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# 3-DEAZA- AND 7-DEAZAPURINES: DUPLEX STABILITY OF OLIGONUCLEOTIDES CONTAINING MODIFIED ADENINE OR GUANINE BASES

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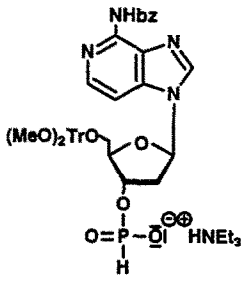
**Abstract**: Oligonucleotide duplexes were destabilized when the purine bases of 2'-deoxyadenosine or 2'-deoxyguanosine were replaced by the corresponding 3-deazapurine (imidazo[4,5-c]pyridine) or 7-deazapurine (pyrrolo[2,3-d]pyrimidine) derivatives. Only $d(AT)_6$ was stabilized by incorporation of $c^7A$ when high salt condition were used. The stability of deazapurine base-protecting groups was also investigated.

The incorporation of 3-deazapurine (imidazo[4,5-c]pyridine) - or 7-deazapurine (pyrrolo[2,3-d] pyrimidine) nucleosides into oligonucleotides changes their physical and biological properties. By this means the fluorescence of ethidium bromide bound to a DNA molecule is quenched[1] and oligonucleotides are protected against phosphodiester hydrolysis by restriction enzymes[2,3]. 3-Deaza-2'-deoxyguanosine has been used in alternating $d(CG)_n$ to reduce the binding capacity of the minor groove[4]. Furthermore, the chemical determinants of bending are studied by incorporation of 3-deaza- as well as 7-deaza-2'-deoxyadenosine into dA-tracts modulating the stacking interaction along the oligonucleotide chain [5,6]. The disaggregation of the $G_4$-oligomers is accomplished by incorporation of $c^7G$ instead of $G^7$. Also the autocatalytic splicing activity of the hammerhead ribozyme changed when $c^7A$ or $c^7G$ were replacing A or G[8,9]. In all cases the substitution of the parent by the deazapurine nucleotide influences the duplex structure. In the following, we report on the duplex stability of oligonucleotides containing 3-deaza- and 7-deazapurines. It will be shown that the modified nucleoside can decrease or increase the $T_m$-value depending on the sequence of the particular oligomer and the salt conditions used.

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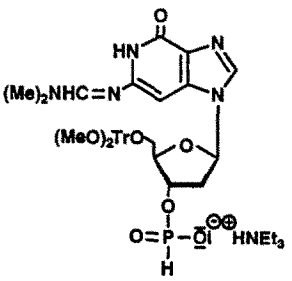


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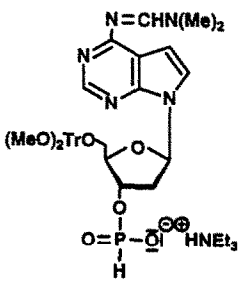


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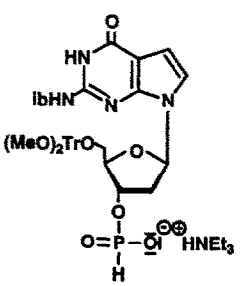


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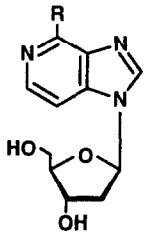
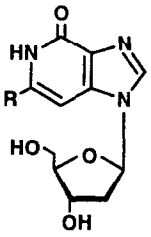
*Synthesis*: The oligonucleotides described in the manuscript have been prepared by solid-phase synthesis using the phosphonates **1-4** or phosphoramidites of $c^3A_d$[10,11], $c^3G_d$[4], $c^7A_d$[12], and $c^7G_d$[13]. The preparation of **4**[14a] has also been reported by others[14b]. For oligonucleotide synthesis the purine $NH_2$-protecting groups had to be changed in the case of 3-deaza or 7-deazapurine nucleosides. This is caused by the absence of one purine nitrogen which increases the stability of the protecting groups significantly. Table 1 summarizes the half-life values of the protected nucleosides **5a-6c** in aqueous ammonia; for the compound

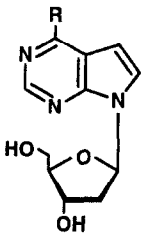
**6a** the $t_{1/2}$ value published earlier[4] is corrected. According to these data the following protecting groups are most suitable for oligoribo- and oligodeoxyribonucleotide synthesis: the dimethylaminomethylidene group for $c^7A_d$ and $c^3G_d$ and the pac group for $c^3A_d$. Only in the case of $c^7G_d$ the same protecting group (ib) could be used as for dG.



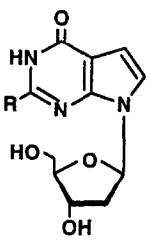
**5a:** R = pacNH
**5b:** R = bzNH



**6a:** R = acNH
**6b:** R = ibNH
**6c:** R = $Me_2NCH{=}N$



**7a:** R = pacNH
**7b:** R = bzNH
**7c:** R = $Me_2NCH{=}N$



**8a:** R = ibNH
**8b:** R = bzNH
**8c:** R = $Me_2NCH{=}N$

Table 1. Half-life Values of Deprotection of Purine, 3-Deazapurine, and 7-Deazapurine 2'-Deoxynucleosides in 25% aq. Ammonia [15a].

| Purine Nucleosides | [°C] | $t_{1/2}$[a] [min] | 3-Deazapurine Nucleosides | | | 7-Deazapurine Nucleosides | | |
|---|---|---|---|---|---|---|---|---|
| ibdG[16] | 40 | 112 | ibc$^3G_d$ (**6b**) | 40 | 500 | ibc$^7G_d$ (**8a**) | 40 | 109 |
| (dma)dG[17] | 40 | 19 | (dma)c$^3G_d$ (**6c**) | 40 | 28 | (dma)c$^7G_d$ (**8b**) | 40 | 22 |
| bzdA [6] | 40 | 175 | bzc$^3A_d$ (**5b**) | 40 | 430 | bzc$^7A_d$ (**7b**) | 40 | 320 |
| pacdA [6] | 40 | 3 | pac$^6$c$^3A_d$ (**5a**) | 40 | 7 | pacc$^7A_d$ (**7a**) | 40 | 25 |
| (dma)dA | 40 | 9 | acc$^3G_d$(**6a**)[15b] | 60 | 550 | (dma)c$^7A_d$ (**7c**) | 60 | 10 |

*Self-complementary Oligonucleotides* [18]: Donor and acceptor properties of nucleobases as well as their electronic structure and their dipole moments influence the stability of an oligonucleotide duplex. Stacking interactions depend on the nearest neighbours of nucleobases. This can be seen e.g. from the melting temperature of 5'd[$(A)_6(T)_6$T] (44°C) and 5'[$(A)_3(T)_3(A)_3(T)_3$T] (40°C): the higher the number of the innerstrand d(AT) segments the lower the $T_m$[19]. Moreover, the solvation and the ionic environment of an oligonucleotide are also of decisive importance. Recently, it was observed by our laboratory that the incorporation of $c^7A_d$ into alternating $d(AT)_n$ increases the stability of the duplex [20]. In order to avoid hairpin formation these experiments were performed at high salt (1M NaCl) in the presence of 100 mM $MgCl_2$ at pH 7. When $T_m$-measurements of $d(AT)_6$ and $d(c^7AT)_6$ were performed at low salt concentration (150 mM NaCl, 10 mM TRIS-HCl, pH 7.0), destabilization of $d(AT)_6$ by 2°C is observed (Table 2). The duplex stabilization found in the case of $d(c^7AT)_6$ is not observed in the case of poly $d(c^7AT)$ which is slightly less stable than poly d(AT)[1] (low salt concentration in the absence of $Mg^{2+}$). Thermodynamic data of $d(AT)_6$ and $d(c^7AT)_6$ duplex formation were calculated in high- as well as low salt concentration buffers from the melting curves according to a two-state model. The following values were obtained for $d(AT)_6$: (a)

(high salt; 14 $\mu$M single strands): $\Delta H$ = -67 kcal/mol; $\Delta S$ = -220 cal/K mol; (b) (low salt; 14 $\mu$M single strand): $\Delta H$ = -48 kcal/mol; $\Delta S$ = -162 cal/K mol. For $d(c^7AT)_6$ the following values were evaluated: (a) (high salt, 15 $\mu$M single strand): $\Delta H$ = -42 kcal/mol; $\Delta S$ = -136 cal/K mol; (b) (low salt; 20 $\mu$M single strands): $\Delta H$ = -86 kcal/mol; $\Delta S$ = -290 cal/K mol. These values show the completely different salt dependence of enthalpic and entropic data in the case of $d(AT)_n$ compared to $d(c^7AT)_n$ oligomers. Meanwhile, it is accepted that alternating $d(AT)_n$ oligomers adopt a particular secondary structure, called 'alternating B-DNA'[21-23] in which a d(AT) core is the repeating element while the alternating d(TA) base pairs are not stacked. The displacement of dA by $c^7A_d$ may result in a more stable uniform structure, resembling a regular B-DNA with almost equal stacking interactions along the oligonucleotide chain. However, the stabilizing effect of $c^7A_d$ can only be observed in buffer solutions with a high $Mg^{2+}$ concentration (100 mM) while it is not found in case of $Mg^{2+}$-free buffers. The $Mg^{2+}$ may compress the sugar-phosphate backbone by coordination at adjacent phosphodiester residues. In the case of $c^3A_d$ the destabilization of short oligomers is much stronger than observed by incorporation of $c^7A_d$ (Table 2).

Table 2: $T_m$-Values of Self-Complementary Oligonucleotides[24a].

| Oligomer | [$\mu$M] | $T_m$ | | | |
|---|---|---|---|---|---|
| poly d(AT)[1] | - | 44 | poly $d(c^7AT)$[1] | - | 42 |
| $d(AT)_6$[20] | 30 | 34 | $d(c^7AT)_6$[20] | 30 | 38 |
| $d(AT)_6$ | 14 | 33 | $d(c^7AT)_6$ | 15 | 36 |
| $d(AT)_6$ | 5 | 30 | $d(c^7AT)_6$ | 4 | 32 |
| $d(AT)_6$[24b] | 14 | 24 | $d(c^7AT)_6$[24b] | 20 | 22 |
| $d[(A)_3(T)_3(A)_3(T)_3T]$ | 8 | 40 | $d(c^3AT)_6$ | 3 | <20 |
| $d[(A)_6(T)_6T]$ | 8 | 44 | | | |
| poly d(GC)[1] | - | 94 | poly $d(c^7GC)$[1] | - | 83 |
| $d(CG)_3$[25] | 10 | 46 | $d(Cc^7G)_3$[25] | 10 | 37 |
| $d(GC)_3$[25] | 10 | 46 | $d(c^7GC)_3$[25] | 10 | 37 |
| $d(GCc^3GCGC)$ | 8 | < 20 | $d(Cc^3GCGCG)$[26] | 8 | 29 |
| $(GC)_3$ | 10 | 50 | $(c^7GC)_3$ | 10 | 38 |
| d(GTAGAATTCTAC) | 3 | 49 | $d(GTAc^3GAATTCTAC)$ | 8 | 38 |
| $d(GTc^3AGAATTCTc^3AC)$ | 3 | 34 | $d(GGAATTCC)^2$ | 30 | 38 |
| d(GTAGAATTCTAC)[12,27] | 4 | 44 | $d(Gc^7GAATTCC)^2$ | 30 | 34 |
| $d(GTAGc^7AATTCTAC)$[12,27] | 4 | 43 | | | |

Alternating oligomers such as $d(CG)_3$ and $d(GC)_3$ show identical $T_m$-values (46°C)[25]. Replacement of all three dG residues by $c^7G_d$ results in a decrease of the $T_m$-value by 9°C in both cases. The corresponding oligoribonucleotide showed a reduction of the $T_m$-value of 12°C. A much stronger destabilization (17°C) occurred when only one dG residue was replaced by $c^3G_d$. This destabilization is sequence dependent: if the first dG of $d(CG)_3$ is substituted by $c^3G_d$ a $T_m$ of 29°C is found while $d(GCc^3GCGC)$ shows a $T_m$-value below 20°C. This demonstrates that the GC base pair in the centre of the duplex provides a higher gain of energy than that at the flanks. Palindromic duplex structures were always destabilized when $c^7A_d$, $c^3A_d$, $c^7G_d$ or $c^3G_d$ were replacing the corresponding purine nucleosides (Table 2). As expected, destabilization was larger for oligomers containing $c^3A_d$ or $c^3G_d$ than for those with $c^7A_d$ or $c^7G_d$.

Table 3. $T_m$-Values of Non-selfcomplementary Oligonucleotides[24a].

| Oligomer | μM | $T_m$ | Oligomer | | |
|---|---|---|---|---|---|
| 5' d(GGCAAAAAAC)<br>3' d(GTTTTTTGCC) | 1.5 | 32 | 5' $d(A)_{12}$<br>3' $d(T)_{12}$ | 14 | 44 |
| 5' d(GGCAAc$^7$AAAAC)<br>3' d(GTTTTTTGCC) | 1.3 | 30 | 5' $d(c^7A)_{12}$<br>3' $d(T)_{12}$ | 14 | 30 |
| 5' d(GGCAAc$^7$Ac$^7$AAAC)<br>3' d(GTTTTTTGCC) | 1.3 | 28 | 5' d(GGCAAc$^3$AAAAC)<br>3' d(GTTTTTTGCC) | 1.5 | 30 |
| 5' d(GGCc$^7$AAAAAc$^7$AC)<br>3' d(GTTTTTTGCC) | 1.3 | 29 | 5' d(GGCc$^3$AAAAAc$^3$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 21 |
| 5' d(GGCc$^7$Ac$^7$Ac$^7$AAAAC)<br>3' d(GTTTTTTGCC) | 1.5 | 28 | 5' d(GGCAAc$^3$Ac$^3$AAAC)<br>3' d(GTTTTTTGCC) | 1.5 | 25 |
| 5' d(GGCAAAc$^7$Ac$^7$Ac$^7$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 28 | 5' d(GGCc$^3$Ac$^3$Ac$^3$AAAAC)<br>3' d(GTTTTTTGCC) | 1.5 | 16 |
| 5' d(GGCAc$^7$AAc$^7$AAc$^7$AC)<br>3' d(GTTTTTTGCC) | 1.3 | 27 | 5' d(GGCAAAc$^3$Ac$^3$Ac$^3$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 16 |
| 5' d(GGCc$^7$Ac$^7$AAAc$^7$Ac$^7$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 25 | 5' d(TAGGAATTCTGA)<br>3' d(ATCCTTAAGACT) | 5 | 51 |
| 5' d(GGCAc$^7$Ac$^7$Ac$^7$Ac$^7$AAC)<br>3' d(GTTTTTTGCC) | 1.5 | 25 | 5' d(TAGc$^3$GAATTCTGA)<br>3' d(ATCCTTAAGACT) | 2 | 40 |
| 5' d(GGCAc$^7$Ac$^7$Ac$^7$Ac$^7$Ac$^7$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 24 | 5' d(TAGGAATTCTGA)<br>3' d(ATCCTTAAc$^3$GACT) | 3 | 42 |
| 5' d(GGCc$^7$Ac$^7$Ac$^7$Ac$^7$Ac$^7$AAC)<br>3' d(GTTTTTTGCC) | 1.5 | 24 | 5' d(TAGc$^3$GAATTCTGA)<br>3' d(ATCCTTAAc$^3$GACT) | 4 | 37 |
| 5' d(GGCc$^7$Ac$^7$Ac$^7$Ac$^7$Ac$^7$AC)<br>3' d(GTTTTTTGCC) | 1.5 | 21 | 5' d(TAc$^3$Gc$^3$GAATTCAc$^3$GA)<br>3' d(ATCCTTAAGTCT) | 2 | 29 |

*Non-selfcomplementary Oligonucleotides*[24a] : The duplex $d(A)_{12}\cdot d(T)_{12}$ shows a much higher $T_m$-value than $d(AT)_6$ (Table 2, 3)[20]. Although the replacement of dA by $c^7A_d$ stabilizes the duplex-structure of alternating d(AT)-units (Table 2), a homooligomer such as $d(A)_{12}\cdot d(T_{12})$ duplex is strongly destabilized when $c^7A_d$ is replacing dA (Table 3). In this relation it is interesting to study the influence of systematic dA-substitutions by $c^7A_d$ and $c^3A_d$ within a $d(A)_6$ tract. For this purpose we have chosen the parent sequence 5'd(GGCAAAAAAC)·3'd(GTTTTTTGCC) which shows an intrinsic curvature and which has already been used for evaluation of the chemical determinants of DNA bending[5,6,28,29].

Table 3 demonstrates that an increasing number of 7-deazaadenine residues within the $d(A_6)$-tracts destabilize the duplex-structure. The $T_m$-value is strongly decreased when $c^3A_d$ is replacing dA. The same tendencies are found in case of $c^3G_d$; according to the oligomer's content of $c^3G_d$ the $T_m$-values are reduced. A $(c^7A_d)_3\cdot(dT)_3$-segment flanked by dC·dG-base pairs shows the same decrease of melting independent of its location at the 5'- or the 3'-site of the dA-tract. A similar behaviour is observed for $c^3A_d$ but with stronger reduced $T_m$-values. This result differs basicly from the bending behavior of these oligonucleotides: here, the location of the modified nucleotide is of decisive influence. Modifications at the 3'-site of the $d(A)_6$ tract reduces bending tremendously while a modification at the 5'-site has only a slight influence.

As it can be seen from oligomers containing $c^3A_d$ the nearest neighbour of the modified base is also of major importance for the stability of the duplex structure. However, from the graphs of figure 1 and 2 an almost linear dependence of the $T_m$-values on the number of modified base residues exist. Within dA-tracts the replacement of one dA-residue by $c^7A_d$ reduces the $T_m$ by about 2°C while $c^3A_d$ leads to a destabilization of nearly 5°C.

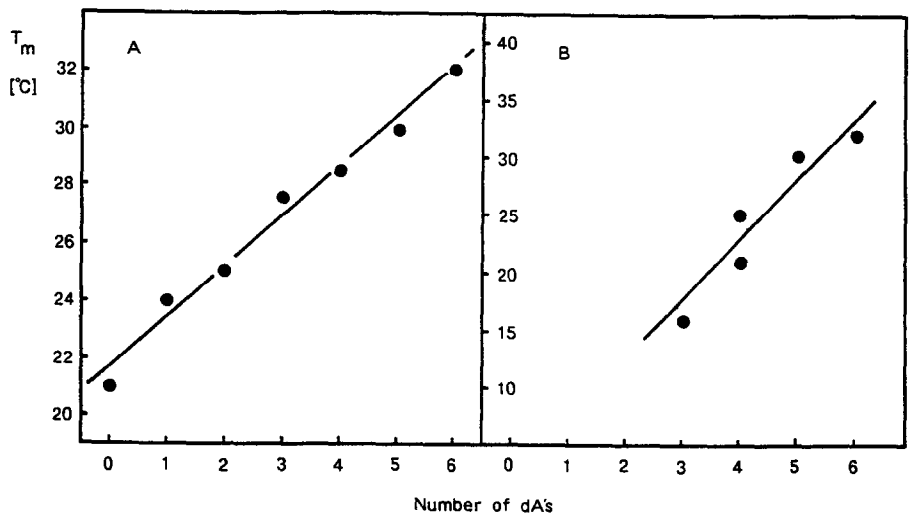



Figure. $T_m$-Values of the oligonucleotide duplexes [5'd(GGCAAAAAAC)·3'd(GTTTTTTGCC)] in which dA residues are replaced by different numbers of $c^7A_d$ (A) or $c^3A_d$ residues (B).

In conclusion, 3-deazapurine 2'-deoxynucleoside residues destabilize oligodeoxynucleotide duplexes much more than 7-deazapurine compounds. This phenomenon is more pronounced in homopurine like tracts than in oligomers, in which the modified base is surrounded by a pyrimidine moiety. Under certain circumstances, e.g. d(A-T) sequences and a high content of $Na^+$ and $Mg^{2+}$ the $c^7A_d$ residue is able to stabilize the duplex structure.

**Acknowledgement**

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**References and Notes**

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14. a) Compound **4** has been synthesized from the DMT-derivative of **8a**[13] as described for **6c**. Colourless foam. $^{31}P$ NMR ((D$_6$)DMSO): 0.86 ($^1J$ (P,H) = 583 Hz, $^3J$ (PH) = 9 Hz. $^1H$ NMR ((D$_6$)DMSO): 11.78, 11.70 (2s, 2 NH); 6.71 (d, PH, J = 583 Hz); 7.04 (d, 5-H, J = 2.3 Hz ); 6.48 (m, 1'-H, 6-H); 3.75 (2s, $CH_3O$); 0.85 - 1.1 (m, ib-$CH_3$, $Et_3N$).
b) Milligan, J.F.; Krawczyk, S.H; Wadwani, S.; Matteucchi, M. *Nucleic Acids Res.* **1993**, *21*, 327.
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24. a) The $T_m$-values were measured in 1 M NaCl and 100 mM $MgCl_2$ buffer solution; b) Measured in 10 mM Tris-HCl, 150 mM NaCl, pH 7.0.
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