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Stabilization of Hoogsteen Base Pairing by Introduction of NH_2 Group at the C8 Position of Adenine

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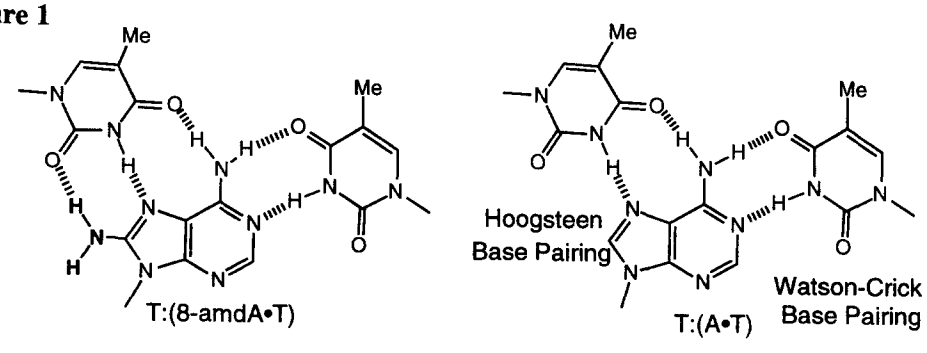
Abstract: The synthesis of 8-aminodeoxyadenosine containing oligonucleotides has been described. The incorporation of an amino group at the C8 position of adenine greatly stabilized the Hoogsteen base pairing in triplex formation.

In addition to the standard Watson-Crick type hydrogen bonding, nucleoside bases can recognize its complementary bases by Hoogsteen-type base pairing,[1] which is typically known to take place in a triplex between the purine strand of the Watson-Crick base pair and a pyrimidine third strand.[2] A variety of base modifications was demonstrated to stabilize the Hoogsteen base pairing in a triplex, but most modifications were made on the third strand of the triplex for their potential therapeutic application.[3] Since ^{1}H-NMR studies on 8-aminodeoxyadenosine (8-amdA) and thymidine (dT) derivatives have shown that they effectively form Hoogsteen base pairs in $CDCl_3$ through an additional H-bonding between the 8-amino group of 8-amdA and the C2 carbonyl of dT,[4] the introduction of an NH_2 group of dA on the purine strand is expected to stabilize Hoogsteen base pairing (Figure 1). We report herein that introduction of an amino group at the C8 position remarkably stabilizes the Hoogsteen-type base pairing in triplex DNA.

Figure 1



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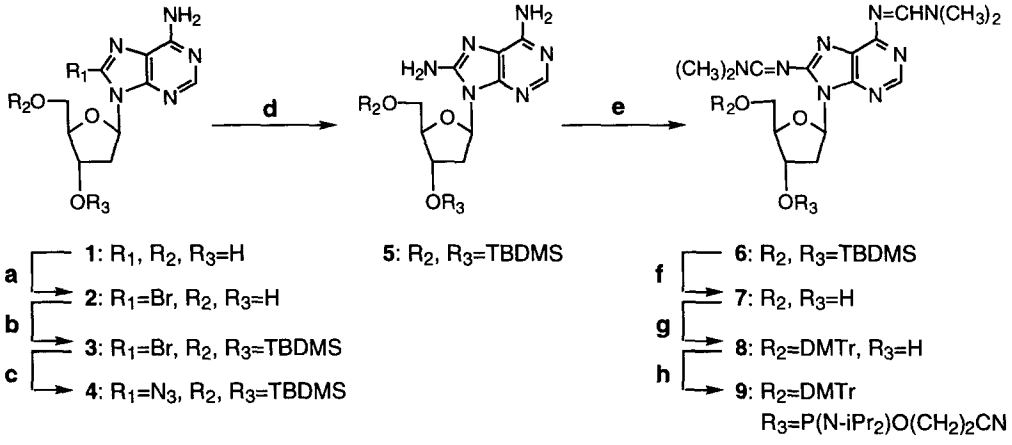
The 8-amdA-containing deoxyoligonucleotides (ODNs) were synthesized according to the standard phosphoramdite chemistry on a DNA synthesizer. The phosphoramidite of protected 8-amdA (**9**) was prepared in 8 steps from deoxyadenosine (Scheme 1). Introduction of an amino group was performed by nucleophilic displacement of 8-bromoadenosine by the reported method.[5] After protecting the N6 and N8 amino groups as *N*,*N*-dimethylformamidine derivative, the *tert*-butyldimethylsilyl groups were removed by treatment with KF in DMF. Standard dimethoxytritylation and phosphophitylation of **7** yielded phosphoramidite **9**.[6] Incorporation of 8-amdA into oligonucleotides was confirmed by enzymatic digestion and ionspray mass spectrometry.[7]

Scheme 1



(a) Br_2 sat. water, pH 4.2 NaOAc buffer, 3 h, 34%; (b) TBDMS-Cl, imidazole, pyridine, 12 h, 81%; (c) NaN_3, DMF, 14 h; (d) H_2, 10% Pd/C, MeOH, 3 h, 85%(2-step yield); (e) DMF-dimethylacetal, pyridine, 3 days, 90%; (f) 18-crown-6, KF, DMF, 15 h, 68%; (g) DMTrCl, NEt_3, pyridine, 15 h, 63%; (h) P(N-iPr)$_2$O$(CH_2)_2$CN, tetrazole, pyridine, 5 h, 98%.

PM3 semi-empirical calculations suggested that Hoogsteen-type base pairing in T:(8-amdA•T) (-6.9 kcal) triplet is more stable than unmodified T:(A•T) (-5.8 kcal) triplet.[8] In order to examine the effects of 8-amdA in a triplex formation, the stabilities of both duplexes and triplexes under neutral (pH 7.0) and acidic conditions (pH 6.0) were investigated. Melting transition data for duplexes and third-strand dissociation of ODNs (**10:11•12**, **10:11•13**, **10:11•14**) are summarized in Table 1. Representative melting curves at pH 7.0 are depicted in Figure 2. The third strand transition temperatures exhibit a marked pH dependence, reflecting protonation of C residues. Introduction of one amino group (**10:11•13**) leads to the increase in Tm of third-strand dissociation relative to the unmodified ODN (**10:11•12**). The triplex was further stabilized by increasing the number of incorporated 8-amdA (**10:11•14**), which contains three 8-amdA residues; this system shows a dramatic increase in Tm of third-strand dissociation. These results clearly indicate that the introduction of an 8-amino group stabilizes triplexes by Hoogsteen base pairing and slightly destabilizes Watson-Crick base pairing in duplex formation.

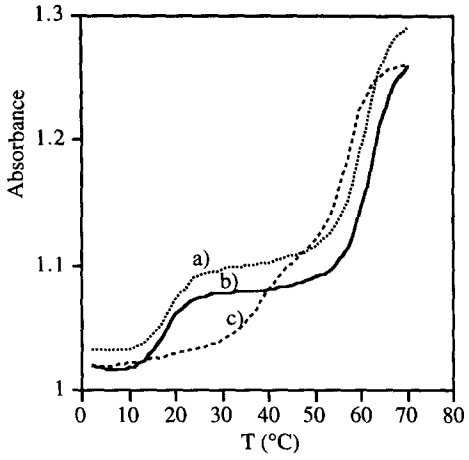


Figure 2. UV-melting curves of (a) 10:11•12, (b), 10:11•13, and (c) 10:11•14. Measurements were conducted at 260 nm in 100 mM Na cacodylate buffer (pH 7.0) containing oligomers 4 μM (each strand) and NaCl 1.0 M.

5'-d(TTCTCTTTCT)-3' **10**
3'-d(CGATTCTCTTTCTAGC)-5' **11**
5'-d(GCTAAGAGAAAGATCG)-3' **12**
5'-d(GCTAAGAGA^{am}AAGATCG)-3' **13**
5'-d(GCTA^{am}AG^{am}AGA^{am}AAGATCG)-3' **14**
5'-d(A^{am}AG^{am}AGA^{am}AAGA)-3' **15**
3'-d(TTCTCTTTCT)-5' **16**

Table 1. Tm Values [°C] for the Dissociation of the Third strand and Duplex[a]

	pH 6.0		pH 7.0	
Triplex	Third strand	Duplex	Third strand	Duplex
10:11•12	33.0	62.2	17.5	62.5
10:11•13	36.5	60.8	18.8	61.0
10:11•14	57.5[b]	57.5[b]	38.3	57.7
10:15•16	43.0[b]	43.0[b]	nd[c]	nd[c]

[a]UV-melting curves were obtained in a 100 mM Na cacodylate buffer containing 1.0 M NaCl at a strand concentration of 4 μM. [b]Cooperative single melting behavior of triplex to single strand was observed. [c]Not determined.

Parallel-stranded duplex with a Hoogsteen base pair was reported in homopurine-homopyrimidine strands[9] and 3'-3' and 5'-5' linked system;[10] therefore, we examined the existence of parallel-stranded duplex formation of **10-15** which forms a stable triplex in the presence of **16**. It was found that **10-15** showed a cooperative single melting behavior (Tm = 38.2 °C); however, UV mixing curve experiment indicated that **10-15** forms a 2:1 complex.[11] The results suggest that **15-16** forms a partially mismatched triplex. Since Wang *et al.* recently showed that 2'-deoxy-3-isoadenine (iA) containing oligonucleotide d(CG[iA]TCG) forms a B-type anti-parallel duplex with central Hoogsteen-type iA:T base pairs;[12] we next studied the Hoogsteen-type base pairing in an anti-parallel duplex. Interestingly, octanucleotide d(amAT)$_4$ showed a remarkably higher thermal stability (Tm = 25.3 °C) compared to the unmodified oligonucleotide (Tm = 8.3 °C).

Considering that (i) triplex formation is difficult in this purine-pyrimidine alternating sequence and (ii) an 8-amino group itself destabilizes the Watson-Crick base-pairing, the present $d(amAT)_4$ system may form an anti-parallel Hoogsteen-type duplex. A detailed analysis of the structure is currently under investigation.

The results clearly demonstrate that one amino group at the adenine C8 position greatly stabilizes the Hoogsteen base pairing in triplex formation by an additional hydrogen bonding. Incorporation of 8-amdA into DNA will provide a useful tool for stabilizing nucleic acid local structures which may have structural and biological significance.

References and Notes

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6. **9**: ^{1}H NMR (400 Mhz, $CDCl_3$) δ 1.10-1.29 (m, 12 H, $CH(\underline{CH_3})_2$), 2.23-2.38 (m, 1 H, H2'), 2.44-2.63 (m, 2 H, $OCH_2\underline{CH_2}CN$), 3.05 (s, 3 H, $N(CH_3)_2$), 3.09 (s, 3 H, $N(CH_3)_2$), 3.13 (s, 3 H, $N(CH_3)_2$), 3.20 (s, 3 H, $N(CH_3)_2$), 3.35-3.76 (m, 7 H, $C\underline{H}(CH_3)_2$, $O\underline{CH_2}CH_2CN$, H2', H5', H5''), 3.74 (s, 6 H, OCH_3), 4.15-4.26 (m, 1 H, H4'), 4.84-4.97 (m, 1 H, H3'), 6.67-6.77 (m, 5 H, methoxyphenyl-*o*, H1'), 7.12-7.43 (m, 9 H, phenyl, methoxyphenyl-*m*), 8.11 (s, 1 H, H2), 8.70 (s, 1 H, $NC\underline{H}N(CH_3)_2$), 8.75 (s, 1 H, $NC\underline{H}N(CH_3)_2$). ^{31}P NMR (121 MHz, $CDCl_3$) δ 149.41, 148.98 (diastereomers). FAB MS: m/e 879 $(M+H)^+$.
7. Ion spray MS (negative); **13**: calcd 4978.3, found 4977.9. **14**: calcd 5008.4, found 5007.9. **15**: calcd 3163.2, found 3162.4.
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