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Stabilization of tandem dG–dA base pairs in DNA-hairpins: replacement of the canonical bases by 7-deaza-7-propynylpurines†

Frank Seela,*[a,b] Simone Budow,[a,b] Khalil I. Shaikh[a,b] and Anup M. Jawalekar[a,b]

[a] *Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastraße 7, 49069 Osnabrück, Germany*

[b] *Center for Nanotechnology, Gievenbecker Weg 11, 48149 Münster, Germany. E-mail: Frank.Seela@uni-osnabrueck.de; Web: http;//www.seela.net; Fax: +49-541-9692370; Tel: +49-541-9692791*

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The stabilizing effect of 7-propynylated 7-deazapurine nucleosides on DNA-hairpins and DNA-duplexes containing d(GA) mismatches was investigated. The corresponding oligonucleotides were synthesized using solid-phase synthesis. For this purpose, the phosphoramidite of 7-deaza-7-propynyl-2′-deoxyadenosine (**3c**) was prepared. The incorporation of **3c** instead of dA into the tandem d(GA) base pair of a DNA-hairpin alters the secondary structure, but has a positive effect on the duplex stability. A complete replacement of the canonical nucleosides of the tandem d(GA) base pair by **3c** and 7-deaza-7-propynyl-2′-deoxyguanosine results in a significant base pair stabilization.

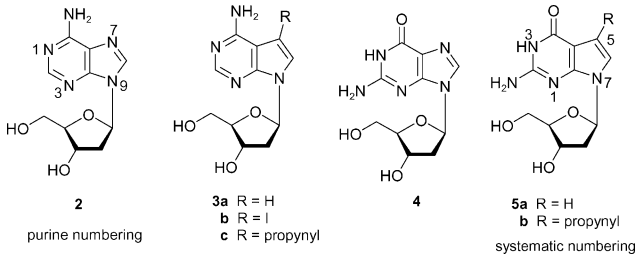
Introduction

The 7-substituted 7-deazapurines are well known as stabilizers of DNA duplexes in comparison to purines. This stabilizing effect has been reported for alkyl, alkynyl and halogeno substituents.[1–5] In this context, the propynyl group deserves particular attention. The propynyl modified nucleosides have a positive effect on DNA duplex stability due to their increased stacking interactions and hydrophobic character.[3–4] However, it is not known whether this is also the case for other base pair motifs which do not follow the Watson–Crick pairing mode. The d(GA) mismatch is a particularly interesting example of one not showing the Watson–Crick motif. It is less efficiently recognized and repaired by the cellular enzymatic repair system than other mismatches.[6] Among the possible mismatches, the tandem d(GA) mispair exhibits a particularly high stability, depending on the sequence context.[7–10] A further stabilization of this unique unit to the level of the canonical base pairs would offer an opportunity to add new letters to the genetic alphabet and to expand the genetic code.

Recent studies on the single stranded oligonucleotide 3′-d(ATCCAGGGATGAATCCAGGGATGA) (**1**) led us to a hairpin structure (Fig. 1) which was confirmed by individual melting experiments showing that its T_m value (70 °C) is independent of concentration. This DNA-hairpin contains three different d(GA) motifs. (i) It forms a d(GA)-overhang at the 5′-terminus, (ii) it possesses an unpaired d(GA) unit in the loop and (iii) it forms adjacent dG–dA pairs of the 5′-purine-GA-pyrimidine-3′ type in the stem region.

Fig. 1 Structure of hairpin **1**.

It has been reported previously that the incorporation of 2′-deoxyinosine in place of 2′-deoxyguanosine within the 5′-pyrimidine-GA-purine-3′ sequence results in a significant base pair destabilization. This is due to the absence of the 2-amino group involved in base pairing.[7,11–12] However, to the best of our knowledge, nothing is known on the stabilization of the tandem dG–dA base pair. The propynyl group introduced in the 7-position of 7-deazapurine 2′-deoxyribonucleosides is well accommodated in the major groove of DNA.[3–4] Therefore it was decided to use this phenomenon, which has been successfully applied to Watson–Crick base pair stabilization, to stabilize adjacent dG–dA base pairs. In this manuscript we report on the thermal stability of modified hairpins derived from the oligonucleotide **1**. For this purpose, the nucleosides **3a–c** and **5a,b** were used as substitutes for compounds **2** or **4** (Scheme 1) in the sequence motif of the tandem dG–dA base pair. For comparison, related duplex structures were also investigated.



Scheme 1 Structures of 2′-deoxyribonucleosides incorporated into tandem dG–dA base pairs.

Results and discussion

UV melting experiments and high-field NMR studies indicate that tandem dA–dG base pairs embedded in particular sequence motifs show the same stability as the canonical dA–dT base pair.[12] Similar observations have been made in the case of the tandem dA–dG mispair of hairpin **1** (Table 1). Several motifs have been described for the tandem d(GA) moiety which are different from the well established dA–dT and dC–dG Watson–Crick base pair motifs. The base pair mode for adjacent dA–dG base pairs strongly depends on the base stacking environment. It was reported that the 5′-pyrimidine-GA-purine-3′ unit adopts the sheared base pair motif (**III**) (Scheme 6) in DNA duplexes, while 5′-purine-GA-pyrimidine-3′ sequences adopt a dG(*anti*)–dA(*anti*) conformation (**I**).[8–9,11–13] In order to investigate the stability of hairpins derived from the structure **1**, a series of oligonucleotides were prepared containing 7-deazapurines instead of purines. For this purpose the phosphoramidites **6a–c**

† Electronic supplementary information (ESI) available: Molecular weights of selected oligonucleotides determined by MALDI-TOF mass spectrometry. See DOI: 10.1039/b510444k

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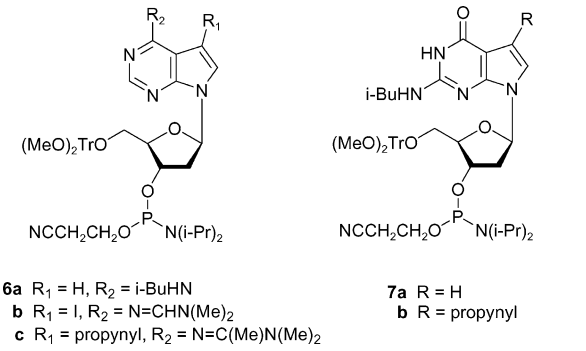
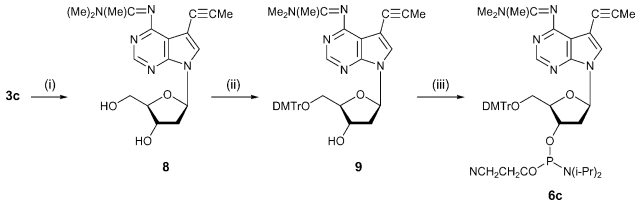
and **7a,b** were employed in solid-phase synthesis (Scheme 2). Compounds **6a,b** and **7a,b** were already known.[1,4,14] The phosphoramidite **6c** was synthesized from **3c** using 7-deaza-7-iodo-2′-deoxyadenosine as the starting material. Compound **3b** was converted to 7-deaza-7-propynyl-2′-deoxyadenosine *via* the Pd-catalyzed cross-coupling reaction as described earlier.[2] The resulting **3c** was protected with the dimethylaminomethylidene group furnishing the amidine **8** (94% yield). Subsequent treatment of **8** with 4,4-dimethoxytrityl chloride in pyridine gave the DMT-derivative **9** (64% yield). Phosphitylation of **9** with 2-cyanoethyl-diisopropylphosphoramido chloridite afforded the phosphoramidite **6c** (86% yield) (Scheme 3). The monomers were characterized by ^{1}H- and ^{13}C-NMR spectroscopy. For details see the Experimental section and Table 4.

Next, a series of oligonucleotides was synthesized, shown in Table 1–3. The oligonucleotides were deprotected and purified by HPLC following the reported procedures.[1,14–15] They were characterized by MALDI-TOF mass spectra (see the electronic supplementary information (ESI)†). The thermodynamic stability of the DNA-duplexes and hairpin **1** and of the nucleobase modified hairpins, shown in Table 1–3, was determined by temperature-dependent UV-melting profiles using curve shape fitting analysis according to McDowell and Turner (MeltWin 3.0).[16] For all the hairpins, the T_m values were found to be independent of the oligonucleotide concentration, confirming intramolecular base pair formation. The composition of hairpin **1** and oligonucleotide **23** was also confirmed by enzymatic hydrolysis using snake venom phosphodiesterase followed by alkaline phosphatase. According to the HPLC profile shown in Fig. 2 the composition analysis is in line with the calculated values.



Scheme 2 Structure of the phosphoramidites **6a–c** and **7a,b**.



Scheme 3 (i) *N,N*-dimethylacetamide dimethylacetal, MeOH, 60 °C, 5 h. (ii) $(OMe)_2$Tr–Cl, pyridine, rt, 30 min. (iii) 2-cyanoethyl-diisopropylphosphoramido chloridite.

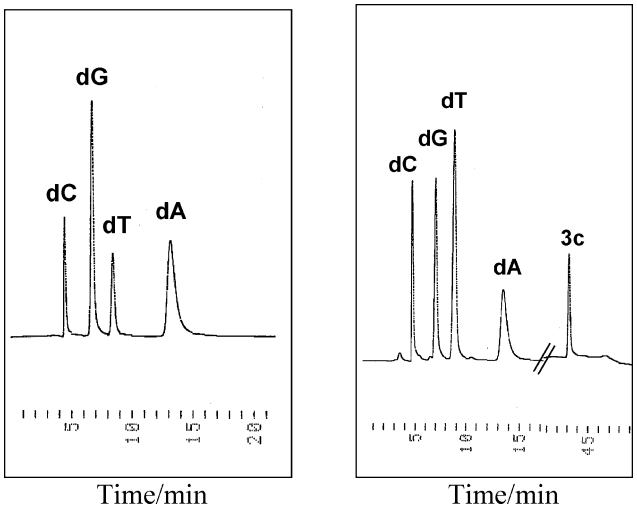


Fig. 2 HPLC profile of the enzymatic analysis of hairpin **1** (left) and oligonucleotide **23** (right) incorporating **3c** by phosphodiesterase followed by alkaline phosphatase in 0.1 M Tris-HCl buffer (pH 8.3) at 37 °C.

While the propynyl group is expected to increase duplex stability, the compounds not carrying 7-substituents would

Table 1 T_m-values and thermodynamic data of hairpin forming oligonucleotides[a]

Sequence	T_m/°C	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1}K^{-1}	$\Delta G°_{310}$/kcal mol^{-1}
A A T C C **A** **G** G G A T G A–5′ G T A G G **G** **A** C C T A –3′ (**1**)	70	−71	−206	−7.0
A A T C C **G** **A** G G A T G A–5′ G T A G G **A** **G** C C T A –3′ (**10**)	58	−55	−166	−3.5
A A T C C **A** **G** G G A T–5′ G T A G G **G** **A** C C T A –3′ (**11**)	71	−76	−219	−7.5
A A T C C **A** **T** G G A T G A–5′ G T A G G **T** **A** C C T A –3′ (**12**)	70	−71	−207	−7.1
A A T C C **C** **G** G G A T G A–5′ G T A G G **G** **C** C C T A –3′ (**13**)	82	−99	−280	−12.5

[a] Measured at 260 nm in 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate buffer, pH = 7.0 with 2.5 μM single-strand concentration.

Table 2 T_m-values and thermodynamic data of hairpins containing modified nucleobases[a]

Sequence	T_m/°C	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1}K^{-1}	$\Delta G_{310}°$/kcal mol^{-1}
A A T C C **X** **Y** G G A T G A−5′ G T A G G **Y** **X** C C T A −3′				
X = **3a**, Y = G (**14**)	70	−71	−206	−7.0
X = **3b**, Y = G (**15**)	76	−75	−214	−8.4
X = **3c**, Y = G (**16**)	78	−75	−215	−8.6
X = A, Y = **5a** (**17**)	68	−64	−188	−6.0
X = A, Y = **5b** (**18**)	72	−68	−196	−7.2
X = **3c**, Y = **5b** (**19**)	80	−83	−237	−9.6

[a] Measured at 260 nm in 100 mM NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate buffer, pH = 7.0 with 2.5 μM single-strand concentration.

Table 3 T_m values and thermodynamic data of oligonucleotides containing 7-propynyl-7-deaza-2′-deoxyadenosine (**3c**)

Duplexes	T_m/°C	$\Delta H°$/kcal mol^{-1}	$\Delta S°$/cal mol^{-1} K^{-1}	$\Delta G_{310}°$/kcal mol^{-1}
5′-d(TAG GTC AAT ACT) (**20**) 3′-d(ATC CAG TTA TGA) (**21**)	47	−89	−253	−10.9
5′-d(TAG GTC **3c**AT ACT) (**22**) 3′-d(ATC CAG TTA TGA) (**21**)	49	−93	−262	−11.3
5′-d(TAG GTC AAT ACT) (**20**) 3′-d(ATC C**3c**G TT**3c** TGA) (**23**)	51	−91	−255	−11.8
5′-d(TAG GTC **3c**AT ACT) (**22**) 3′-d(ATC C**3c**G TT**3c** TGA) (**23**)	53	−82	−227	−11.8
5′-d(TAG G**G**C AAT ACT) (**24**) 3′-d(ATC C**A**G TTA TGA) (**21**)	45	−90	−257	−10.0
5′-d(TAG G**G**C AAT ACT) (**24**) 3′-d(ATC C**3c**G TT**3c** TGA) (**23**)	50	−100	−286	−11.6

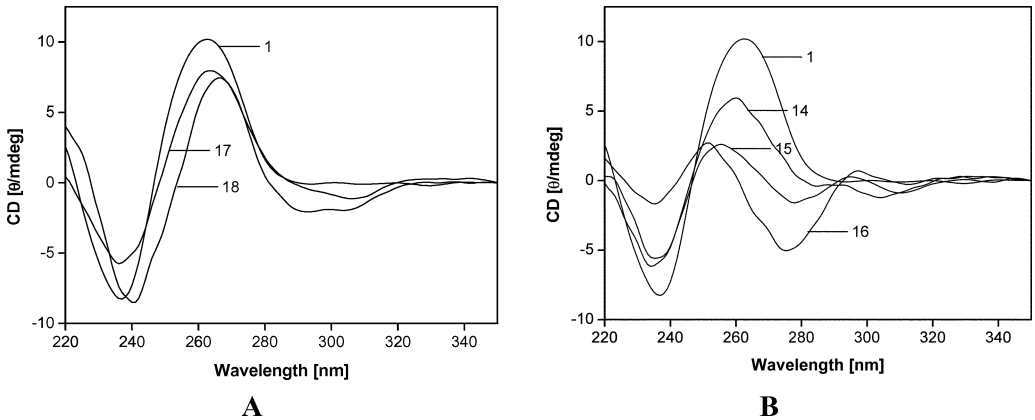
[a] Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$ and 10 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration.

prove whether Hoogsteen interactions are involved during the formation of dG–dA pairs. For these experiments the hairpin **1** was chosen. The hairpin **1**, containing adjacent 5′-d(GA) base pairs (Table 1) within the stem region, showed a T_m of 70 °C. Changing the sequence from 5′-d(GA) to 5′-d(AG) at the same position leads to hairpin **10** with a much lower T_m (58 °C) (hairpin **10** *vs* **1**). This indicates the importance of the sequence order of this structural unit and supports earlier findings observed on duplex DNA.[8] The 5′-dG–dA overhang of hairpin **1** does not stabilize the structure significantly. Its removal did not affect the T_m value (hairpin **11**). The replacement of the two dG–dA base pairs by two dA–dT pairs resulted in identical T_m values (hairpin **12**), while the incorporation of two tridendate dG–dC-pairs increased the hairpin stability significantly (hairpin **13**). As expected, the substitution of the dA residue by the corresponding 7-deaza-2′-deoxyadenosine nucleoside **3a** has almost no effect on the hairpin stability while the replacement of the dG residue by 7-deaza-2′-deoxyguanosine **5a** reduces the tandem base pair stability (Table 2). Such a destabilization has also been observed in the case of dG–dC base pairs in which the guanine moiety was replaced by 7-deazaguanine.[14]

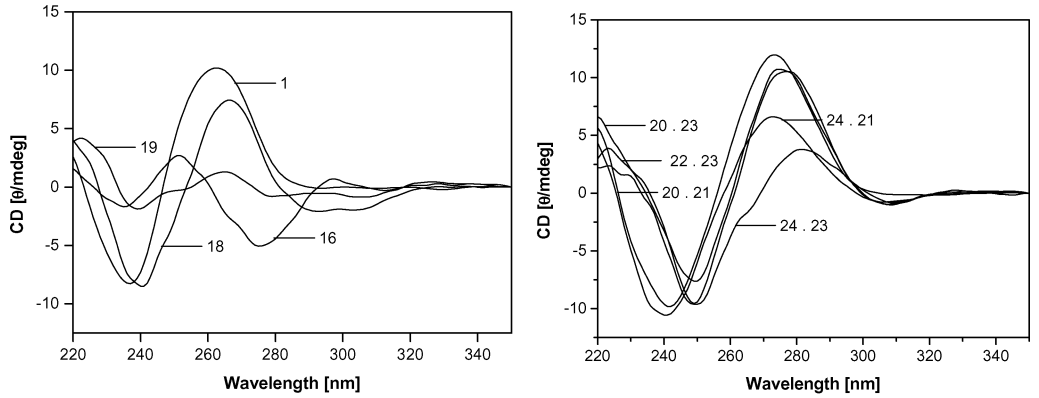
It is expected that 7-substituents of moderate size can stabilize the d(GA) pair. Consequently, the purine residues of the d(GA) pairs were replaced by 7-substituted 7-deazapurines and compared to the corresponding nucleosides, **3a** and **5a**, lacking 7-functionalization. According to the data shown in Table 2, 2′-deoxyadenosine was substituted by compounds **3b** and **3c**. The resulting hairpins **15** and **16** were significantly more stable than the parent hairpins **1** or **14**. The introduction of 7-deaza-7-propynyl-2′-deoxyguanosine (**5b**) instead of dG led to the stabilized hairpin **18** (72 °C of **18** *vs* 68 °C of **17** and 70 °C of **1**). Finally, all four bases of the tandem d(GA) pair were substituted by the propynyl nucleosides **3c** and **5b** in which the average T_m increase was 5 °C for two propynyl modifications per d(GA) pair.

In order to obtain more information regarding the structural influence of the 7-propynyl group in the DNA hairpin structure, circular dichroism (CD) spectra of various hairpins were measured (Schemes 4 and 5).

The unmodified hairpin **1** has a positive lobe at around 265 nm and a negative lobe at around 240 nm. For the modified hairpins **17** and **18** incorporating **5a** and **5b**, only minor changes in the CD-spectrum can be observed, indicating that the propynyl group of 7-deaza-2′-deoxyguanosine fits well into the structural unit of the tandem d(GA) base pair.



Scheme 4 CD-Spectra of modified hairpins incorporating 7-deaza-2′-deoxyguanosine (**A**) and 7-deaza-2′-deoxyadenosine (**B**) analogues (buffer conditions see Table 1).



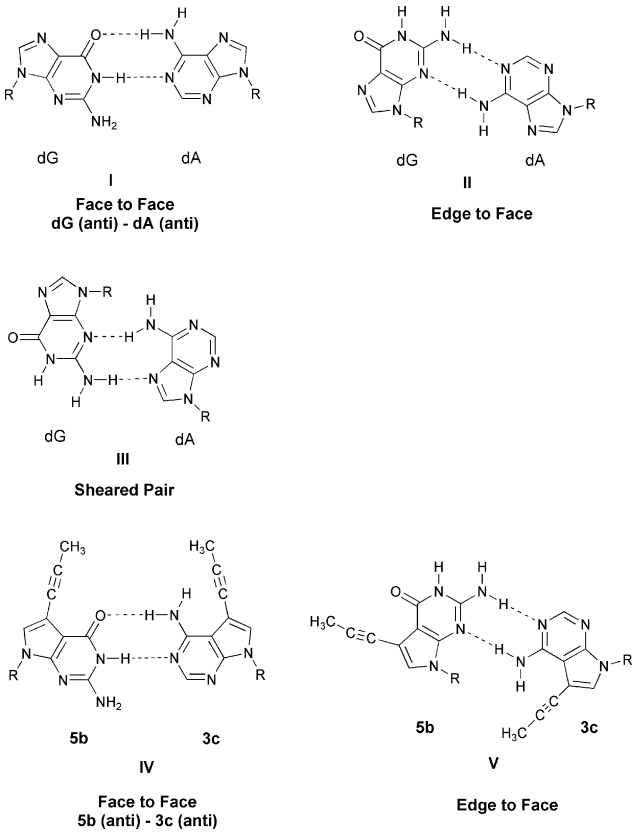
Scheme 5 CD-spectra of 7-propynyl modified hairpins (**A**) and duplexes (**B**) (buffer conditions see Table 1).

The modified hairpins incorporating 7-deaza-2′-deoxyadenosine (**3a**) and their 7-modified analogues (**3b,c**) within the tandem d(GA) unit exhibit CD spectra that look different from the spectra mentioned above. The changes in the CD spectra reflecting structural alterations on the DNA-helices are more pronounced for the 7-substituted 7-deazapurines than for the non-functionalized nucleobases. 7-Deaza-7-propynyl-2′-deoxyadenosine (**3c**) changes the overall B-DNA type conformation. The spectrum adopts a similar shape to that reported for modified single-stranded homooligonucleotides incorporating 8-aza-7-deaza-7-(hex-1-ynyl)-2′-deoxyadenosine residues.[17] In hairpin **19**, the strong influence of 7-deaza-7-propynyl-2′-deoxyadenosine is less pronounced when both **3c** and **5b** substitute the residues of the tandem d(GA) base pair (Scheme 5A). In spite of the significant structural changes of the hairpins **16** and **19** indicated by the CD-spectra, these hairpins exhibit the strongest stabilization.

As we want to know whether a similar structural change observed for the tandem dG–dA motif in the hairpins **16** can also be achieved for a single dA–dG mismatch, the 7-deaza-7-propynyladenosine nucleoside **3c** was introduced into the duplex 5′-d(TAG GTC AAT ACT) (**20**) ·3′-d(ATC CAG TTA TGA) (**21**). From Table 3 it is apparent that the incorporation of either a single propynyl residue or two separated propynyl residues of compound **3c** opposite to dT causes a 2 °C stabilization for a single modification.

Duplex **24**·**21** containing an isolated dA–dG mismatch has a 2 °C lower T_m compared to the standard duplex (**20**·**21**). The introduction of a dG–**3c** mismatch (**24**·**23**) causes only a minor destabilization. The CD spectra (Scheme 5B) of the standard duplex **20**·**21**, the modified duplexes **20**·**23** and **22**·**23**, incorporating **3c** instead of dA, show only a small difference for the negative lobe at around 250 nm. This indicates that the B-DNA structure is only little perturbed, even when three modified residues (**3c**) replace dA. For duplex **24**·**23**, incorporating a single dG-**3c** mismatch pair, a significant change of the positive lobe at around 280 nm can be observed, implying that this mismatch already has a certain effect on the secondary structure of the DNA-duplex. A related but a smaller change is observed on the duplex **24**·**21** containing a single dG–dA base pair.

In the case of DNA-duplexes, several base pair motifs have been described in the literature which strongly depend on the base stacking environment of the tandem d(GA) unit.[7–10] The sheared base pair **III** (Scheme 6) has been established for duplexes containing the 5′-pyrimidine-GA-purine-3′ unit, while 5′-purine-GA-pyrimidine-3′ sequences adopt a dG(anti)–dA(anti) conformation (**I**).[9] The incorporation of the 7-deazapurine nuclesides **3a** and **5a** into the d(GA) unit of hairpin **1**, proves that the sheared base pair motif (**III**) can be excluded for this sequence. From molecular modelling, the edge-to-face base pairing motif **V**, carrying 7-propynyl modifications on both nucleobases, cannot be completely excluded when one of the sugar moieties adopts the *syn*-conformation. Nevertheless, for the 5′-purine-GA-pyrimidine-3′ sequences a dG(anti)–dA(anti) conformation with spiral base stacking properties has been reported.[9] Due to these findings, the propynylated tandem d(GA) base pair present in hairpin **19** is assumed to adopt the base pairing motif **IV**.



Scheme 6 Base pair motifs of dG–dA mismatches.

Conclusions

The following conclusions can be drawn from the data reported above.

(i) The non-canonical tandem dG–dA purine–purine base pairs of hairpin **1** are nearly as stable as dA–dT pairs.

(ii) The replacement of the purine moieties by non-functionalized 7-deaza-2′-deoxyadenosine and 7-deaza-2′-deoxyguanosine did not significantly destabilize the hairpin structure which excludes Hoogsteen base pair formation.

Table 4 ^{13}C-NMR chemical shifts of nucleosides at 298 K[a]

Compound	C(2)[b] C(2)[c]	C(4)[b] C(6)[c]	C(4a)[b] C(5)[c]	C(5)[b] C(7)[c]	C(6)[b] C(8)[c]	C(7a)[b] C(4)[c]	C=N
3c	152.5[d]	157.5	102.3	95.6	125.4	148.9[d]	—
8	152.1[d]	159.1	110.4	97.2	126.9	150.0[d]	162.1
9	152.2[d]	159.1	110.4	97.4	166.6	150.2[d]	162.1
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=C	
3c	83.1	[e]	70.9	87.4	61.9	88.4, 72.6	
8	82.9	[e]	70.9	86.2	61.8	87.4, 73.1	
9	82.5	[e]	70.5	85.4	64.1	86.2, 73.00	

[a] Measured in (D_6) DMSO. [b] Systematic numbering. [c] Purine numbering. [d] Tentative. [e] *Overlapped in DMSO signal.*

(iii) The propynyl group introduced into the 7-position of 7-deazapurine nucleosides is able to stabilize the tandem purine d(GA) base pair significantly. This tandem base pair stability approximates that of a tandem dG–dC pair (hairpin **13**).

(iv) The incorporation of **3c** instead of dA into the tandem d(GA) unit alters the secondary structure of hairpin **16** significantly by causing an extraordinarily high stabilization of this structure (4 °C per modification).

(v) The stabilizing effect of **3c** is stronger for the tandem dA–dG base pairs than for the Watson–Crick base pair **3c**–dT present in duplex DNA.

Experimental

General

All chemicals were purchased from Aldrich, Sigma, or Fluka (Sigma–Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. TLC: aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (VWR International, Darmstadt, Germany). Column flash chromatography (FC): silica gel 60 (VWR International, Darmstadt, Germany) at 0.4 bar; Sample collection with an UltroRac II fractions collector (LKB Instruments, Sweden). UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); λ_{max} (ε) in nm. CD Spectra: Jasco 600 (Jasco, Japan) spectropolarimeter with thermostatically (Lauda RCS-6 bath) controlled 1 cm cuvettes. NMR Spectra: Avance-250 or AMX-500 spectrometers (Bruker, Karlsruhe, Germany), at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm rel. to Me_4Si as internal standard, *J* values in Hz. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany).

The melting temperatures were measured with a Cary-1/3 UV/VIS spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temp. was measured continuously in the reference cell with a Pt-100 resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program.[18]

Synthesis, purification and characterization of the oligonucleotides

The oligonucleotide syntheses were carried out in an ABI 392–08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) at the 1 μmol scale using the phosphoramidites **6a–c** and **7a,b** following the synthesis protocol for 3′-cyanoethylphosphoramidites (user manual for the 392 DNA synthesizer Applied Biosystems, Weiterstadt, Germany). The coupling efficiency was always higher than 97%. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aq. ammonia solution for 14–16 h at 60 °C.[15,18]

Purification of 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, 12 min, 20–50% B in A and 25 min, 20% B in A with a flow rate of 1.0 ml min^{-1}. The solution was dried and treated with 2.5% $CHCl_2COOH$–CH_2Cl_2 for 5 min at 0 °C to remove the 4-4′-dimethoxytrityl residues. The detritylated oligomers were purified by reverse phase HPLC with the gradient: 0–20 min, 0–20% B in A with a flow rate of 1.0 ml min^{-1}. The oligomers were desalted (RP-18, silica gel) and lyophilized on a speed-Vac evaporator to yield colorless solids which were frozen at −24 °C.

The molecular masses of the oligonucleotides were determined by MALDI-TOF Biflex-III mass spectrometry with 3-hydroxypicolinic acid (3-HPA) as a matrix (Bruker Saxonia, Leipzig, Germany) (see the ESI†).

The enzymatic hydrolysis of the oligonucleotides was performed as described by Seela and Becher[19] with snake venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli* from Roche Diagnostics GmbH, Germany) in 0.1 M Tris-HCl buffer (pH 8.3), which was carried out on reverse phase HPLC by gradient: 20 min. 100% A, 40 min. 0–65% B in A; flow rate: 0.7 ml min^{-1}. Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleosides [(ε_{260}): dT 8800, dC 7300, dA 15400, dG 11700, **3c** 5200].

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-*N*^{4}-[(dimethylamino)-ethylidene]-5-(prop-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (8). To a solution of **3c** (300 mg, 1.04 mmol) in MeOH (30 ml) was added *N,N*-dimethylacetamide dimethylacetal (2.2 ml, 15.0 mmol). The reaction mixture was stirred at 60 °C for 5 h and the solvent was evaporated to dryness. The resulting residue was applied to FC (silica gel, column 15 × 3 cm, CH_2Cl_2–MeOH, stepwise gradient, 95 : 5, 9 : 1). Compound **8** was isolated as a colorless solid (350 mg, 94%). TLC (CH_2Cl_2–MeOH, 9 : 1): R_f 0.48. UV (MeOH): λ_{max} 227 (27200), 307 (11500). ^{1}H-NMR ((D_6) DMSO): 1.98 (s, 2 CH_3); 2.04 (m, H_α–C(2′)); 2.16 (m, H_β–C(2′)); 3.09 (2s, $N(CH_3)_2$); 3.52 (m, H_2–C(5′)); 3.81 (m, H–C(4′)); 4.33 (m, H-(3′)); 5.02 (t, OH–C(5′)); 5.25 (d, *J* (3.3, OH–C(3′)); 6.40 (t, H–C(1′)); 7.70 (s, H–C(6)); 8.53 (s, H–C(2)). Anal. calcd. for $C_{18}H_{23}N_5O_3$ (357.41): C 60.49, H 6.49; found: C 59.28, H 6.43.

7-(2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl)-N^4-[(dimethylamino)ethylidene]-5-(prop-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (9). Compound **8** (200 mg, 0.56 mmol) was dried by repeated co-evaporation with anhydrous pyridine (2 × 3 ml) and dissolved in pyridine (3 ml). After addition of 4,4′-dimethoxytrityl chloride (260 mg, 0.77 mmol) the solution was stirred for 30 min at rt. The reaction was quenched by adding 5% aq. $NaHCO_3$ soln. (25 ml) and it was extracted with CH_2Cl_2 (30 ml). The aqueous layer was washed with CH_2Cl_2 (25 × 3 ml). The combined organic phase was dried over Na_2SO_4 and evaporated. The residue was subjected to FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone, stepwise gradient, 9 : 1, 8 : 2, 1 : 1). The main zone afforded compound **9** as a colorless foam (235 mg, 64%). TLC (CH_2Cl_2/MeOH, 9 : 1): R_f 0.48. UV (MeOH): λ_{max} 230 (46200), 283 (11200), 308 (12100). ^{1}H-NMR ((D_6) DMSO): 1.97 (s, 2 CH_3); 2.27 (m, H_α–C(2′)); 2.55 (m, H_β–C(2′)); 3.09 (s, $N(CH_3)_2$); 3.13 (m, H_2–C(5′)); 3.91 (m, H–C(4′)); 4.35 (m, H-(3′)); 5.30 (d, *J* (3.3, OH–C(3′)); 6.55 (t, H–C(1′)); 6.82, 7.20–7.34 (2m, 13H–Ar); 7.56 (s, H–C(6)); 8.35 (s, H–C(2)).

Anal. calcd. for $C_{39}H_{41}N_5O_5$ (659.77): C 71.00, H 6.26, N 10.61; found: C 70.94, H 6.46, N 10.50.

7-(2-Deoxy-5-O-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl)-*N*^{4}-[(dimethylamino)ethylidene]-5-(prop-1-ynyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3′-[(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (6c). To a solution of compound **9** (200 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (5 ml), *N*,*N*-diisopropylethylamine (DIPEA) (67 µl, 0.39 mmol) and 2-cyanoethyl-diisopropylphosphoramido chloridite (115 µl, 0.56 mmol) were added under Ar atmosphere. After stirring for 0.5 h, 5% aqueous $NaHCO_3$ solution was added, and it was extracted with CH_2Cl_2 (2 × 10 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was subjected to FC (silica gel, column 8 × 2 cm, CH_2Cl_2/acetone, 9:1). The main zone afforded compound **6c** as a colorless foam (222 mg, 86%).

TLC (CH_2Cl_2–$(CH_3)_2CO$, 8:2): R_f 0.7. ^{31}P-NMR ($CDCl_3$): 149.78, 150.03.

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