

Use of Custom Synthesized Phosphoramidite Reagents

Before you grab a bottle of acetonitrile to dissolve your custom synthesized phosphoramidite, we suggest you take a few minutes to read these words of advice.

1. Pushing the envelope of scientific knowledge: Most likely very little is known about your custom compound. We do have some suggestions on how your amidite may behave based on our experience with similar compounds. And we can recommend procedures that should work in most cases.

Further, since this particular amidite was synthesized by us only once (and perhaps for the first time by anyone), some of the contaminants that are removed from standard amidites using established protocols may still remain in your compound. At some point it becomes more cost effective to accept the compound as it stands, than to continue to pay for methods of development. This is particularly true of compounds that are very experimental or are merely a means to answer a research question. Purification is usually the most costly step in an amidite synthesis. Multiple purifications can lead to very low yields and high costs. The methods suggested below can help overcome some minor contamination issues by optimizing other parameters.

2. Solvent selection and dryness: Most nucleoside phosphoramidites are soluble in acetonitrile, which is the common solvent for DNA synthesis. However, more lipophilic amidites, such as fatty acid amidites, require dichloromethane. Although dichloromethane can be used in most synthesizers, there are problems with flow rate changes and volatility. Ask your manufacturer if there are any compatibility problems if you decide to use your compound on a synthesizer.

It is also important to use solvent that contains less than 30 ppm water, preferably 10 ppm or less. This can be prepared by using high quality solvent, such as DNA synthesis grade acetonitrile, adding molecular sieves and allowing it to stand at least 24 hours before use. (We found that the Trap-paks from Perseptive Biosystems are the best quality sieves to use for this purpose.) We strongly recommend you use a titration device to test the water content of your solvents. TriLink can supply highly dried solvents over sieves for your amidite if you are unsure of the quality of your supply.

3. More about water: There are two common contaminants arising from the amidite itself that can greatly affect the quality of your synthesis; excess base and water. Neither contaminant is detectable in the phosphorus NMR or HPLC analysis of your compound. The excess base comes from the purification process and is removed using extensive drying at the end of the synthesis. If this is done carefully then excess base will not be a problem. We co-evaporate your compound extensively to remove the base.

Water is less easily eliminated. Some custom amidites tend to cling to water, or are hygroscopic enough to rapidly draw water out of the atmosphere even during fast transfers. The solution to this is to dry the dissolved amidite with high quality molecular sieves (3 Å) just prior to use. The Trap-paks work well here also. The large packs can be cut open and used for several bottles.

Our procedure with any expensive amidite, whether commercially purchased or manufactured by us, is to dissolve the amidite in the appropriate solvent and then add a single layer of sieves to the bottom of the vial. The vial is sealed and allowed to stand overnight. This will drop the water content to acceptable limits.

4. Concentration: Most instruments use between 0.05 M (approx. 50 mg/mL) and 0.1 M solutions of the amidites. In our estimation, the higher the concentration the better. We

recommend using a concentration of 0.1 M for both automated and manual syntheses using modified reagents.

5. Coupling time: The steric hindrance of the amidite dictates the required coupling time. Sometimes only trial and error will provide an answer. We generally use coupling times of 5 minutes for a modified 2' deoxynucleoside and 15 minutes for everything else, including non-nucleosidyl amidites.

If there is a reported reagent that is similar to yours, use that coupling time. Actually, in most cases it is best to double the coupling time. It is very rare to have an amidite that couples worse with extended coupling times, although it is known to occur (methylphosphonates, for example). Another recommendation is to double or even triple couple an important nucleoside. If you are concerned at all about coupling efficiency, this is a good way to boost an 80% coupling to 95% or better. We will describe this process below.

6. Stability of your reagent to the rest of the DNA synthesis process: Another major consideration, and one that should be addressed before a custom synthesis is ordered, is the stability of the reagent to the rest of the coupling cycle chemistries. Is your compound stable to acid, oxidizer or capping reagents? If the answer is no to any one of these reagents, then changes must be made to either the compound or to the synthesis scheme. TriLink can help you develop preliminary stability experiments that will answer some of the questions. However, in the end, an oligonucleotide must be made and tested. A mass spec analysis of your first oligonucleotide is recommended.

7. Deprotection method: This is another stability issue. Many compounds, such as dyes, are not stable to the standard deprotection conditions using conc. NH_4OH at 55° C for 15 hours. There are many alternative methods available. Once again, trial and error may be necessary to optimize this step.

8. Coupling method - automated vs manual: We have learned the most efficient way to use precious amidites is to do the couplings by hand. This is how we couple most of our modified nucleosides. It us gives precise control over the quantity of reagents used and coupling time. An instrument uses more reagents because of priming needs, etc. A manual coupling can be done with just the right amount of material and little to no wastage. This can be critical if you have limited amounts of material.

9. Single coupling vs double or triple coupling: If you really want to make sure your amidite couples well, then it may be best to do multiple couplings. This is done by simply repeating the coupling step one or two times in the cycle before oxidation as illustrated below.

1. Deblock
2. Couple #1 (add amidite and activator to column)
3. Repeat step #2 as many times as needed
4. Oxidize
5. Cap

If your reagent is coupling at 80% efficiency, then the second coupling will get you to 96% (80% of the remaining 20%), etc. Theoretically, although we will never get to 100% as Aristotle explained, we should get extremely close.

10. If all else fails... TriLink can conduct all of the experiments described above and prepare your oligonucleotide for you. Many clients contract to have this done from the beginning of the program. Our goal is to help you succeed with your research.