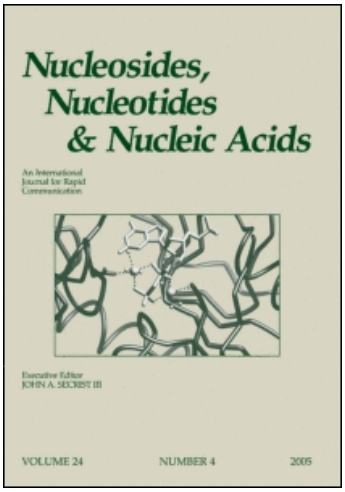

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AN IMPROVED METHOD FOR LARGE SCALE SYNTHESIS OF OLIGONUCLEOTIDES APPLYING THE NPE/NPEOC-STRATEGY

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Abstract: Several oligonucleotides were synthesized in scales up to 60 µmol in a standard 10 µmol cartridge on a standard DNA synthesizer. The advantage of a special phosphoramidite approach using only ß-eliminating protecting groups over the commonly practised automated oligonucleotide synthesis using ammonia-labile blocking groups could be demonstrated by ^{1}H-NMR-spectroscopy.

The growing popularity of the antisense concept[1] initiated the need of larger amounts of synthetic oligodeoxynucleotides tremendously. Furthermore, at least milligram amounts of oligonucleotides are required for structural investigations like NMR-studies[2], X-ray investigations[3], and nucleic acid-protein-interactions.

Recently, Andrus and coworkers reported about the automated synthesis of oligonucleotides in scales up to 1 mmole using a highly loaded polyethyleneglycol derivatized polystyrene support and a special large scale DNA synthesizer[4]. In our opinion, an important problem of that method stems from the commonly practised strategy using phosphoramidite nucleosides with ammonia-labile acyl-protecting groups at the nucleobases (dA^{bz}, dC^{bz} and dG^{ibu}). The disadvantage of this "acyl-strategy" is seen in the deprotection of the oligonucleotide and its simultaneous cleavage from the solid support. Thus, besides possible truncated sequences the desired oligonucleotide is contaminated with all by-products resulting from ammonia deprotection. Especially the application of larger amounts of oligonucleotides as therapeutic agents will require more or less time-consuming and expensive purification procedures ordinarily connected with big losses in yield. For these reasons our group developed in recent years an alternate method for solid-phase syntheses, namely the "NPE/NPEOC-strategy"[5]. The advantage of this phosphoramidite approach using only ß-eliminating protecting groups like the 2-

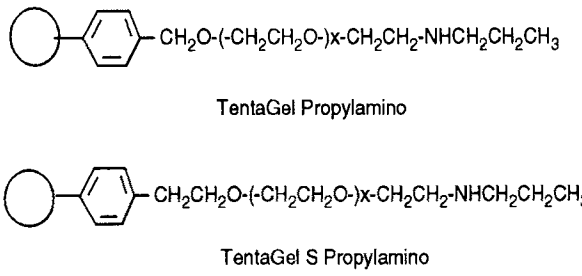

FIG.1: Propylaminopolyethyleneglycol derivatized polystyrene

(4-nitrophenyl)ethyl (NPE) and the 2-(4-nitrophenyl)ethoxycarbonyl (NPEOC) function is seen in the decoupling of the deprotection from the support-cleavage procedure. The two-step approach separates the removal of the protecting groups from the bound oligonucleotide and the final cleavage from the support by ammonia leading to crude products of high purity as demonstrated by several spectroscopic methods.

An effective "large scale" synthesis of oligonucleotides applying the "NPE/NPEOC-strategy" affords a solid support which can be highly loaded with the appropriate starting nucleoside and which must be stable under the deprotection conditions using DBU in aprotic solvents. Based upon previous investigations using low crosslink polystyrene polyethyleneglycol graft copolymers (TentaGel)[6] as well as highly loaded CPG[7] in combination with the "NPE/NPEOC-strategy", we have now chosen new types of TentaGel resins, namely TentaGel Propylamino[8] (FIG.1) fulfilling all required demands. Loading of these supports with appropriately protected 3'-succinyl nucleosides[7] and subsequent acetylation ("capping") led to derivatizations of 150-200 μmole per gram support.

Several oligodeoxyribonucleotides up to 18mers have been synthesized on a standard DNA-synthesizer (ABI Model 392) in scales from 40 up to 60 μmole following a synthetic protocol with a few but important modifications in comparison to standard methods[9]. We can demonstrate, that the crude products resulting from the "NPE/NPEOC-large scale-strategy" are much purer than those derived from the commonly applied "acyl-strategy" as documented clearly by ^{1}H-NMR-spectroscopy.

The tetramer d(GTAC) was synthesized at a 40 μmole scale following the "acyl-strategy" and the "NPE/NPEOC-strategy", respectively, using appropriately protected phosphoramidites and TentaGel supports. After finishing the synthetic part of the chain formation, the "acyl-product" was worked up in the usual manner (support-cleavage and deprotection with concentrated ammonia, 12 hr, 55°C), while in the case of the

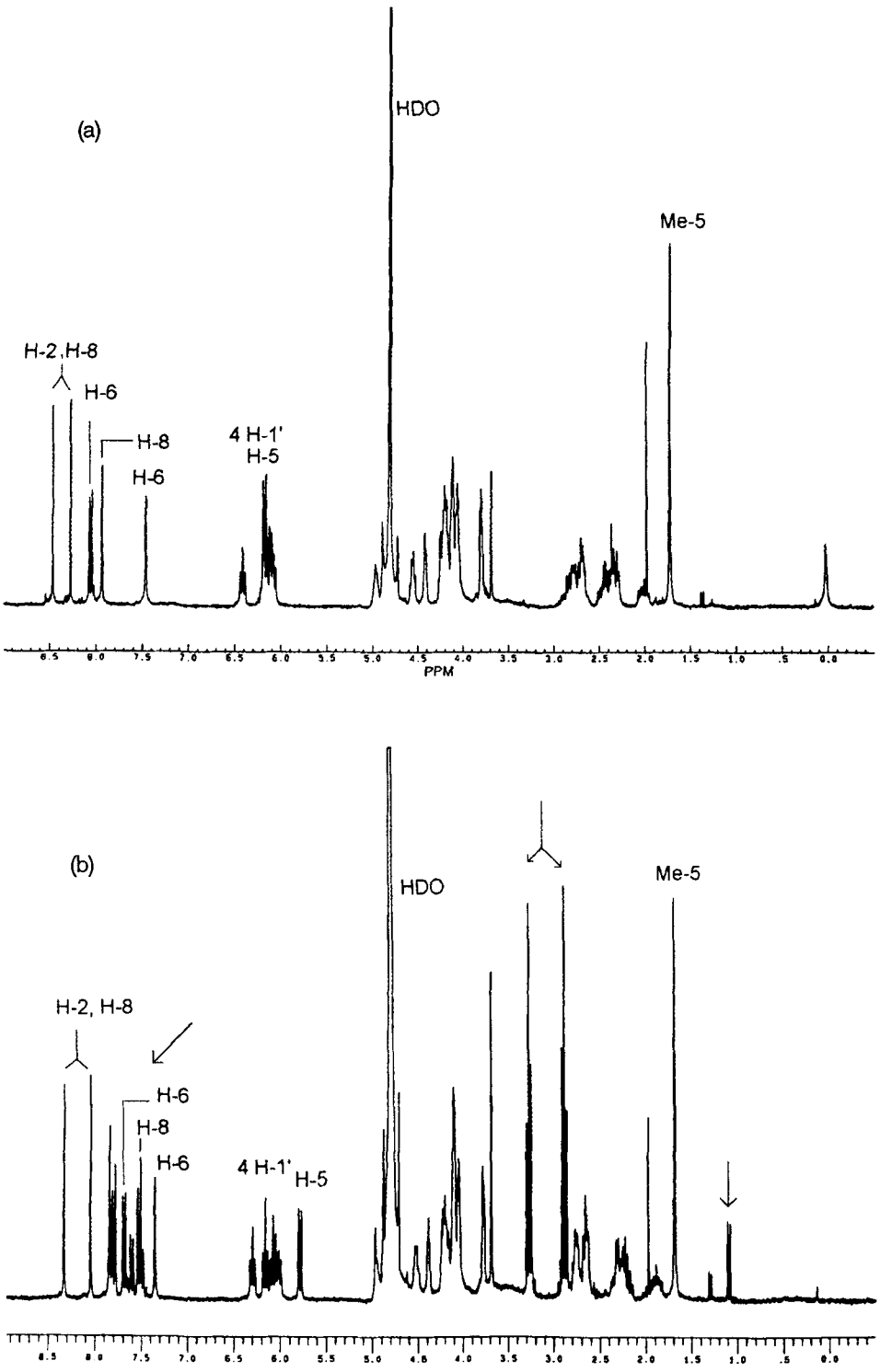

FIG.2: ^{1}H-NMR-spectra (250 MHz, 1,3 mM in D_2O) of the crude tetramers d(GTAC) synthesized in 40 µmol scales by the "NPE/NPEOC-strategy" (above) and by the common "acyl strategy" (below). Signals indicating protecting group residues are marked with arrows

"NPE/NPEOC-product" the support was first treated with 1M DBU in acetonitrile (12 hr), then succesively washed with 1M NH_4HCO_3, water and acetonitrile, and finally support-cleavage was done with concentrated ammonia (2 hr). In both cases the lyophilized crude products were analyzed by HPLC, FAB mass spectrometry and ^{31}P-spectroscopy (data not shown) but only ^{1}H-NMR-spectroscopy showed significant differences between the two products (FIG.2). Thus, the "acyl-product" shows additional signals from protecting group contaminations like benzamide (7.5-7.7 ppm) isobutyrylamide (1.1 ppm) and a reaction-product from ammonia with the acrylonitrile resulting from phosphate deprotection (2.9-3.3 ppm). On the other hand, the ^{1}H-NMR spektrum of the "NPE/NPEOC-product" is free of additional signals indicating the presence of any protecting group residues.

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9. For example, it was found, that a mixture of 10% trichloroacetic acid and 3% N-acetyl-L-cysteine in dichloromethane is a very effective detritylation reagent for TentaGel supports.