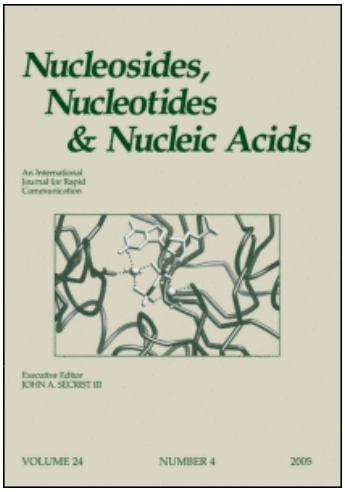

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A New Approach to the Synthesis of Trinucleotide Phosphoramidites - Synthons for the Generation of Oligonucleotide/Peptide Libraries

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A NEW APPROACH TO THE SYNTHESIS OF TRINUCLEOTIDE PHOSPHORAMIDITES - SYNTHONS FOR THE GENERATION OF OLIGONUCLEOTIDE/PEPTIDE LIBRARIES.

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ABSTRACT: Trinucleotide phosphoramidites that correspond to the codons of all 20 amino acids were synthesized in high yield in 5g scale. Precursors of those amidites - trinucleotide phosphotriesters - have been prepared using the phosphotriester approach without protection of the 3'-hydroxyl function. More than 10 oligonucleotides up to 90 bases long have been synthesized by a phosphite-triester approach using new synthons. The 67-mer (12 random codons) has been used to generate a display library of $2x10^8$ complexity.

The key point in drug discovery programs is the screening of large numbers of different molecules for an identification of a lead compound, which possesses the desired properties. Recently, large biologically displayed peptide or antibody libraries have been introduced as a source of potential pharmaceutical leads, offering a tremendous set of diverse molecules[1-3]. Subsets of the 20 amino acids can be introduced at a defined position in the molecule by using oligonucleotides of a mixed composition. Those randomized oligonucleotides are usually synthesized using a mixture of commercially available nucleotides at each step of the synthesis. To achieve a controlled diversity, to avoid the incorporation of undesired amino acids and stop codons into the resulting oligonucleotide, and to construct a desired subset of codons in a defined position trinucleotide synthons may be used.

We developed a method for the synthesis of such trinucleotide phosphoramidites building blocks. The synthetic route is outlined on Fig. 1.

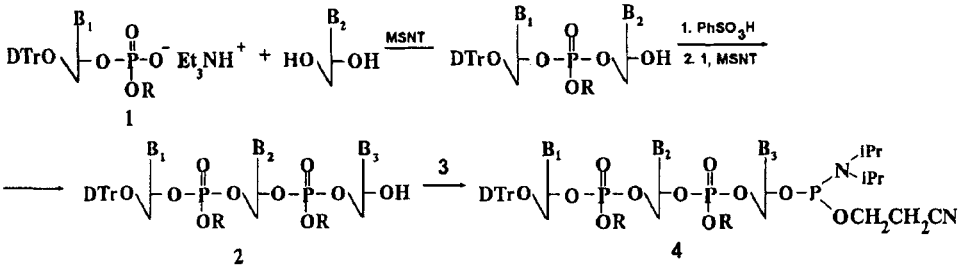

FIGURE 1. The synthetic route of trinucleotide phosphoramidites. B_1, B_2, B_3 - bzA, bzC, ibG, or T, R - o-chlorophenyl, 3 - a mixture of *bis*-(N,N-diisopropylamino)-2-cyanoethoxyphosphine and 1*H*-tetrazole.

Our choice of trinucleotide structures was based on codon usage in *E.coli*[4,5] rather than on simplification of the synthesis strategy[6].

The key substance in the synthesis of 4 was trinucleotide 2 with an unprotected 3'-hydroxyl function (Fig. 1). The main problem in the synthesis of compound 2 was to select a protective group for 3'-hydroxyl. We could not use neither acid-labile protection (because of presence of the acid-labile dimethoxytrityl residue), nor base-labile one (because of presence of the base-labile internucleotide and N-protecting groups). So we decided not to protect the 3'-hydroxyl at all. Such an approach was used earlier[7,8] during synthesis of oligonucleotides by the phosphotriester method.

The experimental details and full description of the synthesis are presented elsewhere[9]. ^{1}H-, ^{31}P-NMR and mass spectra of all intermediates and target products were as expected.

To analyze the codon distribution and overall performance of oligonucleotides synthesized with trinucleotide synthons, we sequenced plasmid DNA from two oligonucleotide syntheses, one with all 20 synthons (#3020), one with the Cys-synthon omitted (#3025). The occurrence of every codon was counted and compared to the expected value. In Fig. 2, the number of the found codons divided by the number of the expected ones is presented. The frequency of codons was within the expected range. Preliminary results indicate, that high affinity peptidic ligands can be isolated from trinucleotide-based libraries.

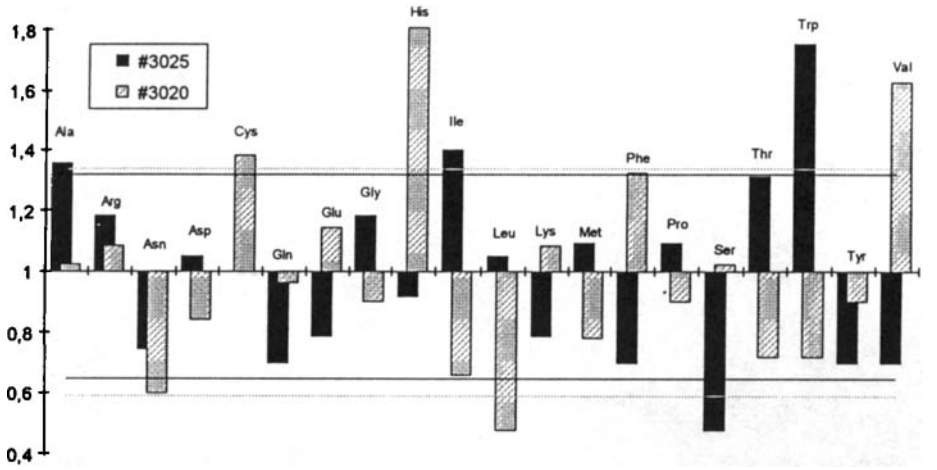

FIGURE 2. Evaluation of two 12-mer libraries. Dotted and solid lines indicate the 95% probability interval for experiments #3020 and #3025, respectively.

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