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Guidelines for Selection and Use of Universal Solid Supports

Version 1.6

Non-nucleosidic universal solid supports have been designed, manufactured and marketed by AM Chemicals[1]. This guideline is intended to assist with the selection and use of appropriate non-nucleosidic universal solid supports in oligonucleotide synthesis. Please feel free to contact us for additional information, comments, or suggestions.

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.

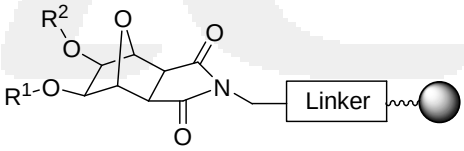
In contrast, a universal solid support is used regardless of the sequence to be synthesized. The standard 2-cyanoethyl nucleoside phosphoramidite respective to the 3'-terminal nucleoside residue is coupled to the universal solid support in the first cycle of oligonucleotide chain assembly. Once the assembly 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 AND completely removes the 3'-terminal phosphate residue to give the standard 3'-OH oligonucleotides. Our supports require no or very minor modifications to the standard protocols of oligonucleotide synthesis. So far, our solid supports 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.

Table. Universal Solid Supports Available from AM Chemicals.[a]

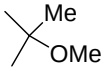
Product #	R^1 and R^2	Linker	Solid Phase
01011-001	H, Ac	Cleavable	CPG 500 Å
01111-001	MED	Cleavable	CPG 500 Å
01211-001	DMT, Ac	Cleavable	CPG 500 Å
01021-001	H, Ac	Stable	CPG 500 Å
01121-001	MED	Stable	CPG 500 Å
01221-001	DMT, Ac	Stable	CPG 500 Å
01012-001	H, Ac	Cleavable	CPG 1000 Å
01112-001	MED	Cleavable	CPG 1000 Å
01212-001	DMT, Ac	Cleavable	CPG 1000 Å
01022-001	H, Ac	Stable	CPG 1000 Å
01122-001	MED	Stable	CPG 1000 Å
01222-001	DMT, Ac	Stable	CPG 1000 Å
01013-001	H, Ac	Cleavable	Polystyrene
01113-001	MED	Cleavable	Polystyrene
01213-001	DMT, Ac	Cleavable	Polystyrene
01023-001	H, Ac	Stable	Polystyrene
01123-001	MED	Stable	Polystyrene
01223-001	DMT, Ac	Stable	Polystyrene

[a]. Consult Figure 1 for chemical structures

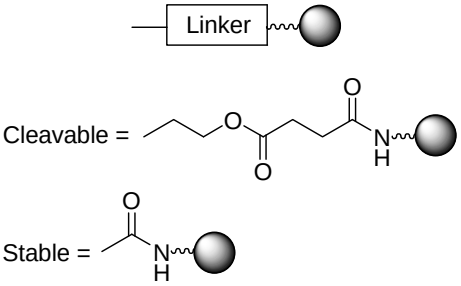
Figure 1. Structures of Universal Solid Supports.



R^1 = H; R^2 = Ac

$R^1 + R^2$ =  (MED)

R^1 = DMT; R^2 = Ac



[1] Patent pending.

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

The structures of the universal supports currently available from AM Chemicals are shown in Fig. 1; the product numbers and the most important features are summarized in the Table. A more detailed discussion of the properties and the use of the supports is given in the text below.

In order to select a universal solid support most suitable for the desired conditions of synthesis and final deprotection of oligonucleotides, three parameters have to be considered:

(i) OH-protecting groups R^1 and R^2;
(ii) attachment of the universal linker to the solid phase;
(iii) the material and the pore size of the solid phase.

OH-Protecting Groups R^1 and R^2. 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 solid supports where one the hydroxy functions is protected with acetyl group, and the other is kept unprotected (products 010XX),[2] or it is protected with the conventional 4,4'-dimethoxytrityl (DMT) group (012XX). Alternatively, the solid supports are protected with an acid-labile methoxyethylidene (MED) group (011XX).

The supports 010XX do not require any initial deprotection. However, following the standard oligonucleotide synthesis cycle which begins with detritylation does not result in any negative effect.

In the supports 011XX, 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 supports 012XX are protected with 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.

Attachment of the Universal Linker to the solid phase. We offer two types of universal solid supports different in the attachment of the universal linker to the solid phase. In the first type, the universal linker is attached via a cleavable ester bond of succinyl linker. These supports (products 01X1X) are perfect for small-scale applications. Similarly to the conventional succinyl solid supports, the oligonucleotides are first released from the solid phase. With AMA and concentrated aqueous ammonium hydroxide at room temperature, this step requires 15 min and 1 h, respectively. The dephosphorylation and the base deprotection are then carried out in solution. 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. 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 rate of dephosphorylation of the 3'-terminus is substantially faster than the deprotection of nucleic bases even when the labile base-protecting groups are used.

In the second type of solid supports (products 01X2X), the universal linker is attached to the solid phase via a chemically inert amide bond. The release of oligonucleotides to solution occurs by dephosphorylation of the solid phase-bound material, which requires 2 h at 60°C and 5 h at 25°C

[2] In the generic product numbers, X stands for a variable digit. For instance, 010XX describes the solid supports 01011, 01021, 01012, and 01022 collectively.

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in concentrated aqueous ammonium hydroxide. These supports are most suitable for large-scale applications where the time of exposure of the solid phase to concentrated aqueous ammonium hydroxide is less critical.

The solid supports 01X2X find another application in preparation of base-labile modified oligonucleotides for which the conventional solid supports are not readily available. In this case, the deprotection is carried out under as mild basic conditions as required by a particular modification. The fact that only the 3'-dephosphorylated material is released to solution substantially simplifies the purification although a lower yield of the product may be expected.

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.[3] 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 cycle of oligonucleotide synthesis, 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 solid support 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.

Pore Size of the Solid Phase. Our studies demonstrated no dependence of the dephosphorylation kinetics on the material of the solid phase (CPG 500 and 1000 Å, low- and high crosslinked polystyrene). Unless the polystyrene is supplied by a customer, we only offer the solid supports based on Controlled Pore Glass (CPG). The solid supports 01XX1 and 01XX2 are prepared by attaching the universal linker to CPG 500 and 1000 Å, respectively. The latter solid support is recommended for the preparation of oligonucleotides longer than 35 nucleotide residues.

Note Regarding the Synthetic Protocol. The most important functional group in the universal linker is a hydroxy group, a site of the coupling of the first, 3'-terminal nucleoside phosphoramidite to the solid phase. 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.

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