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# Guidelines for Selection and Use of Universal Phosphoramidites

Version 1.2

Dear customer,

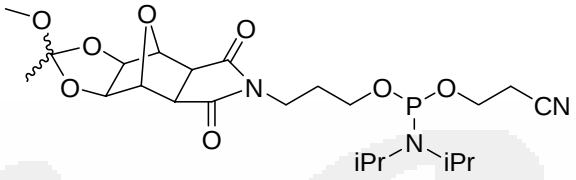
We hope that these guidelines will help you with selecting and using in oligonucleotide synthesis non-nucleosidic universal solid supports manufactured and marketed by AM Chemicals, LLC.[1] Should you have any further questions, comments, or suggestions please feel free to contact us.

The automated synthesis of oligonucleotides by the phosphoramidite method is currently carried out on nucleosidic solid supports that is, the solid supports that contain 3'–terminal nucleosides attached to the solid phase via a readily cleavable ester linkage. One limitation of this approach is that an incorporation of a given nucleoside at the 3'-terminus requires the synthesis of an appropriate solid support. For instance, the preparation of unmodified DNA requires four solid supports. Furthermore, the synthesis of novel oligonucleotide analogs employs a continuously growing number of supports carrying modified nucleosides and other 3'–terminal modifiers.

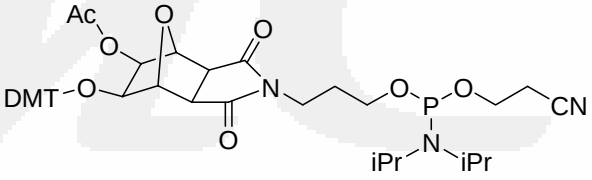
In contrast, a universal phosphoramidite is coupled to any hydroxy- or amino-derivatized solid support suitable for oligonucleotide synthesis to result, upon oxidation, in a universal solid support. The standard 2-cyanoethyl nucleoside phosphoramidite respective to the 3'-terminal nucleoside residue is then coupled to the universal solid support in the second cycle of oligonucleotide synthesis. Once the assembly of an oligonucleotide is complete, the support-bound material is treated with concentrated aqueous ammonium hydroxide or ammonia/methylamine mixture (AMA) under the standard conditions. This releases the oligonucleotides from the solid support by removing the 3'-terminal phosphate residue to give the standard 3'-OH oligonucleotides. Our solid supports and phosphoramidites require no or very minor modifications to the standard protocols of oligonucleotide synthesis. So far, these reagents have been successfully tested with P=O and P=S chemistries in the synthesis of 2'-deoxyoligonucleotides and 2'-OMe and 2'-O-tBDMS oligoribonucleotides.

> The diversity of universal phosphoramidites and solid supports offered by AM Chemicals permits a more convenient preparation of the majority of synthetic oligonucleotides.

The structures and the product numbers of the universal phosphoramidites currently available from our company are shown in Fig. 1. A more detailed discussion of the properties and the use of the phosphoramidites is given below.




Prod No 02120




Prod No 02220

**Figure 1.**

***OH-Protecting Groups.*** The most important functional groups in the universal linker are two vicinal hydroxy groups. One of the hydroxy groups serves as a site of the coupling of the first, 3'-terminal nucleoside phosphoramidite to the solid phase while the other is important for the dephosphorylation reaction in course of the treatment with a base at the end of the synthesis. We offer a choice of two phosphoramidites where the hydroxy groups are protected with an acid-labile methoxyethylidene (MED) group (02120), or it is protected with the conventional 4,4'-dimethoxytrityl (DMT) group (02220).

[1] Patent pending.

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In the phosphoramidite 02120, the hydroxy groups are protected with the MED group. In contrast to the conventional DMT group, no colorful product is formed in the course of deprotection. The treatment with an acid results in the hydrolysis of the orthoester to deprotect one of the hydroxy groups while the other one is left protected as an acetate ester. The MED protecting group is completely removed by treating the solid support for 20 s with 3% dichloroacetic acid or 2% trichloroacetic acid in methylene chloride so that carrying out the standard detritylation subroutine is more than sufficient for the deprotection of the solid support.

The phosphoramidite 02220 is protected with the DMT and acetyl groups. In the chemical environment of the non-nucleosidic universal linker, the DMT group is more stable than at the 5'-terminus of oligonucleotides. An extended detritylation time (4 min) is required for the complete detritylation. The extended detritylation step should only be used in the first cycle of the chain assembly. A more prolonged detritylation of nucleoside residues is neither necessary nor advisable.

***Coupling Protocol.*** No modification to the synthetic protocol is necessary for the attachment of the universal phosphoramidites to solid phases. Once MED or DMT-protecting group is removed, the hydroxy group in the universal linker is available for the coupling with the first nucleoside phosphoramidite. Due to the mechanistic requirements of the postsynthetic dephosphorylation of oligonucleotides, this hydroxy group is slightly more sterically hindered than the 5'-OH group in a nucleoside residue. The increased sterical demand results in a somewhat slower rate of the coupling of nucleoside phosphoramidites to the universal solid supports. On the synthetic scales of 1 µmol and larger where 0.1 M solutions of the nucleoside phosphoramidites are commonly used, this effect is insignificant so that no changes to the coupling protocol is necessary. On smaller synthetic scales employing 0.05 M and more dilute solutions of the nucleoside phosphoramidites, we recommend using the coupling time of the first, 3'-terminal nucleoside phosphoramidite to the universal solid supports extended by 30 to 60 s.

***The release of Oligonucleotides from the Universal Solid Supports.*** In contrast to the standard nucleosidic solid supports, the release of oligonucleotides to solution occurs by dephosphorylation of the solid phase-bound material. The rate of dephosphorylation of the 3'-terminus is dependent on the 3'-terminal nucleoside residue and is the slowest when the 3'-terminal nucleoside residue is 2'-deoxy G. In this case, dephosphorylation of P=O oligonucleotides with concentrated aqueous ammonium hydroxide is complete in 2 h at 60°C and in 5 h at 25°C. In AMA, the dephosphorylation requires 1h at 25°C. With all other bases and 2'-O-substituted nucleosides, the dephosphorylation is more rapid. Thus, we recommend the conditions for dG as the standard dephosphorylation conditions. Importantly, the dephosphorylation of the 3'-terminus is substantially faster than the deprotection of nucleic bases even when the labile base-protecting groups are used.

*Note Regarding the Synthesis of Oligonucleotide Phosphorothioates.* It has been reported previously that a phosphorothioate diester (P=S) linkage between a universal linker and the 3'-terminal nucleoside is substantially more stable towards base-catalyzed hydrolysis than the respective phosphodiester (P=O) group.[2] More recently, we found that the P=S oligonucleotides attached to the universal solid support via phosphodiester moiety were dephosphorylated as fast as the respective P=O oligonucleotides. Based on these observations, we recommend that, in the first two cycles of oligonucleotide synthesis (attachment of the universal phosphoramidite to a solid phase AND the first coupling of nucleoside phosphoramidite), the standard oxidation with iodine or *t*-butyl hydroperoxide solutions be carried out regardless of the chemistry of the oligonucleotide backbone. *Carrying out the synthesis in this manner does not introduce the unwanted P=O moiety into the final product because the 3'-terminal phosphodiester group is removed in the process of dephosphorylation.* If the use of two different cycles in the oligonucleotide chain assembly is not feasible, the phosphite triester linkage between the universal linker and the 3'-terminal nucleoside residue may be sulfurized in the standard manner. In this event, the dephosphorylation time should be extended by a factor of 2.

[2] Guzaev, A. P.; Manoharan, M. *J. Am. Chem. Soc.* **2003**, *125*, 2380-2381.