

A NOVEL PHOTOCLEAVABLE UNIVERSAL SUPPORT FOR OLIGONUCLEOTIDE SYNTHESIS

Emma Anderson,^{a,†} Douglas Picken^a and Tom Brown^b

^a Link Technologies Ltd., 3 Mallard Way, Strathclyde Business Park, Bellshill, Lanarkshire ML4 3BF, UK; www.linktech.co.uk

^b Department of Chemistry, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.

[†] To whom correspondence should be addressed (emma.anderson@linktech.co.uk).

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.

INTRODUCTION

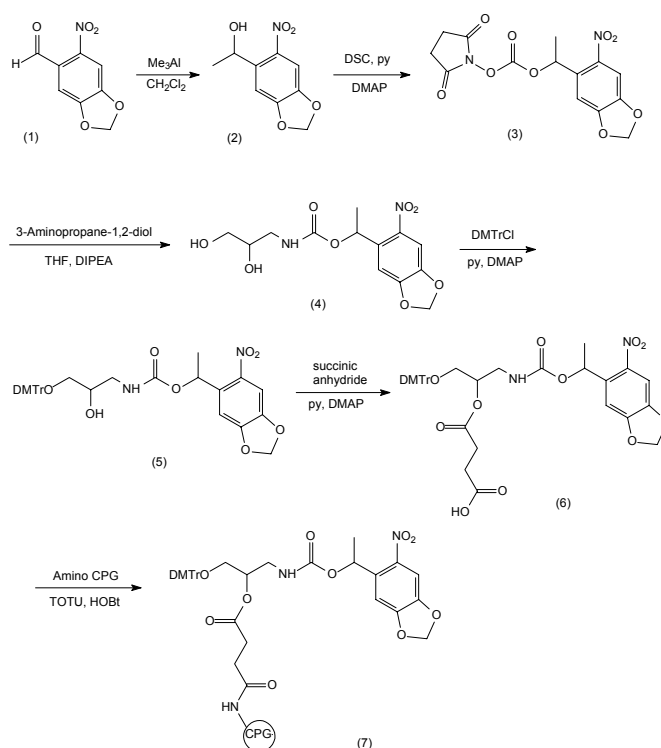
Phosphoramidite methodology is routinely utilised as the preferred route for the chemical synthesis of oligonucleotides. It is based on automated solid-phase chemistry where a growing oligonucleotide chain is produced on an insoluble support. A linker for attachment of an oligonucleotide to the support for this process must be stable during automated synthesis, but labile during cleavage from the support.¹ Conventionally, a succinyl linker has been utilised to attach the 3'-OH of a nucleoside to amino CPG support. This has the disadvantage that before automated synthesis, the support must be pre-derivatised with the first nucleoside in the desired sequence, which can be one of the four DNA bases. The use of a universal support would solve this problem. An important factor in favour of rapid or clean cleavage is that there should be 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 photolabile *eg.* an *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.

EXPERIMENTAL

Synthetic Route—The synthetic route is shown below in Scheme 1. The light sensitive aldehyde **1** was converted to the methyl alcohol **2** in 92% yield on 1-2g quantities. Disuccinimidyl carbonate was utilised to afford **3**. During the first batch synthesis, this material was isolated by column

chromatography in 74% yield however in subsequent runs, the material was used as the crude product in the next step. Coupling of **3** to 3-amino-1,2-propanediol resulted in diol **4** over 2 steps after column chromatography. Tritylation of the primary alcohol afforded the desired material **5** in 55% yield. In the final solution-phase step, succinic anhydride was coupled to the remaining hydroxyl group to furnish **6** in 99% yield after column chromatography in MeOH/EtOAc. Coupling of this acid to the amino support LCAA-CPG was carried out with TOTU and HOBt to give a loading of 25 μmol/g.



Scheme 1—Synthesis of the photocleavable support.

- For a review on linkers for phosphoramidite synthesis see: Guillier, F.; Orain, D.; Bradley, M., *Chemical Reviews*, **2000**, *100*, 2091.
- Lyttle, M.H.; Hudson, D.; Cook, R.M., *Nucleic Acids Research*, **1996**, *24*(14), 2793-2798.
- Hasan, A.; Stengele, K.; Giegrich, H.; Cornwell, P.; Isham, K. R., *Tetrahedron*, **1997**, *53*(12), 4247-4264.

An alternative linker utilised a sarcosine residue to provide a tertiary amide bond and was synthesised as shown in Scheme 2 below. LCAA-CPG was functionalised with Fmoc-sarcosine using standard conditions, then deprotected and coupled to acid **6** to give the support **9** with a loading of 44 μmol/g.

