TECH NOTE

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# 2'-ACE RNA synthesis chemistry

2'-ACE RNA synthesis chemistry is based on a novel protecting group scheme[1]. A new class of silyl ethers is used to protect the 5'-hydroxyl (5'-SIL) in combination with an acid-labile orthoester protecting group on the 2'-hydroxyl (2'-ACE)[2]. This set of protecting groups is then used with standard phosphoramidite solid-phase synthesis technology[3]. The structures of the protected and functionalized ribonucleoside phosphoramidites currently in use are as illustrated in Figure 1.

RNA oligonucleotides are synthesized in a stepwise fashion using the nucleotide addition reaction cycle illustrated in Figure 2. Each nucleotide is added sequentially (3'- to 5'-direction) to a solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are added (step i in Figure 2), coupling the second base onto the 5'-end of the first nucleoside. The support is washed and any unreacted 5'-hydroxyl groups are capped with acetic anhydride to yield 5'-acetyl moieties (step ii). The P(III) linkage is then oxidized to the more stable and ultimately desired P(V) linkage (step iii). At the end of the nucleotide addition cycle, the 5'-silyl group is cleaved with fluoride (step iv). The cycle is repeated for each subsequent nucleotide.

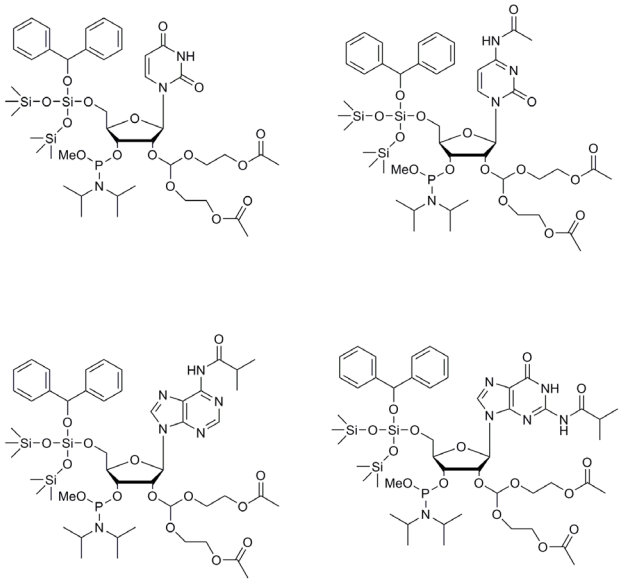

**Figure 1. Protected RNA nucleoside phosphoramidites for Dharmacon 2'-ACE RNA synthesis chemistry.**

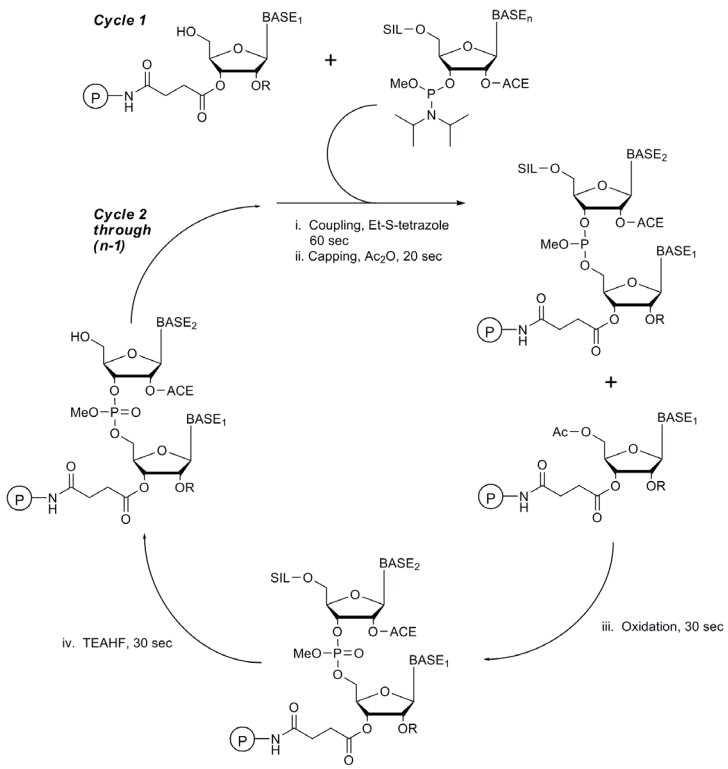


**Figure 2. Outline of the RNA synthesis cycle.**

Following synthesis, the methyl protecting groups on the phosphates are cleaved in 30 minutes utilizing 1 M disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate (S2Na2) in DMF[4]. The deprotection solution is washed from the solid support bound oligonucleotide using water. The support is then treated with 40% methylamine in water for 10 minutes at 55 °C[5]. This releases the RNA oligonucleotides into solution, deprotects the exocyclic amines and modifies the 2'-ACE groups. The oligonucleotides can be analyzed by anion exchange HPLC at this stage. The 2'-orthoester groups are the last protecting groups to be removed. The structure of the 2'-ACE protected RNA immediately prior to 2'-deprotection is as represented in Figure 3.

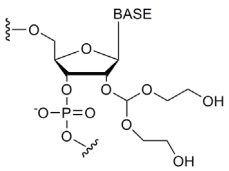


**Figure 3. Structure of 2'-ACE protected RNAimmediately prior to 2'-deprotection**

The ethylene glycol monoacetate orthoester group (Figure 1) that was developed by Dharmacon Research, now part of Horizon Discovery has the following innovative properties. It is stable to the conditions of nucleoside phosphoramidite synthesis and oligonucleotide synthesis. However, after oligonucleotide synthesis the oligonucleotide is treated with methylamine which not only cleaves the oligo from the solid support but also removes the acetyl groups from the orthoesters. The resulting 2-ethyl-hydroxyl substituents on the orthoester are less electron withdrawing than the acetylated precursor. As a result, the modified orthoester becomes more labile to acid-catalyzed hydrolysis (refer to mechanism in Figure 4). Specifically, the rate of cleavage is approximately 10 times faster after the acetyl groups are removed. Therefore, this orthoester possesses sufficient stability in order to be compatible with oligonucleotide synthesis and yet, when subsequently modified, permits deprotection to be carried out under relatively mild aqueous conditions compatible with the final RNA product.

The hydrophilic character of the 2'-protecting groups predicts that 2'-protected RNA of any length or sequence will be readily soluble in water. Base composition and length have been found to have no effect on the solubility of the 2'-protected RNA oligos. To date, all lengths and sequences synthesized with 2'-ACE chemistry have been water soluble. This enables the routine handling of 2'-protected RNA in water. When ready to remove the 2'-protecting groups, the orthoesters are readily hydrolyzed under acid catalysis by the mechanism in Figure 4[6].

As the 2'-protecting groups are very hydrophilic and readily solvated by water, acid-catalyzed hydrolysis proceeds to completion regardless of sequence or length. 5'-SIL-2' ACE oligonucleotide chemistry is a definitive advance in RNA synthesis technology. Nucleoside coupling yields are comparable to DNA and are routinely < 60 seconds. The final acid

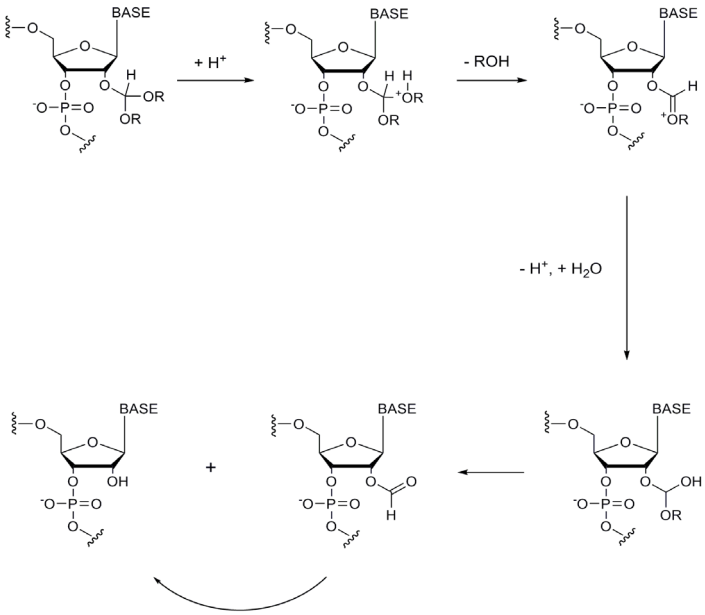


**Figure 4. Generalized mechanism for acid catalyzed hydrolysis of 2'-orthoesters.**

deprotection is mild, fast and amenable to subsequent use with minimal handling. Analysis and purification via PAGE or HPLC are possible with any sequence while in the stable 2'-protected form. This property minimizes opportunities to degrade the RNA, while also making it possible to analyze troublesome sequences with strong secondary structure. 5'-SIL-2'-ACE chemistry has enabled the routine synthesis of RNA oligonucleotides of unprecedented quality.

## References


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