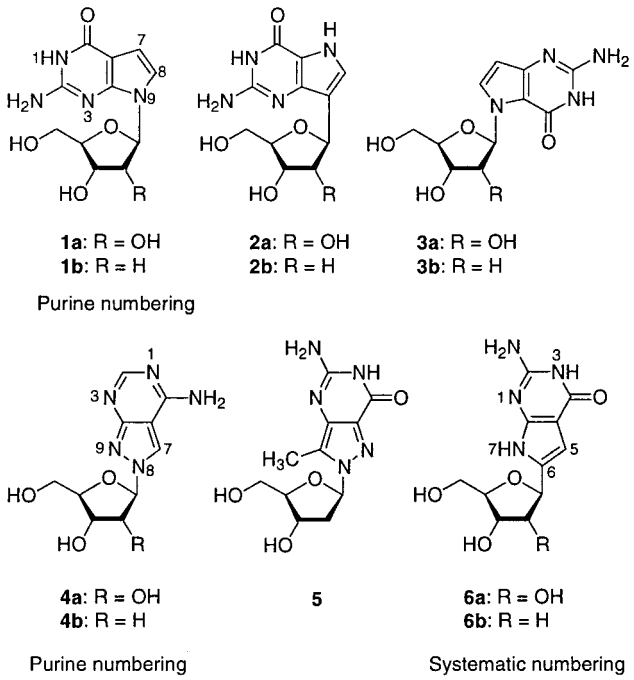
Scheme 1



those. This manuscript reports on the synthesis of the novel pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribofuranoside **6b**, which represents a glycosylic bond stable C-nucleoside having the sugar moiety attached to the 8-position. Compound **6b** was prepared from the corresponding ribonucleoside **6a**[10c] and was converted to its phosphoramidite. The latter was employed in the solid-phase synthesis, and the base pairing properties of oligonucleotides in duplexes with antiparallel and parallel chain orientation were studied.

Results and Discussion

1. Monomers. The glycosylation of pyrrolo[2,3-*d*]-pyrimidines at nitrogen-9 requires the generation of a pyrrolyl anion.[15] This reaction was found to be stereoselective and resulted in the exclusive formation of the *β*-D-ribonucleoside[16] or the 2′-deoxy-*β*-D-ribonucleoside.[15] Nevertheless, glycosylation reactions performed with peracylated ribosugar halides resulted in difficulties as stable orthoamides were formed in many cases.[17] When the glycosylation of pyrrolo[2,3-*d*]pyrimidines is performed in an aprotic solvent using the Vorbrüggen conditions,[18] the sugar cation attacks carbon-7 or -8 of the pyrrolo[2,3-*d*]-pyrimidine moiety. Glycosylation of the unprotected 7-deazaguanine base with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose in nitromethane in the presence of tin(IV) chloride yields the benzoyl protected *C*[8]-ribonucleoside in 56% yield.[10c] This compound was deprotected with saturated methanolic ammonia to give 2-amino-6-(*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**6a**).[10c]

The synthesis of the 2′-deoxyribonucleoside **6b** from the *β*-D-ribofuranoside **6a** made use of the Barton deoxygenation. Prior to this reaction the amino group was protected with an isobutyryl residue employing the protocol of transient protection (Scheme 2).[19] Compound **7** was isolated in 72% yield. As this group had to be removed later from the oligonucleotide, the half-life value of the deprotection was determined in 25% aqueous ammonia (40 °C). From the change of the UV spectra the half-life value was found to be 110 min. According to this, the isobutyryl protecting group is compatible to the deprotection protocol of the canonical bases used in oligonucleotide synthesis (60 °C, 16 h). Prior to the derivatization of the 2′-hydroxyl group, compound **7** was treated with Markiewicz's reagent[20] to give the silyl derivative **8** (57% yield). Treatment of compound **8** with phenoxythiocarbonyl chloride in acetonitrile furnished the 2′-*O*-phenoxythiocarbonyl derivative **9a** (48%) together with the bisphenoxythiocarbonyl compound **9b** (10%) as byproduct. Reductive cleavage of **9a** with tri-*n*-butyltin(IV) hydride in toluene in the presence of α,α′-azoisobutyronitrile (AIBN) yielded the derivative **10** (90%). The desilylation with 0.1 M tetra-*n*-butylammonium fluoride in anhydrous THF gave compound **11** (82%). Next, the 4,4′-dimethoxytriphenylmethyl group was introduced at the 5′-hydroxyl to give compound **12** (72%). The phosphoramidite **13** was prepared under standard conditions from compound **12** (78%).

All compounds were characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectra (see Table 1 and Experimental Section) as well as by elemental analyses. As the ribonucleoside **6a** was not unambiguously assigned with regard to the position of glycosylation as well as to the anomeric configuration it was now done on compound **7**. Since the unprotected nucleoside was difficult to purify as a result of its unfavorable chromatographic properties, compound **7** was used instead. For this assignment the ^{13}C NMR chemical shifts as well as NOE data were used. Upon irradiation of H(1′) a strong NOE of H(7) (4.2%) was observed, while only weak NOEs appeared on H(2′) (1.8%), H(4′) (1.4%), and NH(9) (1.8%) (Scheme 3a). Irradiation on H(7) results in NOEs on H(1′) (3.2%), H(2′) (2.4%); no NOE is observed on NH(9) (Scheme 3b). The NOE on H(2′) is a proof of the *β*-D-configuration, and the NOE on NH(9) ensures that the sugar is connected via the 8-position.

The ^{13}C NMR chemical shifts of the C^{8}-linked nucleosides **6a**−**12** described in this manuscript are summarized in Table 1 and were assigned by gated-decoupled ^{13}C NMR spectra, heteronuclear ^{1}H−^{13}C NMR correlation spectra, and COLOC ^{1}H−^{13}C NMR spectra. For comparison, the spectra of the nucleobases **14a**,**b** and the regularly N^{9}-linked nucleosides **1b** and **15** as well as those of the isobutyryl derivatives **16a**,**b** are given (Scheme 4). From the data shown in Table 1 it is obvious that the C-methylation or C-glycosylation of the heterocyclic bases results in a ∼10 ppm downfield shift of the carbon-8 signals compared to the nonmethylated base **14a** or the regularly linked 7-deazapurine nucleosides

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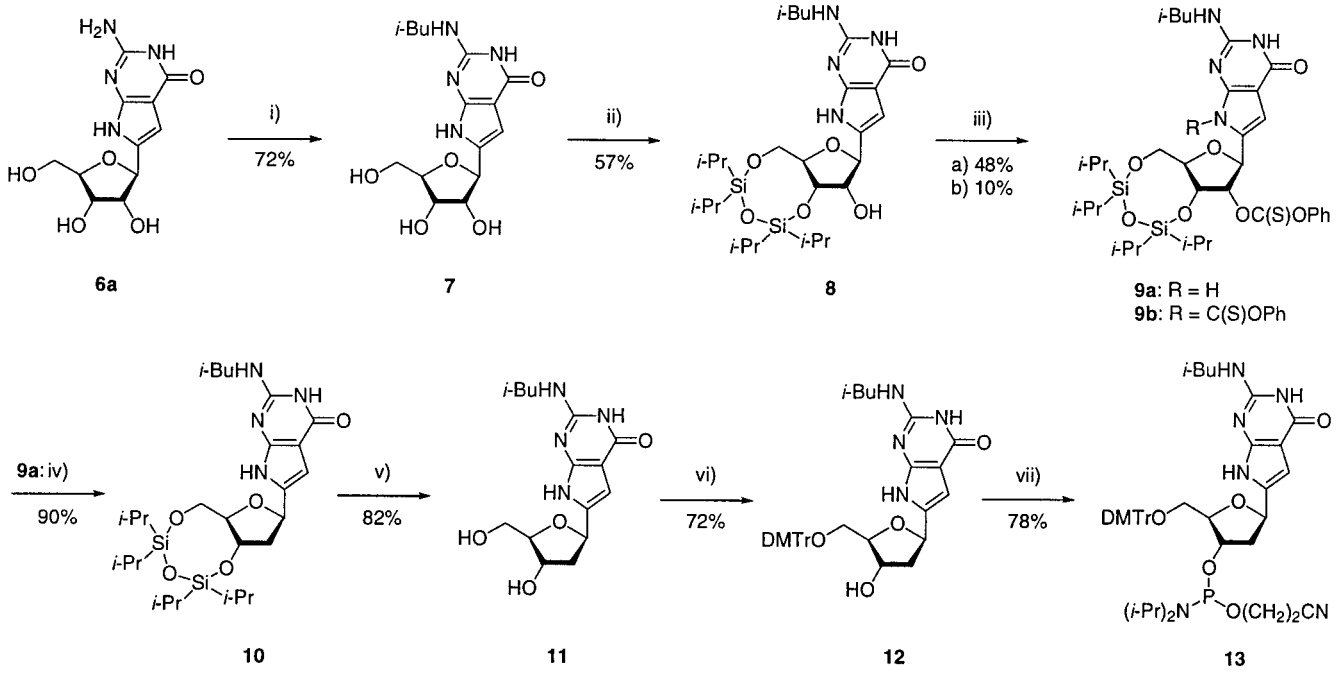
Scheme 2[a]



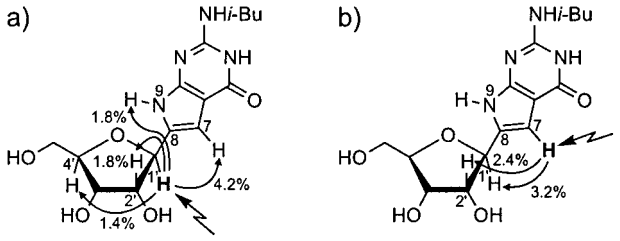
[a] (i) $(i\text{-Bu})_2O$, pyridine, rt, 3 h. (ii) $(i\text{-Pr})_2ClSiOSi(i\text{-Pr})_2Cl$, pyridine, rt, 12 h. (iii) PhOC(S)Cl, acetonitrile, rt, 12 h. (iv) $(n\text{-Bu})_3SnH$, AIBN, toluene, 60 °C, 4 h. (v) 0.1 N TBAF/THF, rt, 3 h. (vi) DMTr-Cl, pyridine, rt, 3 h. (vii) $(i\text{-Pr})_2NP(Cl)O(CH_2)_2CN$, CH_2Cl_2, rt, 20 min.

Table 1. ^{13}C NMR Chemical Shifts of the C[8]-Glycosylated 7-Deazaguanine Derivatives[a]

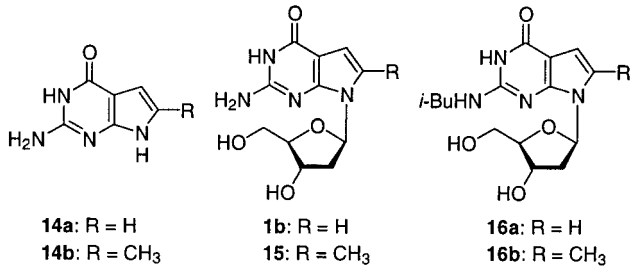
	C(2)[b] C(2)[c]	C(6) C(4)	C(5) C(4a)	C(7) C(5)	C(8) C(6)	C(4) C(7a)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	CO	CH	Me
14a[22]	152.2	158.8	99.9	101.6	116.6	151.1								
14b[22]	151.8	158.4	99.9	98.7	126.2	151.1								
1a[23]	152.6	158.7	100.2	102.3	117.3	151.2	86.1	73.7	70.6	84.6	61.8			
1b[6a]	152.5	158.5	100.1	102.1	116.7	150.5	82.2	39.5	70.8	86.9	61.9			
15[24]	151.7	158.1	99.3	100.9	128.1	150.9	82.7	38.2	70.7	86.7	61.9			
6a	152.3	158.7	99.6	100.3	129.4	151.6	77.9	74.8	71.1	84.8	62.0			
16a[6a]	146.5	156.4	103.4	102.3	119.2	147.3	82.4	39.5	70.7	87.0	61.8	179.6	34.5	18.6
16b[24]	145.9	156.2	103.4	102.0	131.5	148.2	82.6	38.1	70.5	86.7	61.6	180.0		
7	146.3	156.7	103.6	100.5	132.6	148.1	77.5	75.0	70.9	84.8	61.7	179.8	34.6	18.9
8	146.3	156.6	103.6	99.7	132.3	148.2	71.7	74.4	79.3	81.3	61.8	179.7	34.6	18.9
9a	146.8	156.6	103.7	101.1	129.9	148.6	84.6	76.0	70.9	82.0	61.6	179.8	34.6	18.9
9b	148.2	156.1	105.6	104.5	132.9	150.5	85.5	76.5	69.7	80.3	60.2	180.4	34.7	18.8; 18.9
10	146.4	156.6	103.4	100.0	132.7	148.4	74.0	*d*	72.4	85.9	63.7	179.8	34.6	18.9
11	146.3	156.7	103.4	99.7	133.7	148.2	72.9	*d*	72.0	87.7	62.2	179.8	34.6	18.9
12	146.3	156.9	103.5	99.1	133.8	148.2	72.9	*d*	72.3	85.8	64.6	179.8	34.6	18.9

[a] Spectra were measured in d_6-DMSO. [b] First heading row = purine numbering. [c] Second heading row = systematic numbering. [d] Signal is superimposed by DMSO.

Scheme 3



Scheme 4



1a,**b**. The COLOC $^1H-^{13}C$ NMR spectra (delay time (*D*) = 0.1 s) of compound **6b** show cross-peaks to H(7) (6.17 ppm) at 77.9 ppm (C(1′)), 99.6 ppm (C(5)), and 151.6 ppm (C(4)), whereas the spectra at a delay time of 0.25 s show only one cross-peak at 158.7 ppm (C(6)). The chemical shift of the anomeric carbon is influenced by the phenoxythiocarbonyl residue and the silyl clamp (**8**, **9a**,**b**). The chemical shift of the sugar moiety of C^8-ribonucleoside **6a** corresponds to that of the N^9-ribonucleoside **1a** except that the anomeric carbon is shifted. Furthermore, the data are in good agreement with those observed earlier on N^1-methylformycin derivatives.[21]

2. Oligonucleotides. 2.1. Syntheses and Purification. To investigate the base pairing properties of the

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Table 2. T_m Values and Thermodynamic Data of Antiparallel-Stranded Oligonucleotide Duplexes Containing C^8-Linked 7-Deaza-2′-deoxyguanosines (6b, dG*)[a]

aps-duplex		T_m [°C][a]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{310}$ [kcal/mol]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A)	**17** **18**	50 (47)	−84 (−87)	−234 (−245)	−11.5 (−11.0)
5′-d(T-A-**1b**-**1b**-T-C-A-A-T-A-C-T) 3′-d(A-T-**C**-**C**-A-G-T-T-A-T-G-A)	**19**[32] **18**	(46)	(−98)	(−283)	(−10.5)
5′-d(T-A-**6b**-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-**C**-**C**-A-G-T-T-A-T-G-A)	**20** **18**	36 (32)	−52 (−35)	−143 (−93)	−7.9 (−6.7)
5′-d(T-A-**6b**-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-**iC**-**iC**-A-G-T-T-A-T-G-A)	**20** **21**	46 (42)	−82 (−73)	−230 (−205)	−10.2 (−8.9)
5′-d(T-A-G-G-T-**C**-A-A-T-A-**C**-T) 3′-d(A-T-C-C-A-**1b**-T-T-A-T-**1b**-A)	**17**[32] **23**	(46)	(−84)	(−239)	(−10.1)
5′-d(T-A-G-G-T-**C**-A-A-T-A-**C**-T) 3′-d(A-T-C-C-A-**6b**-T-T-A-T-**6b**-A)	**18** **24**	33 (32)	−39 (−46)	−102 (−127)	−7.3 (−7.1)
5′-d(T-A-G-G-T-**iC**-A-A-T-A-**iC**-T) 3′-d(A-T-C-C-A-**6b**-T-T-A-T-**6b**-A)	**25** **24**	49 (45)	−76 (−65)	−210 (−180)	−10.7 (−9.4)
5′-d(T-A-**6b**-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-**Z**-**Z**-A-G-T-T-A-T-G-A)	**20** **28**[33]	42 (38)	−70 (−76)	−200 (−219)	−8.0 (−8.0)
5′-d(T-A-G-G-T-**Z**-A-A-T-A-**Z**-T) 3′-d(A-T-C-C-A-**6b**-T-T-A-T-**6b**-A)	**29**[33] **24**	36 (32)	−57 (−57)	−159 (−168)	−7.5 (−6.7)
5′-d(T-**iC**-A-T-A-A-**iC**-T-**6b**-**6b**-A-T) 3′-d(A-**6b**-T-A-T-T-**6b**-A-**iC**-**iC**-T-A)	**30** **31**	35 (31)	−45 (−45)	−121 (−124)	−7.6 (−6.9)

[a] Measured at 260 nm in 1.0 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. Data in parentheses are measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. iC_d: m^5isoC_d (**22**). dZ: **27**.

C[8]-nucleoside **6b** a series of oligonucleotides was synthesized using the phosphoramidite **13** as building block (Tables 2−4). The solid-phase synthesis of the oligonucleotides was performed on an automated DNA synthesizer using the standard protocol of phosphoramidite chemistry.[25] As the C^8-linked phosphoramidite **13** is poorly soluble in acetonitrile, it was dissolved in a 1:1 mixture of acetonitrile and dichloromethane. The coupling efficiency of the modified phosphoramidite was always higher than 95%. The oligonucleotides were deprotected in concentrated aqueous ammonia (60 °C, 16 h) and were purified by reversed-phase HPLC (RP-18) or OPC cartridges.[26] The homogeneity of the oligonucleotides was proven by reversed-phase HPLC. The nucleoside composition of the oligonucleotides was determined by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase and identified on reversed-phase HPLC.[27] One representative example is shown in Figure 1. Compound **6a** and **6b** show UV spectra with a maximum at 260 nm and a shoulder at 285 nm, being almost identical to the spectra of 7-deaza-8-methylguanine or 7-deaza-2′-deoxyguanosine. A number of oligonucleotides containing the nucleoside **6b** were also characterized by MALDI-TOF mass spectra (see Experimental Section, Table 5).

2.2. Stability of Duplexes with Antiparallel Chain Orientation and Proposed Base Pair Motifs. The base pair recognition and duplex stability of oligonucleotides containing compound **6b** was studied on the oligonucleotide duplex 5′-d(T-A-G-G-T-C-A-A-T-A-C-T)· 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**17•18**) showing antiparallel chain orientation. The thermodynamic data were determined by curve shape analysis of the UV-melting profiles using the Meltwin 3.0 program package.[28] The T_m values and thermodynamic data are summarized in Tables 2−4.

To determine the influence of the modified nucleoside **6b** on the base pair stability the T_m values of the duplex **19•18** containing two regularly linked 7-deaza-2′-deoxyguanosine (**1b**) residues instead of 2′-deoxyguanosines (**17•18**) were compared. From the thermodynamic data of Table 2 it is apparent that the modified pyrrolo[2,3-*d*]pyrimidine with the regularly linked sugar destabilizes the dG−dC base pair only slightly (0.5 °C per modified residue). However, when the unusually linked 7-deazaguanine *C*[8]-nucleoside **6b** was replacing c^7G_d a significant destabilization of 7 °C per modified residue was found (see duplex **20•18**). As studies on N^7-glycosylated purines[12b] have shown, that a change of the glycosylation position can influence the base pair stability in a similar way as the transposition of the substituents of the base, the two dC residues of the oligonucleotide **18** were replaced by two 2′-deoxy-5-methylisocytidine residues (**22**), which showed transposition of the amino and the oxo group, and the oligonucleotide **21** was hybridized with **20**. The 5-methyl derivative of 2′-deoxyisocytidine (**22**) was chosen instead of the 2′-deoxyisocytidine because of the instability of the nonmethylated nucleoside against basic conditions.[29] In this case a rather stable duplex was formed that showed a T_m-decrease of only 2 °C per modified nucleoside (see duplex **20•21** vs **17•18**). The situation is similar when the two **6b** residues are not arranged in a

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(29) (a) Seela, F.; He, Y. *Helv. Chim. Acta* **2000**, *83*, 2527−2540. (b) Roberts, C.; Bandaru, R.; Switzer, C. *J. Am. Chem. Soc.* **1997**, *119*, 4640−4649.

Table 3. T_m Values and Thermodynamic Data of Oligonucleotide Duplexes Containing One C[8]-Linked 7-Deaza-2′-deoxyguanosine (6b, dG*) Hybridized against m[5]isoC$_d$ (22), dT, dG, dC, dA, and dA* (4b)[a]

aps-duplex		T_m [°C]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{310}$ [kcal/mol]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A)	**17** **18**	50 (47)	−84 (−87)	−234 (−245)	−11.5 (−11.0)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**iC**-A-G-T-T-A-T-G-A)	**32** **33**	48 (44)	−90 (−76)	−254 (−215)	−10.9 (−9.4)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**T**-A-G-T-T-A-T-G-A)	**32** **34**	45 (40)	−67 (−72)	−186 (−205)	−9.4 (−8.5)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**G**-A-G-T-T-A-T-G-A)	**32** **35**	43 (40)	−76 (−73)	−214 (−209)	−9.3 (−8.4)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**C**-A-G-T-T-A-T-G-A)	**32** **18**	40 (38)	−39 (−52)	−100 (−140)	−8.2 (−8.3)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**A**-A-G-T-T-A-T-G-A)	**32** **36**	32 (27)	−59 (−62)	−169 (−182)	−6.9 (−5.8)
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**A***-A-G-T-T-A-T-G-A)	**32** **37**	41 (37)	−75 (−73)	−214 (−210)	−8.6 (−7.7)
5′-d(T-A-G-**A**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**T**-A-G-T-T-A-T-G-A)	**38** **34**	48 (43)	−86 (−89)	−241 (−255)	−10.8 (−9.5)
5′-d(T-A-G-**A**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**C**-A-G-T-T-A-T-G-A)	**38** **18**	33	−67	−192	−6.8
5′-d(T-A-G-**iG**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**iC**-A-G-T-T-A-T-G-A)	**39**[34] **33**	54	−102	−286	−13.5
5′-d(T-A-G-**iG**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**T**-A-G-T-T-A-T-G-A)	**39**[34] **34**	45	−79	−223	−10.1
5′-d(T-A-G-**iG**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**G**-A-G-T-T-A-T-G-A)	**39**[34] **35**	44	−71	−199	−9.6
5′-d(T-A-G-**iG**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**C**-A-G-T-T-A-T-G-A)	**39**[34] **18**	44	−73	−205	−9.6
5′-d(T-A-G-**iG**-T-C-A-A-T-A-C-T) 3′-d(A-T-C-**A**-A-G-T-T-A-T-G-A)	**39**[34] **36**	35	−58	−161	−7.6

[a] Measured at 260 nm in 1.0 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. Data in parentheses are measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. iG$_d$: isoG$_d$ (**26**).

Table 4. T_m Values and Thermodynamic Data of Parallel-Stranded Oligonucleotide Duplexes Containing C[8]-Linked 7-Deaza-2′-deoxyguanosine (6b) in Comparison to Parallel-Stranded Duplexes with m[5]isoC$_d$ (22) and isoG$_d$ (26)[a]

ps-duplex		T_m [°C]	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/mol·K]	$\Delta G°_{310}$ [kcal/mol]
5′-d(T-A-G-G-T-**C**-A-A-T-A-**C**-T) 5′-d(A-T-iC-iC-A-**iG**-T-T-A-T-**iG**-A)	**17**[34] **40**	39	−74	−211	−8.8
5′-d(T-A-G-G-T-**C**-A-A-T-A-**C**-T) 5′-d(A-T-iC-iC-A-**6b**-T-T-A-T-**6b**-A)	**17** **31**	31 (27)	−45 (−43)	−125 (−120)	−6.6 (−6.2)
5′-d(T-iC-A-T-A-A-iC-T-**iG**-**iG**-A-T) 5′-d(A-G-T-A-T-T-G-A-**C**-**C**-T-A)	**41**[34] **18**	45 (42)	−79 (−85)	−222 (−245)	−10.0 (−9.4)
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T) 5′-d(A-G-T-A-T-T-G-A-**C**-**C**-T-A)	**30** **18**	35 (31)	−45 (−49)	−122 (−135)	−7.6 (−7.0)
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T) 5′-d(A-G-T-A-T-T-G-A-**G**-**C**-T-A)	**30** **35**	26	−50	−142	−5.7
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T) 5′-d(A-G-T-A-T-T-G-A-**T**-**C**-T-A)	**30** **34**	24	−53	−151	−5.9
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T) 5′-d(A-G-T-A-T-T-G-A-**A**-**C**-T-A)	**30** **36**	26	−41	−113	−6.1
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T) 5′-d(A-G-T-A-T-T-G-A-**iC**-**C**-T-A)	**30** **33**	25	−46	−127	−6.3
5′-d(T-A-**6b**-**6b**-T-**C**-A-A-T-A-**C**-T) 5′-d(A-T-**C**-**C**-A-**6b**-T-T-A-T-**6b**-A)	**20** **42**	20	−55	−163	−4.6
5′-d(T-**C**-A-T-A-A-**C**-T-**6b**-**6b**-A-T) 5′-d(A-**6b**-T-A-T-T-**6b**-A-**C**-**C**-T-A)	**43** **24**	25	−50	−143	−5.8

[a] Measured at 260 nm in 1.0 M NaCl, 100 mM $MgCl_2$, and 60 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. Data in parentheses are measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration.

consecutive way but are dispersed along in the oligonucleotide chain (**24**). In comparison, the T_m-decrease induced by the incorporation of two **6b** residues opposite to dC (**18•24**) was 8.5 °C and only 0.5 °C in the case of

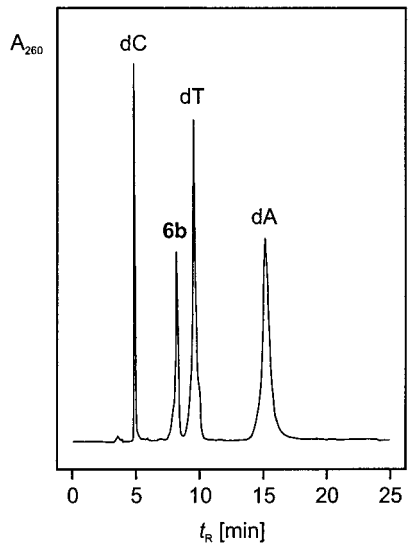
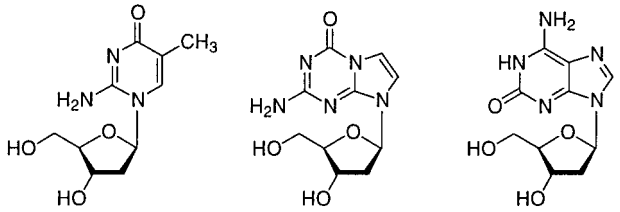


Figure 1. Reversed-phase HPLC (RP-18) profile of the enzyme digestion of the dodecamer 5′-d(T-A-**6b**-**6b**-T-C-A-A-T-A-C-T) (**20**). For the conditions, see the Experimental Section.

Table 5. Molecular Weights Determined by MALDI-TOF Mass Spectroscopy of Some Selected Modified Oligonucleotides Containing C^8-Linked 7-Deaza-2′-deoxyguanosine (dG*, 6b)

oligonucleotide		MH^+ (calc)	MH^+ (found)
5′-d(T-A-**6b**-**6b**-T-C-A-A-T-A-C-T)	**20**	3640.7	3640.3
5′-d(A-**6b**-T-A-T-T-**6b**-A-C-C-T-A)	**24**	3640.7	3639.7
5′-d(T-A-G-**6b**-T-C-A-A-T-A-C-T)	**32**	3641.7	3641.0
5′-d(A-T-iC-iC-A-**6b**-T-T-A-T-**6b**-A)	**31**	3668.7	3667.9
5′-d(T-iC-A-T-A-A-iC-T-**6b**-**6b**-A-T)	**30**	3668.7	3667.1

Scheme 5



m^5isoC_d (**25•24**). Thus, the unusually linked 7-deazaguanine nucleoside **6b** is able to form a strong base pair with m^5isoC_d (**22**) and a weak pair with dC. According to this, compound **6b** develops similar base pairing properties as 2′-deoxyisoguanosine (**26**)[30] but not as 2′-deoxyguanosine in a duplex with antiparallel chain orientation.Earlier investigations confirmed that 5-aza-7-deaza-2′-deoxyguanosine (**27**, dZ), which displays the same Watson−Crick proton donor−acceptor pattern as 2′-deoxy-5-methylisocytidine (**22**), can efficiently replace this nucleoside in DNA duplex structures.[31] Consequently, the 2′-deoxy-5-methylisocytidine residues located opposite to compound **6b** were replaced by 5-aza-7-deaza-2′-deoxyguanosine (**27**). Also in this case stable duplexes were formed (Table 2). As the **27**−**6b** tridentate base pair is a purine−"purine" pair and Hoogsteen pairing is not possible, the double helix should be more distorted than in the case of a pyrimidine−purine pair. In comparison to duplexes with only one compound **6b** incorporation, those with the residue **6b** in both (**30•31**) are further destabilized. Although the sequence of the duplexes **30•31** is different from the others of Table 2, it is apparent that even four incorporations of **6b** residues are tolerated in the 12-mer duplex.

Next, the base recognition selectivity of compound **6b** was tested by placing other bases (dT, dG, dA, and the N^8-glycosylated compound **4b**[13]) opposite to **6b**. According to Table 3, the duplex with one **6b** residue opposite to 2′-deoxy-5-methylisocytidine (**32•33**) results in the most stable base pair (48 vs 50 °C of **17•18**). Moderate T_m decreases were observed when 2′-deoxythymidine (**32•34**, 5 °C), 2′-deoxyguanosine (**32•35**, 7 °C), 2′-deoxycytidine (**32•18**, 10 °C), and the universal nucleoside **4b** (**32•37**, 9 °C) are located opposite to compound **6b**. When compound **6b** is located opposite to 2′-deoxyadenosine (**32•36**), a similar strong T_m decrease (18 °C) is observed as in the case of duplex **38•18** wherein dA is opposite to dC (17 °C). The same series of duplexes containing $isoG_d$ (**26**) instead of compound **6b** are shown for comparison (Table 3).[34] Although the antiparallel-stranded $isoG_d$−m^5isoC_d pair is extraordinarily stable, the base pair discrimination follows the same trend as found for the C^8-linked nucleoside **6b**. It is interesting to note that the duplex **32•33** containing a **6b**-m^5isoC_d base pair is as stable as the duplex **38•34** with a dA-dT base pair at that position.

Then, base pairs were constructed from duplexes exhibiting rather high T_m values in comparison to the duplex **17•18** (Tables 2 and 3; Scheme 6). The base pairs were formed under the following prerequisites: (i) Normal Watson−Crick modes as well as unusual base pairing are allowed. (ii) The distances between the anomeric carbons of nucleoside **6b** and its natural counterparts should approximate those of the Watson−Crick base pairs (11 Å) and the length of the H-bonds was arbitrarily set to 2 Å.[35] In all cases ball-and-stick models (Maruzen, Japan) of the various base pairs were built according to the structures shown in Scheme 6. The corresponding base-paired nucleotide units were compared with those of B-DNA and fitted into the helical structure.

The complementary arrangement of the canonical purine and pyrimidine bases results in the well characterized Watson−Crick bidentate base pair dA−dT (**II**, Scheme 6) and the tridentate dG−dC base pair (**IIIa**). This makes the 1′-carbon of the sugar moiety of the dG−dC base pair (10.8 Å) and the dA−dT base pair (11.1 Å) almost equidistant and minimizes the strain on the DNA backbone.[35] This arrangement also maximizes the number of hydrogen bonds. This principle is also used for the modified base pairs, e.g., the base pair of c^7G_d−dC (**IIIb**) or $isoG_d$−m^5isoC_d (**I**). From the thermodynamic stabilities of the duplexes shown in Table 2 it is obvious that the C^8-glycosylated pyrrolo[2,3-*d*]pyrimidine nucleoside **6b** forms a strong base pair with 2′-deoxy-5-methylisocytidine (**22**). The motif **IV** represents the only possible

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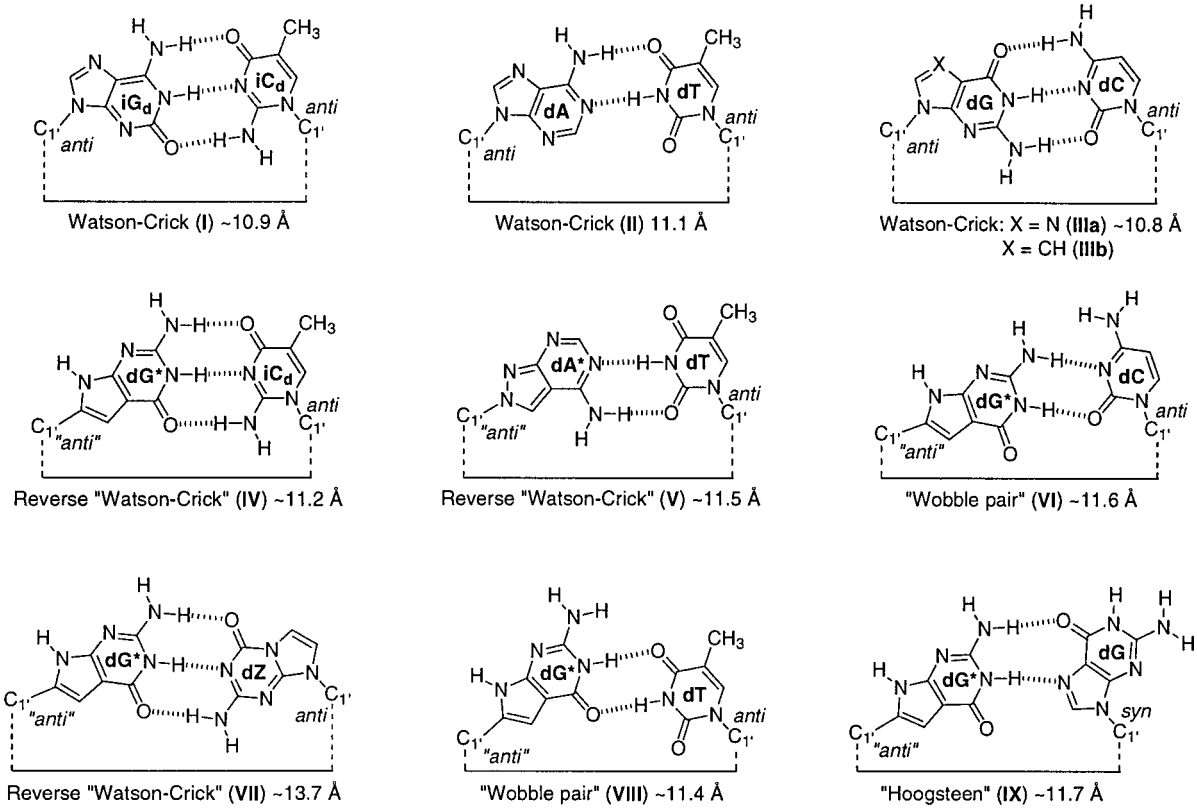
(31) Seela, F.; Melenewski, A. *Eur. J. Org. Chem.* **1999**, 485−496.
(32) Rosemeyer, H. **1999**. Unpublished work.
(33) Seelg, F.; Amberg, S.; Melenewski, A.; Rosemeyer, H. *Helv. Chim. Acta* **2001**, in press.
(34) Seela, F.; Wei, C. *Helv. Chim. Acta* **1999**, *82*, 726−745.
(35) Kennard, O. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: Heidelberg, Germany, 1987; pp 25−52.

Scheme 6

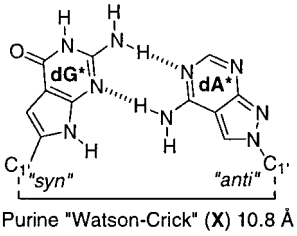


tridentate **6b**−m^{5}isoC$_d$ base pair, which is isomorphous to the **4b**−dT base pair **V**, found earlier.[13] The sugar residues of the base pair **IV** are in the "anti" conformation. The incorporation of such a base pair (**IV**) affects the major groove of the duplex **20•21** or **25•24**. The methyl group of the 5-methylisocytosine moiety can make it more lipophilic, while the pyrrolyl NH located in the major groove increases the hydrophilic character. Moreover, the pyrrolyl hydrogen might act as proton donor within Hoogsteen-type interactions. A bidentate base pair can only be formed by a tautomeric change of the heterocycles.

Contrary to m^{5}isoC$_d$ (**22**), the dC residue forms a rather weak base pair. The bidentate wobble pair **VI** represents a likely structure. Also in this case the distance between the anomeric centers is in the range of the canonical base pairs. When 5-methylisocytosine is replaced by its purine mimic 5-aza-7-deazaguanine (see base pair **VII**) the distance between the anomeric centers is widened by 2.5 Å, which might explain the lower duplex stability. We have also constructed base pairs of compound **6b** with the canonical DNA constituents dT and dG by using the information of the duplex stabilities shown in Table 3. Base pairs such as motifs **VIII** and **IX** are proposed. In addition, the dA-residue which does not form a base pair with compound **6b** was replaced by the universal 8-substituted pyrazolo[3,4-*d*]pyrimidine analogue **4b**.[13] Motif **X** represents a likely **6b**−**4b** base pair (Scheme 7).

2.3. Base Pairing Properties of the Nucleoside 6b in Duplexes with Parallel Chain Orientation. Finally, the stability of duplexes with parallel strand orientation was investigated. For this purpose, the oligonucleotides shown in Table 4 were synthesized and hybridization experiments were performed. To generate those duplexes it is necessary to exchange the dG−dC pairs by isoG$_d$−dC and/or dG−m^{5}isoC$_d$ base pairs. The

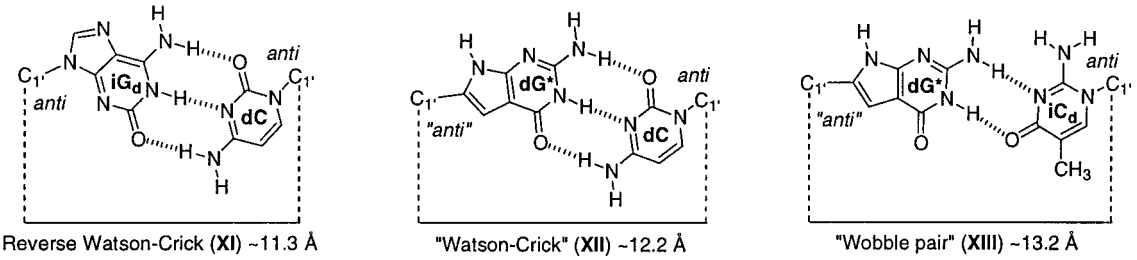
Scheme 7



duplex **17•40** was used as a reference to study the influence of compound **6b** on the base pair stability.[34] When two **6b** residues were incorporated instead of isoG$_d$ (**17•40**) in a dispersed arrangement the T_m value of the duplex **17•31** was decreased by 4 °C per modification. A 5 °C stability decrease per modified residue was observed when a consecutive incorporation was occurring (see **30•18** vs **41•18**). Although the T_m values of duplexes containing compound **6b** were lower than those with 2′-deoxyisoguanosine (**26**), it shows specific base pairing. This is underlined by the fact that the introduction of one of the canonical bases as well as 5-methylisocytosine located opposite to **6b** resulted in significantly lower T_m values (9−11 °C). The incorporation of four unusually linked nucleoside **6b** residues in both strands of the duplex structure opposite to dC still resulted in the formation of duplexes (**20•42**, 20 °C and **43•24**, 25 °C), which were rather labile but showed cooperative melting as found in the other cases.

From the data shown in Table 4 it is apparent that compound **6b** forms a much more stable base pair with dC than with dG, dA, dT, and m^{5}isoC$_d$. The tridentate base pair motif **XII** is suggested for the **6b**−dC interaction. For the hybrid **30•33** with residue **6b** opposite to m^{5}isoC$_d$ the bidentate motif **XIII** is considered, which is in line with the rather low thermodynamic stability of

Scheme 8



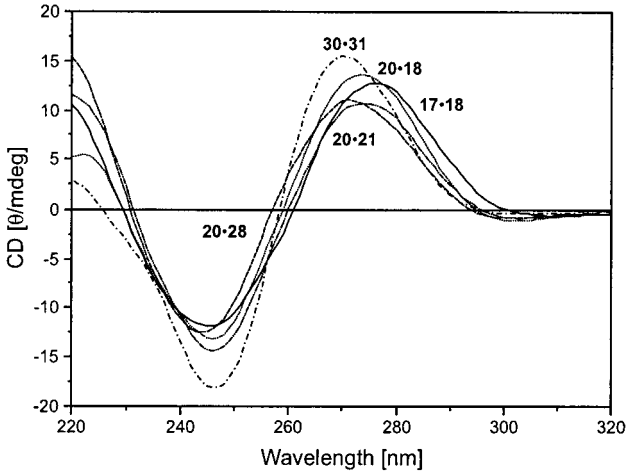


Figure 2. CD spectra of the antiparallel-stranded oligonucleotide duplexes **17•18**, **20•18**, **20•21**, **30•31**, and **20•28**. Measured in 1.0 M NaCl, 100 mM $MgCl_2$, 60 mM Na cacodylate (pH 7.0), with 10 μM oligonucleotide concentration at 10 °C.

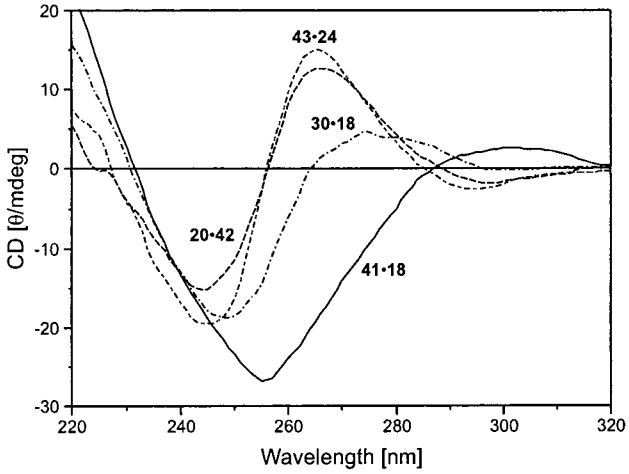


Figure 3. CD spectra of the parallel-stranded duplexes **41•18**, **30•18**, **20•42**, and **43•24**. Measured in 1.0 M NaCl, 100 mM $MgCl_2$, 60 mM Na cacodylate (pH 7.0) with 10 μM oligonucleotide concentration at 10 °C.

this duplex. Also the other duplexes with dG (**30•35**), dT (**30•34**), or dA (**30•36**) opposite to **6b** form base pairs, but as these duplexes are labile, the possible pair motifs will not be given in Scheme 8.

2.4. CD Spectra of the Oligonucleotide Duplexes with Antiparallel and Parallel Chain Orientation Containing Compound 6b. To compare the conformational properties of parallel-stranded DNA containing **6b** with those showing antiparallel chain orientation, the CD spectra of the aps-duplexes **20•18**, **20•21**, **20•28**, and **30•31** were measured at first. The spectrum of **17•18** was used as a reference (Figure 2). All spectra of the antiparallel-stranded duplexes containing compound **6b** show the typical shape of a B-DNA. Nevertheless, an increasing content of compound **6b**−dC base pairs results in a hypsochromic shift of the positive lobe. The CD-spectra of the parallel duplexes containing **6b**−dC pairs show similar Cotton effects (see duplex **20•42** and **43•24** in Figure 3). However, when the amount of $isoG_d$−dC pairs increases, a significant spectral change is observed as it was reported earlier (see duplex **41•18** in Figure 3).[36] This might be interpreted by conformational changes occurring on the duplexes. However, as the CD spectra of the monomeric nucleosides are already different from thoseof the canonical DNA residues, it is difficult to interpret such data.

Conclusion

The incorporation of the unusually linked nucleoside **6b** in oligonucleotide duplexes shows that a change of the glycosylation site from nitrogen-9 to carbon-8 alters the recognition of the nucleobase to its complementary counterpart. Different from the regularly linked 7-deaza-2′-deoxyguanosine (**1b**), which preferentially base pairs with dC in DNA with antiparallel chain orientation (natural DNA), the unusually linked nucleoside **6b** forms a rather strong base pair with 2′-deoxy-5-methylisocytidine (**22**) but not with 2′-deoxycytidine. The nucleoside **6b** shows the base pairing characteristics of 2′-deoxyisoguanosine (**26**). In duplexes with parallel chain orientation compound **6b** pairs much better with dC than with m^5isoC_d. Thus, the recognition of a guanine—or more particularly of a 7-deazaguanine base—is changed into that of isoguanine when the glycosylation site shifts from nitrogen-9 to carbon-8.

Experimental Section

General.[13] All chemicals were purchased from Aldrich, Sigma, or Fluka (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). NMR spectra were measured on Avance DPX 250 or AMX 500 spectrometers (Bruker, Karlsruhe, Germany) operating at proton resonance frequencies of 250.13 and 500.14 MHz (125.13 MHz for ^{13}C), respectively. Chemical shifts are in ppm relative to TMS as internal standard. The *J*-values are given in Hz. UV spectra were recorded on a U 3200 spectrometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on aluminum sheets, silica gel 60 F_{254}, 0.2 mm layer (Merck, Germany) and column flash chromatography (FC) on silica gel 60 (Merck, Germany) at 0.4 bar (4×10^4 Pa).

2-Isobutyrylamino-6-(β-D-ribofuranosyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4(3*H*)-one (7). Compound **6a**[10c] (520 mg,

(36) Seela, F.; He, Y.; Wei, C. *Tetrahedron* **1999**, *55*, 9481−9500.

1.84 mmol) was dried by coevaporation with pyridine (2 × 10 mL). The residue was suspended in pyridine (5 mL), and Me_3-SiCl (3.0 mL, 23.6 mmol) was added at room temperature. After 15 min of stirring, the solution was treated with isobutyric anhydride (0.6 mL, 3.82 mmol) and maintained at room temperature for 6 h. The solution was cooled in an ice bath, H_2O (3 mL) and subsequently (5 min later) a 25% aqueous NH_3 solution (3 mL) was added, and stirring was continued for 15 min. The solution was evaporated to dryness, dissolved in H_2O (20 mL), and washed with Et_2O (2 × 10 mL). The aqueous layer was evaporated to dryness again and coevaporated at first with toluene (2 × 10 mL) and then with MeOH (2 × 10 mL). The residue was applied to FC (column 10 × 2 cm, CH_2Cl_2/MeOH 8:2, v/v) to yield the title compound **7** as a pale yellow powder (470 mg, 72%): TLC (CH_2Cl_2/MeOH 8:2, v/v) 0.3; UV (MeOH) 275 (16600), 294 (15000 sh.); ^{1}H NMR (d_6-DMSO) 1.11 (6H, d, J = 6.8, Me_2), 2.76 (1H, h, J = 6.8, CH), 3.51 (1H, m, 5′-H), 3.57 (1H, m, 5″-H), 3.78 (1H, m, 4′-H), 3.97 (2H, m, 2′-H, 3′-H), 4.62 (1H, d, J = 6.3, 1′-H), 4.91 (1H, d, J = 4.6, 3′-OH), 4.92 (1H, t, J = 5.8, 5′-OH), 5.06 (1H, d, J = 5.8, 2′-OH), 6.39 (1H, d, J = 1.4, 5-H), 11.34 (1H, s, NH), 11.60 (1H, s, NH), and 11.78 (1H, s, NH). Anal. Calcd for $C_{15}H_{20}N_4O_6$: C, 51.13; H, 5.72; N, 15.90. Found: C, 51.23; H, 5.82; N, 15.95.

2-Isobutyrylamino-6-[3,5-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-yl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (8). To a solution of compound **7** (500 mg, 1.42 mmol) in anhydrous pyridine (6 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (0.55 mL, 1.75 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature overnight, and extracted twice with a 5% aqueous $NaHCO_3$ solution (25 mL) followed by brine (25 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and coevaporated twice with toluene (20 mL). The residue was purified by FC (silica gel, 4 × 10 cm, CH_2Cl_2/MeOH 95:5, v/v) to yield the title compound **8** as a colorless foam (485 mg, 57%): TLC (CH_2Cl_2/MeOH 95:5) 0.5; UV (MeOH) 275 (17200), 293 (15700 sh.); ^{1}H NMR (d_6-DMSO) 0.89−1.04 (28H, m, $(CHMe_2)_4$), 1.12 (6H, d, J = 6.7, Me_2), 2.77 (1H, h, J = 6.7, CH), 3.87 (1H, m, 5″-H), 3.91 (1H, m, 5′-H), 3.94 (1H, m, 4′-H), 4.15 (1H, d't′, J = 5.0, J = 3.5, 2′-H), 4.24 (1H, dd, J = 6.0, J = 6.7, 3′-H), 4.71 (1H, d, J = 3.0, 1′-H), 5.03 (1H, d, J = 5.3, 2′-OH), 6.35 (1H, s, 5-H), 11.34 (1H, br. s, NH), 11.67 (1H, s, NH), and 11.74 (1H, br. s, NH). Anal. Calcd for $C_{27}H_{46}N_4O_7Si_2$: C, 54.52; H, 7.79; N, 9.42. Found: C, 54.51; H, 7.88; N, 9.51.

2-Isobutyrylamino-6-[2-*O*-phenoxythiocarbonyl-3,5-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-yl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (9a). To a suspension of compound **8** (1.71 g, 2.88 mmol) and 4-(dimethylamino)pyridine (2.1 g, 17.2 mmol) in anhydrous MeCN (65 mL) was added phenylchlorothionoformiate (0.48 mL, 3.48 mmol) under an argon atmosphere. After the reaction mixture was stirred overnight at room temperature, it was evaporated to dryness and dissolved with CH_2Cl_2 (50 mL). The organic phase was washed twice with cold 1.0 M hydrochloric acid (20 mL), H_2O (20 mL), and a saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was dried (Na_2SO_4), and the solvent was evaporated. The residue, which was a mixture of two major compounds, was purified by FC (silica gel, 4 × 10 cm, CH_2Cl_2/acetone 9:1, v/v). The slower moving major product yielded a colorless foam and was characterized as the title compound **9a** (1.0 g, 48%): TLC (CH_2Cl_2/acetone 9:1) 0.6; UV (MeOH) 274 (18000), 294 (16200 sh.); ^{1}H NMR (d_6-DMSO) 0.95−1.03 (28H, m, $(CHMe_2)_4$), 1.11 (6H, d, J = 6.9, Me_2), 2.77 (1H, h, J = 6.9, CH), 3.90 (1H, m, 5″-H), 4.00 (2H, m, 5′-H, 4′-H), 4.66 (1H, d"t", J = 6.4, 3′-H), 5.14 (1H, d, J = 2.8, 1′-H), 5.90 (1H, "t", J = 4.2, 1′-H), 7.12 (2H, d, J = 7.9, arom. H), 7.33 (1H, t, J = 7.2, arom. H), 7.48 (2H, t, J = 7.5, arom. H), 11.36 (1H, s, NH), 11.81 (1H, s, NH), and 11.87 (1H, s, NH). Anal. Calcd for $C_{34}H_{50}N_4O_8SSi_2$: C, 55.86; H, 6.89; N, 7.66. Found: C, 56.00; H, 6.82; N, 7.60.

2-Isobutyrylamino-7-phenoxythiocarbonyl-6-[2-*O*-phenoxythiocarbonyl-3,5-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-yl)-β-D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (9b). The faster moving byproduct of the synthesis procedure described above was obtained as a yellow powder (250 mg, 10%) and was characterized as compound **9b**: TLC (CH_2Cl_2/acetone 9:1) 0.8; UV (MeOH) 272 (20900), 295 (15600 sh.); ^{1}H NMR (d_6-DMSO) 0.97−1.08 (28H, m, $(CHMe_2)_4$), 1.13 (6H, d, J = 6.4, Me_2), 2.85 (1H, d, J = 6.8, CH), 3.93 (1H, d, J = 8.9, 4′-H), 4.01 (1H, d, J = 12.1, 5′-H), 4.20 (1H, d, J = 12.7, 5″-H), 4.65 (1H, dd, J = 8.9, J = 4.5, 3′-H), 5.78 (1H, s, 1′-H), 6.14 (1H, d, J = 4.5, 2′-H), 6.72 (2H, d, J = 7.6, arom. H), 6.88 (1H, s, 5-H), 7.29 (1H, t, J = 7.3, arom. H), 7.38 (2H, d, J = 8.3, arom. H), 7.41 (2H, t, J = 8.0, arom. H), 7.45 (1H, t, J = 8.3, arom. H), 7.57 (2H, t, J = 7.6, arom. H), 11.44 (1H, br. s, NH), and 12.18 (1H, br. s, NH). Anal. Calcd for $C_{41}H_{54}N_4O_9S_2Si_2$: C, 56.79; H, 6.28; N, 6.46. Found: C, 56.89; H, 6.16; N, 6.41.

2-Isobutyrylamino-6-[3,5-(1,1,3,3-tetraisopropyl-1,3-disiloxan-1,3-yl)-(2-deoxy-β-D-erythro-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (10). To a solution of compound **9a** (1.06 g, 1.45 mmol) in anhydrous toluene (60 mL) was added 2,2′-azobis(isobutyronitrile) (AIBN, 120 mg, 0.73 mmol) and tri-*n*-butyltin(IV) hydride (3.8 mL, 14.1 mmol) under an argon atmosphere. The reaction flask was placed in a preheated oil bath (70 °C). After 2 h, the mixture was cooled to room temperature, and the solvent was evaporated. The oily residue was applied to FC (silica gel, 4 × 10 cm, CH_2Cl_2/MeOH 95:5, v/v) to yield the title compound **10** as a colorless foam (755 mg, 90%): TLC (CH_2Cl_2/MeOH) 0.4; UV (MeOH) 274 (17200), 293 (15500); ^{1}H NMR (d_6-DMSO) 0.99−1.02 (28H, m, $(CHMe_2)_4$), 1.11 (6H, d, J = 6.6, Me_2), 2.18 (1H, m, 2′-H_α), 2.41 (1H, m, 2′-H_β), 2.76 (1H, h, J = 6.5, CH), 3.75 (2H, m, 5′-H_2), 3.95 (1H, m, 4′-H), 4.51 (1H, m, 3′-H), 5.02 (1H, t, J = 7.2, 1′-H), 6.37 (1H, s, 5-H), 11.36 (1H, br. s, NH), 11.70 (1H, br. s, NH), and 11.77 (1H, s, NH). Anal. Calcd for $C_{27}H_{46}N_4O_6Si_2$: C, 56.02; H, 8.01; N, 9.68. Found: C, 56.21; H, 8.15; N, 9.63.

2-Isobutyrylamino-6-(2-deoxy-β-D-erythro-pentofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (11). To a solution of compound **10** (865 mg, 1.5 mmol) in THF (10 mL) was added a 1.0 N solution of $(n\text{-Bu})_4$NF (8 mL, 8 mmol) in THF. The mixture was stirred at room temperature for 3 h, and the solvent was evaporated. The resulting residue was applied to FC (silica gel, 4 × 10 cm, CH_2Cl_2/MeOH 9:1, v/v) to yield the title compound **11** as a colorless powder (410 mg, 82%): TLC (CH_2Cl_2/MeOH 9:1) 0.25; UV (MeOH) 272 (15900), 291 (14200); ^{1}H NMR (d_6-DMSO) 1.11 (6H, d, J = 6.9, Me_2), 2.01 (1H, m, 2′-H_α), 2.11 (1H, m, 2′-H_β), 2.76 (1H, h, J = 7.0, CH), 3.45 (2H, m, 5′-H_2), 3.74 (1H, m, 4′-H), 4.20 (1H, m, 3′-H), 4.79 (1H, t, J = 4.2, 5′-OH), 5.02 (1H, dd, J = 10.1, J = 5.7, 1′-H), 5.08 (1H, d, J = 3.5, 3′-OH), 6.35 (1H, s, 5-H), 11.33 (1H, br. s, NH), 11.63 (1H, br. s, NH), and 11.76 (1H, s, NH). Anal. Calcd for $C_{15}H_{20}N_4O_5$: C, 53.56; H, 5.99; N, 16.66. Found: C, 53.54; H, 5.94; N, 16.63.

2-Isobutyrylamino-6-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (12). Compound **11** (350 mg, 1.04 mmol) was dried by repeated coevaporation with anhydrous pyridine (2 × 10 mL) and dissolved in dry pyridine (15 mL). Then, 4,4′-dimethoxytriphenylmethyl chloride (407 mg, 1.2 mmol) was added, and the solution was stirred for 3 h under argon at room temperature. MeOH (3 mL) was added, and the mixture was stirred for another 30 min. Then the solution was reduced to half of the volume, diluted with CH_2Cl_2 (50 mL), and extracted twice with a 5% aqueous $NaHCO_3$ solution (25 mL) followed by brine (25 mL). The organic layer was dried (Na_2SO_4), filtered, evaporated, and coevaporated with toluene (20 mL twice). The residue was purified by FC (silica gel, 4 × 10 cm, CH_2Cl_2/MeOH 9:1, v/v) to yield the title compound **12** as a colorless foam (480 mg, 72%): TLC (CH_2Cl_2/MeOH 95:5, v/v) 0.2; UV (MeOH) 272 (15700), 294 (13200); ^{1}H NMR (d_6-DMSO) 1.11 (6H, d, J = 6.6, Me_2), 2.10 (1H, m, 2′-H_α), 2.18 (1H, m, 2′-H_β), 2.76 (1H, h, J = 6.6, CH), 3.02 (2H, m, 5′-H_2), 3.72 (6H, s, Me_2), 3.91 (1H, m, 4′-H), 4.16 (1H, m, 3′-H), 5.10 (1H, dd, J = 9.0, J = 6.4, 1′-H), 5.16 (1H, d, J = 3.5, 3′-OH), 6.36 (1H, s, 5-H), 6.82−6.87 (4H, m, arom. H), 7.17−7.39 (9H, m, arom. H), 11.38 (1H, br. s, NH), 11.77 (1H, br. s, NH), and 11.79

(1H, s, NH). Anal. Calcd for $C_{36}H_{38}N_4O_7$: C, 67.70; H, 6.00; N, 8.77. Found: C, 67.70; H, 6.04; N, 8.72.

2-Isobutyrylamino-6-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-erythro-pentofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one 3′-(2-Cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (13). A flask with a solution of compound **12** (530 mg, 0.83 mmol) in anhydrous CH_2Cl_2 (30 mL) was preflushed with argon. Then, $(i\text{-Pr})_2$EtN (0.27 mL, 1.59 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.27 mL, 1.21 mmol) were added under argon. The reaction was monitored by TLC. After stirring for 20 min, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with a 5% aqueous $NaHCO_3$ solution (2 × 15 mL) followed by saturated brine (15 mL). The organic layer was dried (Na_2SO_4), evaporated, and coevaporated with CH_2Cl_2 (2 × 20 mL). The residue was purified by FC (silica gel, 4 × 10 cm, CH_2Cl_2/acetone 8:2, v/v) to yield the title compound **13** as a colorless foam (540 mg, 78%): TLC (CH_2Cl_2/acetone 9:1, v/v) 0.7, 0.75; ^{31}P NMR ($CDCl_3$) 148.8, 150.9.

Synthesis and Purification of the Oligonucleotides. The oligonucleotide syntheses were performed on an ABI 392-08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) in a 1-μmol scale using the phosphoramidite **13** and those of the regular 2′-deoxynucleosides (Applied Biosystems, Weiterstadt, Germany) following the synthesis protocol for 3′-β-cyanoethyl phosphoramidites.[25] The crude oligonucleotides were purified and detritylated on oligonucleotide purification cartridges following the standard protocol for purification.[26] The oligonucleotides were lyophilized on a Speed Vac evaporator to yield colorless solids, which were stored frozen at −18 °C. The enzymatic hydrolysis of the oligomers was performed as described.[27] Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleoside (ϵ_{260} values: dA 15400, dC 7300, dG 11400, dT 8800, **6a** 13200, iG_d 7300, m^5iC_d 6100, dZ 11500, c^7G_d 13100, dA* 6600). Snake-venom phosphodiesterase (EC 3.1.15.1., *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1., *E. coli*) were generous gifts of the Roche Diagnostics GmbH (Penzberg, Germany). MALDI-TOF mass spectra were provided by Dr. J. Gross (Universität Heidelberg, Germany). Some selected MALDI-TOF data of modified oligonucleotides are shown in Table 5. Oligonucleotide analysis was carried out on reversed-phase HPLC (RP-18) with a Merck-Hitachi-HPLC: 250 × 4 mm RP-18 column; gradients of 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (A) and MeCN (B). Gradient I: 50 min 0−50% B in A, flow rate 1.0 mL/min. Gradient II: 20 min 0−25% B in A, flow rate 0.7 mL/min; 30 min 25−40% B in A, flow rate 1.0 mL/min. Gradient III: 20 min 0−25% B in A, flow rate 1.0 mL/min.

Determination of T_m Values and Thermodynamic Data. Absorbance vs temperature profiles were measured on a Cary-1/1E UV/VIS spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temperature was measured in the reference cell with a Pt-100 resistor, and the thermodynamic data ($\Delta H°$, $\Delta S°$, $\Delta G°_{310}$) were calculated using the MeltWin 3.0 program package.[28] The CD-spectra were recorded with a Jasco-600 (Jasco, Japan) spectropolarimeter with a thermostatically (Lauda RCS-6 bath, Germany) controlled 1.0-cm cuvette.

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