Triplex Formation by an Oligodeoxyribonucleotide Containing N^4-(6-Aminopyridinyl)-2'-deoxycytidine

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A number of laboratories have reported on the abilities of nucleoside analogs and their derivatives to support triplex formation between an oligonucleotide third strand and duplex DNA.[1] Here we demonstrate that oligopyrimidine **I** carrying N^4-(6-aminopyridinyl)-2'-deoxycytidine (**1**) can form stable triplexes with DNA duplex **II·III**. The 3',5'-di-*O*-acetyl derivative of **1** was prepared by refluxing 4-(1,2,4-triazol-1-yl)-1-(β-D-3,5-di-*O*-acetylribofuranosyl)pyrimidin-2(1*H*)-one[2] with 2,6-diaminopyridine in pyridine for 3 days.[3] This nucleoside was converted to the *N*-benzoyl-5'-*O*-dimethoxytrityl-3'-*O*-β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite, which was used to prepare **I** by standard phosphoramidite procedures.[4] In addition to nucleoside **1**, oligopyrimidine **I** contained thymidine and 5-methyl-2'-deoxycytidine (C).

Figure 1 shows absorbance versus temperature profiles of solutions containing equimolar concentrations of oligomers **I**, **II**, and **III**. The biphasic curves are indicative of triplex formation, where the first transition corresponds to melting of strand **I**. This interpretation was confirmed by CD spectroscopy. The CD spectra of triplexes **I·II·III**(**1**·A·T) and **I·II·III**(**1**·C·G) at 10 °C showed a characteristic reduction of Δε at 220 and 280 nm relative to the calculated Δε for **II·III** and noninteracting **I**.[1i,5] At 40 °C, a temperature at which the triplex has melted but the duplex is still intact, the observed spectra were identical to the calculated sum of the spectra of **I** and duplex **II·III**.

Triplexes of different stabilities were observed with each base pair combination at position X·Y of duplex **II·III**(X·Y). The T_m's for **I·II·III**(**1**·A·T), 32 °C, and **I·II·III**(**1**·C·G), ~36 °C, were 12–14 °C higher than those observed for **I·II·III**(**1**·T·A) or

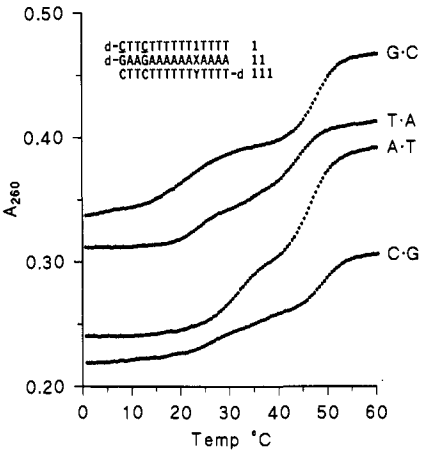


Figure 1. Temperature versus A_{260} profiles of **I·II·III**(**1**·X·Y) measured at pH 7.0 in a buffer containing 0.1 M sodium chloride, 20 mM magnesium chloride, and 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer. The oligomer concentration was 1 μM per strand, and the solutions were heated from 0 °C to 60 °C at a rate of 0.5 deg/min. Base pairs X·Y in **II·III** are shown at the right of each curve, and the curves are offset for clarity.

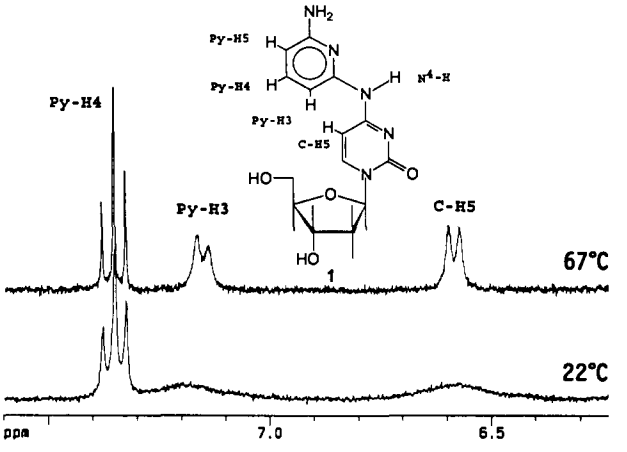
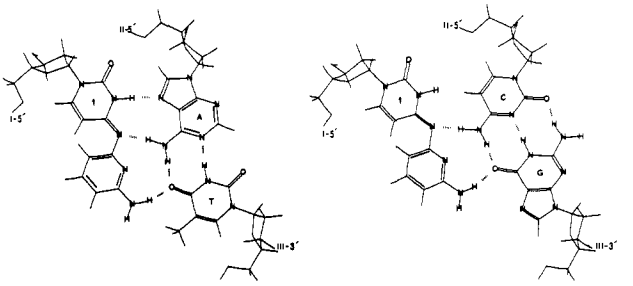


Figure 2. Partial 300-MHz ^{1}H NMR spectra of **1** in DMSO-d_6.

I·II·III(**1**·G·C). In the case of **I·II·III**(**1**·C·G), a broad biphasic transition was consistently observed between 24 °C and 44 °C.

Hydrophobic interactions between the pyridinyl ring of **1** and neighboring bases may contribute to triplex formation by **I**. In the case of **I·II·III**(**1**·G·C), hydrogen bonding between the N-3 (protonated) and N-4 positions of the amino tautomer of the cytosine ring of **1** and G of the G·C base pair appears to be sterically feasible. The observed reduction in T_m of **I·II·III**(**1**·G·C) to 15 °C at pH 8.0 is consistent with such a hydrogen-bonding scheme. The more stable **I·II·III**(**1**·A·T) and **I·II·III**(**1**·C·G) triplexes may involve additional hydrogen-bonding interactions, as shown schematically below. Both triads invoke an unusual imino



tautomeric form of **1**. This tautomer provides a pattern of hydrogen bond donor and acceptor groups which would allow

(1) (a) Young, S. L.; Krawczyk, S. J.; Matteucci, M. D.; Toole, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10023–10026. (b) Ono, A.; Ts'o, P.; Kan, L.-S. *J. Am. Chem. Soc.* **1991**, *113*, 4032–4033. (c) Ono, A.; Ts'o, P. O. P.; Kan, L. S. *J. Org. Chem.* **1992**, *57*, 3225–3230. (d) Griffin, L. C.; Kiessling, L. L.; Beal, P. A.; Gillespie, P.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 7976–7982. (e) Koh, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 1470–1478. (f) Froehler, B. C.; Ricca, D. J. *J. Am. Chem. Soc.* **1992**, *114*, 8320–8322. (g) Krawczyk, S. H.; Milligan, J. F.; Wadwani, S.; Moulds, C.; Froehler, B. C.; Matteucci, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3761–3764. (h) Froehler, B.; Wadwani, S.; Terhorst, T.; Gerrard, S. *Tetrahedron Lett.* **1992**, *33*, 5307–5310. (i) Miller, P. S.; Bhan, P.; Cushman, C. D.; Trapane, T. L. *Biochemistry* **1992**, *31*, 6788–6793. (j) Stilz, H. U.; Dervan, P. B. *Biochemistry* **1993**, *32*, 2177–2185. (k) Milligan, J. F.; Krawczyk, S. H.; Wadwani, S.; Matteucci, M. D. *Nucleic Acids Res.* **1993**, *21*, 327–333. (l) Jetter, M. C.; Hobbs, F. W. *Biochemistry* **1993**, *32*, 3249–3254. (m) Davison, E. C.; Johnsson, K. *Nucleosides Nucleotides* **1993**, *12*, 237–243. (n) Huang, C.-Y.; Cushman, C. D.; Miller, P. S. *J. Org. Chem.* **1993**, *58*, 5048–5049.

(2) This nucleoside was prepared from 3',5'-di-*O*-acetyl-2'-deoxyuridine according to the procedure of Divakar, K. J.; Reese, C. B. *J. Chem. Soc.* **1982**, 1171–1176.

(3) 3',5'-Diacetyl-**1** was isolated in 62% yield following silica gel column chromatography. The mass spectrum, MH^+ 404.2, and ^{1}H NMR spectrum were consistent with the structure of this compound.

(4) Brown, T.; Brown, D. J. S. *Oligonucleotides and Analogues. A Practical Approach*; IRL Press: Oxford, 1991; pp 1–24. The oligomer was deprotected by treatment with concentrated ammonium hydroxide/pyridine (1:1 v/v) at 50 °C for 10 h and purified by C-18 reversed-phase high-performance liquid chromatography. The oligomer gave the expected ratios of nucleosides when digested with a combination of snake venom phosphodiesterase and bacterial alkali phosphatase.

(5) Pilch, D. S.; Levenson, C.; Shafer, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1942–1946.

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formation of two hydrogen bonds with the C·G base pair and three hydrogen bonds with the A·T base pair. Only a single hydrogen bond can be drawn between the pyridinyl amino group of **1** and the O–6 of G or the O-4 of T if 1 is in the amino tautomeric form. This hydrogen-bonding scheme could account for the higher T_m of **I·II·III**(**1**·A·T) relative to that of **I·II·III**(T·A·T), a triplex whose third strand employs two hydrogen bonds per base triad and melts at 22 °C at pH 7.0.[6]

Support for the existence of the imino tautomeric form comes from examination of the ^{1}H NMR and UV spectra of **1**. As shown in Figure 2, resonances corresponding to H-5 of cytosine and the Py-H3 of the pyridinyl ring are unusually broad at 22 °C but appear as sharp doublets at 67 °C.[7] Such broadening could be due to restricted rotation about the C^4–N^4 bond caused by formation of the imino tautomer of **1**. Similar broadening was not observed for O^4-(3-aminophenyl)-2′-deoxyuridine (**2**), an analog of **1** which cannot tautomerize. The UV absorption maxima of **1**, 288 and 327 nm, appear at longer wavelengths relative to those of deoxycytidine, 270 nm, and diaminopyridine, 308 nm.[8] This red shift is consistent with conjugation between the cytosine and pyridine rings, which could occur as a result of tautomerization. The UV spectrum of **2**, on the other hand, does not show a similar red shift.

The broad biphasic transition observed for **I·II·II**(**1**·C·G) suggests the presence of more than one distinct type of triplex. Recent studies by Koshlap *et al.* show that the nonnatural base 4-(3-benzamido)phenylimidazole specifically recognizes T·A and C·G base pairs as a result of intercalation.[9] Intercalation may also play a role in triplex formation by base analog 1.

Our results suggest that **1** and similar base analogs which can potentially contact both bases of a base pair might be used to extend the types of duplex sequences recognized by third-strand oligopyrimidines. Experiments to explore this possibility and to more completely characterize the interactions of **1** are currently in progress.

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(6) Miller, P. S.; Cushman, C. D. *Biochemistry* **1993**, *32*, 2999–3004.
(7) The proton resonances in **1** were completely assigned using 2-D COSY techniques.
(8) UV spectra were recorded in acetonitrile/water (1:1 v/v) at pH 7.
(9) Koshlap, K. M.; Gillespie, P.; Dervan, P. B.; Feigon, J. *J. Am. Chem. Soc.* **1993**, *115*, 7908–7909.