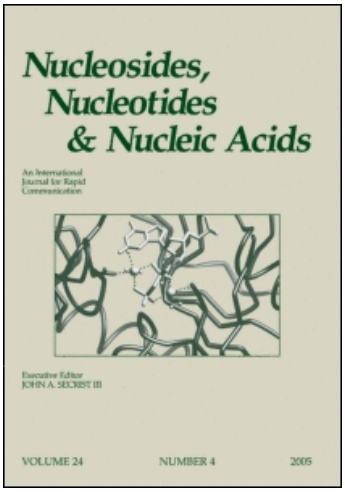

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A Rapid Method for the Functionalisation of Polymer Supports for Solid Phase Oligonucleotide Synthesis

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A RAPID METHOD FOR THE FUNCTIONALISATION OF POLYMER SUPPORTS FOR SOLID PHASE OLIGONUCLEOTIDE SYNTHESIS

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ABSTRACT

A rapid method is described for the covalent anchoring of appropriately protected 2′-deoxyribonucleoside-3′-O-succinates to LCAA-CPG, widely used support for solid phase oligonucleotide synthesis. The method involves the reaction of nucleoside-3′-O-succinates with aminoalkyl functions of the support in the presence of improved and commercially available condensing reagent, TBTU or TPP-DTNP to generate fully functionalised polymer supports with excellent nucleoside loadings.

INTRODUCTION

The solid phase oligonucleotide synthesis has been tremondously simplified in last 10 years. The commercial availability of stable synthons and improved solid supports, labile protecting groups for exocyclic amino functionalities of nucleic bases or the use of novel deprotection conditions[1] for post synthesis work-up have significantly contributed to the development of oligonucleotide synthesis. However, there remains problems still to be resolved by DNA chemists. One of such problems is the time consuming functionalisation of polymer supports, required for oligonucleotide synthesis. A number of protocols[2-6] have been described in the recent past for this purpose. However, there is a need to develop rapid and safe method for the functionalisation of polymer supports, employing commercially available reagents.

We wish to describe here a rapid method in which nucleoside-3′-O-succinates have been anchored to long chain alkylamine-controlled pore glass (LCAA-CPG) using O-(1H-benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium-tetrafluoroborate (TBTU) or triphenylphosphine (TPP) - 2,2′-diothio-bis-(5-nitropyridine) (DTNP) as coupling reagent. The functionalisation of supports proceeds very fast and results in excellent nucleoside loadings in just 5 min with TPP-DTNP as a coupling reagent, while it takes 10 min with TBTU. Though the proposed method involves the use of nucleoside-3′-O-succinates, the preparation of these intermediates is no longer a limiting step. A rapid method[7] for their synthesis has already been reported from this laboratory.

EXPERIMENTAL

Preparation of 5′-O-DMT-2′-deoxynucleoside-3′-O-succinates

The nucleoside 3′-O-succinates were prepared in just 10 min following the procedure[7] reported earlier from this laboratory.

Oligonucleotide synthesis

Oligonucleotides were synthesized on Pharmacia LKB Gene Assembler Plus following manufacturer's recommendations via phosphoramidite chemistry on polymer supports prepared via methods A and B and standard supports (2).

The oligonucleotides were deprotected following standard protocols and purification of oligonucleotides were carried out on preparative Mono Q anion- exchange column (FPLC). The analysis was performed on HPLC using reverse phase C_{18} column.

Functionalisation of polymer supports

Method A: 2,2′-Dithio-bis-(5-nitropyridine) (0.1 mm ol) dissolved in 400 µl of a mixture of acetonitrile-dichloroethane (1:3) was mixed with a solution of an appropriately protected 2′-deoxynucleoside-3′-O-succinate (0.1 mmol) and DMAP (0.1 mmol) in acetonitrile (500 µl). The clear solution so obtained was added a solution of TPP (0.1 mmol) in acetonitrile (200 µl) at room temperature. The mixture was vortexed for few seconds and then added to a vial containing LCAA-CPG (500 mg, 0.05 mmol amino groups) and allowed to react for 5 min at room temperature. The reaction was arrested by adding methanol (500 µl) and the support was recovered on a sintered glass funnel followed by washings with methanol (2x5 ml) and diethyl ether (2x 5 ml). The support was dried first in the air and then under high vacuum. The dried support was subjected to capping of the residual amino functionalities following standard protocol (2). The support was again washed as described above and stored at 4°C. The nucleoside loading on the derivatised supports was determined by acid treatment method (2)

Method B: To a solution of an appropriately protected 2′-deoxynucleoside-3′-O-succinate (0.1 mmol), triethylamine (0.2 mmol) and LCAA-CPG (500 mg, 0.05 mmol amino groups) in dry acetonitrile (1 ml) was added TBTU (0.2 mmol). The mixture was shaken at room temperature for 10 min. After addition of 50% aq. methanol (500 µl), the support was recovered on a sintered glass funnel. The support was washed with methanol (2x5 ml) and diethyl ether (2x 5 ml). The drying of the support and capping of the residual amino functionalities were performed as described in method A. The nucleoside loading on the supports was determined as described above.

RESULTS AND DISCUSSION

The nature of polymer supports, its pore size and the linker arm available on it play vital roles in solid phase synthesis of oligonucleotides. These parameters are relatively easy to control by mere selection of a suitable polymer support. LCAA-CPG has been considered to be an ideal support, which meets all these requirements. The other important parameter is the loading of the leader nucleoside on the supports. For medium to large-sized oligonucleotide synthesis, polymer supports with moderate nucleoside loadings (25- 30 µmol /g support) are considered to be the ideal. the

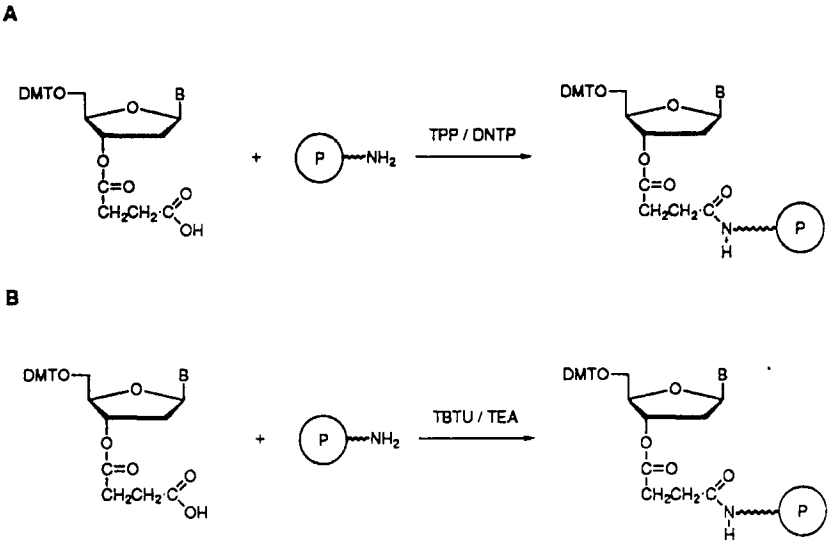

Fig. 1 Functionalisation of LCAA-CPG via methods A and B. A: TPP = tripheylphosphine; DTNP = 2,2′-dithio-bis-(5-nitropyridine) B: TBTU = 1O-(1H-benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium-tetrafluoroborate; TEA = triethylamine. B = T, A(bz), C(bz) or G(ibu)

Table-1 Nucleoside loadings on LCAA-CPG support functionalised following methods A and B

Appropriately protected nucleosides	µMol nucleoside / g LCAA-CPG	
	Method A	Method B
DMT dT	29.8	28.4
DMT dA (bz)	30.1	28.3
DMT dC (bz)	29.7	26.9
DMT dG (ibu)	28.8	28.4

present study was undertaken to develop suitable methods for rapid functionalisation of LCAA-CPG, the widely used polymer support for oligonucleotide synthesis, using commercially available reagents.

The method A involves the oxidation-reduction condensation-based anchoring of appropriately protected 2′-deoxynucleoside-3′-O-succinates onto LCAA-CPG using DTNP-TPP as a condensing reagent[8,9] (Fig 1A). The reaction mechanism involves the generation of an activated species, arylthiotriphenylphosphonium salt, in the first step, which subsequenly reacts with the 3′-O-succinylated nucleoside to give activated acyloxytriphenylphosphonium salt. In the second step of the reaction, amino groups on the support react very fast with this salt to generate fully functionalised polymer supports with excellent nucleoside loadings (Table 1).

The method B involves the use of a commercially available condensing reagent, TBTU. The mechanism[10] involves the generation of an intermediate active ester of benzotriazole of nucleoside-3′-O-succinates, which immediately reacts with the amino groups on the support to give functionalised support with nucleoside loadings 27-30 μMol / g LCAA-CPG (Fig. 1B).

A number of oligonucleotides were synthesized on supports derivatised by the methods A and B. The corresponding oligonucleotides were also synthesized on standard supports under identical conditions. The coupling and isolated yields of the oligos synthesized on the derivatised supports were found to be comparable to the oligos synthesized on the standard supports.

In order to ensure that no modification of nucleic bases occured during the functionalisation of polymer supports, a small quantity (100 mg) of each of the derivatised supports and supports prepared by the standard procedure were subjected to aq, ammonia treatment in sealed vials for 30 min at room temperature. The released 5′-O-DMT-N-protected-2′-deoxynucleosides were extracted in diethyl ether. The solvent was removed and a portion of each of the cleaved nucleoside was dissolved in methanol / ethanol and subjected to UV and HPLC analysis. The released nucleosides were found to identical in all respect (UV analysis, retention time on HPLC etc.) to the corresponding nucleosides released from the standard supports.

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