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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. Copyright © 1996 Elsevier Science Ltd

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 oligonucleotide must have [2] (a) sufficient binding to its target sequence; (b) sufficient specificity; (c) stability towards exo- and endo-nucleases; (d) penetration through cell membrane. To meet these criteria derivatives such as phosphorothioates,[3] phosphoramidates,[4] methylphosphonates,[5] mono,[6] or dithiophosphates,[7] boranophosphates,[8] etc., have been examined,[9] as well as variety of non phosphorous linkages that include formacetal,[9] carbamate,[10] siloxane,[11] sulfur linked,[12] amides,[13] amine,[14] methyl hydroxylamine,[15] and peptide nucleic acid (PNA). [16] However, the quest to develop new and novel modified oligonucleotides, based on sequence specific interactions between complementary nucleic acid, has recently sparked.

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 oligoucleotide analogue derived from serinol is shown in Figure 1, (**2**). The significance of amino acid nucleic acids are many fold. First, serinol nucleosides[17] are simpler to prepare than ribonucleosides. Second, the point of attachement of "-O-P(O)(O)-O-" group is different from normal 3',5'- or 2',5'-linkages that exists in natural DNA/RNA. In this novel class of molecules, the internucleotide linkage originates from 4' of one monomer and ends with 5' of the

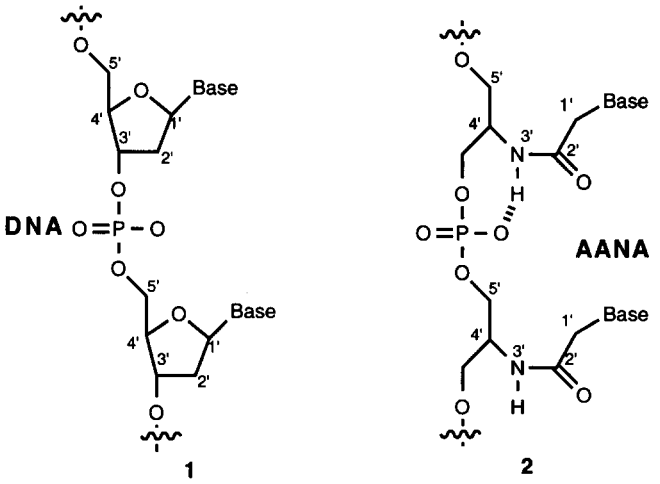


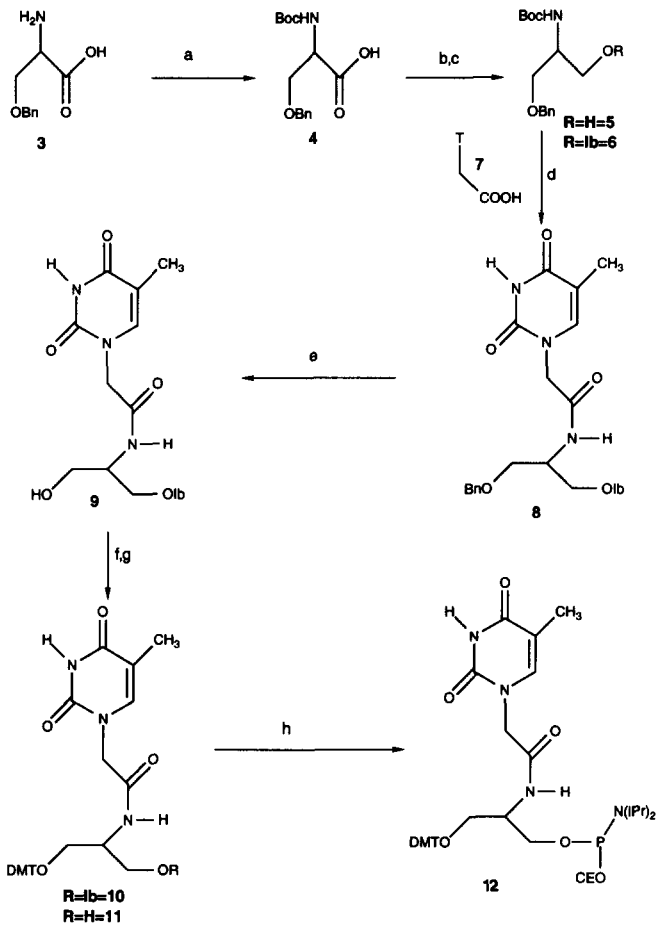

**Figure 1**

adjacent nucleomonomer. Third, we wanted to introduce an intramolecular hydrogen bonding concept within AANA (see Figure 1, structure **2**). We believe, the formation of such hydrogen bonding between the amide "NH" of the acetamide tether and the phosphate "O" is necessary for AANA to adopt a constrained conformation. 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 charge (neutrahydration of negative charge hypothesis)[18] 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. As far as we know, this unique and novel concept is untapped in the paradigm of oligonucleotide therapeutics. Fourth, oligonucleotides built from serinol nucleoside analogues might be stable to nucleases, improving their bioavailability as antisense drugs.

In this communication, we report the synthesis of a novel oligonucleotide building block **12**, constructed for the first time from an optically active L-serine and thymine to give serinol nucleoside (**8**),[17] incorporation of **12** into oligonucleotides and measurement of hybridization of the duplex formed between AANA and natural DNA/RNA.

Optically active amino acid nucleoside analogues bearing thymine were prepared from L-serine-β-benzyl ester by the route shown in Scheme 1. Thymine acetic acid (**7**) was prepared by the literature procedure.[16] Treatment of L-serine-β-benzyl ester with di-*tert*-butyl dicarbonate provided $N^{\alpha}$-BOC-L-serine-β-benzyl ester (**4**) in quantitative yield. Reduction of the α-carboxyl group of **4** using mixed anhydride

**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.

method[19] 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 amino alcohol nucleoside (**8**). Hydrogenation of **8** with $Pd(OH)_2$ in methanol containing cyclohexene furnished 1-O-isobutyryl-2-(thyminylacetyl)amino-3-hydroxy-L-propanol (**9**).[20] Dimethoxytritylation[21] of **9**

followed by the removal of the isobutryl group in **10** with 1N NaOH solution and phosphitylation[22] of **11** afforded amino acid nucleic acid amidite synthon **12** as colorless powder.

**Table 1:** Tm values of Oligonucleotides Containing Amino Acid Nucleic Acid building block[a]

| Sequence | 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' | 59.2 | 66.8 | | |
| 5' ATC TCT CCG CTT CCT TtC 3' | 57.4 | 66.2 | -1.8 | -0.6 |
| 5' ATC TCT CCG CTT CCT ttC 3' | 56.2 | | -1.5 | |
| 5' ATC TCT CCG CTT CCt ttC 3' | 54.7 | | -1.1 | |
| 5' AtC TCT CCG CTT CCT TTC 3' | 58.0 | 66.4 | -1.2 | -0.4 |
| 5' ATC TCT CCG CtT CCT TTC 3' | 50.2 | 60.8 | -9.0 | -6.0 |

[a]Tm is the melting temperature of duplex at midpoint of the melting curve at which half the molecules are duplex and half are single stranded. The concentrations are as follows: oligomer strands, 2 mM each. Melting temperatures (Tm) were determined[23] by measuring change in absorbance at 260 nm (cuvette,1-mm path length) as a function of temperature in sodium phosphate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 0.1mM EDTA. All the values are averaged from at least three experiments. The letter "t" denotes AANA thymidine.

Incorporation of the building block **12** into oligonucleotide sequences was accomplished using ABI 394 DNA synthesizer and protocol,[24] 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.[25] The binding behaviour of these modified oligonucleotides was assayed by examining their ultraviolet (UV) absorbance verses temperature profiles. An 18mer oligonucleotide (5'-ATCTCTCCGCTTCCTTTC-3') was modified with **12** at different locations and hybridized to complementary DNA or RNA. Compared to unmodified DNA, incorporation of **12** at 3'-end of the oligonucleotide decreased the duplex stability by a ΔTm of 1.8 °C per modification. The same type of duplex destability was observed when **12** is substituted at 5'-end too. On the contrary, incorporation of **12** in the middle of the duplex affected the stability of the duplex by 9.0 °C per modification. However, the same modified oligonucleotides showed better hybridizaton behaviour against RNA complement (see Table **1**).

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[26] by 50-fold than natural DNA. On the other hand, 5'-modified AANAs do not show any improved exonuclease property.

It is apparent from this, that if duplex formation occurs with 4',5' linked AANA, it is at best weaker than that for normal DNA. The weaker binding of oligonucleotides containing L-serine amino acid is an indicative that the monomer **12** is not adopting constrained conformation, that of ribose sugar puckering. However, it should be emphasized that it is not yet known what amount of duplex stability the D-serine nucleoside monomer might bring in when incorporated into oligonucleotides *via* 4',5' linkages. Synthesis and biophysical evaluation of oligonucleotides containing D-serine amino acid phosphoramidite building is in progress and will be reported soon. Lack of strong association especially under physiological conditions, may be the disadvantage of using L-amino acid nucleic acids as antisense compounds.

In summary, oligonucleotides containing novel amino acid nucleic acid building block have been synthesized for the first time and studied for their ability to form a stable duplex. Interestingly, AANAs remarkably increase resistance to 3'-exonuclease but disfavor the duplex formation with natural complement.

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