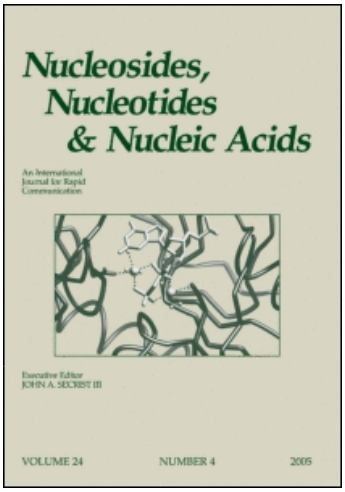


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# A Simple and Stable Silica Gel Support for the Oligodeoxyribonucleotide Synthesis

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# A SIMPLE AND STABLE SILICA GEL SUPPORT FOR THE OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

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ABSTRACT
A simple and efficient synthesis of a solid support with a long chain polyamide spacer has been developed. The spacer was made by successive addition of ethylene-diamine and succinic anhydride. The obtained solid support gives very homogeneous 20 mer oligodeoxyribonucleotides, detected by HPLC and electrophoresis.

## INTRODUCTION

The recombinant DNA technology had stimulated the development of a large number of methods for oligodeoxyribonucleotide synthesis[1,2]. Although with the introduction of the phosphoramidites the tedious manual operations could be replaced by machines, these being expensive, there still exists a need for rapid, simple and inexpensive methods.

Among the less expensive methods is the syringe method which gives quite good results in the synthesis of short oligodeoxyribonucleotides[3]. For long oligodeoxyribonucleotides some difficulties were encountered due to the formation of debris from the supporting material[4]. We describe herein the synthesis of a spacer through which the first deoxyribonucleotide can be bound to the silica gel. Its application for solid phase oligodeoxyribonucleotide synthesis using the syringe method provides excellent results.

## RESULTS AND DISCUSSION

Solid supports containing long alkylamine chains have been reported previously to possess superior physical and chemical properties [5,6,7]. The effectiveness of supports containing spacers may be attributed to the reduction of non-bonded interactions between the solid surface and the entering deoxyribonucleoside molecule thereby increasing the rate and the yield of incorporation of each added monomer in the early coupling steps.





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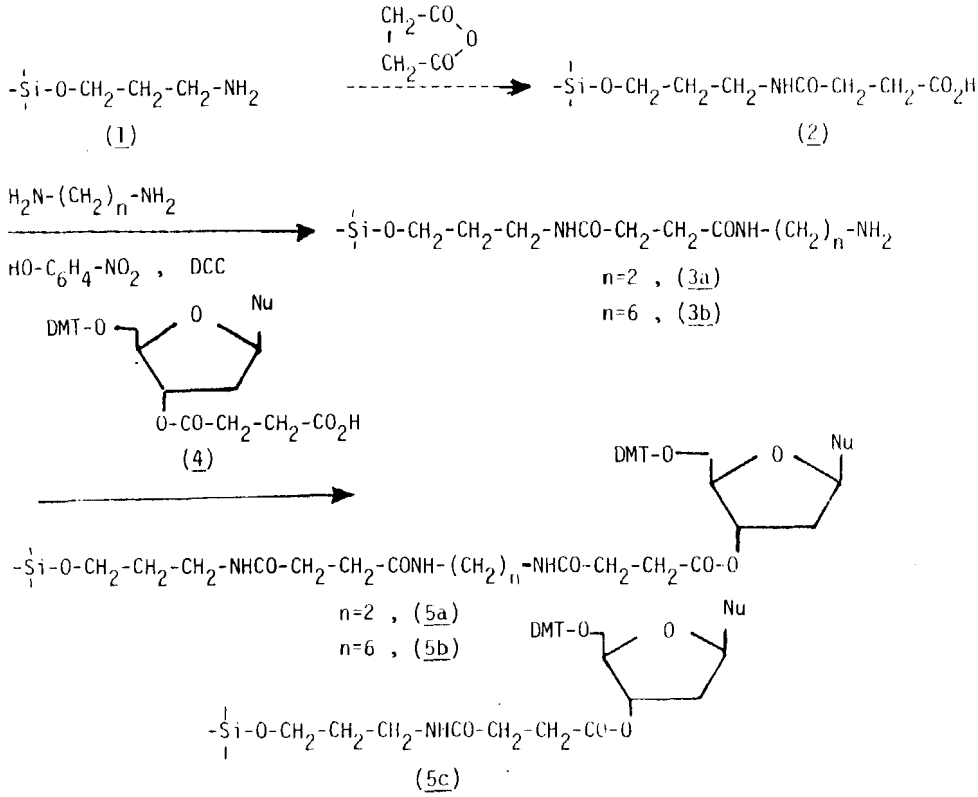


Nu= Protected nucleobase

DCC= N,N'-Dicyclohexylcarbodiimide

DMT= 4,4'-Dimethoxytrityl

SCHEME 1.

The spacer described in this communication was synthesized by succesive addition of succinic anhydride, ethylenediamine and hexamethylenediamine to the alkyamine support according to SCHEME 1. For comparison the support (5c) was also synthesized[1].

One of the advantages in the use of this type of spacer is that ninhydrine provides an easy way to monitor the degree of completion of the reaction in each step by appearance or disappearance of the blue color. Another advantage is the exceptional high yield in each cycle of addition (98%) after only 10 minutes of reaction, resulting in products of very high purity obviating the necessity of capping unreacted hydroxyl groups and working in argon atmosphere in the course of the synthesis.

By this procedure several 15, 18 and 20- oligomers were synthesized for use as hybridization probes. The total yields were 40-50% as calculated from the HPLC pattern (FIGURE 1) and the time needed for each cycle was

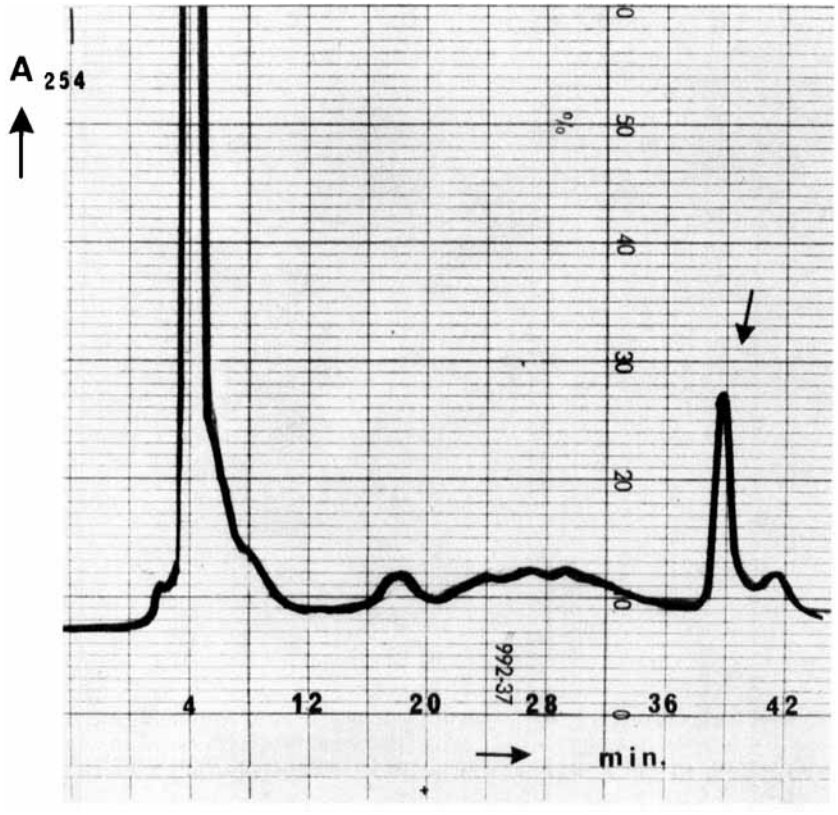


FIGURE 1: HPLC chromatogram of the fully deprotected 20-oligomer d(TTGAGTCCTTTGGGGATCTG) on an IBM Octyl-Endcapped column ( 4.5x250mm) with a flow of 2ml/min. and a gradient of 0-15% acetonitrile in 0.1M triethylammonium bicarbonate, pH 7.0, over 45 minutes.

15 minutes. The products were purified by HPLC and their purity was checked by ($\gamma$-$^{32}$P)-ATP kination followed by electrophoresis. A comparison between oligodeoxyribonucleotides synthesized on the support (5a) prepared as described in SCHEME 1 and oligodeoxyribonucleotides synthesized on two commercial supports (see EXPERIMENTAL) has been done. FIGURE 2 shows that the crude oligodeoxyribonucleotides prepared on support (5a) (lines a and b), are less contaminated by small fragments than those prepared on the commercial supports (lines c and d).

In order to estimate the effect of the spacer length on the synthesis of oligodeoxyribonucleotides we took the same type of silica (see EXPERIMENTAL) and extended the spacer to different lengts and then made the same oligodeoxyribonucleotide (d(TTGAGTCCTTTGGGGATCTG). FIGURE 3 shows that the crude

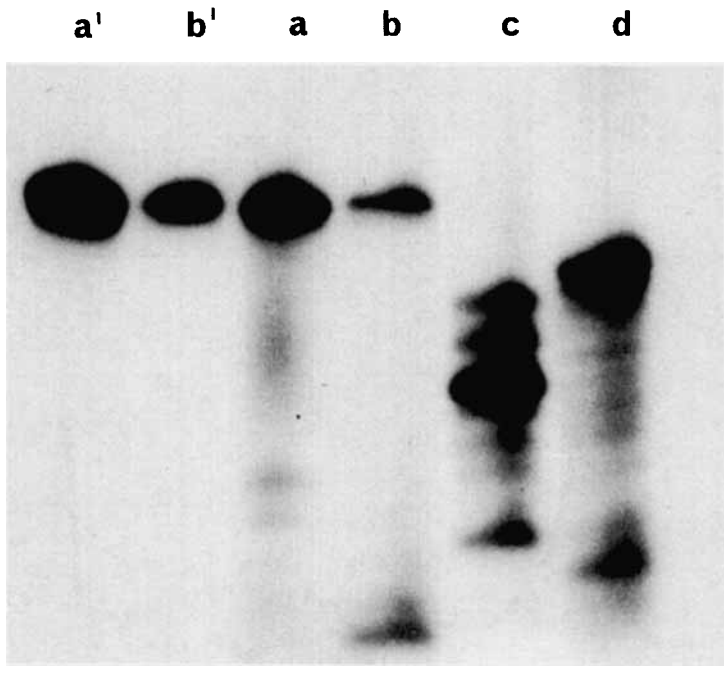


FIGURE 2: Electrophoresis of crude oligodeoxyribonucleotides obtained with the above described support (5a) and with two commercial supports on 20% polyacrylamide gel containing 7M urea; phosphorylation carried out with ($\gamma$-$^{32}$P)-ATP and T4 polynucleotide kinase. Lines a,a' and b,b' are d(TTGAGTCCTTTGGGGATCTG) and d(TTTGAGTCTTTGGGGATCTG) respectively synthesized on support (5a), lines c are pentadecamer and octadecamer d(TTGATCCTTTGGGGA) and d(GATGATACCTAGTGGTCT) respectvely synthesized on commercial supports. Lines a' and b' are purified twenty-mers



oligodeoxyribonucleotide synthesized on the supports with the extended spacers (5a and 5b) are less contaminated by a shorter fragment.

For purposes as hybridization, there was no need to purfy the crude material.

On the basis of our studies and others[5,7], it seems to us that this type of approach holds further promises for modern oligodeoxyribonucleotide synthesis. We believe that many other "spacers" can be designed ( by varying length and structure) to serve as supports for the efficient synthesis of high molecular weight oligodeoxyribonucleotides.

## EXPERIMENTAL

The 5'-protected 3'-phosphoramidites were synthesized according to reported procedures[6,8] . The silica gel support was prepared using Porasil, type C, 80-100 mesh ( Waters Associates, Inc. MILFORD MASS.01757) according to a modification of Caruthers method[1,2] (see SCHEME 1).

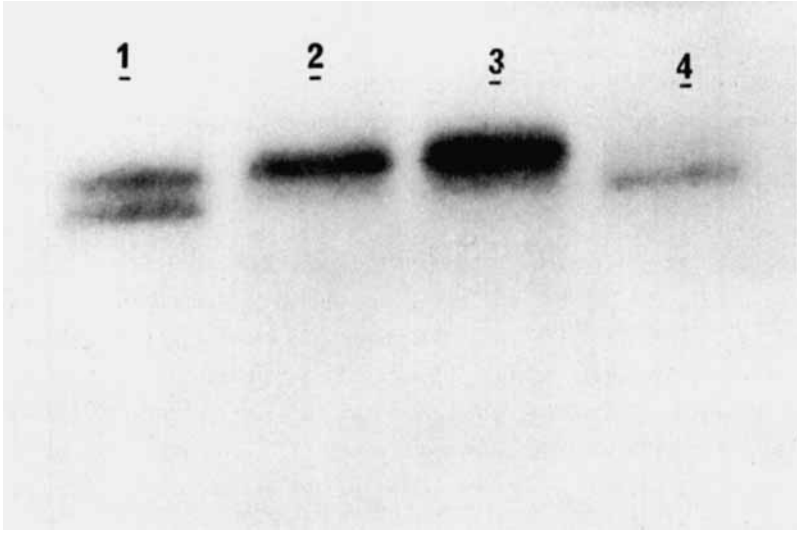


FIGURE 3: Electrophoresis of crude d(TTGAGTCCTTTGGGGATCTG) with the above described supports (5c), (5a) and (5b), (lines 1, 2 and 3 respectively), on 20%polyacrylamide gel containing 7M urea; phosphorylation carried out with ($\gamma$-$^{32}$P)-ATP and T4 polynucleotide kinase. Line 4 corresponds to a purified nonadecamer.



Amino silica (1)[1] (300mg) was agitated with succinic anhydride (400mg) in water (3ml) for 24 hr. The solid product (2) obtained was washed three times with methylene chloride (10ml) and filtered through sintered glass. The dry product was allowed to stand for 2 hr. with p-nitrophenol (80mg), DCC (110mg), pyridine (0.1ml) in dry dioxane (2.5ml), then ethylenediamine (0.2ml) or hexamethylenediamine (0.2gr) was added and the mixture was agitated overnight The product (3a) or (3b) was fitered, washed five times with methanol (10ml) and three times with methylene chloride (10ml), filtered again and dried in air. The incorporation of the amino groups was monitored by a 5% solution of ninhydrine. The first deoxyribonucleoside, to be bound to the support, was activated in the following way: 5'-O-DMT-3'-succinyl nucleoside (4) (100mg) and p-nitrophenol (25mg) in dry dioxane (1ml), was added to a solution of DCC (20mg) in dry dioxane (1ml) and allowed to stand at room temperature for2 hrs till crystals of DCU were formed. Linking of the deoxyribonucleoside (4) to the support (3a) or (3b) was carried out in a test tube sealed with a rubber septum, containing a mixture of the silicagel support (3a) or (3b) (300mg), dioxane (1ml) and triethylamine (0.1ml). The p-nitrophenol ester of the DMT-nucleoside succinate (4) was added through a syringe needle provided with a filter. The mixture was agitated for 24 hrs. The product (5a) or (5b) was washed five times with methanol (10ml) and methylene

chloride (10ml) and filtered. The unreacted hydroxyl and amino groups of the solid support were capped by allowing the dry product (5a) or (5b) to stand for 2 hrs. in a mixture of acetic anhydride (0.6ml) and triethylamine (1ml). The capped product was washed several times successively with methanol and methylene chloride, filtered and dried in air. 300 mg product was obtained. The support (5c) was prepared according to the reported method[1].

The coupling cycles were carried out on 30mg support batches. DMT-deblocking with 2% solution of $Cl_2CHCO_2H$ in methylene chloride gave an absorbance of 2.0 OD/25ml for the trityl cation ($\lambda_{max}$ =500nm) corresponding to a loading of 30umol/g. For each coupling cycle an acetonitrile solution (0.5ml) of phosphoramidite (50mg) and an acetonitrile solution (0.5ml) of tetrazole (20mg) were agitated for 10 minutes in a syringe provided with a filter, together with the DMT-deprotected support. Each cycle gave 96-98% yield measured by the $OD_{500}$ of the trityl cation released. ($OD_{500}$ is the Optical Density at 500 nm).

The syringe ( ONCE made by A-SIK Denmark ) was of 2.0ml volume provided with a home made porous polyethylene filter ( Prover, England). The commercial supports were from BioLabs, New England.

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