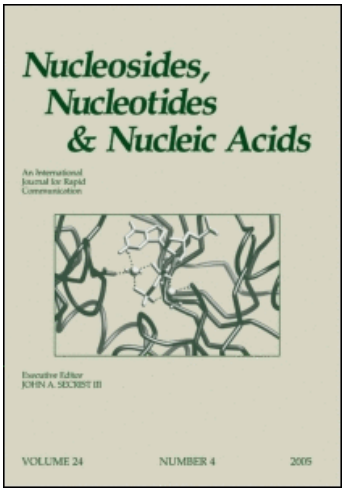

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The 4-Decyloxytrityl Group as an AID in the Affinity Chromatography of Synthetic Oligonucleotides

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THE 4-DECYLOXYTRITYL GROUP AS AN AID IN THE AFFINITY CHROMATOGRAPHY OF SYNTHETIC OLIGONUCLEOTIDES

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Abstract. On substitution with a 5'-terminal (4-decyloxyphenyl-)diphenylmethyl group oligonucleotides are strongly retained on reverse stationary phases. Applications of this affinity separation technique to the purification of genes and gene fragments of up to 147 b are reported.

INTRODUCTION

Chemical synthesis now routinely affords the preparation of oligonucleotides of ca. 60-80 bases. Longer sequences can be constructed, however, their separation from truncated chains as by-products of support synthesis becomes more and more difficult. Extending earlier work on triphenylmethyl protecting groups with long-chain alkyl substituents as tools for the affinity-chromatographic purification of oligonucleotides[1,2] we found that the (4-decyloxyphenyl-)diphenyl-methyl (DTr-) group is optimally geared to separations on modern C_{18} stationary phases. This affinity group effects strong retainment even of long oligonucleotides in an acetonitrile/buffer eluent system, but does not make necessary the admixture of an apolar co-solvent for elution of the shortest oligomers, a phenomenon we find when using the previously[1] recommended (4-hexadecyloxyphenyl-)diphenylmethyl group. In a first publication[3] we have given details on the introduction of the DTr- protecting group and on chromatographic conditions for the purification of DTr-substituted oligonucleotides. Since then, we have routinely used this group in the workup of genes and gene fragments

prepared by the solid-phase technique, and some of these applications will be described here.

RESULTS AND DISCUSSION

Preparation of 5'-DTr-oligonucleotides: Oligonucleotides were synthesized in an automated apparatus using commercial 5'-dimethoxytrityl-nucleoside-3'-ß-cyanoethoxy-phosphoramidites for all chain extensions except the last one. DTr-nucleoside-3'-phosphoramidites were prepared as described[3] and applied in the last cycle. In this way the routine phosphoramidite synthesis program[4] could be used throughout.

Purification of 5'-DTr-oligonucleotides: The immobilized oligonucleotide product was cleaved from the polymer support by ammonia treatment[4] with concomitant deprotection of the internucleotidic bonds and nucleobases. Thus, only the 5'-DTr groups are left on the product.

HPLC was done on an RP-C_{18} column (µ-Bondapak C_{18} (300x7.5 mm I.D., Waters) or LiChroSorb Super RP (E. Merck; 7 µ, 5.0x250 mm)) applying gradients of acetonitrile in 0.1 M triethylammonium acetate (see legend to TABLE 1). The rate of elution gradient was set to ca. 1% acetonitrile per 2 ml eluent, since we had previously found[3] that under these conditions long oligonucleotides could be obtained pure by taking appropriately small fractions (ca. 0.5 ml). The product peak was collected and the oligonucleotide detritylated with 80% acetic acid[4] and desalted. For a double-check of purity samples of the fully deblocked sequence, after labeling with γ-^{32}P-ATP/polynucleotide kinase, were run on a 6% polyacrylamide gel; they usually gave a single spot on autoradiography.

Application to the purification of genes and gene fragments: In the context of a project focussed on the preparation of genes for hypothalamic hormones, particularly human corticotropin[5], several long DNA fragments were prepared[6] and purified according to the scheme described before. These included: A,B) sense and antisense strand of a total

TABLE 1: Conditions for FPLC separation of 5'-DTr-deoxyoligonucleotides Column. PepRPC$_{18}$HR 5/5 (50 x 5 mm I.D.); eluent, a) 20-45% acetonitrile in 0.1 M triethylammoniumacetate (pH 7) in 45 min., b) 20-50% in 36 min., c) 20-60% in 60 min.; flow rate, a) 0.33 ml/min., b) c) 0.5 ml/min.; detection, 254 nm.

Sequence	Elution volume (ml)	Elution gradient (% CH_3CN)
A) 5'- GA ATT CTA TTA GAA CTC CAG CGG AAA GGC CTC CGC AGA CTC GTC CTC GGC GCC GTT GGG GTA AAC TTT AAC TGG ACG ACG TTT TTT GCC CAC CGG TTT ACC CCA ACG GAA GTG TTC CAT GCT GTA GCT CCT ACC CTC GAT ATC TGC A-3' (147 b)	14.8	37[a]
B) 5'- GAT ATC GAG GGT AGG AGC TAC AGC ATG GAA CAC TTC CGT TGG GGT AAA CCG GTG GGC AAA AAA CGT CGT CCA GTT AAA GTT TAC CCC AAC GGC GCC GAG GAC GAG TCT GCG GAG GCC TTT CCG CTG GAG TTC TAA TAG -3' (138 b)	16.8	38[a]
C) 5'- AGA ATT CTA TTA GGG GTA AAC TTT AAC TGG ACG ACG TTT TTT GCC CAC CGG TTT ACC CCA ACG GAA GTG TTC CAT GCT GTA GCT CCT ACC CTC GAT ATC TGC A- 3' (104 b)	13.2	32[b]
D) 5'- GAT ATC GAG GGT AGG AGC TAC AGC ATG GAA CAC TTC CGT TGG GGT AAA CCG GTG GGC AAA AAA CGT CGT CCA GTT AAA GTT TAC CCC TAA TAG AAT TC- 3' (98 b)	18.7	43[a]
E) 5'- A ATT CTG CAG CTA CGG GTA AAC TTT AAC TGG ACG ACG TTT TTT GCC CAC CGG TTT ACC CCA ACG GAA GTG TTC CAT GCT GTA GCT CGG ACC TGC A-3' (95 b)	20.6	35[b]
F) 5'- AA TTC GTT AAC GGC GCC GAG GAC GAG TCT GCG GAG GCC TTT CCG CTG GAG TTC TAA CTG CA- 3' (61 b)	25.6	43[c]
G) 5'- G TTA GAA CTC CAG CGG AAA GGC CTC CGC AGA CTC GTC CTC GGC GCC GTT AAC G-3' (53 b)	29.3	49[c]

human ACTH gene with factor X_a expression linker[7] = 2 fragments of 147 b and 138 b; C,D) sense and antisense strand of a gene for ACTH(1-24) = Synacthen with factor X_a linker = 2 fragments of 103 b and 98 b; E) the antisense strand of a gene for ACTH(1-24) = Synacthen with an expression linker containing a collagenase recognition site[8,9] = 1 fragment of 95 b; F,G) sense and antisense strand of a DNA fragment encoding ACTH(25-39) as a cassette for the synthesis of sequentially variant ACTH-genes constructed on the basis of maximum codon usage by E-coli[10]. The sequences A-G, their separation conditions and elution volumes are listed in TABLE 1. As an example, the elution profile of fragment A of 147 b is given in FIGURE 1. Even in the case of this very

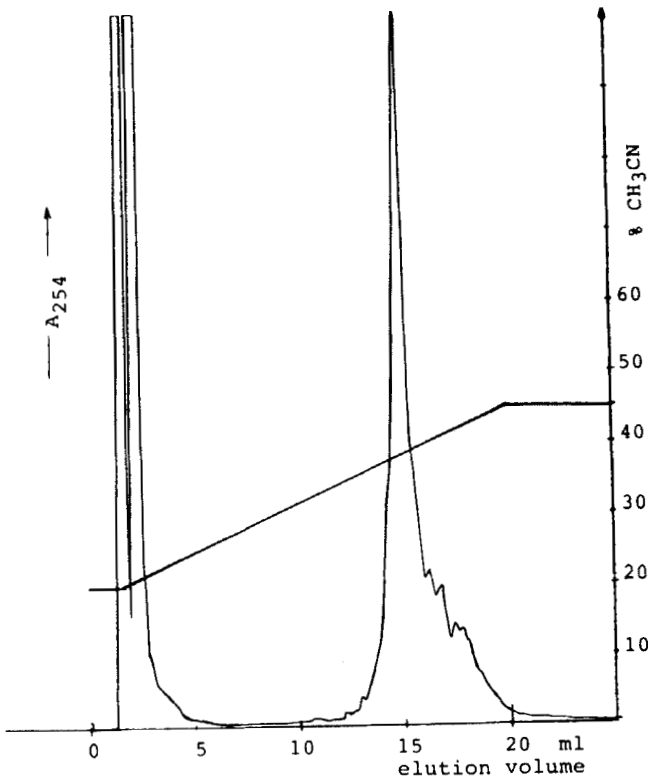

FIGURE 1:

FPLC elution profile of fragment A.

Column: $PepRPC_{18}HR$ 5/5 (50 x 5 mm I.D.);

Eluent: 20 - 45% acetonitrile in 0.1 M triethyl-ammoniumacetate (pH 7) in 45 min.;

Flow rate: 0.33 ml/min.;

Detection: 254 nm (UV).

long fragment the product peak (with a slight tail of truncated DTr-oligonucleotides as by-products of the last condensation or as products of chain scission on workup) is well separated from all the rest of the non-tritylated by-products. These examples demonstrate the applicability of HPAC techniques for routine purifications of oligonucleotides of up to ca. 150 bases, however, the limits of separation, as seen from a study of the separation parameters[3], should be near 180 b or even beyond. The affinity technique described here can be an alternative to, or even an advantage over polyacrylamide gel electrophoresis, particularly in view of the work involved in gel preparation and the generally incomplete recovery of oligonucleotides from gels.

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