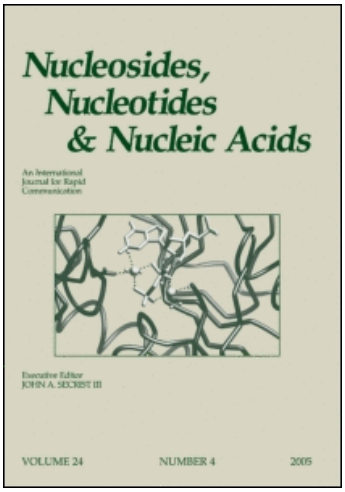

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SYNTHESIS AND PROPERTIES OF PNA OLIGOMERS CONTAINING OROTIC ACID DERIVATIVES

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▫ *We have investigated the incorporation of C6-derivatives of uracil into polypyrimidine peptide nucleic acid oligomers (PNA). Starting with orotic acid (uracil-6-carboxylic acid) we have prepared a PNA monomer containing the methyl orotate nucleobase which is compatible with Fmoc-based synthesis. Treatment of the resin-bound oligomers with hydroxide or amines cleanly converted the ester to an orotic acid or orotamide-containing PNA. Alternatively, the methyl orotate-containing PNA was liberated from the resin by standard acidolysis. PNA bearing a modified nucleobase was found to hybridize to both poly(rA) and poly(dA). Complexes with poly(rA) were more stable than those with poly(dA) but both were destabilized relative to an unmodified PNA. Modification of a terminal residue was tolerated better than modification of an internal position. The type of charge provided by the modification affected the complex stability. In the worst case, an internal modification was nearly as detrimental as a base mismatch.*

Modified nucleobases have been intensively studied in the field of nucleic acids.* Some of our previous work has exploited established chemistry to incorporate modified nucleobases into PNA.[2] This is desirable as it permits the incorporation of functional groups without necessarily introducing chirality, and presents the opportunity to include sites of charge, hydrophilicity, lipophilicity, or sites for further modification can be distributed along the polymer without modification of the base-pairing face of the heterocycle.

To our knowledge orotic acid or orotamides have not been incorporated into oligodeoxynucleotides, thus their effect on PNA structure is speculative. Examination of molecular models from existing structural information suggests that PNA:DNA duplexes[3] and PNA:RNA duplexes[4] may tolerate the substitution better than PNA_2:DNA triple-helical complexes,[5] the latter suffering from unfavorable steric interactions.† However, these three structures also illustrate the

*Reviewed by: Ref. [1].

†HyperChem® 5.1 was used to modify a central thymine or uracil nucleobase in a trimer that was excised from existing structures. The nucleobase was modified to possess a 6-carboxamide group and was geometrically optimized using the MM+ force field while the remainder of the structure was held fixed.

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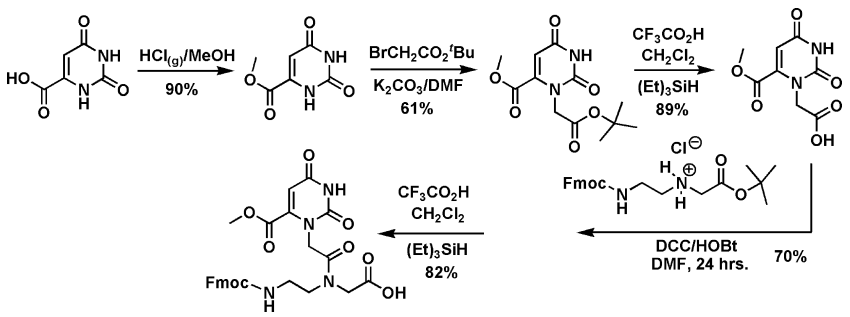


SCHEME 1

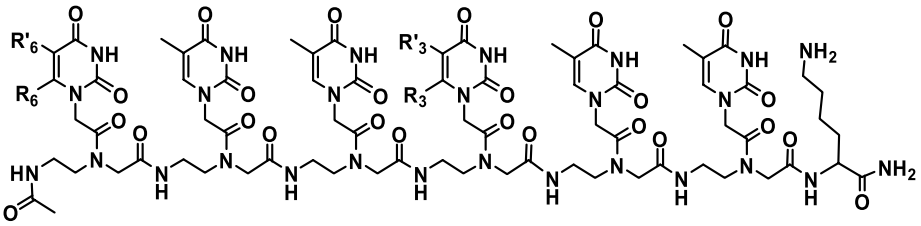
plasticity of PNA conformation. Therefore, we decided to examine all three types of complexes, and the results for triple helices are reported herein.

Our approach to the modified PNA was via the methyl orotate monomer which was synthesized according to Scheme 1. Incorporation of the methyl orotate monomer (MO) into PNA hexamers was done on NovaSyn TGR resin (Rink amide) using the FastMoc chemistry module on an Applied Biosystems 433A synthesizer. The amide linkage to the support permits an on-resin conversion to either an orotamide or orotic acid by the treatment with an amine or hydroxide prior to liberation of the PNA from the support by acidolysis. For example, a typical result is illustrated for sequence 5 (Table 1) incorporating the 3-(*N*,*N*-dimethylamino)propylamide. This compound was prepared by synthesis of the sequence AcTTT(MO)TTK-resin followed by treatment with neat 3-(*N*,*N*-dimethylamino)propylamine at 55°C for 6 hr. After cleavage from the resin, the sequence was isolated as the only significant product (*) by reversed-phase HPLC (Figure 1, panel A) and identified by mass spectrometry (calcd. for $C_{79}H_{111}N_{29}O_{27}$ 1897.82; obs. 1898.83 MH +, 1920.79 MNa+), Figure 1, panel B.

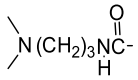
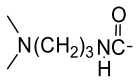
PNA hexamers bearing a single modification, either at the N-terminus or at an internal position were synthesized. An unmodified PNA (AcTTTTTTK-NH_2, sequence 1) and a single mismatch sequence (AcTTTCTTK-NH_2, not shown) were used to gauge the tolerance of the complexes to the modified residue. PNAs were hybridized to poly(rA) or poly(dA) in a 2:1 ratio at a concentration of ~2 mM and in the presence of 150 mM NaCl, 10 mM Na_2HPO_4, 1 mM EDTA at pH 7.0. All compounds were well-behaved and exhibited monophasic cooperative transitions. Inclusion of a modified residue caused a destabilization of the complexes which was least severe for the 3-(*N*,*N*-dimethylamino)propylamide and most severe for the orotic acid substituents which indicates that electrostatic effects are important. However, the substantial loss in complex stability for internal substitution suggests that the substituents also exert a significant steric effect. The worst tolerated substituent was found to be the internal orotic acid modification (sequence 8) which is on par with a complete mismatch (ΔTm (RNA) = −43°C).

The methyl orotate monomer is an effective convertible residue compatible with an on-resin conversion strategy. However, in the context of PNA_2:NA

TABLE 1



Terminal Modification

Entry	R_6	R'_6	R'_3	R_3	T_m (RNA)	T_m (DNA)	ΔT_m(R)	ΔT_m(D)
1	H	$-CH_3$	$-CH_3$	H	62.0	57.0	–	–
2		H	$-CH_3$	H	58.5	47.2	−3.5	−9.8
3		H	$-CH_3$	H	47.2	35.8	−14.8	−21.2
4		H	$-CH_3$	H	46.0	35.0	−16.0	−22.0
Internal Modification								
5	H	$-CH_3$	H		34.0	28.0	−28.0	−29.0
6	H	$-CH_3$	H		22.5	25.5	−39.5	−31.5
7	H	$-CH_3$	H		18.3	24.3	−41.0	−32.7

triple-helices this modification is not well tolerated. It should be noted that the sequences examined contain a modified residue in each PNA domain so its effect was expected to be large. Currently, we are investigating the duplexes formed between PNA sequences containing C6-modified uracil and DNA or RNA.

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