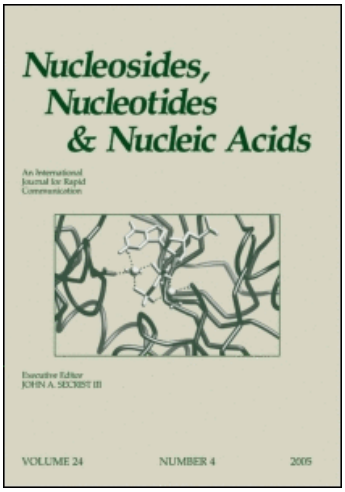

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Amino Acid Nucleic Acids: Synthesis and Hybridization Properties of a Novel Class of Antisense Oligonucleotides

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AMINO ACID NUCLEIC ACIDS: SYNTHESIS AND HYBRIDIZATION PROPERTIES OF A NOVEL CLASS OF ANTISENSE OLIGONUCLEOTIDES

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Abstract: Oligonucleotides containing novel phoshoramidite **12** were synthesized and studied for their hybridization properties for the first time. Interestingly, these modified oligonucleotides showed a remarkable resistance to exonuclease.

Oligonucleotides that specifically recognize messenger RNA[1] present unique opportunities for the treatment of viral diseases, cancer, and for the study of genetic disorders. In order to be pharmacologically useful, the oligonucleotides must have resistance to cellular nucleases and penetrate through cell membrane. To meet these properties several derivatives have been synthesized[2] and examined their biophysical characteristics.

Our interest to design a novel class of antisense oligonucleotides has lead us to envision amino acid nucleic acids (AANA) (i.e., molecules where the individual nucleobases are linked to an amino alcohol through an amide bond and phosphodiester backbone). This isosteric oligonucleotide analogue derived from serinol is shown in Figure 1, (**2**). In this novel class of molecule, we believe, the formation of an intramolecular hydrogen bonding between the amide "NH" of the acetamide tether and the phosphate "O" is necessary for AANA to adopt a constrained conformation (see Figure 1, structure **2**). Thus, the constrained monomers when incorporated into oligonucleotides, may have a force greater than a single hydrogen bond and favor the formation of binding competent nucleic acids. Furthermore, the hydrogen bonding could neutralize the negative of the phosphodiester backbone and this neutralization is expected to enhance the uptake of the AANAs relative to DNAs, rendering them potentially more useful as therapeutic agents.

In this note, we report the synthesis of a novel oligonucleotide building block **12** and biophysical properties of oligonucleotides containing **12**. Optically active amino acid nucleoside[3] analogue bearing thymine was prepared from O-benzyl-D-serine by the route shown in Scheme 1. Thymine acetic acid (**7**) was prepared by the literature procedure.[4] Treatment of O-benzyl-D-serine with di-*tert*-butyl dicarbonate provided N^{α}-BOC-O-benzyl-D-

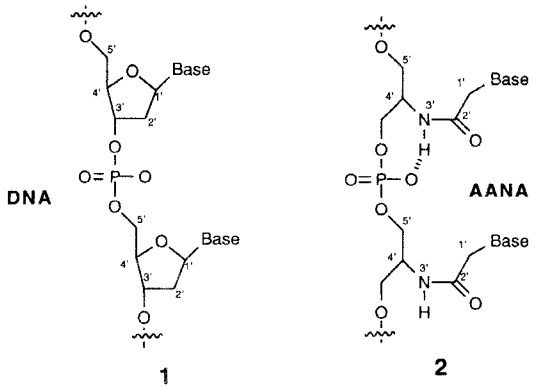
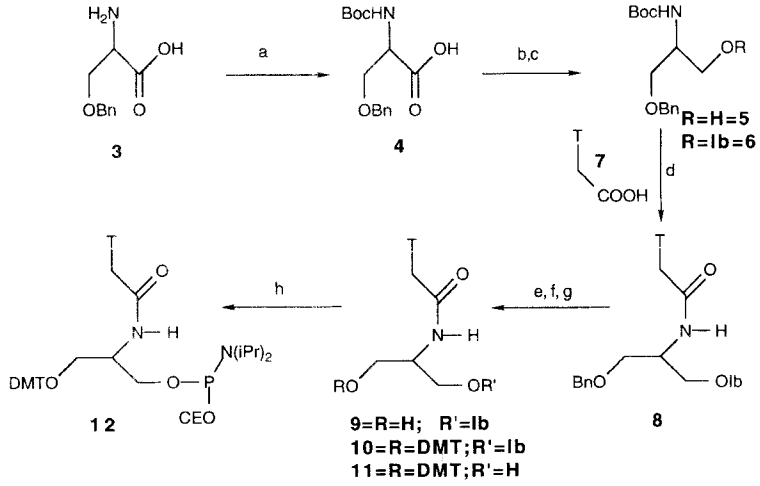


Figure 1



Scheme 1[a]

[a](a) DBDC/THF/water (93%); (b) i. IBCF/TEA/THF; ii.$NaBH_4$THF/Water; (82%); (c) $(IbO)_2O$/Py (84%); (d) i.TFA/CH_2Cl_2, ii. EDC/NMM/HOBt/7/DMF (94%); (e) $Pd(OH)_2$/Cyclohexene (91%); (f) DMTCl/TEA/Py (88%); (g) 1N NaOH/THF/MeOH (99%); (h) $EtN(iPr)_2$/P(Cl)$(iPr)_2OCH_2CH_2CN$ (2 equiv)/CH_2Cl_2 (83%). DBDC = di-*tert*-butyl dicarbonate; IBCF = isobutyl chloroformate; NMM = N-methylmorpholine; HOBt = 1-hydroxybenzotriazole; T = Thymine.

serine (**4**) in quantitative yield. Reduction of the α-carboxyl group of **4** using mixed anhydride method[5] furnished the corresponding alcohol (**5**). Exposure of the alcohol (**5**) to isobutyric anhydride in dry pyridine for 8 h, followed by usual workup produced the diprotected alcohol (**6**). Removal of the t-BOC group with trifluoroacetic acid followed by coupling of the TFA salt with thymine acetic acid (**7**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), as coupling reagent, gave the

Table 1: Tm values Of Modified Oligonucleotides

Sequences	Tm(°C)		ΔTm /modification (°C)	
	DNA	RNA	DNA	RNA
5' GAA AGG AAG CGG AGA GAT 3'				
5' ATC TCT CCG CTT CCT TTC 3'	58.29	66.8		
5' ATC TCT CCG CTT CCT TtC 3'	60.34	69.03	+2.05	+2.23
5' ATC TCT CCG CTT CCt ttC 3'	56.79		- 0.50	
5' AtC TCT CCG CTT CCT TTC 3'	60.52	68.90	+2.23	+2.1
5' ATC TCT CCG CtT CCT TTC 3'	48.45	58.02	- 9.84	- 8.78
5' AtC TCT CCG CTT CCT TtC 3'	56.85	65.8	- 0.72	- 0.50

The letter "t" denotes modified thymidine.

amino alcohol nucleoside (**8**). Hydrogenation of **8** with $Pd(OH)_2$ in methanol containing cyclohexene furnished 1-O-isobutyryl-2-(thyminylacetyl)amino-3-hydroxy-D-propanol (**9**).[6] Dimethoxytritylation[7] of **9** followed by the removal of the isobutryl group in **10** with 1N NaOH solution and phosphitylation[8] of **11** afforded amino acid nucleic acid amidite synthon **12** as colorless powder.

Incorporation of the building block **12** into oligonucleotide sequences was accomplished using ABI 394 DNA synthesizer and protocol,[9] and the coupling efficiency was found to be higher than 99%. Enzymatic degradation and subsequent HPLC analysis of the modified oligonucleotides indicated the expected ratios of the nucleoside components.[10] The binding behaviour of these modified oligonucleotides was assayed by examining their ultraviolet (UV) absorbance verses temperature profiles.[11] An 18mer oligonucleotide (5'-ATCTCTCCGCTTCCTTTC-3') was modified with **12** at different locations and hybridized to complementary DNA or RNA. Incorporation of **12** at the 3'-end of oligonucleotide increased the duplex stability by a ΔTm of 2.05 °C per modification. The same type of duplex stability was observed when **12** is substituted at 5'-end too. On the contrary, incorporation of **12** in the middle of the duplex caused a significant reduction in binding (ΔTm= -9.84 °C per modification). The presence of **12** at 3'-end as well as at 5'-end had very little effect (ΔTm= -0.72 °C) on duplex stability. When the fully modified (4',5' linked) poly T (AANA) 18mer was heated with its complement poly dA (3',5' linked), a steady increase in absorbance (260 nm) is observed but no cooperative transitions are detectable. This confirms the lack of association of poly T (AANA) having 4',5' linkage with poly dA in aqueous solution. Interestingly enough, incorporation of three modifications at the 3'-end of the oligonucleotides, increased their exonuclease stability[12] by 50-fold than natural DNA. On the other hand, 5'-modified AANAs do not show any improved exonuclease property.

It is evident from this study that 4',5' linked AANA forms a weaker duplex than normal DNA. The weaker binding of oligonucleotides containing D-serine amino acid is an indicative that the monomer **12** is not adopting a constrained conformation that of ribose sugar puckering. Although oligonucleotides containing amino acid nucleic acid building block exhibited better stability towards nucleases, their inability to bind strongly especially under physiological conditions, may precludes their use as antisense compounds.

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