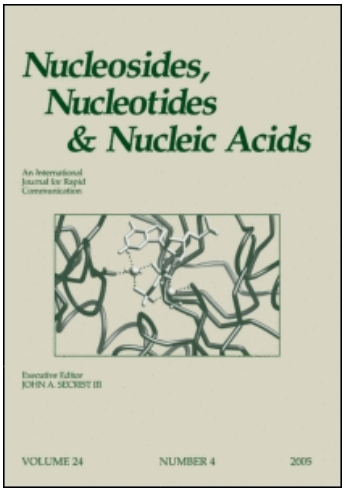


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Propynyl Groups in Duplex, Hairpin and Triplex DNA: 7-Deazapurines and 9-Deazapurines

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**To cite this Article** Seela, Frank , Shaikh, Khalil I. , Budow, Simone and Jawalekar, Anup M.(2005) 'Propynyl Groups in Duplex, Hairpin and Triplex DNA: 7-Deazapurines and 9-Deazapurines', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 851 — 854
**To link to this Article: DOI:** 10.1081/NCN-200059184
**URL:** http://dx.doi.org/10.1081/NCN-200059184

*Nucleosides, Nucleotides, and Nucleic Acids,* 24 (5–7):851–854, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200059184

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# PROPYNYL GROUPS IN DUPLEX, HAIRPIN AND TRIPLEX DNA: 7-DEAZAPURINES AND 9-DEAZAPURINES

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▫ *Oligonucleotides containing 7-deazapurines or 9-deazapurines with propynyl groups at the 7- or 9-position were prepared. The stabilizing effect of the propynyl group was studied on DNA duplexes, hairpins and triplexes.*

**Keywords** 7-Deazapurines, 9-Deazapurines, Oligonucleotides, Duplex, Hairpin, Triplex



## INTRODUCTION

Among the various modifications carried out on the DNA nucleobases the introduction of the propynyl group has been shown to exert a stabilizing effect being useful for antisense technology and oligonucleotides hybridization used for diagnostic purposes. Recently, our laboratory has reported on 8-aza-7-deazapurines carrying 7-propynyl residues.[1] Here, we compare the properties of oligonucleotides containing the 7-deazapurine nucleosides **1,2a–c** as well as the 9-deazapurine nucleosides **3a–c**. The phosphoramidites **4,5,6a–c** were prepared. The influence of the modified nucleosides on the stability of DNA duplexes, hairpins, and triplexes was studied (Scheme 1).

## RESULTS AND DISCUSSION

### Synthesis of the Phosphoramidites

The nucleosides **1c–3c** were synthesized from **1b–3b** using $[(PPh_3)_4Pd(0)]$/CuI catalyzed *Sonogashira* cross-coupling. The amino groups were blocked

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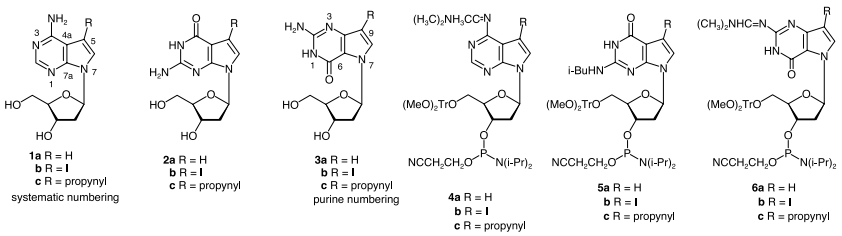



**SCHEME 1**

with either an acetamidine (**1c**), an isobutyryl or formamidine (**2c**),[2] or a formamidine residue (**3c**). The intermediates were converted into the corresponding DMT-derivatives and subsequently phosphitylated. This furnished the phosphoramidites **4c–6c**, which were used in solid-phase synthesis (see Tables 1 and 2).

## The Effect of the 7-Propynyl Group on the DNA Duplex Stability

The displacement of four dG residues by 7-deaza-2′-deoxyguanosine (**2a**) within the duplex **7** · **8** results in a decrease of the $T_m$ value of 3° (**9** · **10**) (Table 1). The introduction of the 7-iodo substituents **2b**[3] instead of **2a** bring the stability to the level of the unmodified duplex (see **11** · **12**, Table 1). Displacement of the 7-iodo by 7-propynyl groups enhances the $T_m$ value further to 53°C (**13** · **14**) which equals to an increase of 1.5°/modification—a value which has been already found for 7-propynyl-substituted 8-aza-7-deaza-2′-deoxyguanosine within the same sequence. The incorporation of the 2′-deoxytubercidin derivative **1c** as well as the 7-iodo compound **1b**[4] results also in a stepwise stability increase, while the non-functionalized **1a** shows the same behavior as dA (Table 1).

**TABLE 1** $T_m$ Values and Thermodynamic Data of Oligonucleotides

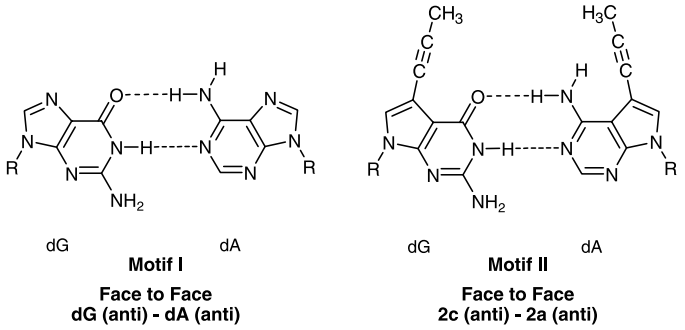
| Duplex | $T_m$ (°C) | $\Delta H°$ (kcal/mol) | $\Delta S°$ (kcal/mol) | $\Delta G_{310}°$ (kcal/mol) |
|---|---|---|---|---|
| 5′-d(TAG GTC AAT ACT) **7** | 47[a] | −87 | −243 | −11.3 |
| 3′-d(ATC CAG TTA TGA) **8** | 50[b] | | | |
| 5′-d(TA**2a2a**TC AAT ACT) **9** | 44[a] | −92 | −284 | −3.6 |
| 3′-d(ATC CA**2a** TTA T**2a**A) **10** | | | | |
| 5′-d(TA**2b2b**TC AAT ACT) **11** | 48[a] | −112 | −348 | −4.1 |
| 3′-d(ATC CA**2b** TTA T**2b**A) **12** | | | | |
| 5′-d(TA**2c2c**TC AAT ACT) **13** | 53[a] | −110 | −314 | −12.8 |
| 3′-d(ATC CA**2c** TTA T**2c**A) **14** | | | | |
| 5′-d(TAG GTC AAT ACT) **15** | 50[b] | −77 | −215 | −10.7 |
| 3′-d(ATC C**1a**G TT**1a** TGA) **16** | | | | |
| 5′-d(TAG GTC AAT ACT) **17** | 54[b] | −85 | −236 | −12.3 |
| 3′-d(ATC C**1b**G TT**1b** TGA) **18** | | | | |
| 5′-d(TAG GTC AAT ACT) **19** | 56[b] | −85 | −233 | −12.5 |
| 3′-d(ATC C**1c**G TT**1c** TGA) **20** | | | | |

[a]10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.
[b]60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl, pH 7.

**TABLE 2** $T_m$ Values of Oligonucleotide Hairpins

| Sequence | $T_m$ (°C) |
|---|---|
| 5′-d(AGT AGG GAC CTA AGT AGG GAC CTA) **21** | 70 |
| 5′-d(AGT AGG G**1a**C CTA AGT AGG G**1a**C CTA) **22** | 70 |
| 5′-d(AGT AGG G**1b**C CTA AGT AGG G**1b**C CTA) **23** | 76 |
| 5′-d(AGT AGG G**1c**C CTA AGT AGG G**1c**C CTA) **24** | 78 |
| 5′-d(AGT AGG **2a**AC CTA AGT AGG **2a**AC CTA) **25** | 68 |
| 5′-d(AGT AGG **2c**AC CTA AGT AGG **2c**AC CTA) **26** | 72 |

[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.




## The Effect of 7-Substituted 7-Deazapurines on the dG-dA Hairpin Base Pairs

The oligonucleotide **21** (Table 2) forms a hairpin shown in Scheme 2. It contains three different d(GA) motifs: 1) a d(G-A) overhang, 2) an unpaired d(G-A) unit in the loop, and 3) two adjacent dG-dA pairs in the stem region. The replacement of dG by 7-deaza-2′-deoxyguanosine (**2a**) within the tandem d(G-A) pair leads to a slight destabilization while the replacement of dA by 7-deaza-2′-deoxyadenosine (**1a**) did not effect the stability (Table 2). Hoogsteen pairing is


**SCHEME 2** Structure of the hairpin **21**.

**TABLE 3** $T_m$ Values of Base-Modified Triplexes

| **X** | $T_m$ (°C) | **X** | $T_m$ (°C) |
|---|---|---|---|
| dC | 39, 65 | **3b** | 41, 64 |
| **3a** | 39, 65 | **3c** | 45, 65 |

10 mM HEPES, 50 mM NaCl, 10 mM $MgCl_2$, 0.5 mM Spermine, 1 μM single-strand concentration, pH 6.5.

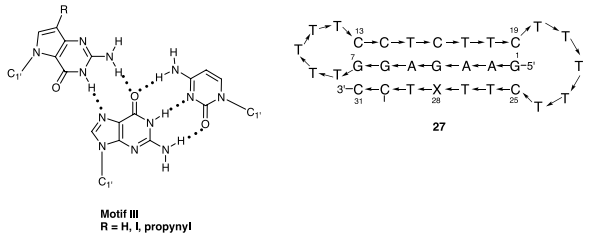



**FIGURE 1**

neither possible for dA nor for dG. Therefore, the most likely base pair motif is that of I. We now replaced the dA residues of the d(G-A) pairs by compounds **1b** and **1c**. The resulting hairpins **23** and **24** are significantly more stable than the hairpins **21** or **22**. Also dG was replaced by **2a** or **2c** leading to a slightly destabilized hairpin **25** (not functionalized at C7) and a stabilized hairpin (**26**) (Table 2). This means that the 7-iodo substituents as well as 7-propynyl groups have a positive effect on the stability of the non-canonical d(G-A) base pair which is represented by the bas pair motif II (propynyl modifications are possible on both sides).

## The Base Pairing Properties of 9-Deaza-2′-Deoxyguanosines in Triplex-DNA

DNA triplexes containing $N^7$-2′-deoxyguanosine were described earlier.[5] Here, we report on triplexes containing the nucleosides **3a–c**. The single strand **27**[6] forms a double hairpin (motif III) with a triplex core. It shows two thermal transitions (Table 3). The lower one results from the melting of the 3′-terminus stabilized by Hoogsteen-pairs, the higher one is represented by the dissociation of the Watson-Crick base pair (duplex melting). Replacement of dC(28) (=X) by 9-deaza-2′-deoxyguanosine (**3a**)[7] does not change the stability of the triplex while the iodo compound **3b** or the propynyl derivative **3c** leads to a stepwise stabilization of the third strand without changing the $T_m$ value of the duplex structure (Figure 1).

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