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Hybridization Properties of Oligonucleotides Bearing a Tricyclic 2'-Deoxycytidine Analog Based on a Carbazole Ring System

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Abstract: A tricyclic carbazole-like 2'-deoxycytidine analog has been synthesized via a Stille biaryl coupling on 5-iodo-2'-deoxyuridine followed by cyclization. The carbazole nucleoside was incorporated in oligonucleotides (ONs) and shown to pair specifically with guanine. Duplexes resulting from the carbazole analog ONs and complementary RNA have elevated T_ms especially when the carbazole nucleosides are clustered. Copyright © 1996 Elsevier Science Ltd

The primary origin of the helix stability of DNA and RNA is the stacking interactions of the aromatic heterocyclic rings.[1] We previously reported the synthesis of tricyclic pyrimidine analogs based on the phenoxazine ring system which are capable of enhanced stacking interactions by virtue of their extended aromatic systems (**1**, Figure 1).[2] These tricyclic structures when incorporated into oligonucleotides (ONs) act as cytosine analogs and result in enhanced helix stability. This enhanced stability is particularly pronounced when the tricycles are in adjacent positions and can stack on their tricycle neighbor.

The phenoxazine ring system is the linear fusion of three six membered rings . A perturbation of this ring system would be the carbazole scaffold (**2**, Figure 1) where the central ring is five membered. Such an analog would be expected to be capable of specific Watson Crick base-pairing and enhanced stacking interactions. We report the synthesis of this carbazole pyrimidine 2'-deoxynucleoside, its incorporation into ONs and the characterization of helix formation with a complementary sequence by Tm analysis.

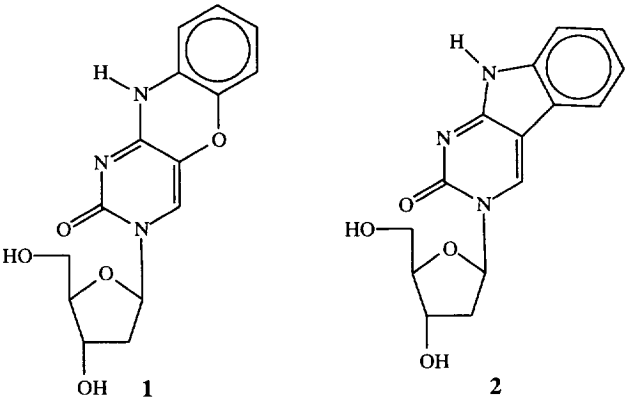
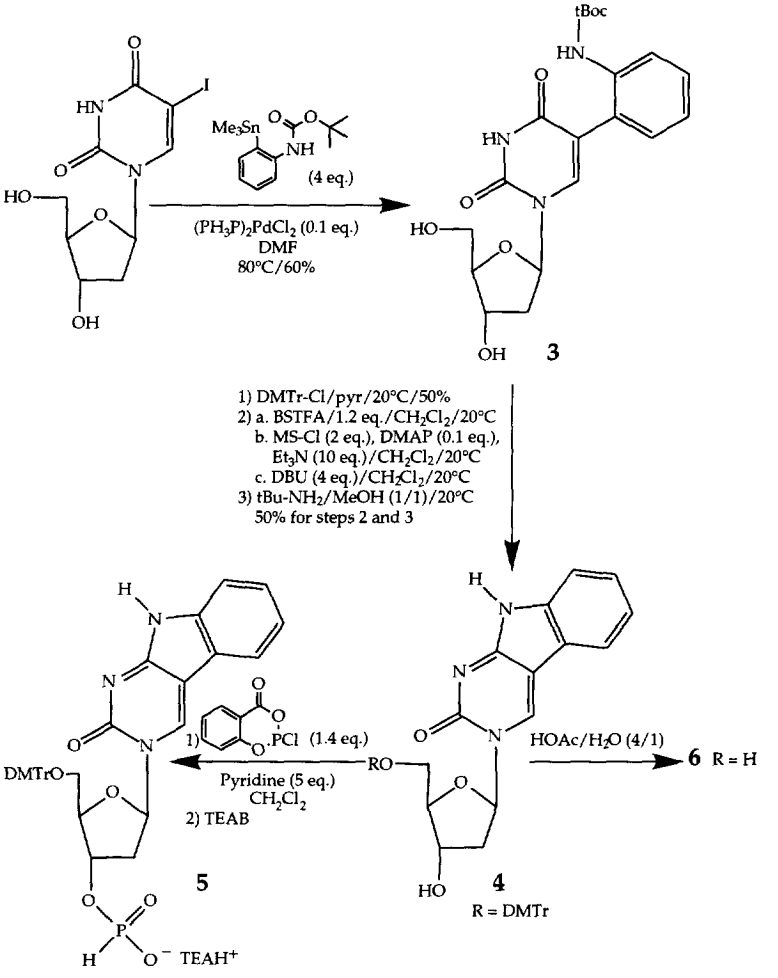


Figure 1.

The synthetic scheme is shown in Scheme 1. The nucleoside is constructed using the Stille biaryl synthesis.[3] 5-Iodo-2'-deoxyuridine and tBOC protected 2-trimethylstannyl aniline[4] were coupled using palladium catalysis yielding the biaryl uracil derivative **3**. The 5' hydroxyl was protected with the dimethoxytrityl group (DMTr). In a one pot reaction, three reactions were performed sequentially. The 3' hydroxyl was transiently protected[5] with the trimethylsilyl group using bis(trimethylsilyl)trifluoroacetamide. The 4 position of the uracil was then activated for displacement using 2-mesitylenesulfonyl chloride[6] followed by the DBU-mediated ring closure to the tricyclic derivative. Following extractive work up, the Boc protecting group was removed from the indole-like nitrogen of the carbazole derivative under mild nucleophilic base conditions yielding **4**.[7] Model studies demonstrated the stability of the unprotected heterocycle to DNA synthesis conditions.[8] The nucleoside was phosphonylated in standard fashion producing **5**,[9] a synthon ready for automated ON synthesis by the H-phosphonate method.[10]

Scheme 1. Synthesis of Carbazole Nucleoside



The ON sequences synthesized are shown in Table 1. ONs were purified by preparative PAGE and characterized by nuclease and phosphatase digestion followed by nucleoside analysis by reverse phase HPLC.[11] All ONs were shown to contain only thymidine, 5-methyl-2'deoxycytidine and the carbazole nucleoside **6** in the correct ratio.

Table 1. T_m Study of Carbazole-Containing ODNs

RNA Target
7 3' AGAGGGAGAGAAAAA

ON	Sequence		T_m°C	Δ T_m°C per Substitution
8	Control	5' dTCTCCCTCTCTTTTT	69	
9	1 Carbazole	5' dTCTCZCTCTCTTTTT	70	1.0
10	3 Apart	5' dTZTCCCTZTZTTTTT	71.5	0.8
11	3 Together	5' dTCTZZZTCTCTTTTT	77	2.6

T_m values were assessed in 140 mM KCl/5 mM Na_2HPO_4/l mM $MgCl_2$, pH=7.2, at 260 nm, and the final concentrations of all ONs and the RNA were approximately 2 μM. T=thymine; C=5-methyl-2'-deoxycytidine; and Z=**2**. T_m values are ± 0.5°C

The carbazole nucleoside-containing ONs were hybridized to the complementary RNA 7[2] and T_m analysis was performed with the data being shown in Table 1. These data show that the clustering of carbazoles in ON 11 results in enhanced stability relative to the non-adjacent context of ON 10. These results are similar to the results observed previously with phenoxazine[2] and are consistent with the postulate that the enhanced stability of the helices results from the increased stacking interactions of the extended aromatic faces of the tricyclic bases.

The specificity for hybridization to a complementary guanine was established by hybridizing the control (ON 8) and the 1 carbazole (ON 9) to a mismatched RNA target (3' AGAGAGAGAGAAAAA) bearing an A in the position opposite the analog. Both the control duplex (53.5°C) and the 1 carbazole duplex (55.5°C) gave T_ms which were significantly lower than those resulting from the hybridization with an opposing G.

This analog is of interest for the application of sequence-specific regulation of gene expression via an antisense mechanism. One can envision using the carbazole as the sole cytosine (C) analog within antisense ONs or in combination with other C analogs bearing extended π bonding faces such as 5-propynyl cytosine or phenoxazine. Different juxtapositioning of such analogs within poly C tracts of ONs will result in perturbations of the stacked conformations between heterocycles. Some of these conformations could result in improved biological potency via an increase in the rate of hybridization of an ON to its target mRNA, a longer hybrid complex lifetime upon formation and/or an enhanced recruitment of cellular enzymes such as RNase H for the destruction of the targeted RNA.

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7. Compound **4**: ^{1}H-NMR δH(300 MHz, $CDCl_3$): 9.00 (s, 1H); 7.60 (d, 1H); 7.49 (d, 2H); 7.38 (d, 4H); 7.30–7.20 (m, 4H); 6.86 (t, 1H); 6.79 (d, 4H); 6.63 (m, 2H); 4.62 (m, 1H); 4.31 (m, 1H); 3.70, 3.69 (2s, 6H); 3.61 (dd, 1H); 3.39 (dd, 1H); 2.97 (m, 1H); 2.76 (br, 1H); 2.43 (m, 1H).
8. The fully deprotected nucleoside **6** was stable to the following conditions: 2.5% dichloroacetic acid/CH_2Cl_2 at 20°C for at least 24 hrs.; 0.1 M I_2 in H_2O/Et_3N/THF (1/1/18) at 20°C for at least 24hrs and conc. NH_4OH at 55°C for at least 24 hrs. The derivative **4** was acetylated using Ac_2O/pyr (1/1) yielding exclusively the monoacetylated 3' ester. This hydroxyl-protected derivative was stable to the H-phosphonate coupling conditions of TEAH salt of ethyl H-phosphonate (0.05M) and pivaloyl chloride (0.25M) in pyridine at 20°C for at least 1 hr.
9. Compound **5**: ^{1}H-NMR δH(300 MHz, $CDCl_3$): 8.96 (s, 1H); 6.89 (d, 1H); 7.51–7.19 (m, 12H); 6.84–6.77 (m, 5H); 6.55 (t, 1H); 6.41 (d, 1H); 5.05–5.03 (m, 1H); 4.40 (m, 1H) 3.69 (2s, 6H); 3.60 (dd, 1H); 3.39 (dd, 1H); 3.07 (m, 6H); 2.96–2.90 (m, 1H); 2.56–2.49 (m, 1H); 1.33 (t, 9H). 31^P-NMR (200MHz, D2O): 3.205.
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