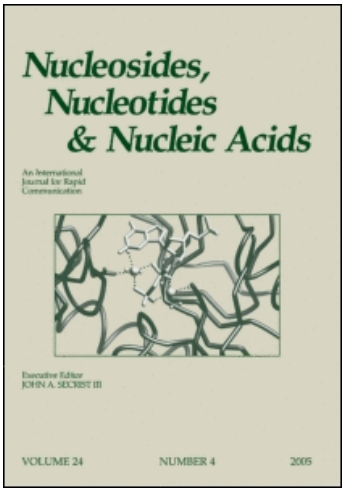

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INVESTIGATIONS ON SARCOSINE AND ISONIPECOTIC ACID CONTAINING PEPTIDE NUCLEIC ACIDS

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ABSTRACT: Two different series of peptide nucleic acids (with the repeating units AlaT-Sar and AlaT-Sar-Inp) were synthesised and evaluated for potential binding to an oligoA/oligoT duplex.

Recently, we synthesised several solid phase libraries containing unnatural amino acids and screened them for affinity against dsDNA.[1] Among several other unnatural amino acids (14 in total), two nucleoside-derived amino acids were used as building blocks in the library i.e. L-alanyl uracil (**1a**) and L-alanyl hypoxanthine (**2**, Fig. 1). Similar nucleo amino acids were synthesised before[2-6] and incorporated into oligopeptides.[3-6] The library was used for selecting peptides with affinity for a 14 base pair dsDNA sequence representing the binding site for NF-IL6 (5'-ACATTGCACAATCT-3' with its complement). A high structure-affinity relationship was observed for peptides with, as

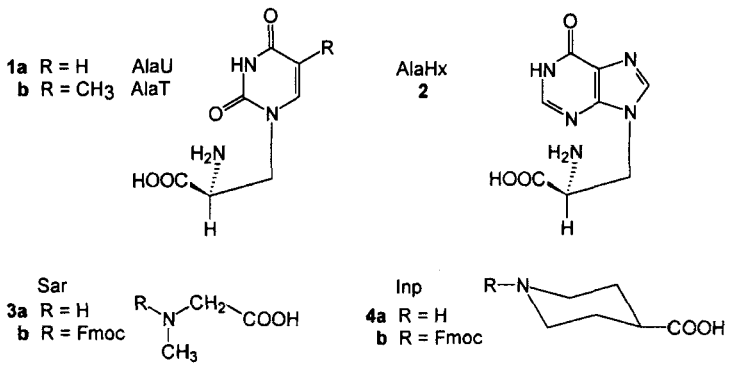


FIG. 1. Structures of L-alanyl-uracil (**1a**), L-alanyl-hypoxanthine (**2**), sarcosine (**3a**) and isonipecotic acid (**4a**).

first amino acid, AlaU (**1a**) and, as second amino acid, AlaU (**1a**) or sarcosine (**3a**, Fig. 1). At the third position, besides AlaU and sarcosine, also high abundance of L-hydroxyproline and isonipecotic acid (**4a**, Fig. 1) was observed. The most remarkable combination isolated from the library was identified as Ac-Arg-AlaU-Sar-AlaU-Sar-AlaU (**5a**, Fig. 2). As the target sequence contains two successive adenine bases (AA.TT sequence), it may be not unrealistic to hypothesise that this may be the binding site of the Arg-AlaU-Sar-AlaU sequence using Hoogsteen-type base pairing.

Therefore we decided to synthesise two different peptide nucleic acids (i.e. with the repeating units AlaT-Sar **5b** and AlaT-Sar-Inp **6**, Fig. 2) and evaluate them for binding to an oligoA/oligoT duplex. All sequences (Table 1) started and ended with an arginine in order to increase binding via electrostatic interactions to the dsDNA and to increase water solubility of the peptide. Because thymine bases generally bind stronger than uracil bases due to an entropic factor, AlaT (**1b**) was substituted for AlaU (**1a**). The synthesis of the building block used to assemble the oligopeptides is shown in Scheme 1.

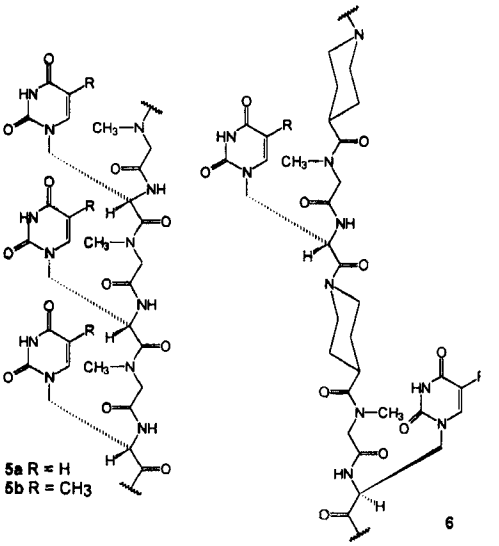


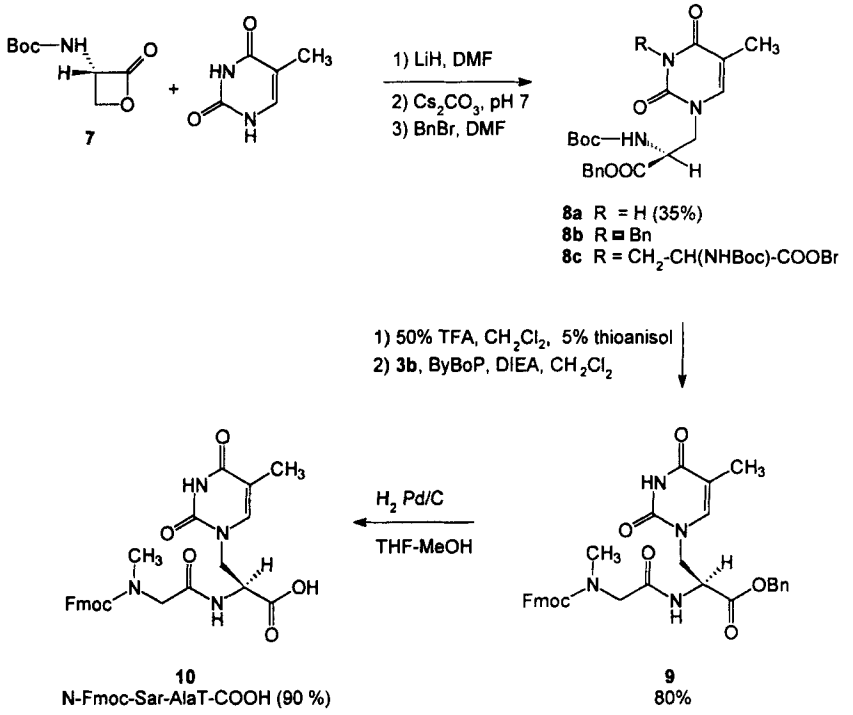
FIG. 2. Unnatural amino acid sequence AlaU-Sar (**5a**) and repeating units AlaT-Sar (**5b**) and AlaT-Sar-Inp (**6**).

TABLE 1. Synthesised sequences

a	AcNH-Arg-AlaU-(Sar-AlaT)$_5$-Arg-CONH$_2$
b	AcNH-Arg-AlaU-(Sar-AlaT)$_{10}$-Arg-CONH$_2$
c	AcNH-Arg-AlaU-(Sar-AlaT)$_{15}$-Arg-CONH$_2$
d	AcNH-Arg-AlaU-Sar-AlaT-(Inp-Sar-AlaT)$_4$-Arg-CONH$_2$

Reaction of N-Boc lactone **7** with the lithium salt of thymine base was immediately followed by benzylation of the intermediate carboxylic acid using benzyl bromide and Cs_2CO_3. An accurate follow-up of the pH is necessary since an excess of Cs_2CO_3 also

deprotonates the N^3-hydrogen atom and leads to more side reactions. Besides **8a**, also the N^3-benzylated compound **8b** and the N,N-dialkylated analogue **8c** were obtained. Removal of the Boc group followed by condensation with N-Fmoc-sarcosine (**3b**) yielded **9**, from which the carboxylic acid **10** was generated by hydrogenation reaction.



SCHEME 1. Synthesis of the oligopeptide building block.

The synthesis of the peptide nucleic acids analogues was carried out on solid support (Tentagel S-NH_2, 0.30 μmol/g) functionalized with a benzhydrylamine linker.[7] After binding of protected arginine, the dimers (**10**) were coupled using 4 eq DIC, 4 eq HOBt in DMF and the coupling was monitored using bromophenol blue and followed by a capping process. Fmoc deprotection was carried out with 20 % piperidine in DMF for 15 min. The first series was finished as follows. After the attachment of five units the resin was divided into three parts. One batch was coupled to Fmoc-Arg(PMC)-AlaU to yield AcNH-Arg-AlaU-(Sar-AlaT)$_5$-ArgCONH$_2$. The two other samples were used to synthesise AcNH-Arg-AlaU-(Sar-AlaT)$_{10}$-ArgCONH$_2$ and AcNH-Arg-AlaU-(Sar-AlaT)$_{15}$-ArgCONH$_2$. For the second series coupling of Fmoc-Sar-AlaTCOOH was followed by coupling of Fmoc-Inp (**4b**) leading to the sequence AcNH-Arg-AlaU-Sar-

AlaT-(Inp-Sar-AlaT)$_4$-ArgCONH$_2$. After deprotection and cleavage from the solid support, the oligomers were purified on reverse phase column and the structure was confirmed using electrospray mass analysis.

The interaction between the synthesised peptides in Table 1 and the oligoA/oligoT duplex was assayed by thermal denaturation experiments, gel mobility shift analyses and a DNase I protection assay.[1] Unfortunately, no stable interaction could be observed between the peptides and the oligoA/oligoT duplex. This may be due to a) an energetically unfavourable binding due to the apolar character of the synthetic peptides; b) the difference between molecular interactions in solid phase and in solution phase; c) the higher sequence selectivity for binding to dsDNA for peptides, generated by the library approach, than hypothesised by the start of this project; d) the non-complementary shape of the synthetic peptides and dsDNA. These factors are further under study in order to better understand interaction between dsDNA and synthetic peptides.

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