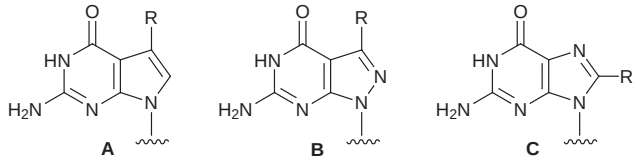
Stabilisation of duplex DNA by 7-halogenated 8-aza-7-deazaguanines

Frank Seela*† and Georg Becher

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

Oligonucleotides containing 7-halogenated 8-aza-7-deaza-2′-deoxyguanosine ($c^7z^8G_d$) derivatives such as d($Br^7c^7z^8$ G-C)$_4$ 8 (T_m = 88 °C) and d($I^7c^7z^8$ G-C)$_4$ 9 (T_m = 84 °C) are significantly more stable than d(G-C)$_4$ 5 (T_m = 59 °C).

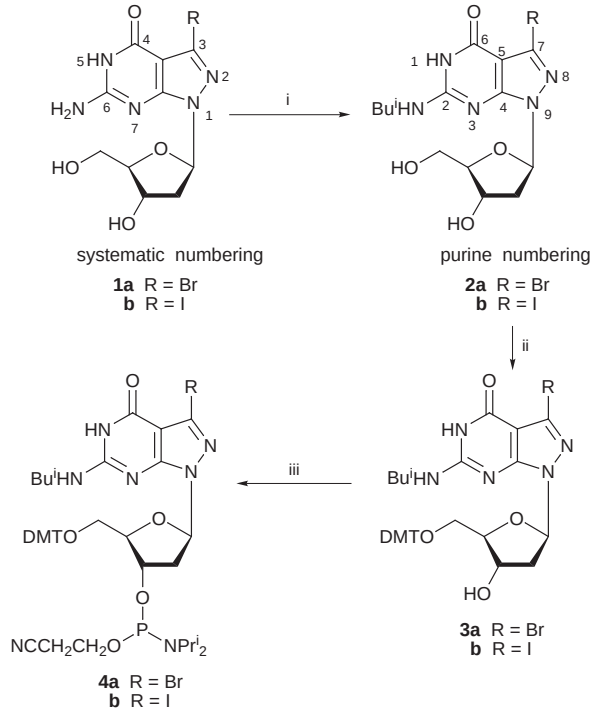
The introduction of 7-halogenated 7-deazapurines (pyrrolo-[2,3-*d*]pyrimidines **A**) into oligonucleotides reveals that these residues are well accommodated in the major groove of the duplex DNA. Furthermore, this modified DNA is stabilised and the particular DNA structure is retained.[1,2] Consequently, the 7 position of 7-deazapurines is an ideal site for the introduction of functional residues into the DNA which can serve later as reporter groups, cleaving agents or residues useful in sequencing by mass spectrometry or by atomic force microscopy.[3] In the series of modified nucleobases related to purines, the 8-aza-7-deazapurines (pyrazolo[3,4-*d*]pyrimidines **B**) depict another heterocyclic system which can be derivatised at the same position while retaining the Watson–Crick recognition site of a purine base (**C**), but not showing the unfavourable properties of 8-substituted purine residues.[4]



Regular pyrazolo[3,4-*d*]pyrimidine nucleosides have already been incorporated into oligonucleotides.[5–7] Also, the monomeric 7-bromo and 7-iodo derivatives of 8-aza-7-deaza-2′-deoxyguanosines (**1a**,**b**) have been synthesised.[8] As we wanted to prove whether compounds **1a**,**b** show the same favourable properties as the corresponding 7-deazapurines, building blocks for oligonucleotide solid-phase synthesis were prepared. For this purpose the isobutyryl residue was introduced as an amino protecting group yielding the protected nucleosides **2a** (64%) and **2b** (66%) (Scheme 1). The protecting group stability was determined UV spectrophotometrically at 300 nm in conc. NH_3 at 40 °C. The half-lives of 7-bromo-8-aza-7-deaza-2′-deoxyguanosine **2a** ($Br^7c^7z^8G_d$; 38 min) and 7-iodo-8-aza-7-deaza-2′-deoxyguanosine **2b** ($I^7c^7z^8G_d$;41 min) were similar to that of the parent 8-aza-7-deaza-2′-deoxyguanosine ($c^7z^8G_d$; 37 min).[7] Subsequently, 4,4′-dimethoxytrityl (DMT) groups were introduced under standard conditions furnishing the 5′-protected compounds **3a** and **3b** (74 and 71% yield), respectively. They were then converted into the phosphoramidites **4a** and **4b** (80 and 76%). All monomeric compounds were characterised by ^{1}H, ^{13}C and ^{31}P NMR spectra as well as by elemental analyses.[9]

Next, the self-complementary hexanucleotides d($Br^7c^7z^8$G-C)$_3$ **13** and d($I^7c^7z^8$G-C)$_3$ **14** as well as the octamers d($Br^7c^7z^8$G-C)$_4$ **8** and d($I^7c^7z^8$G-C)$_4$ **9** were prepared using the building blocks **4a**,**b**. The oligonucleotides were removed from the solid support (conc. aq. NH_3), deprotected and purified on OPC cartridges.[10] Their purity was proven by ion-exchange chromatography on a 4 × 50 mm NucleoPac PA-100 column (DIONEX), and MALDI-TOF mass spectra[11] were obtained. Their base composition was confirmed by enzymatic hydrolysis. Furthermore, their thermodynamic stability was determined by temperature dependent UV–melting profiles. Table 1 summarises T_m values as well as thermodynamic data (MeltWin[12]) of the duplex formation of the self-complementary oligonucleotides **5**, **7**–**9** and **12**–**14** as well as of the corresponding oligomers containing 7-deazaguanine (c^7G_d) (**6**, **10** and **11**).[2]

According to Table 1 it is apparent that the 8-aza-7-deazaguanine moiety which has no 7-substituent stabilises the oligonucleotide duplex [d(c^7z^8G-C)$_4$] **7** (T_m = 72 °C) compared to the parent 2′-deoxyguanosine [d(G-C)$_4$] **5** (T_m = 59 °C, ΔT_m = 13 °C), while the 7-deaza-2′-deoxyguanosine in [d(c^7G-C)$_4$] **6** shows a destabilisation (T_m = 53 °C, ΔT_m = −6 °C).[2] These findings are in agreement with earlier observations made on other oligonucleotides as well as on polynucleotides.[13–15] The octanucleotides with halogenated 8-aza-7-deazaguanine residues [d($Br^7c^7z^8$G-C)$_4$] **8** (T_m = 88 °C) and [d($I^7c^7z^8$G-C)$_4$] **9** (T_m = 84 °C) show considerable duplex stabilisation. The stability of these duplexes was even higher than those of the related oligomers with the 7-halogenated 7-deazaguanine residues (**10** and **11**). Due to the high T_m values of **8** and **9** it was not possible to obtain a complete melting profile, which is necessary to determine the thermodynamic data. Therefore, a set of hexanucleotides was measured showing *ca*. 15 °C lower T_m values. Again, the iodo compound **14**



Scheme 1 *Reagents and conditions*: i, HMDS, Bu^i_2O, DMF, room temp., 13 h, 64% (**2a**), 66% (**2b**); ii, DMTCl, py, room temp., 4 h, 74% (**3a**), 71% (**3b**); iii, (Pr^i_2N)($NCCH_2CH_2O$)PCl, THF, room temp., 30 min, 80% (**4a**), 76% (**4b**)

Table 1 T_m values and thermodynamic data of duplex formation of oligonucleotides[a,b]

Compound	T_m/°C	ΔH/kcal mol^{-1}	ΔS/cal K^{-1} mol^{-1}	ΔG_{298}/kcal mol^{-1}
d(G-C)$_4$·d(G-C)$_4$ **5·5**	59	−67	−179	−11.9
d(c^{7}G-C)$_4$·d(c^{7}G-C)$_4$ **6·6**	53	−65	−178	−10.2
d(c^{7}z^{8}G-C)$_4$·d(c^{7}z^{8}G-C)$_4$ **7·7**	72	−74	−193	−14.5
d(Br^{7}c^{7}z^{8}GC)$_4$·d(Br^{7}c^{7}z^{8}GC)$_4$ **8·8**	88	[c]	[c]	[c]
d(I^{7}c^{7}z^{8}G-C)$_4$·d(I^{7}c^{7}z^{8}G-C)$_4$ **9·9**	84	[c]	[c]	[c]
d(Br^{7}c^{7}G-C)$_4$·d(Br^{7}c^{7}G-C)$_4$ **10·10**	67	−71	−188	−13.0
d(I^{7}c^{7}G-C)$_4$·d(I^{7}c^{7}G-C)$_4$ **11·11**	67	−66	−171	−12.6
d(G-C)$_3$·d(G-C)$_3$ **12·12**	47	−55	−150	−8.4
d(Br^{7}c^{7}z^{8}G-C)$_3$·d(Br^{7}c^{7}z^{8}G-C)$_3$**13·13**	73	−70	−181	−14.3
d(I^{7}c^{7}z^{8}G-C)$_3$·d(I^{7}c^{7}z^{8}G-C)$_3$ **14·14**	71	−63	−162	−12.9

[a] Oligonucleotide conc. is 10 μM. [b] Measured in 10 mM Na cacodylate, 10 mM $MgCl_2$, 0.1 M NaCl. [c] Not measurable.

exhibits a somewhat lower T_m value than the bromo derivative **13** (Table 1). When comparing the thermodynamic data of the duplexes **13·13** and **14·14** with the unmodified duplex **12·12** it is apparent that a more favourable enthalpy term leads to duplex stabilisation. This effect was much more pronounced in the series of oligonucleotides containing 7-halogenated 8-aza-7-deazaguanine than in those containing halogenated 7-deazaguanine.

From the chromatographic behaviour of the 7-halogenated nucleosides **1a**,**b** on RP-18 HPLC (Fig. 1) as well as of the corresponding oligonucleotides **8**, **9**, **13** and **14** it is apparent that the halogen substituents make the compounds more hydrophobic. Thus, the major grooves of such DNA duplexes become hydrophobic and water molecules, normally being present in these grooves, are expelled. This can influence both the enthalpy and the entropy of duplex formation. However, enthalpic changes play the major role.

Another difference which is observed for the 7-halogenated 8-aza-7-deazaguanine nucleosides compared to the non-halogenated compounds is the change of the pK values of deprotonation (7-deaza-8-aza-2′-deoxyguanosine = 9.3; compounds **1a**,**b** = 9.0). This effect also increases the N-glycosylic bond stability[8] and is most likely explained by the electron-withdrawing effect caused by the 7-substituents. As a result, the hydrogen bonds within the G-C base-pair are strengthened and the duplex becomes stabilised. It was also shown that the triphosphates of 8-aza-7-deazaguanine nucleosides are efficiently incorporated into DNA using DNA polymerases;[16] they are useful for introducing reporter groups into a sterically unproblematic position of the DNA molecule.

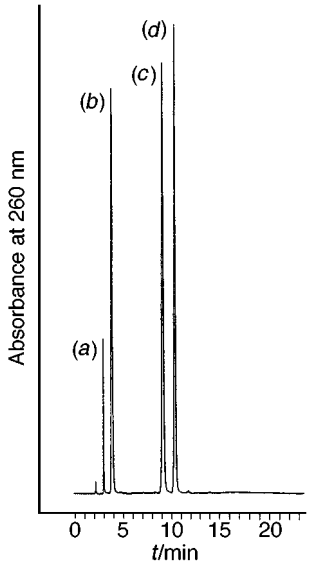


Fig. 1 HPLC profile of (*a*) 6-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one, (*b*) 8-aza-7-deaza-2′-deoxyguanosine, (*c*) **1a** and (*d*) **1b** on a RP-18 (200 × 10 mm) column. The following solvent systems were used: 0.1 M (Et_3NH)OAc (pH 7.0)–MeCN (95:5) (A) and MeCN (B). They were used according to the following profile: 20 min 5–20% B in A, 35 min 20–50% B in A, 35 min 5% B in A.

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Notes and References

† E-mail: Fraseela@rz.uni-Osnabrueck.de

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9 *Selected data* for **3a**: δ_H([$^2H_6$]DMSO) 1.03 [d, *J* 6.7, $(CH_3)_2$], 2.25 [m, H_β-C(2′)], 2.75 [m, H_α-C(2′)], 3.05 [m, H_2-C(5′)], 3.70 (s, 2 CH_3O), 3.95 [m, H-C(4′)], 4.44 [m, H-C(3′)], 5.30 [d, *J* 4.5, HO-C(3′)], 6.37 [t, *J* 6.5, H-C(1′)], 6.77–7.16 (2m, 13 arom. H), 11.86 (s, NH), 11.97 (s, NH). For **4a**: δ_P($CDCl_3$) 148.4, 148.5. For **3b**: δ_H([$^2H_6$]DMSO) 1.12 [d, *J* 6.7, $(CH_3)_2$], 2.25 [m, H_β-C(2′)], 2.77 [m, H_α-C(2′)], 3.03 [m, H_2-C(5′)], 3.70 (s, 2 CH_3O), 3.92 [m, H-C(4′)], 4.44 [m, H- C(3′)], 5.29 [d, *J* 4.2, HO-C(3′)], 6.37 [t, *J* 5.5, H-C(1′)], 6.76–7.33 (2m, 13 arom. H), 11.83 (s, NH), 11.92 (s, NH). For **4b**: δ_P($CDCl_3$) 148.4, 148.5.
10 Oligonucleotide Purification Cartridges, Applied Biosystems, Weiterstadt, Germany.
11 *Selected data* for d(c^{7}z^{8}GC)$_4$ **7**: Calc.: 2412. Found (M^+): 2412. For d(Br^{7}c^{7}z^{8}GC)$_4$ **8**: Calc.: 2727. Found (M^+): 2728. For d(I^{7}c^{7}z^{8}GC)$_4$ **9**: Calc. 2918. Found (M^+) 2915.
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