Efficient Synthesis of 2′-Deoxyformycin A Containing Oligonucleotides and Characterization of Their Stability in Duplex DNA

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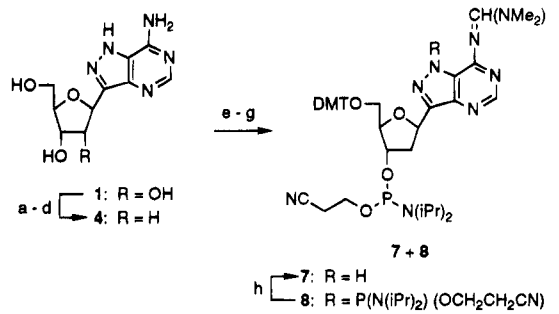
Received July 19, 1995 (Revised Manuscript Received September 21, 1995)

Formycin A (**1**) and 2′-deoxyformycin A (**4**) are C-nucleoside analogs of adenosine and 2-deoxyadenosine.[1] Both **1** and **4** show antitumor activity,[2] and formycin also exhibits antiviral activity.[3] *In vitro*, formycin A and its derivatives can effectively substitute for the corresponding adenosine substrates in a variety of enzymatic reactions.[4] Though formycin A and 2′-deoxyformycin A effectively mimic the recognition and hydrogen-bonding properties of adenosine, the presence of the ribosyl-carbon linkage provides stability against enzymatic cleavage.[5] Oligodeoxynucleotides incorporating **4** would be expected to represent unique substrate based inhibitors of *N*-glycosylase enzymes specific for 2′-deoxyadenosine within duplex DNA. In particular, *E. Coli* MutY is an adenine glycosylase which participates in the repair of G·A, 8-oxo-G·A, and C·A mismatches in DNA by removal of the adenine base.[6,7] DNA *duplexes* incorporating 2′-deoxyformycin opposite G, 8-oxo-G, or C may retain specific recognition by MutY to form a stable MutY–DNA complex which would be amenable to structural studies.

The potential usefulness of C-nucleoside-containing oligonucleotides underscores the need for efficient syntheses and characterization of these molecules. A synthesis of a N^7-benzoyl protected 2′-deoxyformycin A phosphoramidite and its incorporation into a triple helix forming oligonucleotide has been reported.[8] However, the phosphoramidite was obtained in low overall yield of 13%, and the effects of the presence of 2′-deoxyformycin in duplex DNA were not characterized. Herein, we report an efficient synthesis of a 2′-deoxyformycin A phosphoramidite (Scheme 1) and its incorporation into DNA via automated solid phase methods.[9] In addition, we present a thermodynamic characterization of the effect on duplex stability of F·X base pairs (X = T, G, C, or A) and of substitution of 2′-deoxyformycin for 2′-deoxyadenosine in defined G·A mismatch structures.

Scheme 1[a]



[a] Key: (a) $TIPDSiCl_2$, pyr, 20 °C, 2.5 h; (b) PhOC(S)Cl, DMAP, CH_3CN, 20 °C, 6.5 h, 48% (two steps); (c) polymer-supported tin hydride, AIBN, toluene, 80 °C, 1.5 h, 92%; (d) TBAF, THF, 20 °C, 1 h, 94%; (e) $Me_2NCH(OMe)_2$, DMF, 20 °C, 1 h, 94%; (f) DMTCl, cat. DMAP, pyr, 20 °C, 1 h, 68%; (g) $iPr_2NP(Cl)OCH_2CH_2CN$, Et_3N, CH_2Cl_2, 20 °C, 1 h, 47% (**7**) + 48% (**8**); (h) 1*H*-tetrazole, iPr_2NH, CH_3CN, 20 °C, 24 h, 81%.

Our synthesis of a 2′-deoxyformycin A phosphoramidite (**7**) was performed as outlined in Scheme 1. The established deoxygenation procedure[10] was modified in the reduction step by using a polymer-supported organotin hydride instead of the commonly used monomeric tributyltin hydride.[11] This procedure facilitates the isolation of the deoxygenated product **4** since the immobilized organotin reagent can simply be filtered off after its use and recycled. Using this method, **4** was obtained from **1** in 42% overall yield. Dimethylformamidine protection[12] of the exocyclic amino group followed by protection of the 5′-hydroxyl group as the 4,4′-dimethoxytrityl ether and conversion of the 3′-hydroxyl group to the phosphoramidite resulted in formation of **7**. Additionally, the bisphosphitylated product **8** was obtained. However, we were able to convert **8** in 81% yield to **7** by treatment with 1*H*-tetrazole and *N*,*N*-diisopropylamine in anhydrous acetonitrile.[13] Thus, the desired phosphoramidite **7** was obtained in an overall yield of 22% starting from **1**. The phosphoramidite **7** was incorporated with >95% coupling efficiency into oligonucleotides using standard phosphoramidite chemistry. Base composition analysis of the modified oligonucleotides was performed by HPLC after digestion with snake venom phosphodiesterase and alkaline phosphatase,[14] confirming that the 2′-deoxyformycin had been incorporated without modification during DNA synthesis. In addition, synthesis and analysis of a short oligomer of the sequence T–F–T by FAB-MS gave a strong parent ion at the expected molecular weight of m/z 860 $(M + H)^+$.

We have incorporated 2′-deoxyformycin A into DNA duplexes across from all four natural bases to form F·G, F·C, F·A, and F·T base pairs. UV absorbance versus temperature measurements were used to compare the resulting stability of these DNA duplexes with their A·G-, A·C-, A·A-, and A·T-containing counterparts (Table 1).[15] For all four base pairs in the sequences 1–8, those containing 2′-deoxyformycin instead of 2′-deoxyadenosine

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(15) All measurements were performed in triplicate (see supporting information for details).

0022-3263/95/1960-7094$09.00/0

Table 1. Melting Temperatures and Thermodynamic Parameters for Modified and Unmodified Oligonucleotide Duplexes[a]

No.	Sequence	pH	T_m(°C)	ΔH°	ΔS°	$\Delta G°_{298}$
1[b]	5'-GAGCT**G**GTGGC-3'	7	47.3	73.9	206	12.7
	3'-CTCGA**A**CACCG-5'	5	45.9	75.5	211	12.6
2	5'-GAGCT**G**GTGGC-3'	7	45.2	63.7	174	11.7
	3'-CTCGA**F**CACCG-5'	5	45.1	68.3	189	12.0
3	5'-GAGCT**C**GTGGC-3'	7	39.4	67.3	190	10.8
	3'-CTCGA**A**CACCG-5'	5	45.3	78.8	222	12.7
4	5'-GAGCT**C**GTGGC-3'	7	39.0	63.0	177	10.5
	3'-CTCGA**F**CACCG-5'	5	44.6	75.3	211	12.3
5	5'-GAGCT**A**GTGGC-3'	7	42.3	72.0	201	12.0
	3'-CTCGA**A**CACCG-5'					
6	5'-GAGCT**A**GTGGC-3'	7	39.9	68.3	193	10.9
	3'-CTCGA**F**CACCG-5'					
7	5'-GAGCT**T**GTGGC-3'	7	57.9	94.1	259	17.0
	3'-CTCGA**A**CACCG-5'					
8	5'-GAGCT**T**GTGGC-3'	7	54.9	82.5	226	15.2
	3'-CTCGA**F**CACCG-5'					
9[b]	5'-CG**G**GAATTC**A**CG-3'	7	47.9	86.5	244	13.8
	3'-GC**A**CTTAAG**G**GC-5'	5	46.6	77.2	216	12.9
10	5'-CG**G**GAATTC**F**CG-3'	7	44.1	76.9	217	12.3
	3'-GC**F**CTTAAG**G**GC-5'	5	45.2	77.8	219	12.6
11[c]	5'-AT**GA**GC**GA**ATA-3'	7	47.6	80.7	226	13.4
	3'-ATA**AG**CG**AG**TA-5'					
12	5'-AT**GF**GC**GF**ATA-3'	7	<15	—	—	—
	3'-ATA**FG**CG**FG**TA-5'					

[a] Conditions: 10 mM phosphate buffer containing 1 M NaCl and 1 mM EDTA with a total oligonucleotide concentration of 3-10 μM. ΔH and ΔG units are kcal mol^{-1} and ΔS units are cal K^{-1} mol^{-1}. [b] G·A structure A at pH 7 and B at pH 5; see ref. 17 and 18. [c] G·A structure C at pH 7; see ref. 20.

exhibit slightly lower T_m values (<3 °C), indicating a slightly lower duplex stability. In contrast, substitution of the T·A base-pair in duplex 7 with a mismatched base-pair X·A (X = G, C, or A) results in a much larger decrease in the T_m value of at least 10.6 °C. The pH dependence of the T_m values of the G·A and C·A mismatch containing duplexes 1, 3, and 9 is also similar to the corresponding 2'-deoxyformycin-containing duplexes 2, 4, and 10. For example, in the C·F-containing duplex 4, the pH dependence of the T_m values indicates increased stability at low pH in a manner similar to that for the corresponding C·A containing duplex 3. This is expected since formation of two hydrogen bonds in a C·A mismatch requires protonation of adenine at N-1, and therefore, **4** can readily adopt a similar structure at low pH.[16]

The effects of substitution of **4** for 2'-deoxyadenosine in G·A mismatches was investigated in sequences with defined structures for the G·A mismatch. Structural studies in solution have characterized three different conformations for G·A mismatches (Figure 1).[17-20] The NMR studies of sequences 1 and 9 indicate that the mismatch at pH 7 adopts the G(anti)-A(anti) conformation (structure A) while the G(syn)-A(anti) conformation (structure B) predominates at acidic pH.[17,18] 2'-Deoxyformycin substitution for 2'-deoxyadenosine in these sequences causes a small decrease in duplex stability (ΔT_m ~2 °C per mismatch). These results can be rationalized by the fact that, in structures A and B, substitution of **4** for 2'-deoxyadenosine would not adversely effect the ability to form two hydrogen bonds.

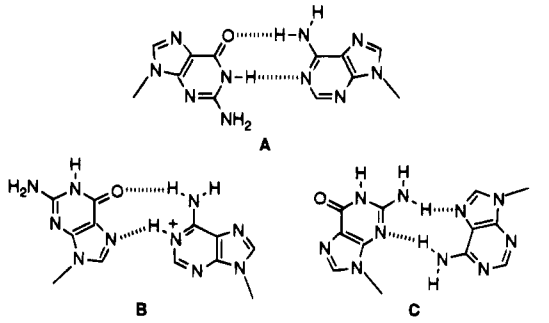

Figure 1. Forms of the G·A mismatch which have been characterized in different duplexes in solution. (A) G(anti)–A(anti). (B) G(syn)–A$^+$(anti). (C) G(anti)–A(anti) conformation (in tandem GA mismatches).

In contrast to the G·A mismatch sequences discussed above, a dramatic duplex destabilization is observed for an A → F substitution in sequence 11 which contains unusually stable tandem G·A mismatches (structure C).[20] The destabilization of this structure by the A → F substitution is expected since 2'-deoxyadenosine contains a hydrogen bond acceptor at the N7 position which is involved in hydrogen bonding in structure C while the predominate NH tautomer of 2'-deoxyformycin A provides a hydrogen bond donor at this position. Thus, in sequence 12, 2'-deoxyformycin A cannot form the corresponding G·F structure to the G·A mismatch structure C. This result is strikingly consistent with the unusual structure C for the tandem G·A mismatches in sequence 11 and suggests that 2'-deoxyformycin oligonucleotides could be used to determine whether or not the N7–N6 (Hoogsteen) bonding face of 2'-deoxyadenosine is employed for stabilizing DNA structures in solution.

In conclusion, we have developed a convenient and efficient synthesis of a 2'-deoxyformycin phosphoramidite which can be site-specifically incorporated into DNA. Our results indicate that DNA duplexes containing isolated F·X base pairs (X = A, T, C, or G) have similar stability to their A·X counterparts. Furthermore A → F substitution into the common G·A mismatch structures (A and B) causes minimal disruption in duplex stability, suggesting that the DNA repair enzyme MutY which recognizes G·A mismatches may also recognize G·F base pairs in DNA. Investigations into the ability of G·F containing duplexes to serve as inhibitors for MutY are in progress.

Acknowledgment. This work was supported by a Young Investigator Award to S.S.D. from the Arnold and Mabel Beckman Foundation, a UC Cancer Research Coordinating Committee Grant, and the donors of the Petroleum Research Fund (ACS-PRF, Type G), administered by the American Chemical Society.

Supporting Information Available: Experimental procedures and characterization data (6 pages).

JO9512970

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