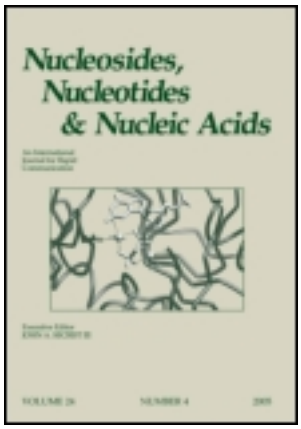

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Novel Photocleavable Universal Support for Oligonucleotide Synthesis

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Novel Photocleavable Universal Support for Oligonucleotide Synthesis

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ABSTRACT

A novel photocleavable universal support for the automated solid phase synthesis of oligonucleotides is described. The linker between the growing oligonucleotide chain and CPG support contains a nucleophilic amine protected with a photocleavable group. On exposure to UV light, this group is detached and the free amine affords cleavage of the oligonucleotide from the support. The use of long wavelength UV light avoids damage to the DNA.

Key Words: Photocleavable support; Universal support; Oligonucleotide synthesis.

INTRODUCTION

Conventionally, a succinyl linker has been utilised to attach the 3′-OH of a nucleotide to amino CPG support.[1] This has the disadvantage that before

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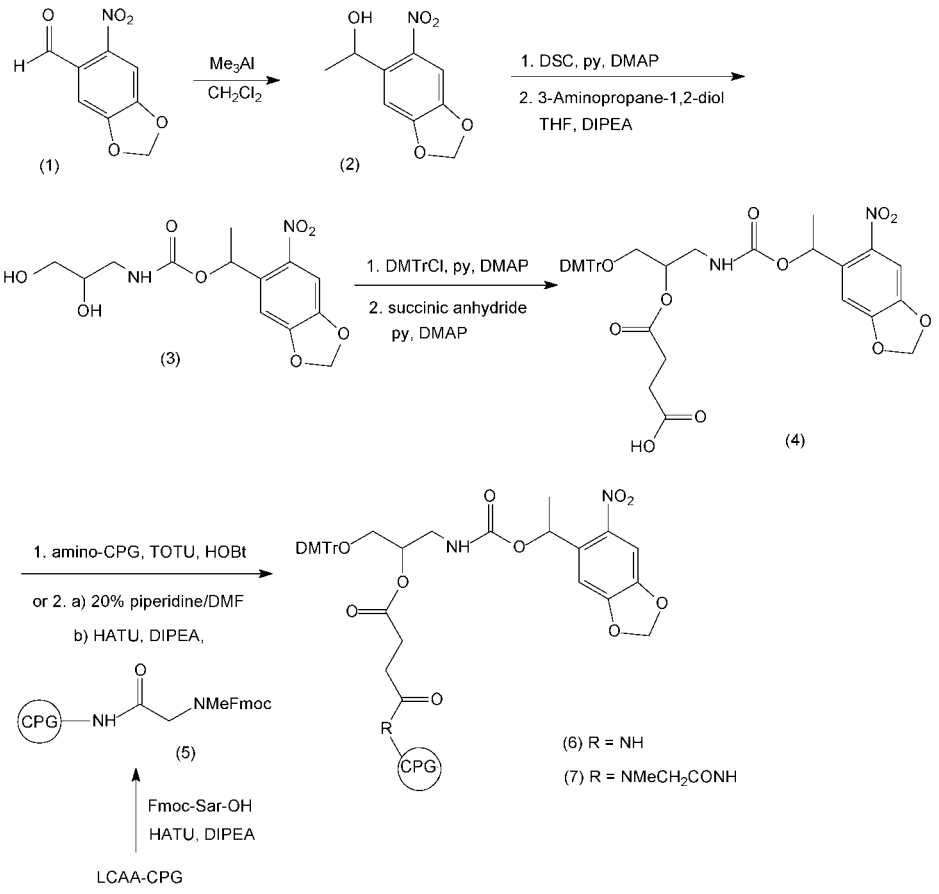
automated synthesis, the support must be pre-derivatised with the first nucleotide in the desired sequence, which can be one of the four DNA bases. Use of a universal support would solve this problem. Rapid or clean cleavage should then leave no trace of the linker left on the 3′-OH as this free hydroxyl is essential for applications such as sequencing and PCR.

Our approach is based on a substituted aminopropanediol,[2] where the amine protecting group is a photolabile *o*-nitrobenzyl group.[3] The advantages of this type of group are its stability under the synthesis conditions and that no reagents are required for deprotection, only UV radiation. The nitroso by-product from the UV treatment is then easily removed by washing of the CPG support.

RESULTS AND DISCUSSION

The synthetic route is shown in Sch. 1. The aldehyde **1** was converted to methyl alcohol **2** in 92% yield. Disuccinimidyl carbonate provided an activated carbonate,



Scheme 1. Synthesis of the photocleavable supports.

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which was used crude in the next step. Coupling of this to 3-amino-1,2-propanediol resulted in 51% of diol **3** over 2 steps after chromatography. Tritylation of the primary alcohol and addition of succinic anhydride afforded the desired material **4** in 54% yield after chromatography. Coupling of this acid to amino CPG support was carried out with TOTU and HOBt to give a loading of 25 μmol/g for **6**. An alternative linker utilised a sarcosine residue to provide a tertiary amide bond. Amino-CPG was functionalised with Fmoc-sarcosine using standard conditions, then deprotected and coupled to acid **4** to give the support **7** with a loading of 44 μmol/g.

Initial experiments on support **6** in MeCN or 0.1 M TEAA suggested that UV absorbance could be used to follow the cleavage of the nitroso ketone.

Supports **6** and **7** were used in standard oligonucleotide synthesis to provide solid supported T6 and mixed 19 mer G GGT GAA TTA CAA GCT CCG. For support **6** + T6, photocleavage at 365 nm for 1–3 h at a distance of 5 cm from the light source was attempted while the material was still in the oligo synthesis column with a small quantity of MeCN. Cleavage of the oligonucleotide from the support was achieved using 3.5% NH_3 in 0.1 M TEAA to give an HPLC purity of 51%, while MS data confirmed that no other 3′-modified products were observed.

Research was then performed in open topped fritted columns, whose frits were around 3.5 cm from the UV source. Trials were carried out with dry support, dry support with agitation, dry support with wash cycle, MeCN, MeCN with agitation and 20% 0.1 M TEAA in MeCN over various time periods. Optimal results for support **6** were from the dry support with 30 min wash cycle over 3 h and 20% TEAA in MeCN over 6 h, so these conditions were applied to the supported oligonucleotides.

Moving on to study photocleavage of T6 and 19 mer oligonucleotides, the functionalised support was removed from the synthesis column and added to a fritted open topped column. For support **6** + T6: photocleavage was attempted in MeCN, 20% 0.1 M TEAA in MeCN and dry support with a wash cycle at regular time intervals. Following cleavage from the support, the best HPLC result (72% desired product) came from the dry support that was washed every 30 mins over 3 h. For support **7** + T6: photocleavage and oligonucleotide cleavage was carried out as above and the MeCN for 2 h or the wash cycle over 3 h both gave 61% by HPLC. For support **7** + 19 mer: all the attempted conditions gave similar results ~42% by HPLC.

CONCLUSIONS

These novel photocleavable supports can be utilised to provide oligonucleotides with no 3′-modification. The photocleavage has been shown to occur under a variety of conditions and will be further optimised.

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