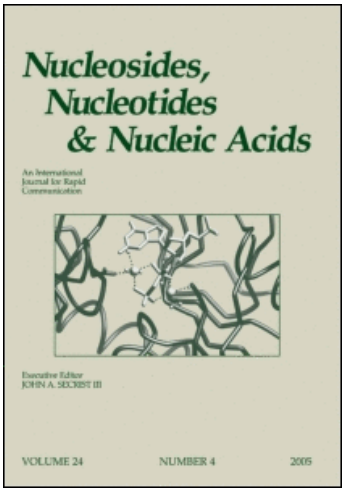

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CG BASE PAIR RECOGNITION WITHIN DNA TRIPLE HELICES USING *N*-METHYL-3*H*-PYRROLO[2,3-*d*]PYRIMIDIN-2(7*H*)-ONE NUCLEOSIDE ANALOGUES

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□ *Triplex-mediated recognition of Py.Pu base pairs in DNA is a greater challenge than for Pu.Py base pairs as fewer hydrogen bonds are presented for binding in the major groove. Initial studies on* m*-aminophenyl-modified analogues of the bicyclic nucleoside* N*-methyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one suggest that selective recognition of the CG base pair is possible.*

Keywords Triple helices; CG recognition; triplex; fluorescence melting; UV melting

INTRODUCTION

Mixed-sequence recognition of duplex DNA by triplex-forming oligonucleotides (TFOs) is an essential requirement for their use in medicinal and biotechnological applications.[1–4] Achieving strong, yet specific binding to pyrimidine.purine base pairs (CG, TA) by TFOs is a greater challenge than to Pu.Py base pairs (GC, AT). The purine bases present two hydrogen-bonding sites in the major groove, yet the pyrimidine bases offer only one.[5,6] In efforts to find a replacement for T (the only natural base capable of recognizing CG), 5-methyl-1*H*-pyrimidin-2-one (^{4H}T) has been prepared and shown to be selective for CG.[7] The proposed interaction occurs via one weak C–H···O hydrogen bond and a conventional N–H···N hydrogen bond (Figure 1).

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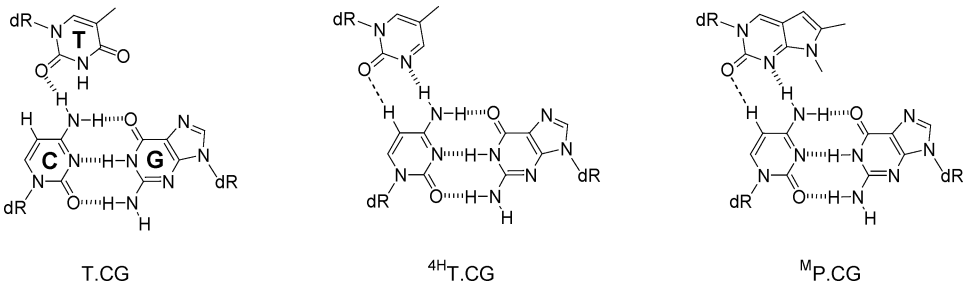


FIGURE 1 T.CG, ^{4H}T.CG and ^{M}P.CG triplet models.

Previous studies indicate that it may be possible to utilize additional interactions across the CG base pair to form more stable, selective triplets than those formed by the natural base T.[8] We have now synthesized *m*-aminophenyl-modified analogues of the core bicyclic nucleoside ^{M}P (Figure 1), using more rigid yet better-positioned linkers than the aminoalkyl modifications already evaluated. It was anticipated this would lead to enhanced binding affinity and selectivity due to hydrogen bonding to the C^6=O and N^7 of guanine and (in certain sequence contexts) additional base-stacking interactions (Figure 2).

The TFOs containing *N*-methyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one were obtained by incorporation of the corresponding furano-pyrimidine phosphoramidite into the oligonucleotide, followed by post synthetic modification (methylamine insertion, cyclization). Triplex-forming oligonucleotides for UV melting contained only a trace amount of uncyclized material and were otherwise clean. TFOs for fluorescence melting contained the *N*-methylpyrrolo-dC modification as the major product, but with a significant amount of uncyclized material.

RESULTS AND DISCUSSION

Three phosphoramidite monomers were synthesized, with *m*-trifluoroacetamido-, ureido-, and acetamido-phenyl modifications at the

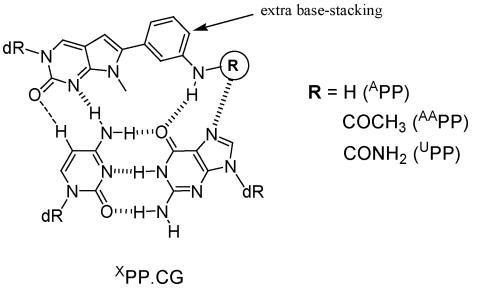
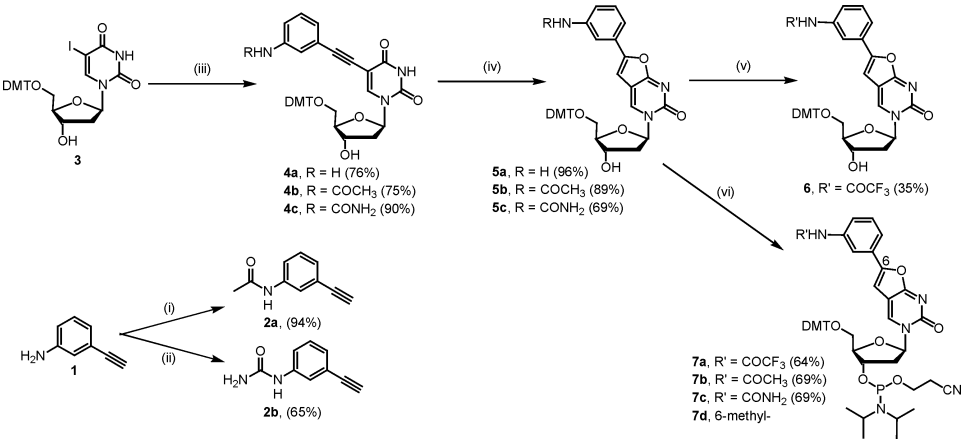


FIGURE 2 *m*-Aminophenyl-modified 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-ones (^{X}PP) in a putative ^{X}PP.CG triplet.

6-position. The heterocyclic core was constructed by Sonogashira cross-coupling[9] to form the derivatized 5-alkynyluridine derivative, followed by triethylamine/CuI-catalyzed cyclization.[8,10] Alkynes **2a** and **2b** were obtained from 3-ethynylaniline **1** by reaction with either phenyl carbamate or acetylation using acetyl chloride. The alkynes were reacted with 5′-*O*-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxyuridine **3** via Pd-catalysed cross-coupling[9] to afford three nucleosides **4a–c**. Following cyclization, the aniline moiety in nucleoside **5a** was protected as the trifluoroacetamide using ethyl trifluoroacetate and subsequent phosphitylation gave the desired furano-pyrimidine phosphoramidite monomers **7a–c**. The 6-methyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one modification (^{M}P) was obtained by incorporating the commercially available 6-methyl-3*H*-furo[2,3-*d*]pyrimidin-2(7*H*)-one phosphoramidite **7d** into oligonucleotides followed by post-solid-phase synthetic modification (described below).



SCHEME 1 Synthesis of 3*H*-furo[2,3-*d*]pyrimidin-2(7*H*)-one nucleoside phosphoramidite monomers: i) phenyl carbamate, 100°C; ii) AcCl, TEA, Et_2O, 0°C-rt; iii) alkyne **1,2a/b**, $Pd(PPh_3)_4$, CuI, TEA, DMF, rt; iv) CuI, MeOH, TEA, 80°C; v) CF_3COOEt, DMAP, TEA, THF, 80–85°C; vi) 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite, DIPEA, THF, rt.

Following incorporation into oligonucleotides under standard solid-phase DNA synthesis conditions, the furanopyrimidine bases were converted to the *N*-methyl-pyrrolopyrimidines by deprotection in 30% aqueous methylamine, followed by recyclization with DOWEX 50WX8-400 ion-exchange resin (H^+ form).

UV melting studies were conducted on triple helices containing modified nucleotides ^{M}P and ^{A}PP, opposite a single CG inversion in a homopurine tract. At both pH 6.1 and 6.4, ^{A}PP showed enhanced binding affinity to CG compared to ^{M}P ($\Delta T_m = 4.0$°C). This represents a significant improvement over T (Table 1).

Fluorescence melting studies were carried out to evaluate modifications ^{M}P, ^{A}PP, ^{AA}PP, and ^{U}PP, opposite a CG inversion (Table 2), using a different

TABLE 1 T_m values (°C) obtained from UV/melting curves ($\lambda = 260$ nm) of singly substituted 15-mer TFOs 5′-TTTTT^{m}CT**X**T^{m}CT^{m}CT^{m}CT^{a,b} with target duplex 5′-GCTAAAAAGA**C**AGAGAGATCG/3′-CGATTTTTCT**G**TCTCTCTAGC (average over four experiments). Duplex melting temperatures are shown in parentheses. Concentration 5 μM:1 μM (TFO:duplex) in 10 mM sodium phosphate buffer with 200 mM NaCl and 1 mM Na_2EDTA

	X =	
pH	^{M}P	^{A}PP
6.07	31.7 (62.3)	35.7 (63.7)
6.40	25.2 (62.5)	29.2 (63.9)

^{a}^{m}C = 5-methyl-2′-deoxycytidine.
^{b}MALDI-TOF MS of modified TFO: X = ^{A}PP found m/z 4612.0 (expected 4611.2).

and longer duplex target. Under these conditions ^{U}PP showed the strongest binding affinity for CG as expected, but it was slightly less selective than ^{M}P for CG relative to AT and TA. All ^{X}PP modifications were more selective for CG relative to GC than the control ^{M}P. Although UV melting studies indicated that ^{A}PP binds more strongly to CG than ^{M}P, fluorescence melting indicated the opposite. This is probably due to the nature of the neighbouring triplets (UV melting ..T**X**T.., fluorescence melting ..T**X**P..); the P.AT triplet is very stable compared to T.AT and will present a different base-stacking environment.

TABLE 2 T_m values determined at pH 6.0 from fluorescence melting curves of singly substituted 15-mer TFOs 5′-**D**-P^{m}C^{m}CTP^{m}CT**X**PTPTPT^{m}CPT^{a,b} with target hairpin duplex 5′-**F**-GTGTTAGGAAGA **Y**AAAAAAGAACTGGT–HEG_2–ACCAGTTCTTTTTT**Z**TCTTCCTAACAC (average over two or four runs). Concentration 2.5 μM:0.25 μM (TFO:duplex) in 20 mM NaOAc solution with 200 mM NaCl

YZ	^{M}P	^{A}PP	^{AA}PP	^{U}PP
CG	53.0	51.9	52.0	53.9
GC	40.6	38.1	38.8	39.8
AT	40.3	40.3	40.6	42.4
TA	39.9	42.3	44.7	44.6

^{a}^{m}C = 5-methylcytidine, P = propargylamino-dU, **D** = DABCYL (fluorescence quencher), **F** = FAM (fluorescent marker).
^{b}MALDI-TOF MS of modified TFO: X = ^{M}P found m/z 5807.4 $[M+H]^+$ (expected 5806.1), X = ^{A}PP found m/z 5884.7 $[M+H]^+$ (expected 5884.2), X = ^{AA}PP found m/z 5926.7 $[M+H]^+$ (expected 5926.3), X = ^{U}PP found m/z 5927.3 $[M+H]^+$ (expected 5927.3).

CONCLUSION

Three modified 3*H*-furo[2,3-*d*]pyrimidin-2-one nucleoside phosphoramidites have been synthesised, incorporated into oligonucleotides and post-synthetically converted to *N*-methyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one nucleosides. Triplex melting studies suggest that the base-stacking environment plays an important role in triplet stability. The order of affinity of the modified bases (^{M}P, ^{A}PP) for CG was reversed on replacement of the neighbouring T.AT triplet with propargylamino-dU.AT. Studies are underway to synthesise *N*-methylpyrrolo-dC phosphoramidites for incorporation into oligonucleotides.

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