Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 289–292

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Influence of Modified Purine Bases on the Stability of Parallel DNA

Frank Seela,* Changfu Wei, Georg Becher, Matthias Zulauf and Peter Leonard

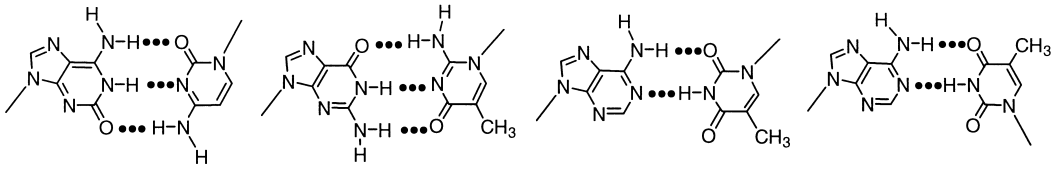
Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie, Universität Osnabrück, Barbarastr. 7, D-49069 Osnabrück, Germany

Received 24 September 1999; accepted 6 December 1999

Abstract—The stability of the parallel-stranded (ps) DNA duplexes is increased when the dA-residues are replaced by the 7-substituted 7-deaza-2′-deoxyadenosine derivatives **3a**,**b** or the dG-residues by the 8-aza-7-deazapurine 2′-deoxynucleosides **6** and **7a**,**b**. Also the *N*-7-glycosylated adenine **5** forms stable base pairs in ps-DNA while it destabilizes oligonucleotide duplexes with antiparallel chain orientation. The presence of a 2-amino group as in compound **4b** is critical for the DNA-structure, leading to a much greater destabilization of the ps-hybrids than of aps-DNA.

Parallel-stranded (ps) DNA is formed when the guanine–cytosine Watson–Crick base pair of antiparallel-stranded (aps) DNA is replaced by the isoguanine–cytosine pair (**I**) and/or isocytosine– or 5-methylisocytosine–guanine pairs (**II**).[1–5] The adenine–thymine pair has not to be displaced as it shows ambiguous base pairing (**III** and **IV**) which allows the formation of parallel and antiparallel duplexes without modification. Nevertheless, the stability of the base pair **III** is lower than that of **IV** resulting in less stable ps-hybrids when the number of adenine–thymine pairs (**III**) is higher than that of the base pairs **I** and **II**.[6] In order to overcome this problem it was of interest to stabilize ps-DNA by the incorporation of modified bases which strengthen aps-DNA.[7,8]

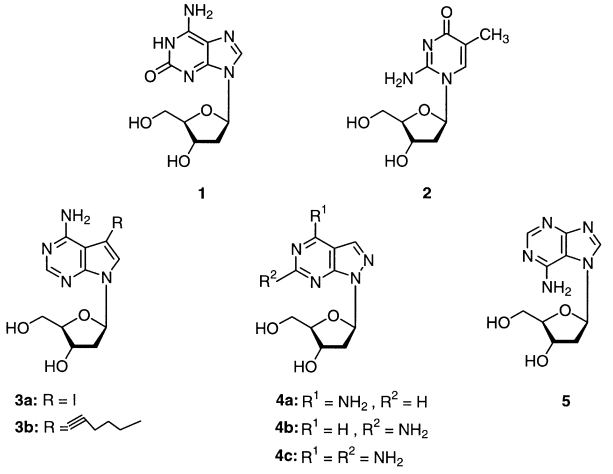
In a first set of experiments, the 2′-deoxyadenosine residues were replaced by the base-modified nucleosides **3**–**5** (Table 1). The oligonucleotides of Tables 1–3 were synthesized on solid-support using phosphoramidite chemistry.[9,10] The composition of the oligomers was proven by enzymatic hydrolysis[7] and MALDI-TOF spectra. As standard duplexes with parallel chain orientation the hybrids **9·10** and **11·12** containing 2′-deoxyisoguanosine (iG_d, **1**) and 2′-deoxy-5-methylisocytidine (m^5iC_d, **2**) were chosen. The T_m-values of the duplexes are shown in Table 1.



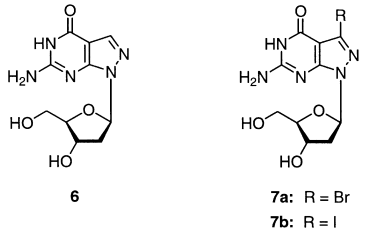
Keywords: DNA; nucleic acids; nucleosides; substituents effects.
*Corresponding author. Tel.: +49-541-969-2791; fax: +49-541-969-2370; e-mail: fraseela@rz.uni-osnabrueck.de

0960-894X/00/$ - see front matter
PII: S0960-894X(99)00679-4

As can be seen, the incorporation of the 7-substituted 7-deazaadenosine derivatives **3a**,**b** (synthesis of the nucleosides[11–17]) stabilizes the ps-duplex (**9·13** and **9·15** versus **9·10**). A slight destabilization is observed when the 8-aza-7-deazaadenosine **4a** is introduced (**9·17** versus **9·10**). Compared to the standard duplexes **9·10** and **11·12** the 2-amino-8-aza-7-deaza-2′-deoxyadenosine (**4c**) has no significant influence on the duplex stability (→ **9·21** and **11·22**), while the amino-8-aza-2-7-deazapurine nucleoside **4b** leads to a strong decrease of the T_m-value (**9·19** versus **9·10**). The *N*-7-glycosylated adenine **5** is well accommodated in ps-DNA.[18,19] The most probable base pair motifs for the duplexes are shown in the motifs **V**–**VIII**.



In a second series of experiments, the influence of modified guanine derivatives on the stability of ps-duplexes was studied. For this purpose compounds **6**, **7a**,**b** were chosen which stabilize aps-DNA.[8] They were incorporated opposite to 2′-deoxy-5-methylisocytidine (**2**), thereby replacing 2′-deoxyguanosine. The incorporation of 8-aza-7-deaza-2′-deoxyguanosine (**6**) stabilizes the base pair (**IX**) as demonstrated in the case of the ps-duplexes **9·25** and **11·26** (Table 2). A further stabilization is observed when the 7-halogenated derivatives **7a** or **7b** are incorporated instead of 2′-deoxyguanosine (**9·27**, **9·29** versus **9·10**; **11·28**, **11·30** versus **11·12**). Depending on the sequence the stabilization is 2° per base pair for compound **7a** and almost 3° per base pair in the case of the ps-duplex **9·29** containing compound **7b**.



In a third set of experiments (Table 3), the modified nucleosides **3a**, **4b**,**c**, **5** and **7b** were incorporated into parallel as well as into antiparallel dodecamers, and the stability of these duplexes was investigated. According to Table 3, the 7-substituents of the 7-deaza-2′-deoxyadenosine (**3a**) or 8-aza-7-deaza-2′-deoxyguanosine (**7b**) stabilize both ps- and aps-duplexes. The ΔT_m in the ps-DNA is +1.5° per base pair for compound **3a** and +1° for **7b**. The influence of the modified bases on the duplex stability is stronger in aps-hybrids than in ps-DNA. Here, the T_m-value is increased by 2.5° per modified base for **3a** and 3° per base pair for **7b**. The incorporation of the 8-aza-7-deaza-2,6-diaminopurine nucleoside **4c** increases the stability of the aps-duplex (**12·21** versus **12·10**) while it does not alter the stability of a ps-hybrid (**9·21** versus **9·10**). A strong destabilization is found, however, when the 2-amino-8-aza-7-deazapurine nucleoside **4b** was replacing dA in a duplex with parallel chain orientation (**9·19** versus **9·10**). In contrast to that, the T_m-decrease in the aps-duplex is rather small (**12·19** versus **12·10**). The *N*-7-glycosylated adenine **5** is well accommodated in parallel DNA while its incorporation in aps-DNA results in a rather labile duplex (**9·31** versus **12·32**).

Pattabiraman has constructed a parallel right-handed DNA from dA–dT reverse Watson–Crick base pairs on the basis of molecular mechanics calculations.[20] Corresponding duplexes have been prepared by Jovin.[21] One outcome of this model was the equal size of the grooves, in contrast to the distinct grooves of B-DNA. Recently, a parallel decamer duplex containing iG_d–dC, m^5iC_d–dG and dA–dT base pairs with all pairs in the reverse Watson–Crick pairing mode was investigated by NMR spectroscopy.[22] In general, this work supports the model of Pattabiraman,[20] but more detailed structural information was given. Furthermore, it was shown that intercalators are effectively bound to ps-DNA.

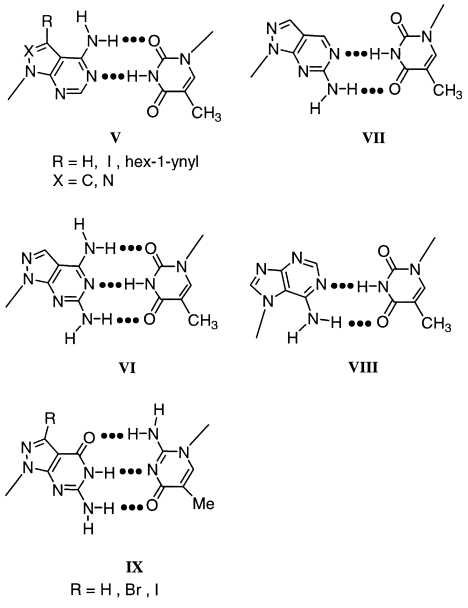


Table 1. T_m-values of oligonucleotides modified by iG_d, m^5iC_d and 2′-deoxyadenosine derivatives[a,b]

ps-Duplexes [c]	T_m (°C)	ps-Duplexes [c]	T_m (°C)
5′-d(ATiCiCAiGTTATiGA) (**9**) 5′-d(TA G GT CAATAC T) (**10**)	39	5′-d(TiCATAAiCTiGiGAT) (**11**) 5′-d(A GTATT GA C CTA) (**12**)	44
5′-d(AT iCiCAiGTTAT iGA) (**9**) 5′-d(T**3a** G GT CAAT**3a** CT) (**13**)	43	5′-d(TiCAT AAiCT iGiGAT) (**11**) 5′-d(A GT**3a**TT G**3a** C C TA) (**14**)	48
5′-d(AT iCiCAiGTTAT iGA) (**9**) 5′-d(T**3b** GGT CAAT**3b** CT) (**15**)	41	5′-d(TiCAT AAiCT iGiGAT) (**11**) 5′-d(A GT**3b**TT G**3b** C CTA) (**16**)	43
5′-d(AT iCiCAiG T TA TiGA) (**9**) 5′-d(T**4a** G GT C**4a4a**T**4a** CT) (**17**)	37	5′-d(TiCAT AAiCT iGiGAT) (**11**) 5′-d(A GT**4a**TT G**4a** C CTA) (**18**)	44
5′-d(AT iCiCAiGT T AT iGA) (**9**) 5′-d(T**4b** GGT C**4b4b**T**4b** CT) (**19**)	25	5′-d(TiCA TAAiCT iGiGAT) (**11**) 5′-d(**4b**GT**4b**TT G**4b** C CTA) (**20**)	27
5′-d(ATiCiCAiG T TATiGA) (**9**) 5′-d(TA G GT C**4c4c**TA CT) (**21**)	38	5′-d(TiCAT AAiCTiGiGAT) (**11**) 5′-d(A GT**4c**TT G**4c** C CTA) (**22**)	45
5′-d(ATiCiCAiGT T ATiGA) (**9**) 5′-d(TA GG T C **5 5** TA C T) (**23**)	37	5′-d(TiCATAAiC TiGiGAT) (**11**) 5′-d(A GT**5** TT G **5** C CTA) (**24**)	38

[a]UV-measurements (260 nm) were performed in 1 M NaCl, 0.1 M $MgCl_2$ and 60 mM Na-cacodylate buffer, pH 7.0. Single strand concentration is 5 μM.
[b]Numbers refer to the related nucleosides.
[c]d(iC) = m^5iC_d = compound **2**.

Table 2. T_m-values of oligonucleotides modified by iG_d, m^5iC_d and 2′-deoxyguanosine derivatives[a,b]

ps-Duplexes[c]	T_m (°C)	ps-Duplexes[c]	T_m (°C)
5′-d(ATiCiCAiGTTATiGA) (**9**) 5′-d(TA**GG** T C AATA CT) (**10**)	39	5′-d(TiCATAAiCTiGiGAT) (**11**) 5′-d(A **G**TATT **G**A C CTA) (**12**)	44
5′-d(ATiCiCAiGTTATiGA) (**9**) 5′-d(TA **6 6** T CAATA CT) (**25**)	40	5′-d(TiCATAAiCTiGiGAT) (**11**) 5′-d(A **6** TATT **6** A C CTA) (**26**)	47
5′-d(ATiC iCAiGTTATiGA) (**9**) 5′-d(TA**7a7a** T CAATA CT) (**27**)	43	5′-d(TiCATAAiCTiGiGAT) (**11**) 5′-d(A**7a**TATT**7a**A C CTA) (**28**)	48
5′-d(ATiC iCAiGTTATiGA) (**9**) 5′-d(TA**7b7b**T CAATA CT) (**29**)	45	5′-d(TiCATAAiCTiGiGAT) (**11**) 5′-d(A**7b**TATT**7b**A C CTA) (**30**)	48

[a–c]See Table 1.

Table 3. Comparison of the T_m-values of ps- and aps-oligonucleotide duplexes containing modified bases[a,b]

ps-Duplexes[c]	T_m (°C)	$(\Delta T_m)^d$ (°C)	aps-Duplexes[c]	T_m	$(\Delta T_m)^d$
5′-d(ATiCiCAiGTTATiGA) (**9**) 5′-d(TA G GT CAATA CT) (**10**)	37		3′-d(ATCCAGTTATGA) (**12**) 5′-d(TAGGTCAATACT) (**10**)	46	
5′-d(ATiCiCAiGTTATiGA) (**9**) 5′-d(T**3a**GG T CAAT**3a**CT) (**13**)	40	(+3)	3′-d(AT CCAGTTA TGA) (**12**) 5′-d(T**3a**GGTCAAT**3a**CT) (**13**)	51	(+5)
5′-d(ATiCiCAiGT T ATiGA) (**9**) 5′-d(T**4b**GG T C**4b4b**T**4b**CT) (**19**)	20	(−17)	3′-d(AT CCAGT T AT GA) (**12**) 5′-d(T**4b**GGTC**4b4b**T**4b**CT) (**19**)	42	(−4)
5′-d(ATiCiCAiGT T ATiGA) (**9**) 5′-d(TAG G T C**4c4c**TA CT) (**21**)	37	(0)	3′-d(ATCCAGT T ATGA) (**12**) 5′-d(TAGGTC**4c4c**TACT) (**21**)	50	(+4)
5′-d(ATiCiCAiGT TATiGA) (**9**) 5′-d(TA**7b7b**T CAATA CT) (**29**)	39	(+2)	3′-d(AT C C AGTTATGA) (**12**) 5′-d(TA**7b7b**TCAATACT) (**29**)	52	(+6)
5′-d(ATiCiCAiGT TATiGA) (**9**) 5′-d(T **5**G G T C **5 5** T**5** CT) (**31**)	35	(−2)	3′-d(ATCCAGT TATGA) (**12**) 5′-d(T **5**GGTC **5 5** T**5**CT) (**32**)	26	(−20)

[a]Measured UV-spectrophotometrically (260 nm) in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate buffer, pH 7.0. Single strand concentration is 5 μM.
[b,c]See Table 1.
[d]The ΔT_m is the T_m difference compared to the parent duplex.

Combining the data of structural investigation[20–22] with the present study on the incorporation of modified purine bases leads to the conclusion that (i) 7-deazapurines such as pyrazolo[3,4-*d*]pyrimidines (8-aza-7-deazapurines) form stable reverse Watson–Crick base pairs in ps-DNA and have a similar influence on the duplex stability as in aps-DNA, (ii) the 7-substituents, such as halogens or alkynyl residues of pyrrolo[2,3-*d*]pyrimidines or pyrazolo[3,4-*d*]pyrimidines are still well accommodated in the groove of ps-DNA and stabilize the duplex structure. This might be caused by an increase of hydrophobicity and/or stacking interactions of the helix; (iii) an *N*-7-glycosylated adenine (**5**) forms a rather stable ps-DNA while aps-DNA becomes labile; thus the reversal of the glycosylation sites has an analogous effect on the base pair recognition as the exchange of substituents ($dG \rightarrow iG_d$); (iv) a 2-amino group, e.g. in the 2-amino-8-aza-7-deazapurine nucleoside **4b** destabilizes a ps-duplex (**9·10**→**9·19**; −17 °C) much more strongly than in a duplex with antiparallel chain orientation. (**10·12**→**12·19**; −4 °C) indicating the autonomous structure of ps-DNA.

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft and the Roche Diagnostics GmbH is gratefully acknowledged.

References and Notes

1. Seela, F.; Gabler, B.; Kazimierczuk, Z. *Collect. Czech. Chem. Commun.* (Special Issue) **1993**, *58*, 170.
2. Seela, F.; Wei, C. *Helv. Chim. Acta* **1997**, *80*, 73.
3. Sugiyama, H.; Ikeda, S.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 9994.
4. Seela, F.; He, Y.; Wei, C. *Tetrahedron* **1999**, *55*, 9481.
5. Seela, F.; Wei, C. *Helv. Chim. Acta* **1999**, *82*, 726.
6. van de Sande, J. H.; Ramsing, N. B.; Germann, M. W.; Elhorst, W.; Kalisch, B. W.; v. Kitzing, E.; Pon, R. T.; Clegg, R. C.; Jovin, T. M. *Science* **1988**, *241*, 551.
7. Seela, F.; Zulauf, M. *Chem. Eur. J.* **1998**, *4*, 1781.
8. Seela, F.; Becher, G. *Chem. Commun.* **1998**, 2017.
9. Beaucage, S. L. In *Methods in Molecular Biology, Protocols for Oligonucleotides and Analogs, Synthesis and Properties*; Agrawal, E. S., Ed.; Humana Press: Totowa, NJ, 1993; Vol. 20, pp 33–61.
10. Applied Biosystems, In *User Bulletin*; 1990; pp 6–15.
11. **3a**,**b**: Seela, F.; Zulauf, M. *Synthesis* **1996**, 726.
12. **4a**: Seela, F.; Kaiser, K. *Helv. Chim. Acta* **1988**, *71*, 1813.
13. **4b**: Seela, F.; Driller, H. *Helv. Chim. Acta* **1988**, *71*, 1191.
14. **4c**: Seela, F.; Becher, G. *Collect. Czech. Chem. Commun.* (Special Issue) **1999**, *2*, 320.
15. **5**: Seela, F.; Winter, H. *Helv. Chim. Acta* **1994**, *77*, 597.
16. Seela, F.; Steker, H. *Helv. Chim. Acta* **1986**, *69*, 1602.
17. **7a**,**b**: Seela, F.; Becher, G. *Synthesis* **1998**, 207.
18. Seela, F.; Leonard, P. *Helv. Chim. Acta* **1998**, *81*, 2244.
19. Seela, F.; Wei, C.; Melenewski, A.; Leonard, P. *Nucleic Acids Symposium Series* **1997**, *37*, 149.
20. Pattabiraman, N. *Biopolymers* **1986**, *25*, 1603.
21. Jovin, T. M. In *Nucleic Acids and Molecular Biology*; Eckstein, F.; Lilley, D. M. J., Eds.; Springer–Verlag: Berlin, Heidelberg, 1991; Vol. 5, pp 25–38.
22. Yang, X.-L.; Sugiyama, H.; Ikeda, S.; Saito, I.; Wang, A. H.-J. *Biophysical J.* **1998**, *75*, 1163.