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UNIVERSAL SUPPORT II AND III

INTRODUCTION

With the introduction of high throughput DNA synthesizers, the relevance of universal supports is more important than ever. A universal support is used in place of a synthesis support that has the first base attached to the support. This permits the use of one support for all syntheses.

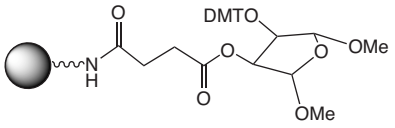
A universal support offers the following advantages:

- eliminates the need for an inventory of nucleoside supports.
- minimizes the possibility of error in the selection of the correct support type.
- reduces time and eliminates the possible error in the generation of an array of nucleoside supports in high throughput synthesizers.
- allows the preparation of oligonucleotides containing a 3'-terminal nucleoside which is not available as a support.

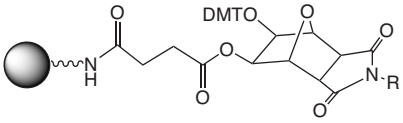
UNIVERSAL SUPPORT / GLEN UNYSUPPORT

Our first Universal support, introduced in 1997, the McLean support[1], was based on the ribonucleoside elimination model. The main impediment to the universal adoption of Universal Support (1) was the aggressively basic conditions required to complete the elimination (dephosphorylation) reaction to release the terminal hydroxyl group. Our original Universal Support (1) is compatible with deprotection using anhydrous methylamine gas and has been used extensively. However, the time for complete dephosphorylation is longer than most companies wish to allocate. A recent development has been the use of a support based on a molecule which is "conformationally preorganized" to accelerate the dephosphorylation reaction.[2,3] By using a rigid bicyclic molecule on the support (2), it was hoped that the molecule's conformation would facilitate the formation of the cyclic phosphate transition state, thereby stimulating the rate of dephosphorylation. And, indeed, it was found that the rate of elimination is markedly faster than the original Universal Support. Glen UnySupport™ is the methyl version of support (2), which is preferred for high throughput oligonucleotide synthesis.

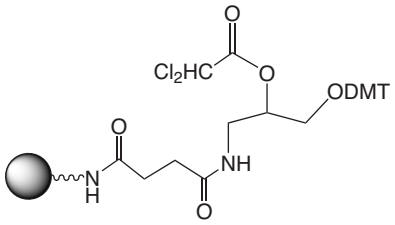
FIGURE 1: **UNIVERSAL SUPPORT STRUCTURES**



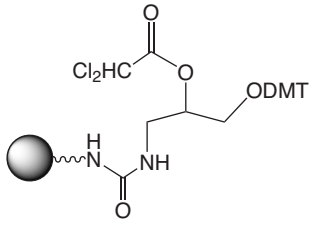
Universal Support (1)



UnyLinker™ (R=Ph)
Glen UnySupport™ (R=Me) (2)



Universal Support II (3)



Universal Support III (4)

UNIVERSAL SUPPORT III

Classic supports have the first nucleoside attached to the support through the 3' hydroxyl with a long chain alkyl amine (lcaa) group on the support. Elongation occurs by reaction of a 3' cyanoethyl phosphoramidite with a 5' hydroxyl group generated on the support. Subsequently, when using a universal support, terminal 3'-dephosphorylation is required to remove the phosphate from the first phosphoramidite addition to the support. Work by Azhayev's group in 2001 used a novel (±)-3-Amino-1,2-propanediol linker[4] that took advantage of an amide group's ability to form weak hydrogen bonds leading to an amide assisted dephosphorylation under mildly basic conditions. The dephosphorylation reaction eliminates the desired 3'-OH oligonucleotide into solution as shown in Scheme

1. This strategy was first employed on our Universal Support II and further improved upon on Universal Support III.

The differences and advantages of Universal III over Universal II were reviewed in our May 2008 Glen Report (20.1). Universal Support II (3) contains the same functional linker as Universal Support III (4), with the main difference being the attachment to the support. For practical purposes, the supports perform in an identical manner. Summarizing the Glen Report, Universal Support III uses an improved method for preparing the support while providing better control of the loading and quality of the support. For more information see: http://www.glenresearch.com/GlenReports/GR20-110.html

Using our improved Universal Support III (4), an oligo yield of >95% can be achieved with purity equivalent to the same oligo prepared using conventional supports. Conditions for Cleavage and Deprotection are outlined below. Universal Support III has been shown to generate oligonucleotides with the same efficacy in polymerase extension reactions as regular oligos. Despite the mild elimination reaction, oligonucleotides up to 75mer in length can be prepared routinely without loss of oligo during the synthesis cycles.

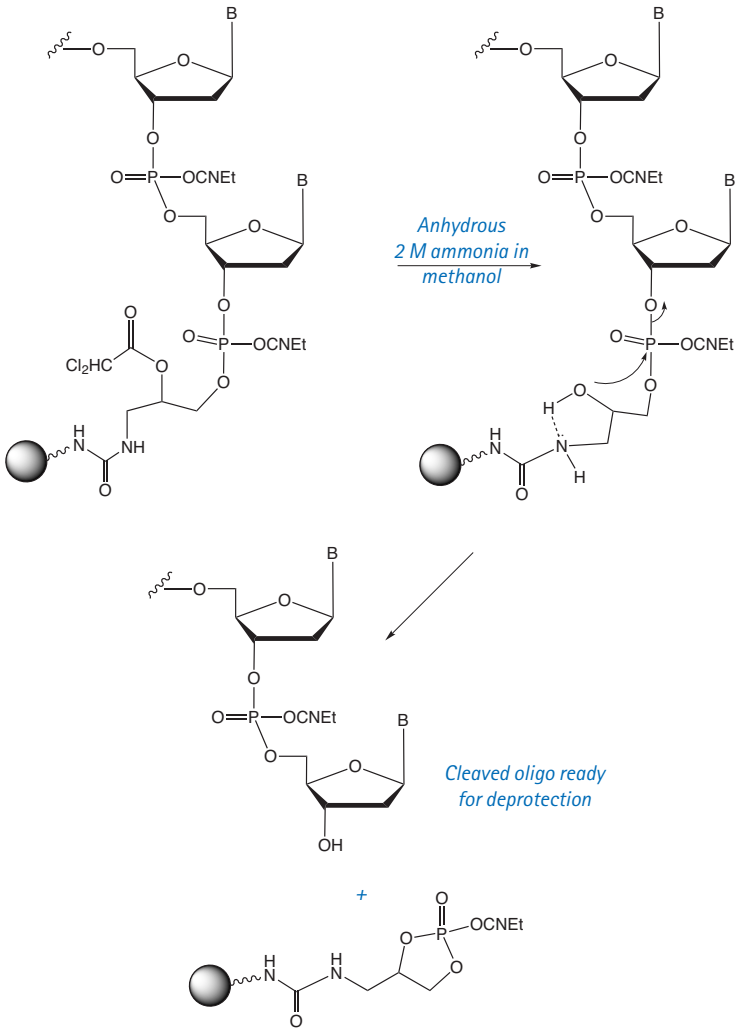
Caution: Universal Support III is not compatible with preliminary elimination of cyanoethyl deprotecting groups using hindered bases such as diethylamine (DEA) or DBU.

After cleavage and dephosphorylation with anhydrous ammonia in methanol, standard deprotection using the conditions appropriate for removal of the protecting groups on the nucleobases is then used to generate the oligonucleotides.

References:

1. S. Scott, P. Hardy, R.C. Sheppard, and M.J. McLean, in A universal support for oligonucleotide synthesis, Innovations and Perspectives in Solid Phase Synthesis, 3rd International Symposium, 1994; R. Epton, Ed. Mayflower Worldwide: 1994; pp 115-124.
2. A.P. Guzaev, and M. Manoharan, ***J Am Chem Soc***, 2003, **125**, 2380-2381.
3. R.K. Kumar, A.P. Guzaev, C. Rentel, and V.T. Ravikumar, *Tetrahedron*, 2006, **62**, 4528.
4. A.V. Azhayev, and M.L. Antopolsky, *Tetrahedron*, 2001, **57**, 4977-4986.
   A. Azhayev, M. Antopolsky, T.M. Tennila, H. Mackie, and J.B. Randolph, *GEN*, 2005, **25**.

SCHEME 1: **AMIDE ASSISTED ELIMINATION OF UNIVERSAL SUPPORT III**



SYNTHESIS

Synthesize oligos on Universal Support II or III using standard conditions. Do not treat the support with DEA or DBU as this will cause irreversible binding of the oligo to the support.

CLEAVAGE

For standard and UltraFast deprotection protocols, cleave the oligo from the support using 2 M ammonia in methanol at room temperature for 30 - 60 minutes. With longer oligos, the 2M ammonia in methanol solution may become hazy during cleavage. This is due to the lower solubility of long oligos in methanol. When this is observed, we suggest an additional rinse of the support using 2M ammonia in methanol with a few drops of water added.

DEPROTECTION

Standard

Add 1 volume of 30% ammonium hydroxide, seal and deprotect using the conditions appropriate for removal of the protecting groups on the nucleobases.

UltraFast

Add 1 volume of AMA (ammonium hydroxide/40% aqueous methylamine 1:1) seal and deprotect at 65°C for 10 minutes.

UltraMild Using Ammonium Hydroxide

Add 1 volume of ammonium hydroxide, seal and leave at room temperature for 8 hours.

Cleavage and Deprotection - UltraMild Using Potassium Carbonate in Methanol

Cleave and deprotect the oligo using anhydrous 50 mM potassium carbonate in methanol at room temperature for 4 hours if using UltraMild Cap Mix A or overnight if using regular capping.