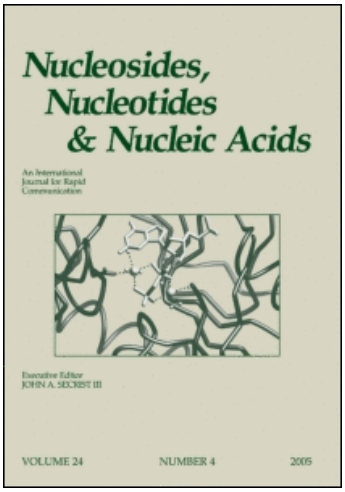

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LARGE-SCALE, PEG-SUPPORTED DNA SYNTHESIS

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ABSTRACT. Using polyethylenglycol (PEG) as soluble supporting polymer, oligonucleotides have been prepared in high yield by an easy and fast procedure. The method looks promising for rapid synthesis of grams of short oligomers at reasonable cost.

The solid-phase synthesis of oligonucleotides, by eliminating tedious and time-consuming purification steps and replacing the phosphate diesters with the more reactive phosphite derivatives, led to a very fast production of the desired compounds. Nontheless, this widely used procedure is, to our knowledge, unsatisfactory when grams of pure material are required. To overcome this difficulty many attempts have been made to develop methodologies which hopefully would combine some of the advantages of liquid- and solid-phase approaches (1-5). The same problem existed in the peptide synthesis, and a possible solution was to employ a supporting polymer soluble in the reaction media (6). Turning back to oligonucleotides, the presently foreseen possibility that some natural and modified "antisense" sequences may find therapeutic applications (7) stresses this need even more. Toward this aim, we propose a new liquid-phase procedure, called **H.E.L.P.** (**H**igh **E**fficiency **L**iquid **P**hase), based on the utilization of polyethylenglycol (PEG) as soluble polymeric support (8). This idea was presented in some papers in the early seventies (9-10), but was not pursued any further. The peculiar and convenient feature of PEG is its solubility in the reaction mixtures, from which it precipitates nicely by addition of diethyl ether. Hence, the polymer-bound oligonucleotide is rapidly freed from excess

	Method A (12,14)	Method B (13,15)
Phosphorylating agent	5'-DMT-O-nucleoside (3 X) 2-chlorophenylphosphate	5'-DMT-O-nucleoside (3 X)
Condensing agent	2-(mesitylbenzenesulphonyl) 3-nitro-1,2,4-triazole (6 X)	2-chlorophenyl-O,O-bis (1-benzotriazolyl)-phosphate (3 X)
Catalyst	N-methylimidazole NMI (10 X)	N-methylimidazole NMI (5 X)

Scheme 1

reagents, catalysts and by-products, just as it occours in the filtration step of the solid-phase approach.

The advantage of **H.E.L.P.** over this last method stays in that both the phosphorilatying nucleotide and the 5'-OH growing chain are in solution. Coupling yields higher than 90 % are obtained in less than 60 min by the phosphotriester technique using about twice the amounts of phosphorilating monomer, condensing agent and nucleophilic catalyst commonly employed in the standard solution method. Like in the solid-phase procedure it appears convenient to treat the PEG-bound oligomer with a capping mixture before the detritylation step. For easier purification of the final deprotected product, the polymer-bound sequence can be recrystallized form methylene chloride/dietyl ether or ethyl alcohol. Working with the PEG-5000 monomethyl ether the precipitates are always clean and easily filtrable, at least up to the octamer level (maximum length tested). As reported below, 150 mg of a 97 % pure (HPLC titre) octadeoxynucleotide were obtained, in four days, starting from 1.6 g of PEG-nucleoside (deprotection and purification excluded).

In our opinion the present method has the following positive aspects: 1) **hundreds of mgs** of **good quality** product can be prepared in **one run**, in an ordinary chemistry laboratory; 2) significant **time saving** in respect to standard solution synthesis; 3) significant **cost saving** in respect to solid-phase synthesis; 4) **easy monitoring** of the reaction by combined spectrophotometric techniques; 5) **scaling-up**, at least up to grs amount, can be reasonably foreseen, as well as synthesis of longer sequences (15-20mers, possibly by using larger PEG).

The starting material was polyethylenglycol monomethylether of average molecular weight 5000. The PEG was subjected to reaction with the 5'-O-DMT-2'-deoxynucleotide-3'-O-succinates as in the solid-phase (11). The unraected OH groups of PEG were capped with a 10% solution of acetic anhidride in pyridine. The extent

Table 1. PEG-supported synthesis of homo-dideoxynucleotides.

samples	yield (%)	
	method A	method B
d(TpT)	96	98
d(CpC)	85	82
d(GpG)	105	95
d(ApA)	90	90

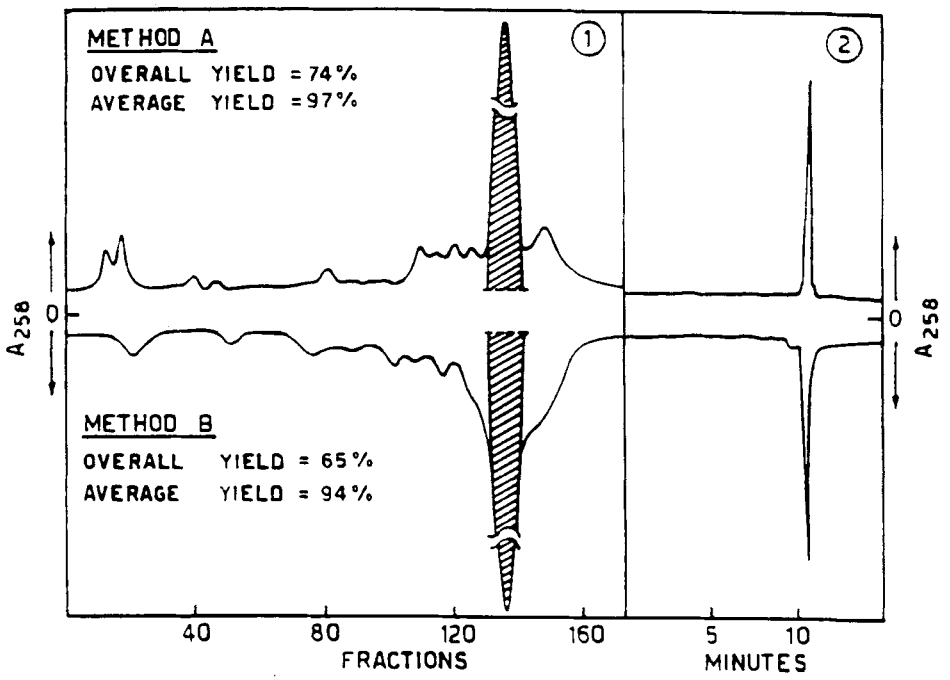

Figure 1. Purification of d(TAGCGCTA). 1) Ion-exchange purification of crude products. 2) Reverse-phase analytical HPLC of purified octamers.

of the functionalisation was measured by DMT absorption at 498 nm ; loading of up to 180 umoles of nucleoside per gram of polymer were obtained.

The efficiency of the coupling reaction on PEG was tested by preparation of the four natural homo-dideoxynucleotides. After removal of DMT group in 3 % TCA solution in methylene chloride, the formation of 3',5'-phosphotriester linkage was achieved by two different approaches: methods A and B are summarized in the Scheme 1.

The complete removal of reagents was easily achieved by crystallization of PEG-nucleoside from methylene chloride/diethyl ether or by absolute ethanol and confirmed by TLC analysis.

The coupling yields are generally higher than 90 % (Table 1) and the crude deprotected products resulted very pure as shown by reverse phase HPLC analysis. In the case of d(GpG) a yield higher than 100 % are observed with method A, indicating the formation of side-products, as confirmed by HPLC. This drawback is essentially absent with the method B. The unambiguous identification of products was made by comparison with the same homodimers independently synthesized.

The feasibility of these methods was tested in the synthesis of the octanucleotide d(TAGCGCTA). The final products were identified by comparison with the same octamer independently synthesized. The oligomers were removed from the PEG and deprotected following the standard procedures (16). The products were purified by ion-exchange chromatography. The elution profiles are shown in Figure 1, together with the HPLC of the purified octamers. With these methods we were able to obtain very high quantities of purified TEA salts of d(TAGCGCTA). With method A we obtained 85 mg of pure product from 1.0 g of functionalised PEG and with method B 150 mg of the same product from 1.6 g of functionalised PEG.

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