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8-Aza-7-deazapurine–pyrimidine base pairs: the contribution of 2- and 7-substituents to the stability of duplex DNA

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Abstract—The 8-aza-7-deazapurin-2,6-diamine 2′-deoxyribonucleoside (**2a**)–dT base pair is more stable than the 2-amino-2′-deoxyadenosine (**1**)–dT pair. Halogeno or propynyl substituents introduced in the 7-position of the 8-aza-7-deazapurine system (**2b**–**d**) lead to an additional duplex stabilization. Pyrazolo[3,4-*d*]pyrimidine nucleosides related to 2′-deoxyadenosine or 2′-deoxyguanosine show a similar behavior. The base pair stabilization is comparably small when identical substituents are present in the 5-position of 2′-deoxyuridine. The extraordinary stability of the **2b**–**d**–dT base pairs is caused by better proton donor properties of the two amino groups of the 8-aza-7-deazapurin-2,6-diamine moiety and the better base stacking induced by the 7-substituents compared to that of 2-aminoadenine.

1. Introduction

2-Amino-2′-deoxyadenosine (**1**, n^2A_d) (Scheme 1) has attracted much attention because of its capability to form a tridentate base pair with dT. The base pair is expected to show a similar stability as that of dG–dC but retaining the recognition characteristics of the dA–dT system.[1–6] Thus, it was suggested that the n^2A_d–dT base pair makes a similar contribution to the DNA duplex stability as the tridentate dG–dC pair. Surprisingly, the T_m-values of DNAs incorporating compound **1** are not increasing linearly by an increasing number of n^2A residues.[7–13] Also, they are generally lower than expected for a tridendate base pair. This was verified on synthetic polynucleotides as well as on naturally occurring *S*-2*L* DNA. This DNA—containing 70% dG–dC and 30% n^2A_d–dT—shows a similar T_m-value as an oligomer incorporating dA in place of n^2A_d.[3,14–16] According to these discrepancies, the influence of the **1**—dT pair has been studied extensively by CD and NMR spectroscopy as well as by thermodynamic data analysis.[3,11,12,15,17–20]

Our laboratory has reported that the duplexes are significantly more stable when the purin-2, 6-diamine nucleoside **1** is replaced by the 8-aza-7-deazapurin-2, 6-diamine nucleoside **2a** (Scheme 1) (purine numbering is used throughout the discussion).[21] The base pair of **2a** with dT becomes even more stable when halogeno substituents are introduced in position-7. Then, the base pairs between the 8-aza-7-deazapurin-2,6-diamine nucleosides **2b**–**d** with dT approach the stability of the tridentate dG–dC base pair.[21,22] They show a similar base selectivity as dA–dT and do not form stable odd base pairs as it was reported for the pyrrolo[2,3-*d*]pyrimidine analogue of **1**.[23] This manuscript reports on the duplex stability of oligonucleotides incorporating various 8-aza-7-deazapurine 2′-deoxyribonucleosides. The unusual contribution of the 2-amino group of the 8-aza-7-deazapurin-2,6-diamine nucleosides will be discussed by comparing their 7-substituted derivatives and the nucleosides **1** and **3** (Scheme 1).[24] Also, the stabilizing effects of the 7-substituents in the series of nucleosides containing 8-aza-7-deazapurin-2,6-diamine (**2b**–**d**), 8-aza-7-deazaadenine (**4a**–**c**),[25,26] and 8-aza-7-deazaguanine (**5a**–**c**)[26] will be reported. Finally, the behavior of the major groove directed 7-substituents of the 8-aza-7-deazapurine nucleosides will be related to the 5-substituents of the pyrimidine compounds **6a**–**d**.

2. Results and discussion

2.1. Synthesis and properties of the monomers

The phosphoramidites of the nucleosides **2a**–**c** have been described earlier.[21,22] The synthesis of the phosphoramidite of the propynyl compound **2d** is now reported (Scheme 2). The iodo nucleoside **2c** was used as starting material for the synthesis of the propynyl compound which was obtained by the Pd(0)-catalyzed cross-coupling reaction.[27,28] Afterwards, **2d** was protected to give the *N*,*N*′-bis-isobutyryl derivative **7**.[29] Further conversion afforded the DMT

Keywords: pyrazolo[3,4]pyrimidine; purin-2,6-diamine; nucleosides; oligonucleotides; major groove substituents; base pair stability.

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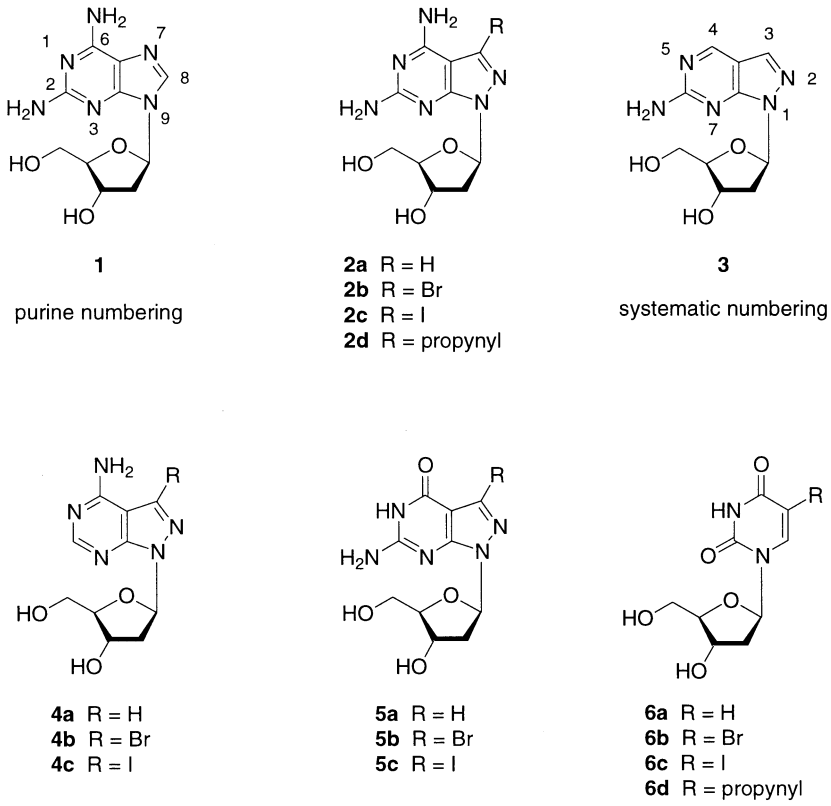
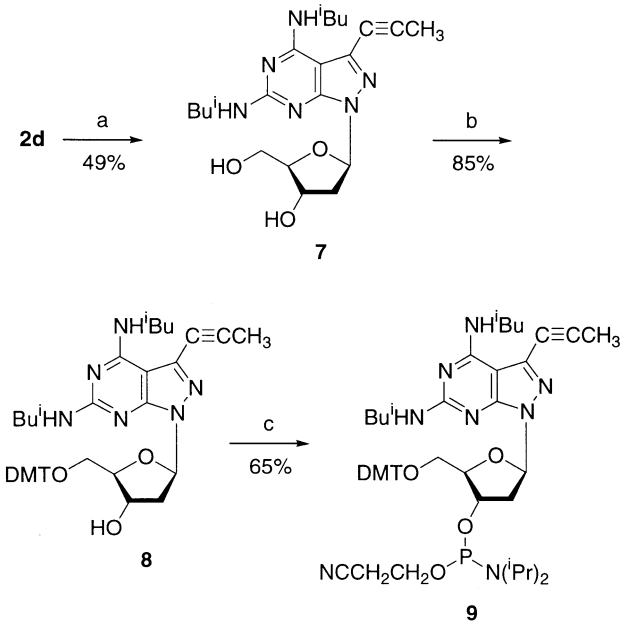


Scheme 1. Nucleosides.

compound **8**.[21] Phosphitylation gave the phosphoramidite **9**, which was then employed in solid-phase oligonucleotide synthesis. The ^{13}C NMR data of the monomers are summarized in Table 1. For other details, see Section 4.



Scheme 2. (a) Me_3SiCl, isobutyric anhydride, pyridine, room temperature, (b) DMT-Cl, pyridine, room temperature, (c) $(NCCH_2CH_2O)(iPr)_2NPCl$, $(iPr)_2EtN$, CH_2Cl_2.

2.2. The stability of duplexes incorporating the base pairs of 2-amino-2′-deoxyadenosine (1) or of the 8-aza-7-deazapurin-2,6-diamine 2′-deoxyribonucleoside (2a) with dT

It has been reported that 2-amino-2′-deoxyadenosine (**1**) contributes very little to the DNA–DNA or DNA–RNA duplex stability.[30] Six incorporations of the nucleoside **1**[21] into the duplex **10**·**11** increase the T_m value by only 3°C (→duplex **12**·**13**) which corresponds to a +0.5°C T_m increase per **1**–dT base pair (Table 2). From this observation, it is obvious that the presumed tridentate base pair **I** (Scheme 3) is rather weak or even not formed. However, when the nitrogen-7 of compound **1** is shifted to position-8 (**2a**), six incorporations of **2a** result in a T_m increase of +10°C, which averages to +1.7°C per **2a**–dT base pair (→duplex **14**·**15**, bp **IIa**, Scheme 3). This indicates that the altered nitrogen pattern of the pyrazolo[3,4-*d*]pyrimidine system causes a duplex stabilization.

In order to elucidate the role of the 2-amino group of the 7-substituted pyrazolo[3,4-*d*]pyrimidin-2,6-diamine 2′-deoxynucleoside **2a**–**d**,[22] the related '2′-deoxyadenosine' derivatives **4a**–**c**[25] lacking the 2-amino group were incorporated in oligonucleotide duplexes at exactly the same positions (Table 2). The replacement of one or several dA-residues by compound **4a** (→duplex **24**·**25**, bp **IVa**, Scheme 4) has no influence on the T_m values even in the case of six incorporations.[26] The T_m value of **24**·**25** is only 3°C lower than that of **12**·**13** with six incorporations of nucleoside **1**, but 10°C lower than that of the duplex **14**·**15**

Table 1. ^{13}C NMR chemical shifts of pyrazolo[3,4-*d*]-pyrimidin-4,6-diamine 2′-deoxyribonucleosides and derivatives[a]

	C(2)[a,b] C(6)[b,c]	C(4)[a,b] C(7a)[b,c]	C(5)[a] C(3a)[c]	C(6)[a,b] C(4)[b,c]	C(7)[a] C(3)[c]	C≡C	CH_3
2a[21]	156.9	158.3	95.5	162.7	133.3		
2c[22]	157.0	157.6	91.2	162.2	98.3		
2d	157.8	156.7	95.6	162.7	127.3	72.0, 91.5	4.2
7	155.5	153.6	104.6	155.5	129.2	72.5, 91.0	4.2
8	153.6	155.5	104.5	157.9	126.4	72.7, 90.8	4.2
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O	
2a[21]	83.3	38.0	71.3	87.4	62.7		
2c[22]	83.1	37.6	70.9	87.3	62.4		
2d	83.0	37.6	71.0	87.3	62.5		
7	83.5	37.5	70.8	87.6	62.5	175.2,176.0	
8	83.5	37.9	70.5	85.5	64.1	175.2,175.8	

Measured in (D_6) DMSO at 298 K.
[a] Purine numbering.
[b] Tentative.
[c] Systematic numbering.

containing **2a**. This means that the interchange of N-7 and C-8 of 2′-deoxyadenosine exerts no influence on the base pair stability of dA–dT pair, a finding which is different from the effect of the 2-amino group-containing nucleosides **1** vs **2a**.

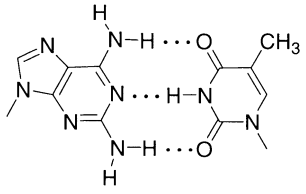
When H-7 of compound **4a** is substituted by a bromo or iodo substituent, the T_m increase of the duplex is in the same range as with **2a** (+18°C for **2b**, and +15°C for **2c** compared to **2a**); the 7-propynyl residue of **2d** increases the T_m value by 18°C (Table 2). The incorporation of **4b** and **4c** enhances the T_m values by 10 and 11°C compared to **4a**. This indicates that the 7-substituents have a positive effect in both series of pyrazolo[3,4-*d*]pyrimidine nucleosides, no matter whether they carry a 2-amino function or not. A similar phenomenon is observed in the series of pyrrolo[2,3-*d*]pyrimidine nucleosides.[31–33] However, a comparison of the two series of oligonucleotide duplexes shows that those containing the 2-amino functionalized nucleosides **2a–c** exhibit 10–18°C higher T_m values than those incorporating compounds **4a–c**. Only a tridentate base pair of **2a–d** with dT can explain this finding, while such a base pair is rather weak in the case of **1**–dT.

In order to evaluate the contribution of the 2-amino group separately, the effect of the nucleoside **3** containing the 2-amino group as the only base substituent was studied (bp **III**, Scheme 4). Here, a T_m decrease of −5°C (**22**·**23**, 42°C) (Table 2) was observed compared to compound **4a** carrying a 6-amino group (**24**·**25**).[26] This phenomenon indicates that the presence of a 2-amino group is sterically unfavorable in compound **3**. The incorporation of a 2-aminopurine instead of an adenine moiety results in only a slight increase in T_m. However, when a **2a**–dT base pair is formed both amino groups of the 8-aza-7-deazapurin-2,6-diamine **2a** are able to form hydrogen bonds not observed in the case of the purin-2,6-diamine nucleoside **1**.

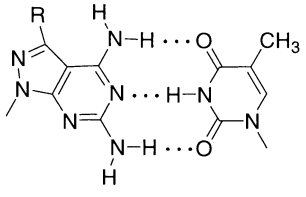
Table 2. T_m values of duplexes containing six modified nucleoside residues (**1–4**)

Duplex	T_m (°C)
5′-d(TAG GTC AAT ACT) (**10**) 3′-d(ATC CAG TTA TGA) (**11**)	47
5′-d(T**1**G GTC **11**T **1**CT) (**12**)[21] 3′-d(ATC C**1**G TT**1** TGA) (**13**)	50
5′-d(T**2a**G GTC **2a2a**T **2a**CT) (**14**) 3′-d(ATC C**2a**G TT**2a** TGA) (**15**)	57
5′-d(T**2b**G GTC **2b2b**T **2b**CT) (**16**)[22] 3′-d(ATC C**2b**G TT**2b** TGA) (**17**)	75
5′-d(T**2c**G GTC **2c2c**T **2c**CT) (**18**) 3′-d(ATC C**2c**GT T**2c** TGA) (**19**)	72
5′-d(T**2d**G GTC **2d2d**T **2d**CT) (**20**) 3′-d(ATCC**2d** G TT**2d** T GA) (**21**)	75
5′-d(T**3**G GTC **33**T **3**CT) (**22**)[25] 3′-d(ATC C**3**G TT**3** TGA) (**23**)	42
5′-d(T**4a**G GTC **4a4a**T **4a**CT) (**24**) 3′-d(ATC C**4a**G TT**4a** TGA) (**25**)	47
5′-d(T**4b**G GTC **4b4b**T **4b**CT) (**26**) 3′-d(ATC C**4b**G TT**4b** TGA) (**27**)	57
5′-d(T**4c**G GTC **4c4c**T **4c**CT) (**28**) 3′-d(ATC C**4c**GT T**4c** TGA) (**29**)	58

Measured at 260 nm in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5+5 μM oligonucleotide concentration.



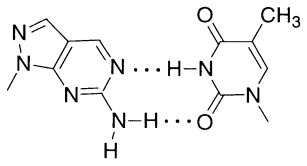
I: **1**-dT



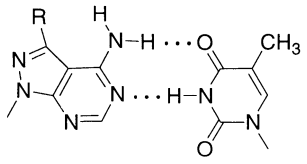
IIa: **2a**-dT
IIb: **2b**-dT
IIc: **2c**-dT
IId: **2d**-dT

a: H; b; Br; c: I; d: Propynyl

Scheme 3. The base pairs **I** and **II**.



III: **3**-dT



IVa: **4a**-dT
IVb: **4b**-dT
IVc: **4c**-dT
a: H; b; Br; c: I.

Scheme 4. The base pairs **III** and **IV**.

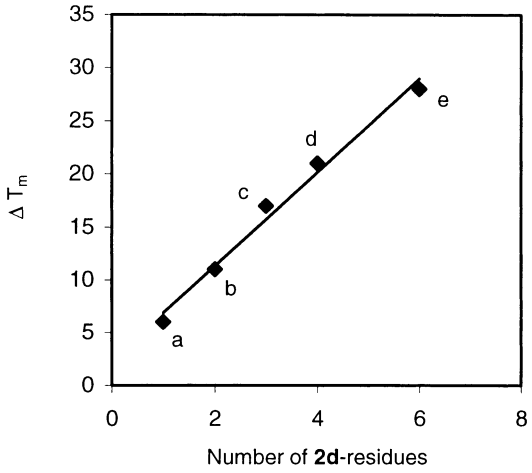
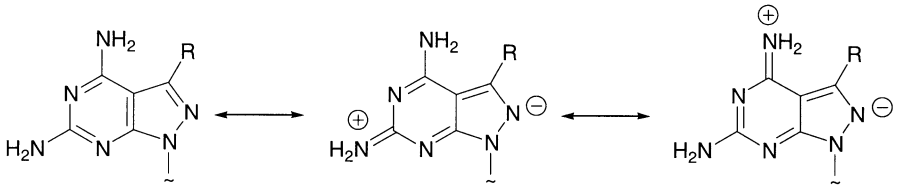


Figure 1. Graph of the ΔT_m vs. the number of nucleoside **2d** incorporations. (a) 5′-d(TAG GTC **2d**AT ACT)·3′-(ATC CAG TTA TGA), (b) 5′-d(TAG GTC AAT ACT)·3′-(ATC C**2d**G TT**2d** TGA), (c) 5′-d(TAG GTC **2d**AT ACT)·3′-(ATC C**2d**G TT**2d** TGA), (d) **35**·**21**, (e) **20**·**21**.

The reason for the different behavior of 2-amino groups present in the purine nucleoside **1** and the pyrazolo[3,4-*d*]-pyrimidine nucleosides **2a**–**d** results from the differences of steric and electronic properties of the nucleobases. The 2-amino group of compound **1** is out of plane of the heterocyclic system.[16] Moreover, this group which is located in the minor groove of B-DNA can cause steric strain onto the helix in the case of multiple incorporations. As a result, the formation of the tridentate base pair is problematic. These unfavorable properties are overcome by the use of the pyrazolo[3,4-*d*]pyrimidine nucleosides **2a**–**d**. The presence of nitrogen-8 allows the formation of mesomeric structures (Scheme 5) increasing the amide character of the two amino groups. As a result, they are now both in plane with the heterocycle and in a more favorable situation for base pairing and/or stacking. The amide character of the amino groups is also in line with the ease of the removal of amino protecting groups (acyl groups). They are much more difficult to be split off from the purin-2,6-diamine nucleoside **1**[21,22] than from **2a**–**d**.[21,22,34,35]

The contribution of the 7-propynyl substituent of **2d** to the duplex stability goes along with the formation of tridentate base pair with dT as found for **2b**, **c** (Table 2).[22] Each incorporation of a **2b**–dT, **2c**–dT or **2d**–dT base pair contributes +3.5°C (bp **IIb**, Scheme 3), +3.8°C (bp **IIc**), or +4°C (bp **IId**) to the T_m increase (six incorporations→ duplexes **16**·**17**, **18**·**19**, and **20**·**21**) (Table 2). The



Scheme 5. Mesomeric structures.

Table 3. T_m values of duplexes containing four **2a**–**d**/dT base pairs in place of dG–dC

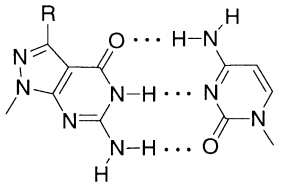
Duplex	T_m (°C)	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol K)	$\Delta G_{310}{}^0$ (kcal/mol)	$\Delta\Delta G_{310}{}^0$ (kcal/mol)
5′-d(TAG GTC **2a2a**T ACT) (**30**)[22] 3′-d(ATC C**2a**G TT**2a** TGA) (**15**)	51	−99	−279	−12.3	
5′-d(TAG GCC **GG**C ACT) (**31**) 3′-d(ATC C**G**G CC**G** TGA) (**32**)	66	n.d.	n.d.	n.d.	
5′-d(TAG GTC **2b2b**T ACT) (**33**) 3′-d(ATC C**2b**G TT**2b** TGA) (**17**)	67	−105	−285	−17.0	−4.7
5′-d(TAG GTC **2c2c**T ACT) (**34**) 3′-d(ATC C**2c**GT T**2c** TGA) (**19**)	66	−104	−285	−16.6	−4.3
5′-d(TAG GTC **2d2d**T ACT) (**35**) 3′-d(ATC C**2d**G TT**2d** TGA) (**21**)	68	−111	−300	−18.0	−5.7

Conditions see Table 2. Thermodynamic parameters are calculated from the program Meltwin 3.0; the standard error for ΔH^0 and ΔS^0 is 15%; n.d.=not detected.

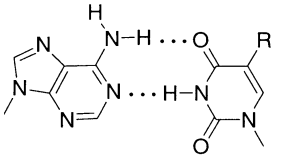
Table 4. T_m values of duplex formation of oligonucleotides containing the **5a**–**c**–dC base pairs

Duplex	T_m (°C)
5′-d(TA**G** **G**TC AAT ACT) (**10**) 3′-d(ATC CA**G** TTA T**G**A) (**11**)	47
5′-d(TA**5a** **5a**TC AAT ACT) (**36**)[25] 3′-d(ATC CA**5a** TTA T**5a**A) (**37**)	50
5′-d(TA**5b** **5b**TC AAT ACT) (**38**) 3′-d(ATC CA**5b** TTA T**5b**A) (**39**)	55
5′-d(TA**5c** **5c**TC AAT ACT) (**40**) 3′-d(ATC CA**5c** TTA T**5c**A) (**41**)	54

Conditions see Table 2.



Va: 5a-dC
Vb: **5b**-dC
Vc: **5c**-dC



VIa: dA-**6a**
VIb: dA-**6b**
VIc: dA-**6c**
VId: dA-**6d**

a: H; b; Br; c: I; d: Propynyl

Scheme 6. The base pairs **V** and **VI**.

replacement of four dA–dT pairs of the duplex **10**·**11** by either four **2b**–dT, four **2c**–dT or four **2d**–dT pairs (duplexes **33**·**17**, **34**·**19**, **35**·**21**, bp **IIb**–**d**, Scheme 3) enhances the T_m values to that of the duplex **31**·**32** containing eight tridentate dG–dC pair (Table 3). In other words, these pyrazolo-[3,4-*d*]pyrimidine–dT base pairs reach the stability of the dG–dC pair.[19] Also, a linear relationship between the number of **2d**-incorporations vs the ΔT_m is observed as it is found for dG–dC (Fig. 1).

2.3. The stability of the base pairs between 8-aza-7-deaza-2′-deoxyguanosine (5a) and its 7-substituted derivatives 5b–c with dC

The stabilizing effects of the 8-aza-7-deazapurine nucleosides **2a**–**d** and **4a**–**c** prompted us to undertake similar studies on duplexes containing the 7-substituted pyrazolo[3,4-*d*]pyrimidine analogues of 2′-deoxyguanosine (**5a**–**c**).[25] The T_m values of duplex melting are listed in Table 4. Contrary to the **4a**–dT base pair, the introduction of the **5a**–dC base pair (bp **Va**–**c** in Scheme 6) has a positive influence on the duplex stability (**→36**·**37**) when compared to that of dG–dC. An averaged T_m increase of +0.75°C per base pair modification is observed (Table 4). This means that the shift of nitrogen-7 to position-8 strengthens the tridentate base pairs as it is observed for the **2a**–dT pair. Apparently, in both cases, the 2-amino group becomes a better proton donor thereby increasing the base pair stability.

2.4. The duplex stability increase induced by the pyrimidine nucleosides 6a–d vs the 'purine' nucleoside 4a–c

The substituents at the 5-position of pyrimidine nucleosides **6a**–**d** protrude into the major groove of B-DNA as it is found for the 7-substituents of the 8-aza-7-deazapurine nucleosides **4a**–**c** (bp **VIa**–**d** vs **IVa**–**c**, Schemes 4 and 6). Thus, it was expected that the hydrophobic character of the major groove would be increased in both series of compounds. However, the T_m data of Table 5 clearly show that the T_m increase caused by one incorporation is significantly smaller for **6a**–**c** (0–0.75°C) than for **4a**–**c** (0.75–1.75°C).[36,37] Therefore, other factors have to be taken into account. From the shape of the nucleobases it is obvious that the overlap of a 'purine' moiety is larger than for a pyrimidine base. Moreover, it was shown that the polarizibility[38] is related to the T_m-increase. The polarizabilities of the 5-substituted pyrimidine bases are 9.91×10^{-24} for **6a**, 12.99×10^{-24} cm^3 for **6b**, 15.32×10^{-24} cm^3 for **6c**, 14.92×10^{-24} cm^3 for **6d**,[39] while those values for the 7-substituted dA-derivatives are 14.68×10^{-24} cm^3 for **4a**, 17.73×10^{-24} cm^3 for **4b**, and 19.79×10^{-24} cm^3 for **4c**. The corresponding values for the diamino compounds **2a**–**d** are 16.36×10^{-24} cm^3 for **2a**, 19.41×10^{-24} cm^3 for **2b**, 21.47×10^{-24} cm^3 for **2c** and 20.12×10^{-24} cm^3 for **2d**). This indicates that a high polarizibility goes along with a high T_m-value. Apart from that, the substitution at the 7-position of the nucleobases induces a stereoelectronic effect on the sugar moiety which can alter its conformation, making the

Table 5. T_m values of duplex formulation of oligonucleotides containing nucleosides **6a**–**d**

Duplex	T_m (°C)	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol K)	$\Delta G_{310}{}^0$ (kcal/mol)	$\Delta\Delta G_{310}{}^0$ (kcal/mol)
5′-d(TAG G**6a**C AA**6a** ACT) (**42**)[22] 3′-d(ATC CAG **6a6a**A TGA) (**43**)	46	−86	−245	−10.3	
5′-d(TAG G**6b**C AA**6b** ACT) (**44**) 3′-d(ATC CAG **6b6b**A TGA) (**45**)	49	−94	−268	−11.3	−1.0
5′-d(TAG G**6c**C AA**6c** ACT) (**46**) 3′-d(ATC CAG **6c6c**A TGA) (**47**)	50	−99	−282	−11.9	−1.6
5′-d(TAG G**6d**C AA**6d** ACT) (**48**) 3′-d(ATC CAG **6d6d**A TGA) (**49**)	52	−89	−247	−12.0	−1.7

Conditions see Table 2.

nucleosides more or less favorable for base stacking interactions. The high-anti conformation of the pyrazolo[3,4-*d*]-pyrimidine nucleosides demonstrated by X-ray data analyses[40] might also play a role in the stability of those base pairs. However, it is yet not known how these phenomena effect base stacking in detail.[41]

Next, the thermodynamic data (ΔH^0, ΔS^0, ΔG^0) of the duplex formation were calculated using the program Meltwin 3.0.[42] The free energies correspond to the T_m-values. The entropy change is always unfavorable during duplex formation. By comparing the thermodynamic parameters of duplexes formed by the halogenated nucleosides **2b**, **c** with that of the propynyl compound **2d**, the latter shows a more favorable enthalpy change. This might result from the more lipophilic character of the propynyl group and/or the conjugation of the triple bond with the heterocyclic base. The latter will enlarge the total surface area of the heterocycle. This mesomeric effect is not possible with the bromo or iodo substituents.

3. Conclusion

The replacement of the purine nucleoside 2-amino-2′-deoxyadenosine (**1**) by the pyrazolo[3,4-*d*]pyrimidine analogues **2a**–**d** increases the dA–dT base pair stability extraordinarily because of the formation of tridentate base pairs and the influence of the 7-substituents. The effect of stabilization is not observed for the base pair of 2-amino-2′-deoxyadenosine (**1**) with dT. The stabilizing effects of the 7-substituents do not depend on the particular Watson–Crick recognition site of the base. The tridentate base pairs **2b**–**d**–dT reach the stability of the tridentate dG–dC pair. The contribution of identical 5-substituents of 2′-deoxyuridine residues on the base pair stability is comparably small or can even be neglected.

4. Experimental

4.1. General

Monomers. Thin-layer chromatography (TLC) was performed on aluminum sheets, silica gel 60 F_{254} (0.2 mm, Merck, Germany). Flash chromatography (FC) was carried out (0.4 bar) on silica gel 60 H (Merck, Darmstadt, Germany). NMR Spectra were measured on an Avance -DPX-250 spectrometer (Bruker, Germany), at 250.13 MHz for ^{1}H and 125.13 MHz for ^{13}C, δ values are in ppm relative to internal $SiMe_4$ (^{1}H, ^{13}C) or external H_3PO_4. Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). Chemicals were purchased from ACROS, Fluka, or Sigma-Aldrich. Solvents of technical grade were distilled before use.

Oligonucleotides. The oligonucleotides were synthesized on an ABI 392-08 synthesizer in the trityl-on mode. The syntheses followed the standard protocol. Complete deprotection was conducted in 25% aq. NH_3 for 12–18 h at 60°C. After deprotection the DMT-containing oligonucleotides were purified by reversed-phase HPLC (RP-18); the gradients are: 3 min 20% B in A, 12 min 20–40% B in A with a flow rate of 1.0 ml/min. (*A*) 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; (*B*) MeCN. Then the mixture was evaporated to dryness and the residue was treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ 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. The oligomers were desalted (RP-18, silica gel) and lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at −24°.

The oligonucleotides were characterized by: (i) enzymatic hydrolysis as described[21] with snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamenteus*) and alkaline phosphatase (EC 3.1.3.1, *E. coli* from Roche Diagnostics GmbH, Germany) and (ii) by MALDI-TOF-spectra measured on a Biflex III spectrometer (Bruker Saxonia, Leipzig, Germany). The melting temperatures were measured with a Cary-1/3 UV/VIS spectrophotometer (Varian, Australia) equipped with a Cary thermo electrical controller.

4.1.1. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-propynyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-diamine (2d). To a solution of compound **2c** (0.6 g, 1.54 mmol)[22] in anhydrous DMF (3.2 ml) which was kept under Ar atmosphere. CuI (66 mg, 0.36 mmol), tetrakis(triphenyl-phosphine)palladium(0) (207 mg, 0.29 mmol), and anhydrous Et_3N (0.77 ml, 5.5 mmol) were added. The reaction mixture was cooled (ice-water bath) and saturated with propyne (30 min). After stirring for 24 h at room temperature, a second portion of the reagents (same amounts) was added to the mixture. The propyne treatment was repeated for another 30 min under cooling and the reaction was kept for another 24 h at room temperature. Then, the mixture was adsorbed on silica gel (25 g) and applied to FC (CH_2Cl_2/MeOH 20:1). A white solid (0.43 g, 92%) was obtained after evaporation. R_f (CH_2Cl_2/MeOH 9:1) 0.38. UV (MeOH): λ_{max} 269 (12,000), 289 (8400). ^{1}H NMR ((D$_6$)DMSO), 2.12 (s, CH_3); 2.14, 2.67 (2m, H_2–C(2′)); 3.43 (m, H_2–C(5′)); 3.76 (m, H–C(4′)); 4.36 (m, H–C(3′)); 4.77 (t, J=5.7 Hz, OH–C(5′)); 5.20 (d, J=4.3 Hz, OH–C(3′)); 6.28 (s, NH_2); 6.32 (t, J=6.5 Hz, H–C(1′)). Anal. calcd for $C_{13}H_{16}N_6O_3$ (304.21): C 51.32, H 5.30, N 27.63; found: C 51.28, H 5.16, N 27.55.

4.1.2. 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4,6-diisobutyrylamino-3-propynyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (7). Compound **2d** (0.64 g, 2.1 mmol) was co-evaporated three times with anhydrous pyridine and then dissolved in anhydrous pyridine (10 ml), followed by addition of Me_3SiCl (1.34 ml, 10.6 mmol). After 15 min, isobutyric anhydride (1.75 ml, 7.5 mmol) was added to the solution. After 3 h the reaction mixture was cooled in an ice-bath and diluted with H_2O (5.7 ml); 5 min later aq. ammonia (25%, 5.7 ml) was added and the stirring was continued for another 30 min. The reaction mixture was evaporated to dryness, the residue was purified by FC (CH_2Cl_2/MeOH 20:1) furnishing a colorless solid (0.46 g, 49%); R_f (CH_2Cl_2/MeOH 9:1) 0.40. UV (MeOH): λ_{max} 241 (32,200), 280 (13,500). ^{1}H NMR ((D$_6$)DMSO), 1.14 (m, CH(CH_3)$_2$); 2.07 (s, CH_3); 2.28, 2.76 (2m, H_2–C(2′)); 2.85 (m, CH(CH$_3$)$_2$); 3.48 (m, H_2–C(5′)); 3.82 (m, H–C(4′)); 4.42 (m, H–C(3′)); 4.72

(m, OH–C(5′)); 5.31 (d, J=4.3 Hz, OH–C(3′)); 6.56 (t, J=6.4 Hz, H–C(1′)); 10.49, 10.60 (2s, NHCO). Anal. calcd for $C_{21}H_{28}N_6O_5$ (444.39): C 56.76, H 6.31, N 18.92; found: C 56.66, H 6.46, N 18.82.

4.1.3. 1-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4,6-diisobutyrylamino-3-propynyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (8). Compound **7** (0.42 g, 0.94 mmol) was co-evaporated with anhydrous pyridine (three times) and then dissolved in pyridine (1.5 ml). To this solution DMT-Cl (0.48 g, 1.41 mmol) was added, and the mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of MeOH, and the mixture was evaporated to dryness. It was dissolved in CH_2Cl_2 (5 ml) and subjected to FC (CH_2Cl_2/MeOH 20:1) to give a colorless foam (0.6 g, 85%). R_f (CH_2Cl_2/MeOH 9:1) 0.7. UV (MeOH): λ_{max} 237 (48,800), 282 (13,100). ^{1}H NMR ((D$_6$)DMSO), 1.15 (m, CH(CH_3)$_2$); 2.07 (s, CH_3); 2.30, 2.77 (2m, H_2–C(2′)); 2.85–3.06 (m, CH(CH$_3$)$_2$, H_2–C(5′)); 3.71 (s, CH_3O); 3.93 (m, H–C(4′)); 4.49 (m, H–C(3′)); 5.34 (d, J=4.7 Hz, OH–C(3′)); 6.57 (m, H–C(1′)); 6.75–7.33 (m, arom. H); 10.45, 10.63 (s, NHCO). Anal. calcd for $C_{42}H_{46}N_6O_7$ (746.23): C 67.56, H 6.17, N 11.26; found: C 67.64, H 6.30, N 10.96.

4.1.4. 1-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4,6-diisobutyrylamino-3-propynyl-1*H*-pyrazolo[3,4-*d*]pyrimidine 3′-[(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (9). Compound **8** (0.57 g, 0.76 mmol) dissolved in anhydrous CH_2Cl_2 (20 ml) under Ar atmosphere was reacted with the 2-cyanoethyl diisopropylphosphoramidochloridite (0.56 ml, 2.4 mmol) in the presence of (iPr)$_2$NEt (0.56 ml, 0.56 mmol) at room temperature. An hour later, the reaction mixture was diluted with CH_2Cl_2, and the solution was washed twice with a 5% aq. $NaHCO_3$ solution, followed by brine. The organic solution was dried (Na_2SO_4), concentrated, and the residue was submitted to FC (CH_2Cl_2/acetone 85:15) yielding a colorless foam (0.45 g, 64.7%). R_f (E): 0.44, 0.52. ^{31}P NMR ($CDCl_3$): 149.77, 149.84. ^{1}H NMR ((D$_6$)DMSO), 1.29 (m, CH(CH_3)$_2$); 2.24 (s, CH_3); 2.49, 2.69 (2m, H_2–C(2′)); 3.05–3.80 (m, CH(CH$_3$)$_2$; H_2–C(5′)); 3.79 (s, CH_3O); 4.23 (m, H–C(4′)); 4.82 (m, H–C(3′)); 6.69 (m, H–C(1′)); 6.75–7.42 (m, arom. H); 8.27, 8.65 (2s, NHCO).

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