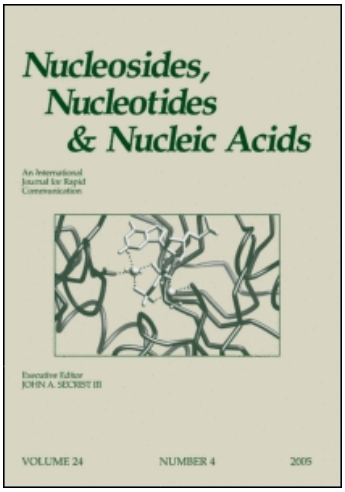

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Reusable Solid-Phase Supports for Oligonucleotide Synthesis Using Hydroquinone-*O,O'*-diacetic Acid ("Q-Linker")

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REUSABLE SOLID-PHASE SUPPORTS FOR OLIGONUCLEOTIDE SYNTHESIS USING HYDROQUINONE-*O,O'*-DIACETIC ACID ("Q-LINKER")

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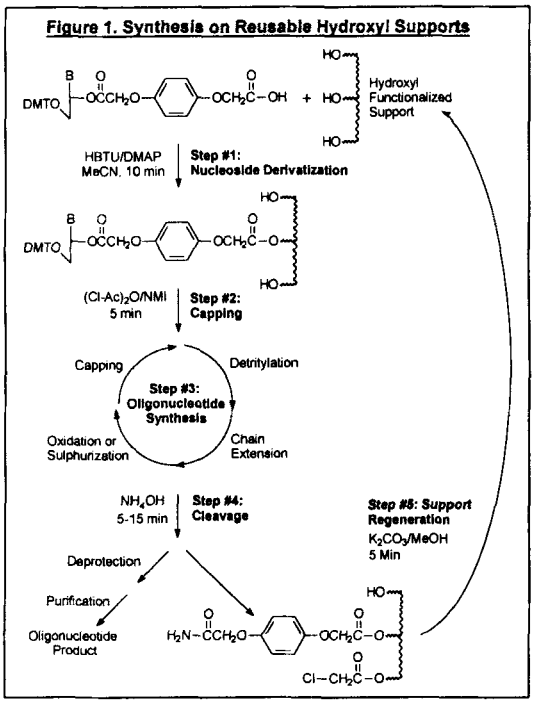
ABSTRACT

Reusable solid-phase supports for large scale oligonucleotide synthesis have been prepared by converting amino derivatized supports into hydroxyl supports. Rapid nucleoside attachment, via a *Q-linker* arm, was automatically performed on the DNA synthesizer using HBTU and DMAP as the coupling reagents. All steps were suitable for automation and ~ 1.5 h was required to prepare the supports for reuse. Up to twelve consecutive syntheses of a 20-mer phosphorothioate were performed on a synthesis column.



A method to reuse solid-phase supports is required to reduce the costs involved in large scale solid-phase synthesis. However, conventional amino derivatized supports are not suitable for reuse because of the great stability of amide linkages. Instead, hydroxyl derivatized supports were used to form hydrolyzable *ester* linkages via a Q-linker[1] attached to the first nucleoside. We evaluated 12 different hydroxyl derivatized supports, prepared by adding 12-dimethoxytrityl dodecanoic acid[2] or 1,4-butanediol diglycidyl ether[3] linker arms to various amino or hydroxyl supports.

The support recycling strategy (FIG. 1) was based upon the following features:

1, Rapid (≤ 10 min) automated support derivatization using HBTU and DMAP[4].

2, Use of labile chloroacetyl[5] groups to cap off unreacted surface hydroxyl sites.

3, A short NH_4OH cleavage time to minimize damage to the support's surface.

4, Support regeneration with K_2CO_3/MeOH after removal of the oligonucleotide.

5, All steps were performed without removing the support from the synthesis column.

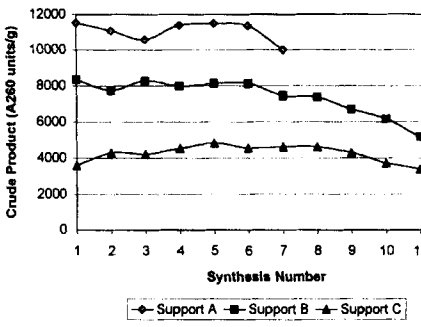


Figure 2. Crude product obtained from reusable supports with high (> 100 μmol/g), medium (~ 70 μmol/g), and low (~ 30 μmol/g) capacities.

The scheme shown in FIG. 1 was performed on PE/ABD 394 (1 μmole scale) and Pharmacia Oligo Pilot II (200 & 1,000 μmole scales) DNA synthesizers. Up to 12 consecutive 20-mer phosphorothioate syntheses were performed on the same column. The amount of crude product produced during each use, for three of the supports tested, is shown in FIG. 2. The best supports could be reused ~8-10 times and others ~5-7 times, before any significant decrease in product yield. Analysis of the products by either CE, AX-HPLC, or ES-MS showed only the expected product mixture and no impurities, attributable to the support recycling, were found.

The support recycling strategy was applicable to a wide range of support materials. However, further investigation into the factors which contribute to the eventual decrease in product yield is still required. Further process development to optimize pilot scale synthesis conditions for different support materials is presently in progress.

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