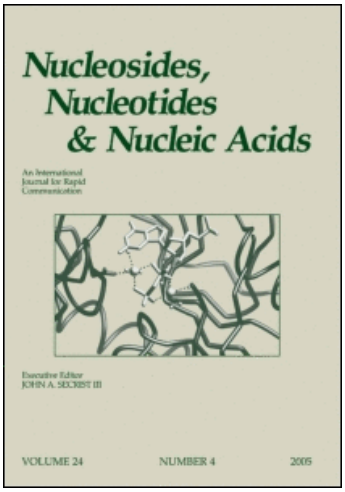

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A Versatile Polymer Support Useful for Large-Scale Synthesis of DNA for Biomedical Applications

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A VERSATILE POLYMER SUPPORT USEFUL FOR LARGE-SCALE SYNTHESIS OF DNA FOR BIOMEDICAL APPLICATIONS

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ABSTRACT: A suitably derivatized Merckogel[R] **1** as an efficient solid support for the large-scale synthesis of DNA for biomedical applications, is described. Partial hydrolysis of these polyvinylacetate resins with 0.5 M NaOH solution[1] at room temperature for 30 - 80 min. yielded carriers **1** that could be loaded with 120, 150, 258, and 368 μmoles of nucleosides g^{-1}.

Introduction

Although a large number of new polymer supports have been developed[2], considerable efforts are still necessary to search for new polymer supports for the synthesis of oligonucleotides in large scale with high purity, especially in view of potential applications of structurally modified oligonucleotides as therapeutics, but also with special requirements of structural, material, and antisense[3] research. A synthesis on solid support has the advantage that all purification steps necessary in solution chemistry can be replaced by simple washing procedures, thus drastically decreasing the time needed to

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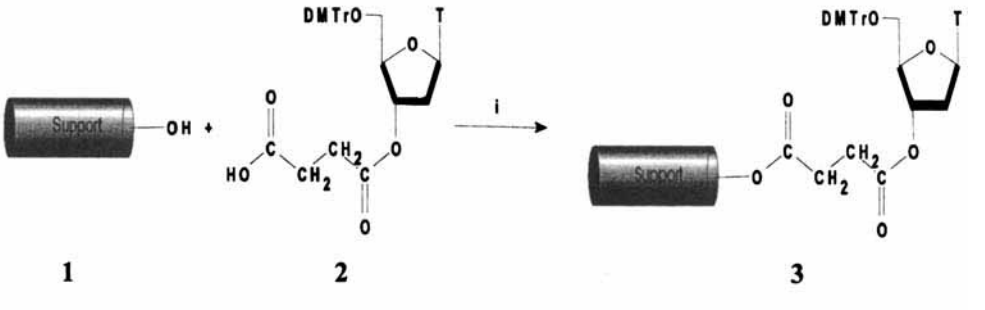
assemble DNA fragments. Most solid phase methods, on the other hand, suffer from the fact that relatively small quantities of oligonucleotides can be reacted per unit weight of polymer and from problems created by the separation of the crude product released from the carrier. These problems can be surmounted, if solid supports were available, which have high nucleoside loading capacity, yet give excellent yields of chain elongation. This will give a maximum quantity of oligonucleotides of desired length with high purity. Thus, highly loaded solid supports are ideally suited for large-scale oligonucleotide synthesis, because the major part of the mass is growing oligonucleotide, not polymer support. Furthermore they combine high yields through solution - like kinetics with the advantage of easy handling and washing, as inherent to solid support synthesis.

Results and Discussion

As an approach, we have been studying a support system based on commercial Merckogel OR 1000000^{R} (E.Merck, Darmstadt, Germany)[1,4] by using actual chemistry of internucleotide bond formation on a high capacity carrier. Partial hydrolysis of these polyvinylacetate resins with 0.5 M NaOH solution in dioxan-water (1:1 mixture)[1] at room temperature for 30 - 80 min. yielded different carriers **1** that could be loaded with 120, 150, 258, and 368 μmoles of nucleoside per gram support as determined by the standard DMTr assay procedure[5]. The loading of first nucleoside on to carriers **1** was achieved by treating 5'-*O*-DMTr-dT-3'-*O*-succinate (**2**) in dry pyridine and N-methylimidazole, and the derivatized Merckogel **1** in presence of MSNT as coupling agent to give mononucleoside loaded support **3** (Scheme 1). To the best of our knowledge, this is higher than the highest loading ever reported from other laboratories. The capping of unreacted OH groups in monomer loaded support **3** was done with acetic anhydride in dry pyridine containing a small amount of DMAP[5].

A series of preparations of dT_{20} were done by using the monomer loaded support **3** under standard DNA synthesizer conditions, using reagents for 1 to 10 μmole scale. The average stepwise yield of chain elongation by trityl cation assay was found to be more than 98%. The capillary electrophoretic analysis of crude synthetic oligomer indicated good quality of the product in each case, as shown in FIG. 1 and FIG. 2.



Scheme 1 : Loading of monomer 5'-*O*-DMTrdT-3'-*O*-succinate (**2**) on derivatized Merckogel **1**.
Reagents and Conditions : (i) Pyridine (dry), MSNT (1-(2-mesitylene-sulfonyl)-3-nitro-1H-1, 2, 4-triazole), N-methylimidazole, 24h, r.t.

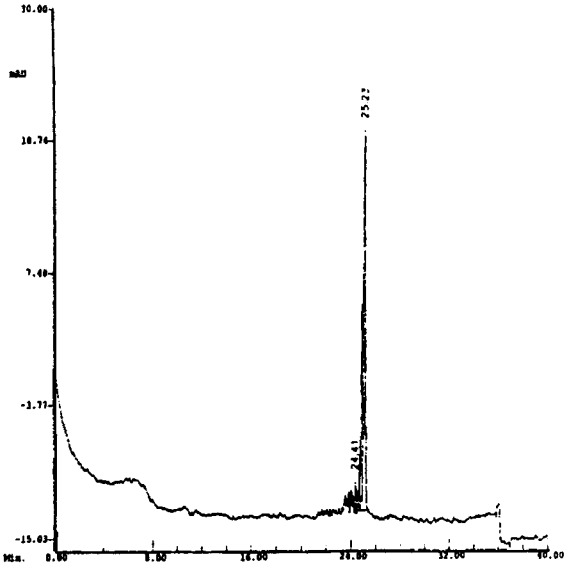


FIG. 1: C.E. profile of crude dT_{20}
(1 μmole scale; Abs. 260 nm)

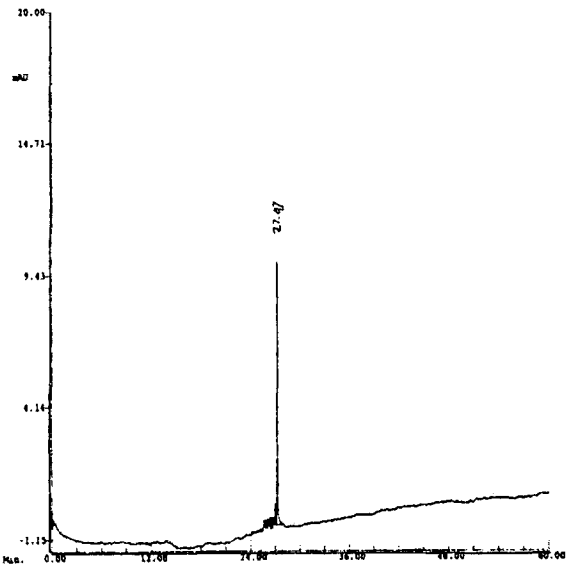


FIG. 2: C.E. profile of crude dT_{20}
(10 μmole scale; Abs. 260 nm)

Experimental

The synthesis of oligonucleotides was done on a DNA synthesizer (Applied Biosystems 381 A DNA synthesizer) using standard automated phosphoramidite chemistry. The UV spectra were recorded on a "Beckman DU 7500 spectrophotometer" using fixed wave length at 498 nm. The IR spectra of the support were recorded on a "Bruker IFS 113v FT-IR spectrometer". Purity of the synthesized oligomers after treatment with 30% ammonia solution for 15 hours at 55° C, followed by desalting over a NAP column, was checked by capillary electrophoresis using a "Bio-Rad BioFocus 2000 Capillary Electrophoresis System". The partial hydrolysis of Merckogel OR 1000000 ^{R} was done according to the reported procedure[1]. Preparation of the monosuccinylated nucleoside **2** was done by using the standard methods[5].

*Stepwise loading of nucleoside **2** on to derivatized Merckogel^{R} **1**:*

To a solution of succinylated deoxyribonucleoside **2** (100 mg, 0.16 mmol) in dry pyridine (2.5 ml) was added MSNT (125 mg, 0.42 mmol) and N-Methylimidazole (500

μl) followed by partially hydrolysed Merckogel OR 1000000 ^{R} **1** (100 mg). The reaction mixture was kept at room temperature overnight with occasional shaking. Then the support was filtered, washed extensively with pyridine, methanol, and then ether. The support was dried under vacuum.

Conclusion

This investigation describes the development of a suitably derivatized Merckogel^{R} as an efficient solid support for large-scale synthesis of DNA for biomedical applications. The Merckogel^{R} system is highly variable in its capacity, which can be simply regulated by the extent of partial hydrolysis and stepwise loading of nucleosides up to 368 μmole/g. Work is in progress to optimize the yield of synthesized oligonucleotides for antisense applications[3].

Acknowledgments

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