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NUCLEIC ACID ANALOG PEPTIDE (NAAP) 2. SYNTHESES AND PROPERTIES OF NOVEL DNA ANALOG PEPTIDES CONTAINING NUCLEOBASE LINKED β-AMINOALANINE.

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Abstract: As substitutes for antisense and triplex oligonucleotides, oligopeptides containing N^{β}-(thymin-1-ylacetyl) β-aminoalanine and N^{β}-(cytosin-1-ylacetyl) β-aminoalanine moieties were synthesized on solid support by using standard Boc-chemistry. The obtained peptide containing ten thymine bases was shown to form a hybrid duplex with a complementary oligo DNA, dA_{10}, with a melting temperature (Tm) of 36.5°C at pH 7.0. © 1997 Elsevier Science Ltd. All rights reserved.

The artificial control of gene expression by synthetic molecules has been of special interest from the medicinal and biological aspects.[1] Particularly, nucleic acids and their analogs such as antisense or triple-helix forming oligonucleotides, ribozymes and decoy RNAs are promising reagents for genetic medicines. Toward a medicinal application of such nucleic acid reagents, however, some problems remain to be resolved, which include degradation by cellular nucleases, impermeability through cell membranes, low hybridization affinity caused by electrostatic repulsion between the anionic phosphate backbones in the duplex or in the triplex. Recently, a great number of chemical modifications have been made on such synthetic DNAs and RNAs in order to overcome such drawbacks of natural oligonucleotides.[2] Among those, introduction of a peptide backbone into such nucleic acid analog molecules seems to be attractive because peptide compounds can be expected to have such desirable properties as nuclease resistance, membrane permeability, and good affinity and specificity to nucleic acids as can be seen in a number of DNA binding proteins in cellular systems. In fact, P. Nielsen and his coworkers proved that peptide nucleic acid (PNA) can bind to DNA and RNA in a sequence specific manner with extraordinary affinity.[3]

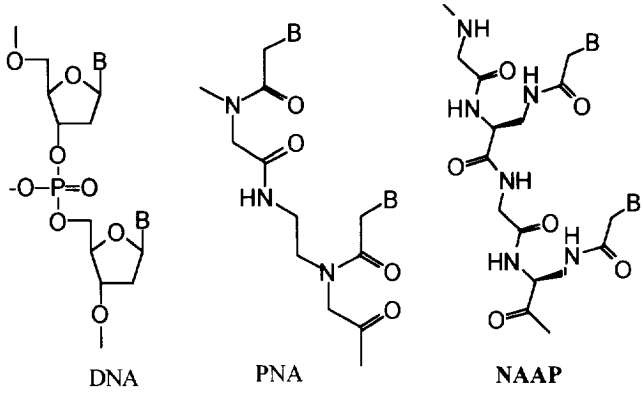
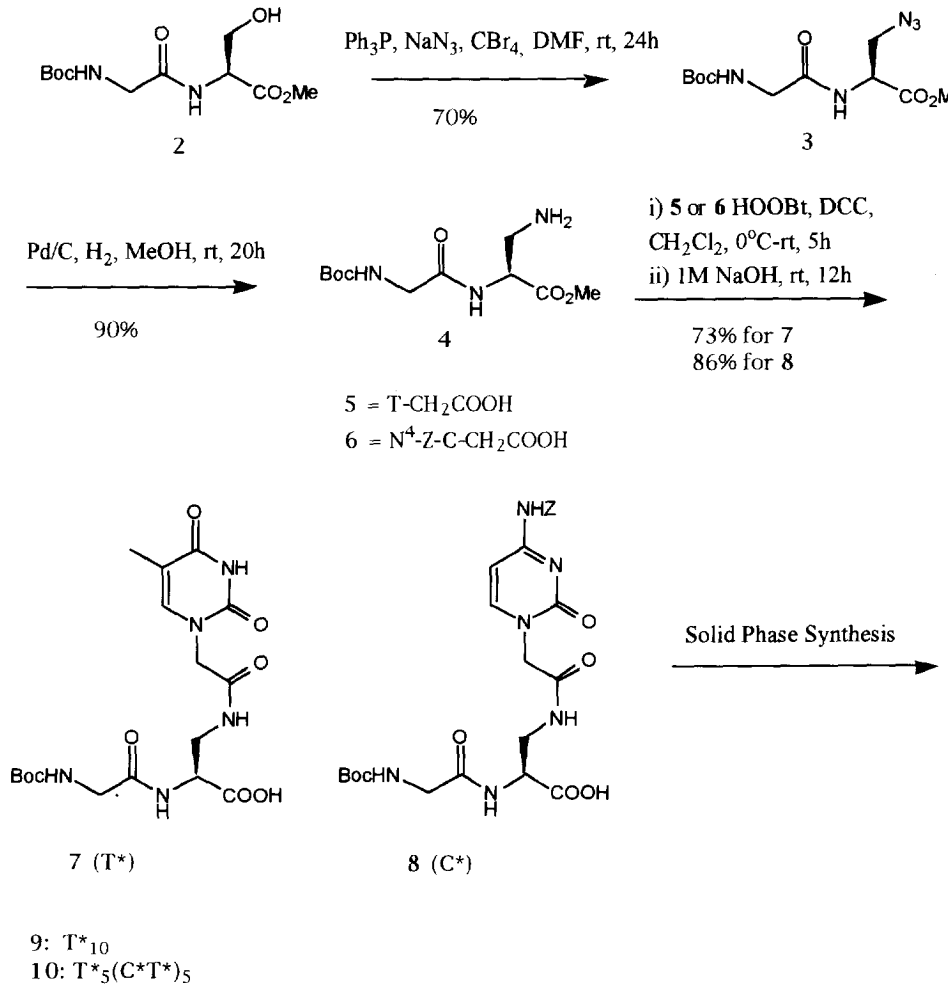


Figure 1. Structure of Nucleic Acid Analog Peptide (NAAP)

In the course of our studies to explore novel DNA binding molecules, we reported a sequential synthesis of a novel nucleic acid analog peptide (NAAP) bearing 3-amino-β-D-riboheptanofuranuroate as a monomer unit on solid support.[4] In the present study, we designed and synthesized a genuine peptide compound composed of glycine and nucleobase linked β-aminoalanine which bears nucleobases at intervals of six atoms just as in DNA. Some hybridization properties of the NAAP with the complimentary DNA are also described.

Preparations of *N*-*t*-butoxycarbonylglycyl-N^{β}-(thymin-1-ylacetyl)-L-β-amino- alanine (**7**, T*) and *N*-*t*-butoxycarbonylglycyl-N^{β}-(cytosin-1-ylacetyl)-L-β-amino- alanine (**8**, ^{Z}C*) were achieved as shown in Scheme 1. The protected glycyl-L-serine methyl ester (**2**) was reacted with NaN_3, Ph_3P and CBr_4 in DMF at room temperature to give azide compounds **3** in 70 % yield. The compound **3** was hydrogenated into the amino derivative **4**. Condensation of the β-aminoalanine derivative **4** with thymin-1-ylacetic acid (**5**) and (N^4-benzyloxycarbonylcytosin-1-yl)acetic acid (**6**) in the presence of DCC and HOOBt[5] followed by hydrolysis with sodium hydroxide afforded the desired compound **7** in 73 % yield and **8** in 86 % yield, respectively. These protected amino acids **7** and **8** were readily applicable to solid phase peptide synthesis using standard Boc-chemistry on MBHA resin.[6] Cleavage and deprotection were performed with TFMSA-TFA-DMS-*m*-cresol (1:5:3:1).[7] Purification by RP-HPLC (Megapak SIL C18-10, 10 mm, 10 x 250 mm) eluted with linear gradients of acetonitrile in 0.1 % TFA gave 11.2 μg (3.5 μmol) of 20 mer peptide T^*_{10} (**9**) and 42.2 μg (9.1 μmol) of 30 mer peptide $T^*_5(C^*T^*)_5$ (**10**). The purified peptides were confirmed by FAB mass spectrometry; mass for **9** $C_{122}H_{154}N_{52}O_{51}$ m/z calcd 3164.9, found 3165.9 [$(M+H)^+$], and for **10** $C_{177}H_{224}N_{82}O_{71}$ m/z calcd 4636.2, found 4637.2 [$(M+H)^+$]

In the preliminary study on the hybridization properties of the NAAPs, it was shown that the oligopeptide **9** containing ten thymine bases could bind to the complementary oligo DNA dA_{10} with T_m of 36.5 °C at pH 7.0 (Measured in a buffer containing 50 mM Tris, pH 7.0, 20 mM $MgCl_2$, 100 mM NaCl, [Strand] = [Target] = 0.5 mM). It should be noted that the thermal stability of the hybrid duplex of **9** and dA_{10} was nearly constant regardless of the salt concentration, i.e., Tm was 36.2 °C at 20 mM $MgCl_2$ and 1.0 M NaCl, and 36.4 °C at 0 M $MgCl_2$ and 0 M NaCl . It can be considered that this independent stability of the duplex from the cationic strength of the solution is due to the electrostatically neutral property of NAAP **9** .



Scheme 1. Synthesis of DNA Analog Peptides Containing Nucleobase Linked β-Aminoalanine

The oligopeptides **9** and **10** were designed to have nucleobase moieties at intervals of six atoms on the backbone, which was previously demonstrated by P. E. Nielsen and his coworkers[8] to be critical for the hybrid formation with DNA or RNA. The linkage between the nucleobase and the backbone in **9** and **10** is longer than that in DNA or PNA by two atoms. It was also pointed out by P. E. Nielsen's group that the length of the linkage could be a little flexible. Moreover, the favorable orientation of the base moieties in **9** and **10** may be fixed by an intramolecular hydrogen bond as shown in Figure 2.[9] Therefore, the NAAPs synthesized in the present study can be expected to form a hybrid duplex with ssDNA and ssRNA and a hybrid triplex with dsDNA. In addition to the obvious resistance against cellular nucleases, the analogs can be expected to have greater hybridization affinity with DNA and RNA, and permeability through the cell membrane because of their electrostatically neutral backbones. More detailed studies to reveal hybridization and biological properties of the DNA Analogs **9** and **10** are now in progress in our laboratory.

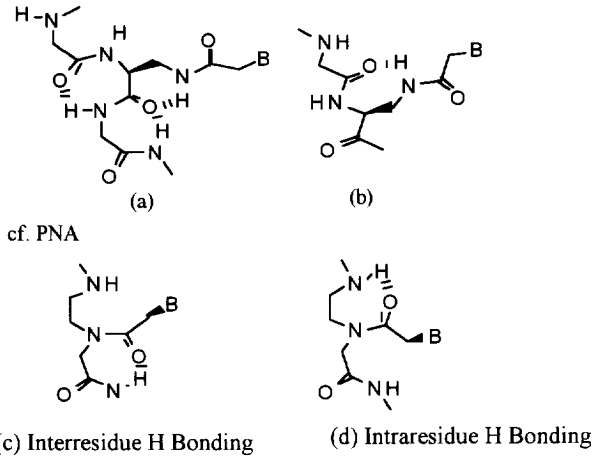


Figure 2. Intramolecular Hydrogen Bonding in NAAP (a and b) and PNA[9] (c and d)

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ABBREVIATIONS: MBHA: 4-methylbenzhydrylamine, TFA: trifluoroacetic acid, DCC: 1,3-dicyclohexylcarbodiimide, DMS: dimethylsulfide, TFMSA: trifluoromethanesulfonic acid, HOOBt: 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine

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