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Oligonucleotide duplexes containing N^8-glycosylated 8-aza-7-deazaguanine and self-assembly of 8-aza-7-deazapurines on the nucleoside and the oligomeric level †

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The 8-aza-7-deazaguanine N^8-(2′-deoxy-β-D-ribofuranoside) (**1**) was synthesized, converted into the phosphoramidite **4** and incorporated into oligonucleotides. Nucleoside **1** forms stable base pairs with 2′-deoxy-5-methylisocytidine in DNA with antiparallel chain orientation (aps) and with 2′-deoxycytidine in duplexes with parallel chains (ps). According to the CD spectra self-complementary oligonucleotides d(**1**-m^5isoC)$_3$ and d(**1**-C)$_3$ form autonomous DNA-structures. Neither the nucleoside **1** nor the regularly linked 8-aza-7-deaza-2′-deoxyguanosine form G-like tetrads while the regularly linked 8-aza-7-deaza-2′-deoxyisoguanosine gives higher molecular assemblies which are destroyed by bulky 7-bromo substituents. This was verified on monomeric nucleosides by ESI-MS spectrometry and on oligonucleotides by HPLC analysis.

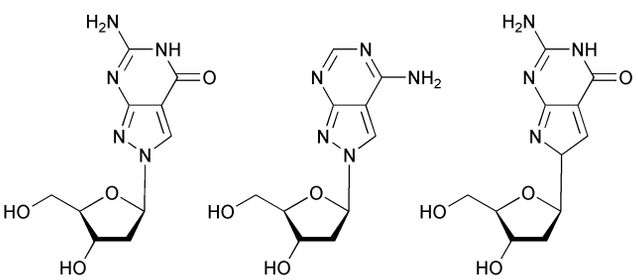
Introduction

Nucleosides with unusual glycosylation positions are naturally occurring; pseudouridine is found in tRNA,[1] the N^7-glycosylated α-D-ribofuranoside of adenine is a constituent of vitamin B_{12}. Various others have been isolated as side products of glycosylation reactions.[2] Oligonucleotides incorporating nucleosides with unusual linkages between the nucleobases and the sugar phosphate backbone show unexpected base pairing properties.[3] In order to extend our knowledge of the recognition motifs formed by non-natural bases mimicking the shape of natural ones we have already investigated the base pairing properties of 8-aza-7-deazapurine nucleosides related to the canonical DNA constituents.[4] This led us to duplex structures with enhanced duplex stability and other favorable properties.

For most of the functions of DNA the nucleobases differ in their structure and therefore in their recognition properties while the sugar phosphate backbone remains unchanged. A wider view of possible biopolymers resembling the characteristics of a DNA molecule is possible by changing the heterocyclic moieties of the nucleobases, their substituent pattern and/or their arrangement within the DNA-duplex. This can lead to novel DNA-like molecules suitable for various applications in molecular biology and nanotechnology.

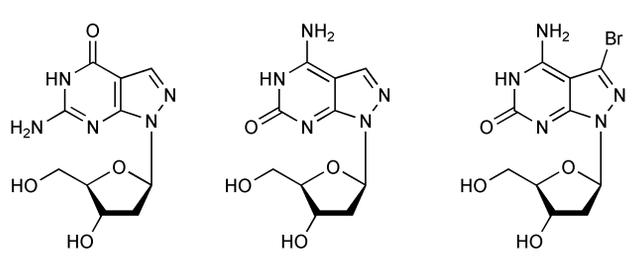
As the pyrazolo[3,4-*d*]pyrimidine (8-aza-7-deazapurine) nucleobases contain a nitrogen at position-8, they became targets for nucleosides with unusual glycosylation sites. Earlier, we have shown that the 8-aza-7-deazaadenine N^8-(2′-deoxyribofuranoside) (**2**, Scheme 1) or its 2-amino derivative develop base pairing ambiguity against the four canonical DNA constituents.[5,6] The 7-deazaguanine C^8-(2′-deoxyribofuranoside) (**3**) forms stable base pairs in duplexes mimicking the properties of 2′-deoxyisoguanosine.[7]

This manuscript reports on the synthesis and the base pairing of the 8-aza-7-deazaguanine N^8-(2′-deoxy-β-D-ribofuranoside) **1** which was converted into the phosphoramidite **4** and incorporated in oligonucleotides. The investigation includes duplexes with antiparallel and parallel chain orientation and compares them with oligonucleotides containing the regularly linked molecules. Also the possible formation of higher molecular assemblies incorporating the nucleosides **1**, **5**–**7** (Scheme 2) will be reported. For identification ESI-mass spectrometry is used on the monomeric nucleosides and ion exchange HPLC on the oligonucleotides.



1 **2** **3**

Scheme 1 Unusually linked 2′-deoxyribonucleosides.



5 **6** **7**

Scheme 2 Regularly linked 8-aza-7-deazapurine 2′-deoxyribonucleosides.

Results and discussion

1. Monomer synthesis and oligonucleotides containing N^8-glycosylated 8-aza-7-deazaguanine (1)

Earlier, the 8-aza-7-deazaguanine N^8-(2′-deoxy-β-D-ribofuranoside) (**1**) was synthesized from 2-amino-6-methoxy-8-aza-7-deazapurine 2′-deoxyribofuranoside (**8**) by treatment

† Electronic supplementary information (ESI) available: expanded versions of Tables 2–5 and figure showing HPLC profiles of the enzymatic hydrolysis of oligonucleosides 5′-d(**1**-C)$_3$ and 5′-d(TAG **1** TC AAT ACT). See http://www.rsc.org/suppdata/ob/b3/b309485p/

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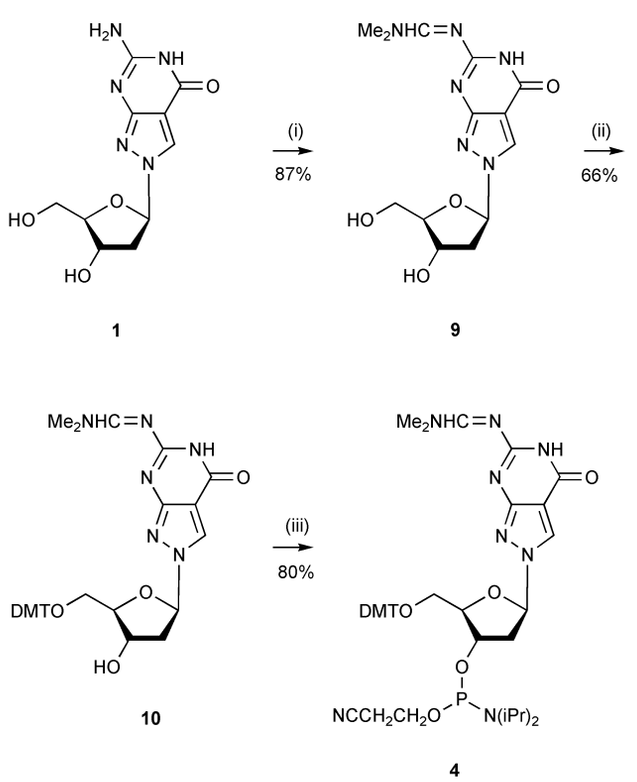
Table 1 ^{13}C NMR Chemical shifts of 8-aza-7-deazapurine nucleosides (d_6-DMSO, 25 °C)

Compound	C(3)[a] C(7)[b]	C(3a)[a] C(5)[b]	C(4)[a] C(6)[b]	C(6)[a] C(2)[b]	C(7a)[a] C(4)[b]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	HC=N
1[9]	127.8	102.2	159.3[d]	153.5[d]	160.7	89.7	40.0	70.5	88.1	61.9	
5[9]	134.9	99.7	157.4[d]	154.6[d]	155.2	83.1	37.9	71.0	87.3	62.4	
6[11]	134.7	92.6	157.0[d]	155.2[d]	[e]	84.3	[c]	71.2	88.2	62.5	
9	128.0	104.1	160.2[d]	158.1[d]	160.0[d]	89.9	39.8	70.6	88.1	62.0	158.0
10	128.1	104.4	160.2[d]	158.0[d]	160.1[d]	89.2	[c]	70.4	85.6	64.1	157.9
11	136.0	92.9	157.4[d]	155.3[d]	[e]	83.7	[c]	73.5	87.6	64.2	
12	138.1	93.3	156.7[d]	154.2[d]	[e]	82.9	37.4	72.2	86.7	62.9	
13	128.3	102.5	159.3[d]	153.5[d]	160.8[d]	89.5	[c]	72.2	87.4	63.0	
14	135.3	99.8	157.7[d]	154.9[d]	155.7[d]	82.9	37.5	72.6	86.8	63.3	

[a] Systematic numbering. [b] Purine numbering. [c] Superimposed by DMSO. [d] Tentative assignment. [e] Not detected.

with aqueous potassium hydroxide[8,9] (purine numbering is used throughout this manuscript). For solid-phase oligonucleotide synthesis the phosphoramidite **4** was prepared. The amino group of compound **1** was protected with a dimethylaminomethylidene residue leading to compound **9** (Scheme 3). Regarding oligonucleotide synthesis, the stability of the protecting group was investigated and kinetic measurements were performed in 25% aqueous ammonia solution at 40 °C. The reaction was monitored UV-spectrophotometrically. The determined half life value for compound **9** was 5 minutes which is suitable for further manipulations. Compound **9** was converted into the 5′-*O*-(dimethoxytriphenylmethyl) protected (DMT) derivative **10** employing standard conditions.[10] Finally, the phosphoramidite **4** was prepared from **10** using chloro(2-cyanoethoxy)(diisopropylamino)-phosphine in the presence of *N*-ethyldiisopropylamine. All compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy (see Table 1 and experimental part) as well as by elemental analysis. As it has been shown earlier that the change of the glycosylation position from N^9 to N^8 results in a significant downfield shift of C(5), C(6) and C(4) and in an upfield shift at C(7), the glycosylation position of the corresponding N^8-derivatives **9**, **10** and **13** was established.[8]



Scheme 3 Protecting group strategy for the synthesis of the phosphoramidite **4**. *Reagents and conditions*: (i) *N*,*N*-dimethylformamide dimethylacetal–methanol; rt; 48 h. (ii) DMT-Cl–pyridine; rt; 4.5 h. (iii) chloro(2-cyanoethoxy)(diisopropylamino)phosphine–^{i}Pr$_2$EtN; rt; 30 min.

The oligonucleotides were prepared by solid-phase synthesis on a 392 DNA-synthesizer (Applied Biosystems, Weiterstadt, Germany) using the standard protocol of phosphoramidite chemistry.[12] The coupling efficiency of the modified phosphoramidite **4** was always higher than 98%. The nucleoside composition of the oligonucleotides was proven by MALDI-TOF mass spectrometry (Table 7) as well as by enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase and RP-HPLC.

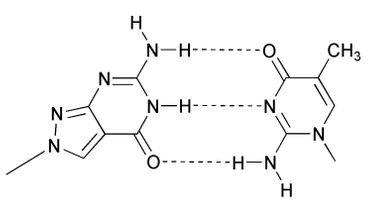
1.1. Non-self-complementary oligonucleotide duplexes with antiparallel or parallel chain orientation. In order to investigate the base pairing properties of the nucleoside **1** it was incorporated in the antiparallel duplex 5′-d(TAGGTCAATACT)·3′-d(ATCCAGTTATGA) (**15·16**) replacing dG residues in one or both strands. As we have recognized from experiments performed on the related 7-deazaguanine C^8-nucleoside **3**[7] that a change of the glycosylation site from nitrogen-9 to position-8 resulted in a change of the nucleobase recognition pattern (guanine behaves like isoguanine), oligonucleotide duplexes were constructed in which compound **1** was located opposite to isoC$_d$. Because of the low stability of isoC$_d$ under alkaline conditions the 5-methyl derivative was used instead.[13] From Table 2 it is apparent that the incorporation of compound **1** opposite to m^{5}isoC$_d$ results in duplexes which are only slightly less stable than those containing dG–dC pairs (**15·16**), while incorporation of compound **1** opposite to dC leads to a strong destabilization. Thus compound **1** shows the base pairing properties as iG$_d$. Evidently, the change of the glycosylation site from N^9 to N^8 (**5** *vs.* **1**) leads to a similar change of the base pair recognition as the interchange of the substituents of the base moiety (dG ⟶ iG$_d$).

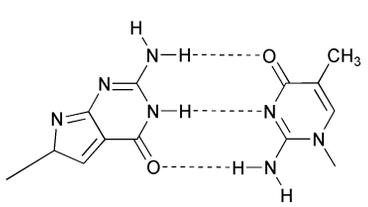
With regard to T_m-data of oligonucleotide duplexes containing 2′-deoxyisoguanosine as reference, the incorporation of one nucleoside **1**-residue shows a reduction of the T_m value of 3 °C for **23·18** and 6 °C for **19·24**, compared to the isoguanine containing duplexes **17·18** and **19·20**, demonstrating that the duplex stability depends on the nearest neighbours. Incorporation of four modified nucleoside residues leads to a ΔT_m of −5 °C per modification (**25·26**). Oligonucleotide duplexes of the same length containing an abasic site—which neither develop stacking interactions nor hydrogen bonding—were shown to induce a much stronger T_m-decrease (ΔT_m = −15 °C per modification)[5] than the change of the glycosylation site from base position-9 to position-8. This indicates that the incorporation of compound **1** stabilizes duplex DNA by base stacking and the formation of a tridentate base pair according to the motif **I** (see duplexes **23·18** and **19·24**). The motif **I** is related to the already published motif **II**[7] and shows also relationships to motif **III** with regard to the formation of a base pair with m^{5}isoC$_d$. The motifs **I**–**III** are all of that of a tridentate base pair similar to the canonical motif **IV** (Scheme 4).

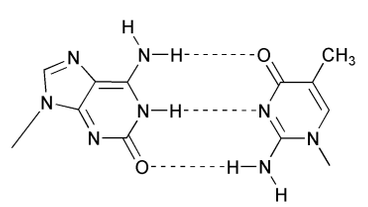
Table 2 T_m Values and thermodynamic data of non-self-complementary antiparallel-stranded oligonucleotide duplexes containing N^8-linked 8-aza-7-deaza-2′-deoxyguanosine (**1**) hybridized against d(iC) and dC[a,b]

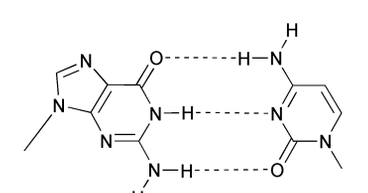
Duplex	Compound no.	T_m/°C
5′-d(TAG GTC AAT ACT)	**15**	51
3′-d(ATC CAG TTA TGA)	**16**	
5′-d(TAG **iG**TC AAT ACT)[14]	**17**	54
3′-d(ATC **iC**AG TTA TGA)	**18**	
5′-d(TAG GT**iC** AAT ACT)[14]	**19**	54
3′-d(ATC CA**iG** TTATGA)	**20**	
5′-d(AT**iC iC**A**iG** TTA T**iG**A)	**21**	60
3′-d(TA**iG iG**T**iC** AAT A**iC**T)	**22**	
5′-d(TAG **1** TC AAT ACT)	**23**	48
3′-d(ATC **iC**AG TTATGA)	**18**	
5′-d(TAG GT**iC** AAT ACT)	**19**	51
3′-d(ATC CA **1** TTA TGA)	**24**	
5′-d(AT**iC iC**A**1** TTA T**1**A)	**25**	40
3′-d(TA **1 1**T**iC** AAT A**iC**T)	**26**	
5′-d(TAG **1** TC AAT ACT)	**23**	36
3′-d(ATC **C**AG TTA TGA)	**16**	
5′-d(TAG GT**C**AAT ACT)	**15**	35
3′-d(ATC CA**1** TTA TGA)	**24**	

[a] Measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM sodium cacodylate buffer, pH 7. 5 μM + 5 μM oligonucleotide concentration. [b] d(iC) = m^5isoC_d = 2′-deoxy-5-methylisocytidine.









Scheme 4 Base pair motifs of duplexes with antiparallel strand orientation (aps).

Next, the base pair discrimination of compound **1** against the canonical DNA-constituents dG, dT or dA was studied (Table 3). While duplexes with compound **1** opposite to dA are rather labile, the ones with **1** opposite to dG (**23·27**; **31·24**) and dT (**23·30**; **33·24**) are surprisingly stable. This observation was also made on duplexes incorporating those compounds at a different position of the oligomer. Also rather stable duplexes are found when compound **1** was facing itself (**23·28**; **32·24**). Thus compound **1** shows ambiguous base pairing against dG, dT and **1** but shows base pair discrimination against dA. This behaviour shows similarities to that of the C^8-linked 7-deazaguanine 2′-deoxyribonucleoside.[7]

Table 3 T_m Values and thermodynamic data of non-self-complementary antiparallel-stranded oligonucleotide duplexes containing N^8-linked 8-aza-7-deaza-2′-deoxyguanosine (**1**) hybridized against dG, **1**, dT and dA[a]

Duplex	Compound no.	T_m/°C
5′-d(TAG **1** TC AAT ACT)	**23**	48
3′-d(ATC **G**AG TTA TGA)	**27**	
5′-d(TAG **1** TC AAT ACT)	**23**	46
3′-d(ATC **1** AG TTA TGA)	**28**	
5′-d(TAG **1** TC AAT ACT)	**23**	44
3′-d(ATC **T** AG TTA TGA)	**29**	
5′-d(TAG **1** TC AAT ACT)	**23**	33
3′-d(ATC **A**AG TTA TGA)	**30**	
5′-d(TAG GT**G** AAT ACT)	**31**	43
3′-d(ATC CA **1** TTA TGA)	**24**	
5′-d(TAG GT**1** AAT ACT)	**32**	45
3′-d(ATC CA**1** TTA TGA)	**24**	
5′-d(TAG GT**T** AAT ACT)	**33**	41
3′-d(ATC CA**1** TTA TGA)	**24**	
5′-d(TAG GT**A** AAT ACT)	**34**	30
3′-d(ATC CA**1** TTA TGA)	**24**	

[a] Measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM sodium cacodylate buffer, pH 7. 5 μM + 5 μM Oligonucleotide concentration.

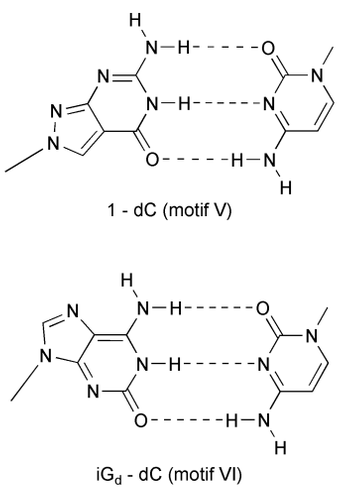
According to the observation that compound **1** forms rather base pairs with 2′-deoxy-5-methylisocytidine in duplexes with antiparallel chain orientation, it was expected that stable duplexes with parallel chain orientation are also formed when compound **1** is incorporated in such duplexes and is located opposite to dC. Hybridization experiments according to Table 4 were performed with the parallel-stranded isoguanine containing duplex **35·16**.[14] Isoguanine was replaced by compound **1** and the four regular nucleosides as well as 2′-deoxy-5-methylisocytidine were located on the opposite side in order to investigate the duplex stabilities. Compared to **35·16** the duplex **26·16** shows a T_m decrease of 4.5 °C per modification with two consecutive **1**–dC pairs. Although there is a lower duplex stability when compound **1** was introduced in the case of non-self-complementary duplexes—which might be due to a heterogeneous DNA structure—still a specific base pairing could be observed. Nevertheless the duplex **26·16** is significantly more stable than those incorporating dA, dT and dG opposite to compound **1**, while that of **26·36** still shows a rather high T_m-value.

1.2 Self-complementary duplexes. Earlier it has been demonstrated, that duplexes with alternating residues of the 8-aza-7-deazaadenine N^8-(2′-deoxyribofuranoside) (**2**) and dT are extraordinarily stable with T_m-values higher than those of oligonucleotides of the same length but incorporating alternating dA–dT.[15] This prompted us to study self-complementary hexanucleotides with alternating residues of the nucleoside **1** and dC or m^5isoC_d. From the results shown on self-complementary duplexes it is very likely that the chain orientation of this duplex is parallel forming a base pair according to motif **V** (Scheme 5). As a consequence base overhangs are expected to be present at the 3′ and 5′-termini. Interestingly, the duplex 5′-d(**1**-C)$_3$ (**37·37**) is rather stable and shows a similar T_m to that of 5′-d(iG-C)$_3$ (**38·38**) (Table 5). The related duplex containing pyrazolo[3,4-*d*]pyrimidine isoguanine analogue 5′-d(**6**-C)$_3$ is more stable while that of the 5′-d(**40**-C)$_3$ contain-

Table 4 T_m Values and thermodynamic data of parallel-stranded non-self-complementary oligonucleotide duplexes containing N^8-linked 8-aza-7-deaza-2′-deoxyguanosine (**1**)[a,b]

Duplex	Compound no.	T_m/°C
5′-d(TiCA TAA iCT**iG iG**AT)[13]	**35**	45
5′-d(AGT ATT GA**C C**TA)	**16**	
5′-d(TiCA TAA iCT**1 1**AT)	**26**	36
5′-d(AGT ATT GA**C C**TA)	**16**	
5′-d(TiCA TAA iCT**1 1**AT)	**26**	32
5′-d(AGT ATT GA**1** CTA)	**36**	
5′-d(TiCA TAA iCT**1 1**AT)	**26**	23
5′-d(AGT ATT GA **G** CTA)	**27**	
5′-d(TiCA TAA iCT**1 1**AT)	**26**	25
5′-d(AGT ATT GA**T** CTA)	**29**	
5′-d(TiCA TAA iCT**1 1**AT)	**26**	23
5′-d(AGT ATT GA**A** CTA)	**30**	
5′-d(TiCA TAA iCT**1 1** AT)	**26**	28
5′-d(AGT ATT GA**iC** CTA)	**18**	

[a] Measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM sodium cacodylate buffer, pH 7. 5 μM + 5 μM Oligonucleotide concentration. [b] d(iC) = m^5isoC_d = 2′-Deoxy-5-methylisocytidine.



Scheme 5 Base pair motifs of ps-duplexes containing compound **1** or 2′-deoxyisoguanine.

ing the corresponding pyrrolo[2,3-*d*]pyrimidine base shows a lower stability. These findings correspond well to those observed on 6-mer duplexes with regularly linked "guanine" nucleosides such as 5′-d(G-C)$_3$ *vs.* 5′-d(**44**-C)$_3$ and 5′-d(**46**-C)$_3$.

It is apparent that the duplexes with parallel chain orientation contain only five base pairs, while those formed by antiparallel chains are hold together by six pairs. Thus the parallel duplexes are expected to show a lower stability. Nevertheless the terminal overhangs will stabilize the five base pair duplex structure by additional stacking interactions which will cause a positive effect on the duplex stability.[16]

Next the stability of the duplex 5′-d(**1**-m^5isoC)$_3$ **42·42** was studied. In this case the chain orientation is expected to be antiparallel. This duplex showed a slightly higher T_m-value as that of **37·37**. Thus, the recognition pattern of guanine is changed into that of isoguanine when the glycosylation site is shifted from position-9 to position-8. Compound **1** is able to form duplexes with parallel and antiparallel chain as it is observed for isoguanine nucleosides. Such changes can be also caused by changing the anomeric configuration which has been recently investigated on anomeric 5-aza-7-deazaguanine mimicking the base pair recognition site of isocytosine.[19] In order to obtain some information on the particular duplex structure of the duplexes **37·37** and **42·42** with the unusually linked backbones their CD-spectra were measured. According to Fig. 1 this duplex forms an autonomous DNA-structure being different to that from A- or B-DNA. A related CD-spectrum of **37·37** has been observed for a 12-mer duplex d(**2**-T)$_6$.[6] Moreover the CD-spectrum of **42·42** shows similarities to a left handed Z-DNA.[3a]

Table 5 T_m Values and thermodynamic data of self-complementary oligonucleotides[a] with parallel and antiparallel chain orientation

Duplex	Compound no.	T_m/°C
5′-d(**1**-C-**1**-C-**1**-C)-3′	**37**	34
5′-d(**1**-C-**1**-C-**1**-C)-3′	**37**	
5′-d(iG-C-iG-C-iG-C)-3′ [17]	**38**	33
5′-d(iG-C-iG-C-iG-C)-3′	**38**	
5′-d(**6**-C-**6**-C-**6**-C)-3′ [17b]	**39**	41
5′-d(**6**-C-**6**-C-**6**-C)-3′	**39**	
5′-d(**40**-C-**40**-C-**40**-C)-3′ [17c]	**41**	22
5′-d(**40**-C-**40**-C-**40**-C)-3′	**41**	
5′-d(**1**-iC-**1**-iC-**1**-iC)-3′	**42**	37
3′-d(iC-**1**-iC-**1**-iC-**1**)-5′	**42**	
5′-d(G-C-G-C-G-C)-3′ [18]	**43**	46
3′-d(C-G-C-G-C-G)-5′	**43**	
5′-d(**44**-C-**44**-C-**44**-C)-3′ [18d]	**45**	62
3′-d(C-**44**-C-**44**-C-**44**)-5′	**45**	
5′-d(**46**-C-**46**-C-**46**-C)-3′ [18e]	**47**	37
3′-d(C-**46**-C-**46**-C-**46**)-5′	**47**	

[a] Measured in 1 M NaCl, 0.1 M $MgCl_2$, 60 mM sodium cacodylate buffer, pH 7.0. 5 μM + 5 μM Oligonucleotide concentration [b] **6**: $c^7z^8iG_d$: 8-Aza-7-deaza-2′-deoxyisoguanosine [c] **40**: c^7iG_d: 7-Deaza-2′-deoxyisoguanosine [d] **44**: $c^7z^8G_d$: 8-Aza-7-deaza-2′-deoxyguanosine [e] **46**: c^7iG_d: 7-Deaza-2′-deoxyguanosine

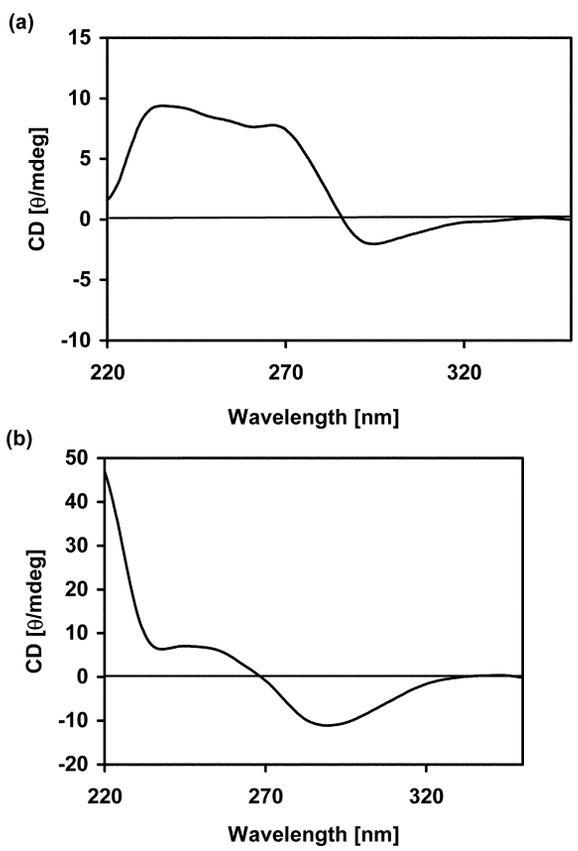
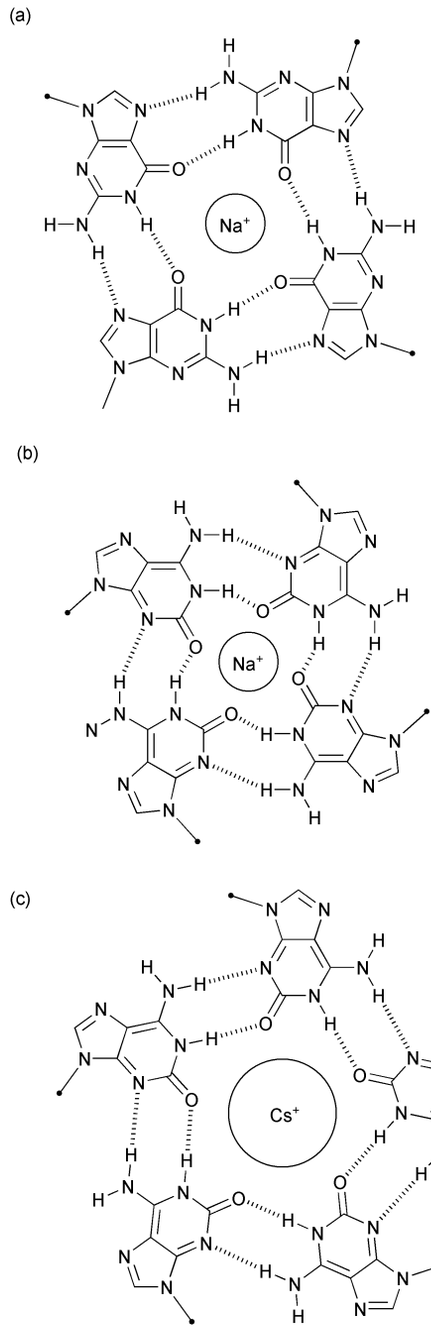


Fig. 1 CD-spectrum of the duplex (a) 5′-d(1-C)$_3$ and (b) 5′-d(1-m^5iC)$_3$.

2. Supramolecular assemblies of 8-aza-7-deazapurine 2′-deoxyribonucleosides

Several investigations have shown that guanine and isoguanine nucleosides as well as the corresponding base or sugar-modified oligonucleotides are able to form supramolecular assemblies. In the case of guanine nucleosides tetrameric structures (Scheme

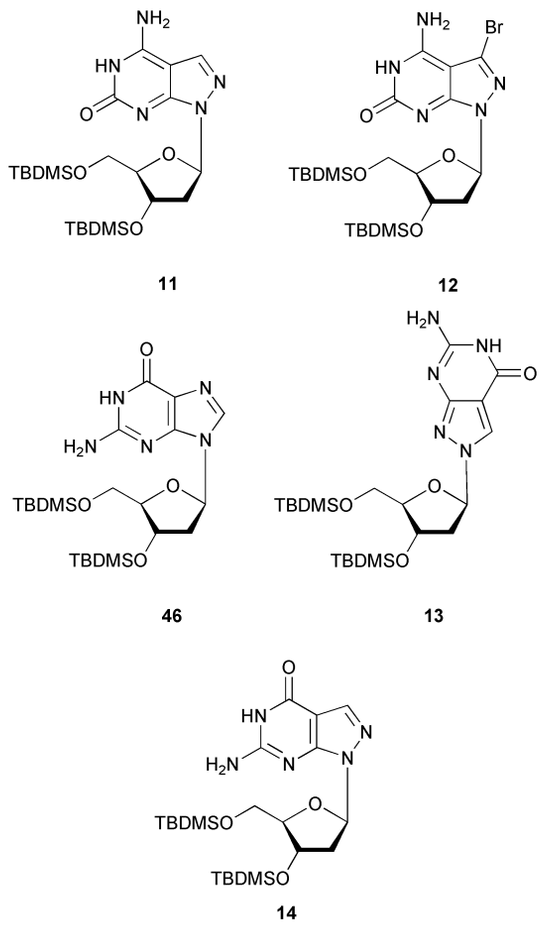


Scheme 6 Structures of (a) dG-tetraplex, (b) iGd-tetraplex, and (c) iGd-pentaplex.

6a) have been identified while isoguanine compounds tend to form tetrameric (Scheme 6b) as well as pentameric structures (Scheme 6c).[20–22]

In order to prevent such an aggregation, which obscures data collection during the sequencing performed by gel electrophoresis or on arrays with immobilized DNA, base modified guanine nucleosides have been used to impair the undesired self-aggregation. Compounds showing this capability are 7-deaza-2′-deoxyguanosine and 8-aza-2′-deoxyguanosine.[23,24] They can not form Hoogsteen base pairs due to the lack of the nitrogen at position 7 thereby destroying the G-quartet. The situation is different in the case of isoguanine assemblies.[25] As nitrogen-7 is not participating in the formation of the supramolecular aggregates 8-aza-7-deaza-2′-deoxyisoguanosine (**6**) forms high molecular assemblies.[22]

It has been already shown on a sugar protected isoguanine ribonucleoside by electrospray-ionization mass-spectroscopy (ESI-MS) that both tetrameric as well as the pentameric assemblies are formed simultaneously in the presence of sodium cations.[26] Now we have performed similar experiments using 8-aza-7-deazapurine 2′-deoxynucleosides (**1**, **5**–**7**). These phenomena were studied by ESI-MS spectrometry (ESI-MS; HP LC/MS 1100 Series with Chemstation Software) employing the lipophilic 3′, 5′-TBDMS-protected nucleosides **11**–**14**,**48** (Scheme 7). The nucleoside derivatives were prepared from the corresponding nucleosides by silylation with *tert*-butyldimethylsilyl chloride (TBDMS-Cl). The sugar protected nucleosides were dissolved in $CHCl_3$ together with sodium tetraphenylborate. The suspensions were stirred for 30 minutes at room temperature, stored over night at −23 °C, and employed for ESI-MS studies.



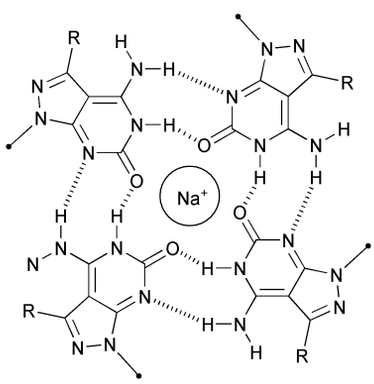
Scheme 7 3′, 5′-TBDMS protected 8-aza-7-deazapurine 2′-deoxyribonucleosides.

Table 6 summarizes the outcome of these experiments. The nucleoside **11** is able to build up a tetrameric aggregate with one sodium cation complexed to four compound **11** residues (m/z = 2004.8). The sodium is expected to be located in the center of the assembly. For steric reason the tetramer should form a bowl shaped structure.[27] A pentameric aggregate as found for the isoguanine ribonucleoside[20] was not detected for the pyrazolo[3,4-*d*]pyrimidine nucleoside,[22] which can be traced back to the structural differences of compound **11** to the isoguanine derivative. It has been reported that 8-aza-7-deazapurine nucleosides show a particular glycosylic bond conformation (high-*anti*) compared to the anti-conformation of purine nucleosides which can affect supramolecular aggregation. Although compound **11** formed a tetrad, such a formation was not observed when 8-aza-7-deaza-2′-deoxyisoguanosine was carrying a 7-bromo substituent (**12**). Apparently, the higher molecular assembly shown in Scheme 8 can not accommodate the bulky bromo residues. It might be possible that the amino group of compound **12** forms a hydrogen bond with the bromine substituent. Such a bond has been detected in the case of a derivative lacking the 2-oxo group.[28]

Table 6 Relative peak areas (%) of 3′, 5′-TBDMS-protected nucleosides (N*) by ESI mass spectrometry[a]

Compound	Monomer $[N^* H]^+$	Dimer $[N^*_2 H]^+$	Dimer $[N^*_2 Na]^+$	Tetramer $[N^*_4 Na]^+$
11		100	14	100 (*m*/*z*: 2004.8)
12	100	69		
48			25	100 (*m*/*z*: 2005.4)
13	100	9		
14	100			

[a] Nucleoside concentration: 10 mM in $CHCl_3$, $NaBPh_4$.



Scheme 8 8 Structure of the tetraplex formed by 8-aza-7-deaza-2′-deoxyisoguanosine (**6**) (R = H), but does not occur in the case of **7** (R = Br).

The same experiments as performed with the N^9 compounds **11** and **12** were undertaken with the N^8-linked 8-aza-7-deaza-2′-deoxyguanosine derivative (**13**) as well as with the regularly linked nucleoside **14**. In both cases aggregates are not formed, which results from the absence of a nitrogen-7 as Hoogsteen acceptor position in the case of compound **14** and/or the unusual glycosylation site of compound **13** (Table 6). In comparison, the purine nucleoside 2′-deoxyguanosine in form of derivative **48** was shown to create a tetrameric species at 2005.4 *m*/*z* containing one sodium ion per four dG-residues.

In order to confirm these phenomena also on the oligonucleotide level, the nucleosides of Scheme 2 were incorporated in oligonucleotides. The following oligonucleotides were prepared by solid-phase synthesis: 5′-d($T_4G_4T_2$) (**49**), 5′-d(T_4**5**$_4T_2$) (**50**), 5′-d(T_4**6**$_4T_2$) (**51**), and 5′-d(T_4**7**$_4T_4$) (**52**). Then the oligonucleotides were investigated with regard to aggregate formation by ion exchange chromatography (experiments performed on compounds **49** and **51** were already reported earlier).[22] For this purpose the solutions were injected onto an ion-exchange Gen-Pak™ Fax (**49**–**51**) or Dionex PA-100 (**52**) column thermostated at 30 °C.

Earlier experiments performed on 5′-d($T_4G_4T_2$) (**49**) have shown that the peak with the short retention time represents the single-stranded oligomer while the slower migrating peak results from the high molecular assembly (Fig. 2a). In contrast to that, the 8-aza-7-deaza-2′-deoxyguanosine-containing oligonucleotide **50** shows only one fast migrating peak indicating that an aggregation does not take place. This is in accordance with the mass spectrometric experiments performed on the nucleoside derivatives dG and **14** (see above).

While oligonucleotides incorporating short runs of 8-aza-7-deaza-2′-deoxyguanosine do not form tetrameric assemblies, 8-aza-7-deaza-2′-deoxyisoguanine (**6**) builds up such structures (Fig. 2c).[21,22] However, the oligonucleotide incorporating the 7-bromo derivative **7** (**52**) does not aggregate (Fig. 2d, the retention time is slightly faster due to Dionex PA-100 column). These observations correlate well with the findings on the monomeric nucleoside level (see above). From this one can deduce that the aggregation of guanine nucleosides is prevented both by regularly linked 8-aza-7-deazaguanine (**5**) as well as by

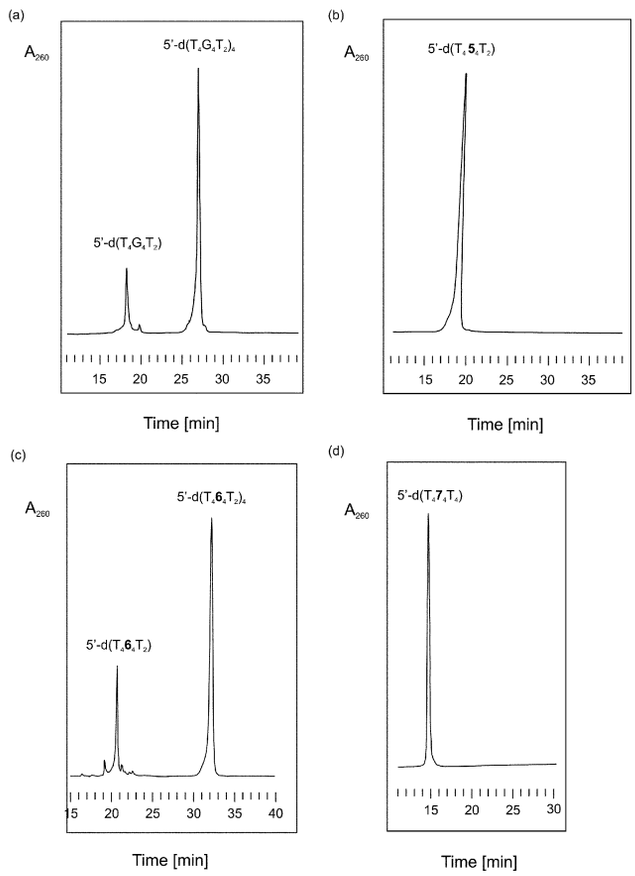


Fig. 2 Ion exchange HPLC elution profile (a) of 5′-d($T_4G_4T_2$) (**47**), (b) 5′-d(T_4**5**$_4T_2$) (**48**), (c) 5′-d(T_4**6**$_4T_2$) (**49**), and (d) 5′-d(T_4**7**$_4T_4$) (**50**) at 30 °C; conditions: see Experimental.

the N^8-glycosylated compound **1**, as is similarly found for 7-deaza-2′-deoxyguanosine.[23] In the case of "isoguanine" nucleosides, such as **7**, aggregation is prevented by bulky 7-substituents.

Conclusions

Introduction of the 8-aza-7-deazaguanine N^8-(2′-deoxy-β-D-ribofuranoside) **1** into oligonucleotide duplexes with antiparallel chain orientation leads to a change of the base recognition, which is now comparable with that of 2′-deoxyisoguanosine. Antiparallel duplexes are rather stable when compound **1** forms base pairs with 2′-deoxy-5-methylisocytidine; towards dG and itself it shows ambiguous base pairing. In parallel duplexes the most stable base pair is formed when the nucleoside **1** base pairs with dC. These properties observed on non-self-complementary duplexes with random base pair composition can be also realized on self-complementary oligonucleotide duplexes such as the hexanucleotides 5′-d(**1**-C)$_3$ and 5′-d(**1**-m^{5}isoC)$_3$.

Investigation of supramolecular aggregates of pyrazolo[3,4-*d*]-pyrimidine nucleosides related to dG indicate that aggregation

is prevented when nitrogen-7 is absent as electron donating site both on the N^9 and N^8-glycosylated isomer **1** and **5**, as shown earlier for other 7-deazapurine nucleosides such as 7-deaza-2′-deoxyguanosine. Even, the weakly basic 7-nitrogen of 8-aza-2′-deoxyguanosine nucleoside prevents the formation of supramolecular assemblies.[24] Isoguanine nucleosides such as 8-aza-7-deaza-2′-deoxyisoguanosine still forms self-assembled structures. However, the presence of a bulky 7-bromo substituent prevents aggregate formation also in this case.

Experimental

General

Thin-layer chromatography (TLC) was performed on TLC aluminium sheets silica gel 60 F_{254} (0.2 mm, VWR International, Germany). Reversed-phase HPLC was carried out on a 4 × 250 mm RP-18 (10 μm) LiChrosorb column (VWR International) with a Merck-Hitachi HPLC pump (model 655 A-12) connected with a variable wavelength monitor (model 655-A), a controller (model L-5000), and an integrator (model D-2000). UV-spectra were recorded on a U-3200 spectrophotometer (Hitachi, Japan), $\lambda_{max.}$ in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. Half-life values (τ) were measured on U-3200 spectrophotometer (Hitachi, Japan) connected with a temperature controller (Lauda, Germany). NMR spectra were measured on an Avance DPX 250 and an AMX 500 spectrometer (Bruker, Germany); chemical shifts (δ) are in ppm downfield from internal TMS (^{1}H, ^{13}C) or external 85% H_3PO_4 (^{31}P). The *J*-values are given in Hz. The solvents were purified and dried according to standard procedures. MALDI-TOF was recorded on Biflex-III spectrometer (Bruker, Leipzig, Germany) in the reflector mode. The average power of the nitrogen laser (337.1 nm) at 20 Hz was 3–4 mW (150–200 μJ/pulse) with a delay time of 600 ns. The enzymatic hydrolysis of the oligomers was performed as described below using the following extinction coefficients: ε_{280}: dA: 2400, dC: 6900, dT: 6300, m^5isoC_d: 1600, **1**: 6600. Snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli*) were generous gifts from Roche Diagnostics GmbH, Germany.

Ion-exchange HPLC

The ion-exchange chromatography was performed on a 4.6 × 100 mm Gen-Pak™ Fax Column (Waters, WAT015490, USA) or a 4 × 250 mm NucleoPac™-column (Dionex P/N 043010, USA) using a Merck-Hitachi HPLC apparatus with a pump (model L-7100), a UV-detector (model L-4250) connected with an integrator (model D-2000). A column oven (model L-7350, Merck, Germany) was used to control the temperature of the ion-exchange column. The oligonucleotides were prepared as follows. A sample of 0.1 A_{260}-units was dissolved in 20 μL 1.0 M NaCl-buffer. The solution was heated to 60 °C for 5 min., brought to room temperature and kept in a refrigerator (−23 °C) for 16 h. Then, the sample was brought to room temperature, diluted with 100 μL buffer A and injected into the system, which had been preheated to 30 °C. The column was eluted using the following systems: 0–40 min with 0–80% B in A with a flow rate of 0.5 mL min^{-1} (A: 25 mM Tris-HCl, 1.0 mM EDTA, pH 8.0; B: 1.0 M NaCl, 25 mM Tris-HCl, 1.0 mM EDTA, pH 8.0).

Electrospray-ionization mass spectrometry

The ESI-MS measurements were performed using HP LC/MS 1100 with Chemstation Software. To 1 mL of a 10 mM solution in $CHCl_3$ 0.05 mmol $NaBPh_4$ was added. The suspension was stirred for 30 minutes and incubated at −23 °C overnight. The solid was removed, the solution filtered and used for the ESI-MS measurements.

Table 7 Molecular weights determined by MALDI-TOF mass spectroscopy of modified oligonucleotides

Oligonucleotide		MH^+ (calc)	MH^+ (found)
5′-d(TAG**1**TCAATACT)	**21**	3645.4	3645.5
5′-d(AGTATT**1**ACCTA)	**22**	3645.4	3648.5
5′-d(AT**iCiC**A**1**TTAT**1**A)	**23**	3673.5	3673.4
3′-d(ATC**iC**AGTTATGA)	**18**	3657.7	3657.4
5′-d(TAGGT**iC**AATACT)	**19**	3657.7	3659.8

Oligonucleotides

The oligonucleotide syntheses were carried out on an ABI 392–08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) in a 1 μmol scale using the phosphoramidite **4**, those of the regular 2′-deoxynucleosides (Applied Biosystems, Weiterstadt, Germany) together with the DPC- and formamidine-protected phosphoramidite of 8-bromo-8-aza-7-deaza-2′-deoxyisoguanosine [19] as well as the isobutyryl-protected phosphoramidite of 8-aza-7-deaza-2′-deoxyguanosine following the synthesis protocol for 3′-β-cyanoethyl phosphoramidites. Oligonucleotide **42** was synthesized with a Universal support column carrying a 3′-terminal ribose moiety (Universal support 500, Glen Research, Sterling, VA, USA). After cleavage of the oligonucleotides from the solid support, the former were deprotected in 25% aqueous ammonia solution for 12–16 h at 60 °C. Purification of the 5′-dimethoxytrityl-oligomers was performed by reversed-phase HPLC (*RP-18*) with the following solvent gradient system (A, 0.1 M $(Et_3NH)OAc$ (pH 7.0)–MeCN 95 : 5; B, MeCN): 3 min 20% B in A with a flow rate of 1.0 mL min^{-1}, 12 min 20–40% B in A with a flow rate of 1 mL min^{-1}. Treatment with 2.5% $CHCl_2$-$COOH/CH_2Cl_2$ for 5 min at room temperature to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC with the gradient: 20 min 0–20% B in A with a flow rate of 1 mL min^{-1}. Oligonucleotides for investigation of aggregate formation were synthesized in the trityl-off mode, deprotected in 25% aq. ammonia solution at 60 °C for 16 h followed by purification by ion exchange chromatography (NucleoPac™-column, 4 × 250 mm) with the following solvent system (C: 1.5 M LiCl in aq. NaOH (pH 12.0)–D: aq. NaOH (pH 12.0): 5 min 5% D in E, 25 min 5–30% D in E, 10 min 30–5% D in E with a flow rate of 1.0 ml min^{-1}. The oligomers were desalted and lyophilized on a Speed-Vac evaporator to yield colorless solids. MALDI-TOF mass spectra of modified oligonucleotides are shown in Table 7.

The composition of oligonucleotides was proven by enzymatic hydrolysis: The oligonucleotides were dissolved in 0.1 M Tris-HCl buffer (pH 8.3, 200 μl), and treated with snake-venom phosphodiesterase (3 μl) at 37 °C for 45 min and alkaline phosphatase (3 μl) at 37 °C for another 30 min. The reaction mixtures were analyzed by HPLC (RP-18, at 260 and 280 nm, solvent system A, 0.7 ml min^{-1}).

2-(2-Deoxy-β-D-erythro-pentofuranosyl)-6-{[(dimethylamino)-methylidene]amino}-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-one 9

To a stirred suspension of **1** (200 mg, 0.75 mmol) in MeOH (10 mL) *N*,*N*-dimethylformamide dimethylacetal (1 mL, 7.53 mmol) was added. The mixture was stirred at room temperature for 48 h and evaporated to dryness. The residue was applied to flash chromatography (FC; column 2 × 10 cm, CH_2Cl_2–MeOH 9 : 1 to 8 : 2) and eluted stepwise. Colorless foam of **9** (210 mg, 87 %) (Found: C, 48.36; H, 5.70; N, 26.01. $C_{13}H_{18}N_6O_4$ requires C, 48.44; H, 5.63; N, 26.07%); TLC (CH_2Cl_2–MeOH 8 : 2): R_f 0.77; UV (MeOH): 233 (24300); 278 (13000); 338 (15500). δ_H (250 MHz; d_6-DMSO): 2.21 (1H, m, 2′-H_α), 2.50 (1H, m, 2′-H_β), 3.03, 3.16 (6H, 2m, $2NCH_3$), 3.55 (2H, m, 5′-H_2), 3.85 (1H, m, 4′-H), 4.37 (1H, s, 3′-H), 4.91 (1H, 5′-OH), 5.27 (1H, m, 3′-OH), 6.51 (1H, 't', *J* = 6.0, 1′-H), 8.42 (1H, s, 3-H) and 8.56 (1H, s, HC=N).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-erythro-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-one 10

Compound **9** (210 mg, 0.65 mmol) was dried by repeated co-evaporation from anhydrous pyridine and dissolved in anhydrous pyridine (1.5 mL). The solution was treated with dimethoxytrityl chloride (286 mg, 0.84 mmol) at room temperature under stirring (4.5 h). MeOH (1 mL) was added, and the stirring was continued for 5 min. The mixture was poured into 5% aqueous $NaHCO_3$ solution (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried (Na_2SO_4). After evaporation of the solvent, the residue was applied to FC (silica gel, column 2 × 15 cm), which was pre-washed with CH_2Cl_2 and eluted with CH_2Cl_2–acetone (stepwise elution from 98 : 5 to 8 : 2) to give **10** (270 mg, 66%) as a colorless foam (Found: C, 65.31; H, 5.76; N, 13.28. $C_{34}H_{36}N_6O_6$ requires C, 65.37; H, 5.81; N, 13.45%); TLC (CH_2Cl_2–acetone 9 : 1): R_f 0.60; UV (MeOH): 236 (31200), 319 (44800); δ_H (250 MHz; d_6-DMSO): 2.28 (1H, m, 2′-H_α), 2.67 (1H, m, 2′-H_β), 3.03, 3.16 (6H, m, NCH_3), 3.34 (2H, m, 5′-H_2), 3.69 (3H, m, OCH_3), 3.92 (1H, m, 4′-H), 4.43 (1H, m, 3′-H), 5.31 (1H, d, J = 4.9, 3′-OH), 6.18 (1H, m, 1′-H), 6.74–7.34 (m, arom. H), 8.48 (1H, s, 3-H), 8.66 (1H, s, HC=N) and 10.96 (1H, s, NH).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-6-{[(dimethylamino)methylidene]amino}-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-one 3′-(2-cyanoethyl-*N*, *N*-diisopropyl)phosphoramidite 4

A solution of compound **10** (250 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was preflushed with argon and kept under argon atmosphere. Then, 2-cyanoethyldiisopropylphosphoramidochloridite (130 μL, 0.58 mmol) and *N*,*N*-diisopropylethylamine (130 μL, 0.75 mmol) were added at room temperature. Stirring was continued for 30 min. An aqueous solution of 5% $NaHCO_3$ (10 mL) was added. The mixture was shaken, the layers separated, and the aqueous layer extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4), filtered, evaporated, and applied to FC (silica gel, column 2 × 10 cm, CH_2Cl_2–acetone 8 : 2) yielding a colorless foam (265 mg, 80%). TLC (CH_2Cl_2–acetone 8 : 2): R_f 0.95. ^{31}P NMR ($CDCl_3$): 149.6, 149.9.

4-Amino-1-[2-deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-erythro-pentofuranosyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one 11

200 mg (0.75 mmol) Anh. **6** was dissolved in pyridine (3 ml). 710 mg (4.7 mmol) *tert*-Butyldimethylsilyl chloride and 532 mg (7.81 mmol) imidazole were added and the reaction mixture was stirred at room temperature for 48 h. Purification was performed by flash chromatography (silica gel, column 3 × 15 cm, CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 9 : 1) yielding a colorless solid (210 mg, 57%) (Found: C, 53.41; H, 8.07; N, 14.52. $C_{22}H_{41}N_5O_4Si_2$ requires C, 53.30; H, 8.34; N 14.13%); TLC (CH_2Cl_2–MeOH 9 : 1): R_f 0.50; UV (MeOH): 251 (5300), 290 (4500); δ_H (250 MHz; d_6-DMSO): 0.02 (12H, m, CH_3), 0.86 (18H, m, CH_3), 2.13 (1H, m, 2′-H_α), 2.76 (1H, m, 2′-H_β), 3.63 (2H, m, 5′-H2), 3.76 (1H, m, 4′-H), 4.56 (1H, m, 3′-H), 6.27 (1H, m, 1′-H), 7.92 (2H, s, NH_2), 8.56 (1H, s, 3-H) and 10.83 (1H, s, NH).

4-Amino-3-bromo-1-[2-deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-erythro-pentofuranosyl]-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-one 12

100 mg (0.29 mmol) Anh. 4-amino-3-bromo-1*H*-pyrazolo-[3,4-*d*]pyrimidin-6-one was dissolved in pyridine (3ml). 355 mg (2.4 mmol) *tert*-Butyldimethylsilyl chloride and 266 mg (3.91 mmol) imidazole were added and the reaction mixture was stirred at room temperature for 48 h. Purification was performed by flash chromatography (silica gel, column 3 × 15 cm, CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 9 : 1) yielding a colorless solid (120 mg, 72%) (Found: C, 46.02; H, 7.14; N, 12.10. $C_{22}H_{40}N_5O_4Si_2Br$ requires C, 45.98; H, 7.02; N 12.19%); TLC (CH_2Cl_2–MeOH 9 : 1): R_f 0.70; UV (MeOH): 231 (18500), 296 (2600); δ_H (250 MHz; d_6-DMSO): 0.04 (12H, m, CH_3), 0.86 (18H, m, CH_3), 2.17 (1H, m, 2′-H_α), 2.68 (1H, m, 2′-H_β), 3.58 (2H, m, 5′-H2), 3.75 (1H, m, 4′-H), 4.55 (1H, m, 3′-H), 6.25 (1H, m, 1′-H), 7.62 (2H, s, NH_2) and 10.83 (1H, s, NH).

6-Amino-2-[2-deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-erythro-pentofuranosyl]-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-one 13

150 mg (0.56 mmol) Anh. **1** was dissolved in pyridine (2 ml). 530 mg (3.5 mmol) *tert*-Butyldimethylsilyl chloride and 400 mg (5.9 mmol) imidazole were added and the reaction mixture was stirred at room temperature for 48 h. Purification was performed by flash chromatography (silica gel, column 3 × 15 cm, CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 9 : 1) yielding **13** (180 mg, 65%) as a colorless solid. (Found: C, 53.26; H, 8.28; N, 14.10. $C_{22}H_{41}N_5O_4Si_2$ requires C, 53.30; H, 8.34; N 14.13%); TLC (CH_2Cl_2–MeOH 9 : 1): R_f 0.50; UV (MeOH): 244 sh (6300), 319 (5600); δ_H (250 MHz; d_6-DMSO): 0.04 (12H, m, CH_3), 0.82 (18H, m, CH_3), 2.27 (1H, m, 2′-H_α), 2.64 (1H, m, 2′-H_β), 3.56 (br., 5′-H_2, 4′-H), 4.54 (1H, m, 3′-H), 6.09 (1H, m, 1′-H), 6.23 (2H, s, NH_2), 8.39 (1H, s, 3-H) and 10.96 (1H, s, NH).

6-Amino-1-[2-deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-erythro-pentofuranosyl]-2*H*-pyrazolo[3,4-*d*]pyrimidin-4-one 14

250 mg (0.94 mmol) Anh. **5** was dissolved in pyridine (2.5 ml). 885 mg (5.87 mmol) *tert*-Butyldimethylsilyl chloride and 668 mg (9.8 mmol) imidazole were added and the reaction mixture was stirred at room temperature for 48 h. Purification was performed by flash chromatography (silica gel, column 3 × 15 cm, CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 9 : 1) yielding a colorless solid (230 mg, 50%) (Found: C, 53.26; H, 8.28; N, 14.10. $C_{22}H_{41}N_5O_4Si_2$ requires C, 53.30; H, 8.34; N 14.13%); TLC (CH_2Cl_2–MeOH 9 : 1): R_f 0.50; UV (MeOH): 253 (13300); δ_H (250 MHz; d_6-DMSO): 0.04 (12H, m, CH_3), 0.86 (18H, m, CH_3), 2.18 (1H, m, 2′-H_α), 2.80 (1H, m, 2′-H_β), 3.56 (2H, m, 5′-H_2), 3.97 (1H, m, 4′-H), 4.56 (1H, m, 3′-H), 6.30 (1H, m, 1′-H), 6.71 (2H, s, NH_2), 7.82 (1H, s, 3-H) and 10.60 (1H, s, NH).

Acknowledgements

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