The Chemical Synthesis of Long and Highly Modified RNA using 2'-ACE Chemistry

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Abstract

Due to increased uses of various RNAs in understanding critical structural, functional, and regulatory roles of RNA in biology, rapid, reliable, and cost-efficient methods of RNA oligonucleotide synthesis are in demand. Traditional methods of RNA synthesis based on 2'-silyl (TBDMS or TOM) protection strategies are limited in their ability to construct oligos longer than 40 nucleotides in length (far smaller than important biologically active RNA molecules). A significant improvement in RNA synthesis technology, 5'-silyl-2'-acetoxy ethyl orthoester (2'-ACE) chemistry, results in faster coupling rates, higher yields, greater purity, and superior ease of handling. Using 2'-ACE chemistry, we have developed convenient and efficient protocols to synthesize: (1) long RNA sequences in excess of 100 bases, (2) transfer RNAs (tRNAs) with natural modifications such as pseudouridine, m2A, and m2G, (3) RNA oligonucleotides highly modified with virtually any chemical modification; and (4) long RNA with dual-labeled fluorescent dyes. Our results clearly demonstrate that 2'-ACE chemistry is the method of choice for long RNA synthesis applications.

Introduction

Dharmacon has previously developed a novel RNA synthesis chemistry making RNA synthesis as reliable, accessible and of comparable quality as routinely observed in DNA synthesis. The chemistry employs a new 5´-silyl protecting group in conjunction with a unique acid-labile 2´-orthoester protecting group, 2´-bis(acetoxyethoxy)-methyl ether (2´-ACE).[1,2] The 2´-protecting groups are rapidly (< 30 minutes) and completely removed under mild conditions in aqueous buffers. 2´-ACE technology enables the routine synthesis of RNA oligos (in particular long RNA) in high yield and of unprecedented quality.

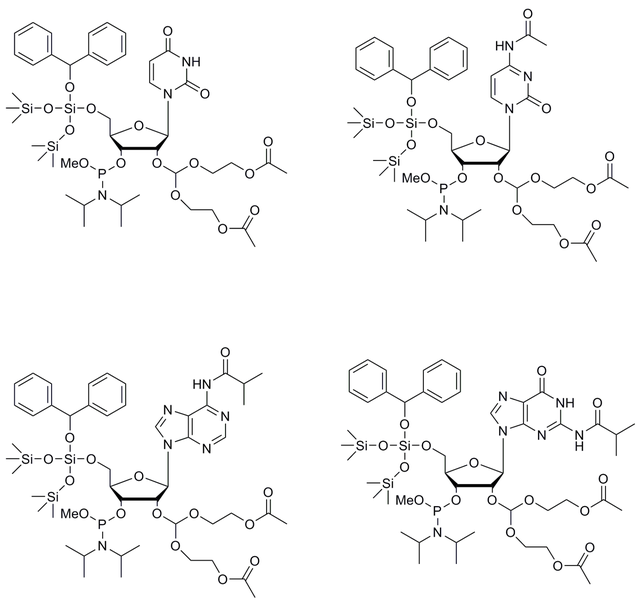

Figure 1. Protected RNA nucleoside phosphoramidites for Dharmacon 2'-ACE RNA synthesis chemistry.

Dharmacon 2´-ACE chemistry features the following advantageous characteristics:

1. 2´-ACE amidites produce high stepwise coupling yields (>99%) and the protected oligo requires minimal post synthesis handling. These factors maximize yields of the full length RNA product.
2. 2´-ACE-protected RNAs are water soluble and nuclease resistant. The 2´-groups also minimize secondary structure. These properties permit the accurate analysis of every oligonucleotide using HPLC or PAGE, regardless of the sequence.
3. The ability to work with the RNA while it is in the 2´-protected form best ensures the stability, purity and homogeneity of every RNA oligonucleotide.
4. 2´-ACE-protected RNA oligos are substrates for some enzymes. For example, it is possible to 5´-label 2´-ACE-protected RNA oligos using T4 Kinase and ATP. This property maximizes yields and purity of the RNA by allowing one to work with the 2´-stable form as long as possible prior to the final application.
5. The 2´-ACE groups are completely removed under extremely mild conditions (pH 3.8, 60 °C, 30 minutes) using acid-catalyzed hydrolysis in aqueous buffers.

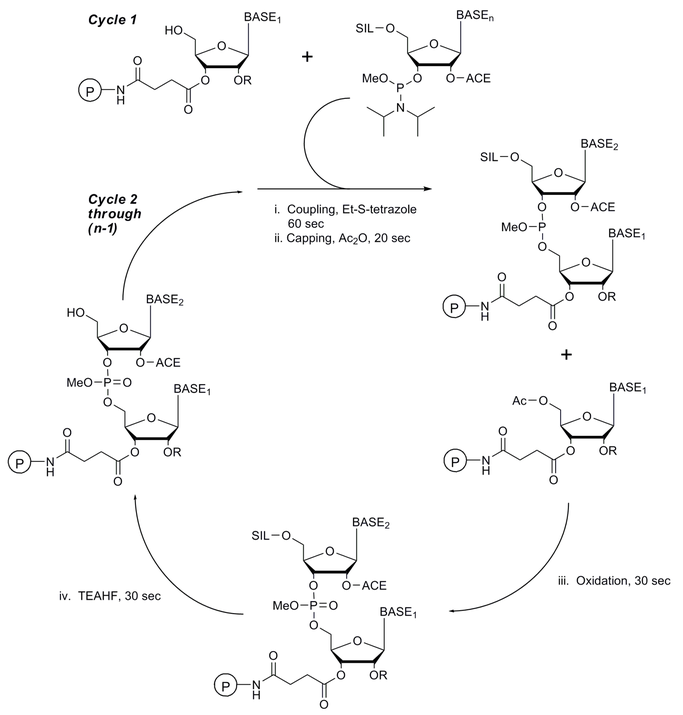

Figure 2. Outline of the Dharmacon 2'-ACE RNA synthesis cycle.

Methods

Each RNA oligonucleotide (**Table 1**) was chemically synthesized on a MerMade synthesizer (Bioautomation Corporation) using polystyrene solid support and 2'-ACE phosphoramidites (**Figure 1**). After completion of synthesis cycles, the oligonucleotide on the support was treated with Na_2S_2 solution at room temperature followed by washing with water. The oligonucleotide was cleaved from the support with 40% of aqueous N-methylamine (NMA) and then heated to 55 °C followed by lyophilization to dryness. The crude RNA was purified by PAGE (hm-RNA-73) or HPLC (dl-RNA-5011, d-RNA-02, l-RNA-06, b-RNA-17). 2'-ACE RNA was de-protected and desalted, then the quality and identity of the RNA product was confirmed by LC-MS.

Name	RNA sequence (5'>3')	Length
dl-RNA-5011	GGAGCUDY550CGCUUCGGCGAGGUCGUGCCAGCUCUUCGGAGCAAUDY647ACUCGAmC	49
hm-RNA-73	C18*mG*mG*mC*mA*mA*mU*mG*mU*mU*mG*C3*mC*mG*CGUCUGAGGGAUCUCUAGUUACC*C*GA*A*G*A*G*C*U*C*A*U*C3*mC*mA*mA*mC*mA*mU*mU*mG*mC*mC*C18*idT	58
d-RNA-02	DY647AAGCUGACCCUGAAGUUCAUCUGCACCUGGGCCGAAAAAGACCUGACUUCUAUACUAAGUCUACGUCCC	69
t-RNA-11	GAAGACCCCGUGAACCUCUUCGGAGGUACUCCGm2GGGAdhUAACAGGC~UGAUACCGUGAGGUGUUUGGCAmCCUC	71
l-RNA-06	**AUGAUGGGCUGGCAUUCUUGAGGCAUCUCAGUGUUUGAAUUGGAAGAAUGUGUGGUGAAUGGCACUGAUUGACAUUGUGCCUCUAAGUCACCUAUUCAAUUAGGGCGACCGUGU**	114
b-RNA-17	BiotinCAACCUAAAACUUACACCCGGUAAGGAAAUAAAAAUGAAGGGAGCAGAUAAAGCAAAAAACAGUGAUGGGGCGUGACGCUACCCGCGUCACGCCCCAGAAUAACGCUGCGCUG	115

Table 1. Chemically synthesized long and highly modified RNAs by 2'-ACE chemistry. mC: 2'-OMe-cytidine; C18: C18 spacer; *: phosphorothioate; mG: 2'-OMe-guanosine; mA: 2'-OMe-adenosine; mU: 2'-OMe-uridine; C3: C3 spacer; idT: inverted deoxythymidine; m2G: N^2-methylguanosine; dhU: 5,6-dihydro-uridine; ~U: pseudo-uridine.

Results

Name	Purification	FL Pure	Mass Calculated	Mass Found
dl-RNA-5011	HPLC D/D	65%	17386.2	17388.9
hm-RNA-73	PAGE D/D	-	20446.1	20442.6
d-RNA-02	HPLC D/D	88%	22505.5	22499.6
t-RNA-11	HPLC D/D	75%	22891.8	22903.6
l-RNA-06	HPLC D/D	86%	36709.9	36719.4
b-RNA-17	HPLC D/D	90%	37608.2	37609.8

Table 2. Summary of LC-MS analysis on the long and highly modified RNAs synthesized by 2'-ACE chemistry. D/D: Desalting/Deprotection of 2'-ACE.

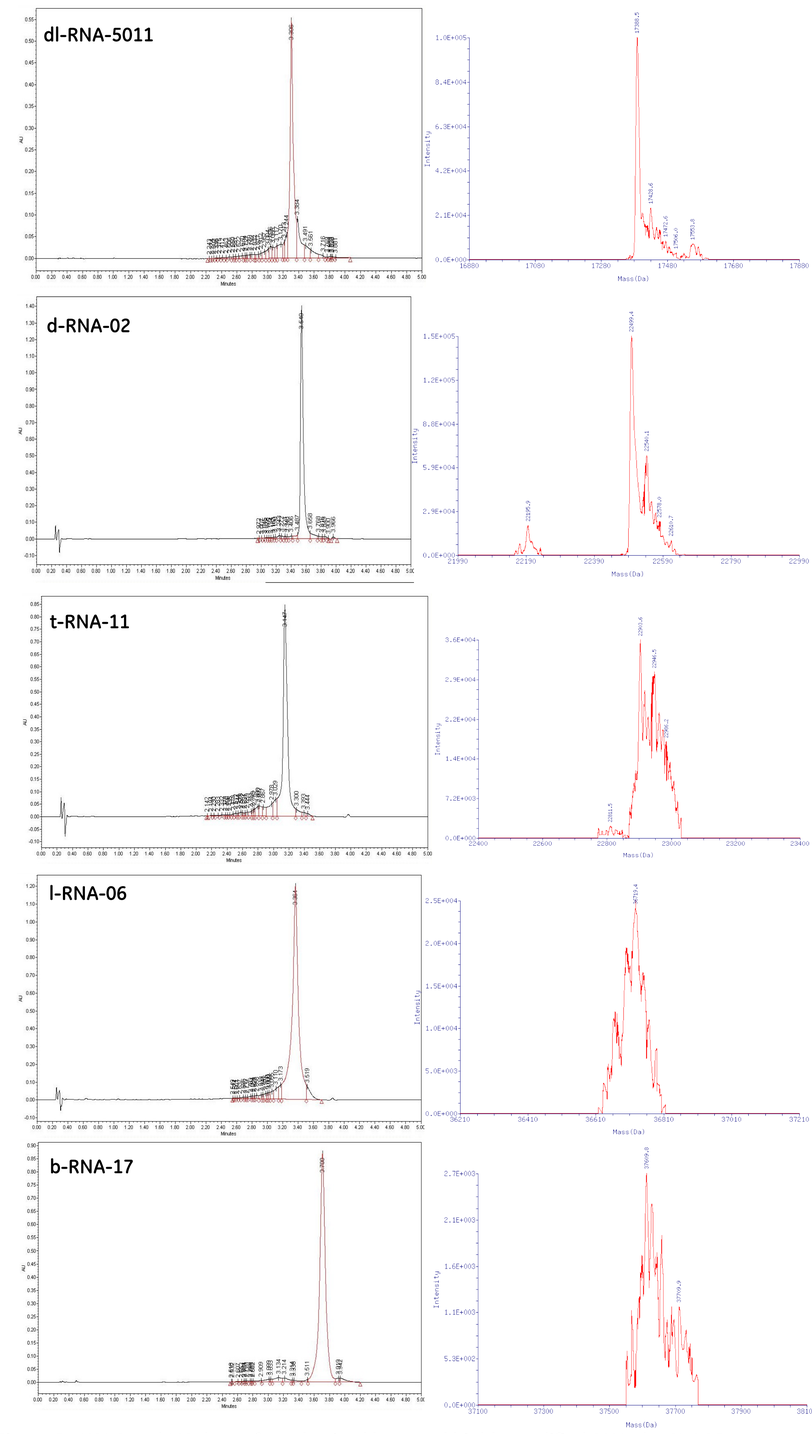

Figure 3. UPLC and MS data of synthesized long and highly modified RNAs by 2'-ACE chemistry.

Conclusion

The innovative properties of 2´-ACE chemistry have transformed RNA oligonucleotide synthesis. Exceptional quality and high yields are consistent and reliable, including for long RNAs. RNA-oligonucleotide dependent applications can be executed with greater ease and confidence by utilizing the powerful advantages of 2´-ACE technology.

Further Information

To learn more about GE Healthcare Dharmacon proprietary RNA synthesis, chemical modifications, dye labels, purification and processing options and *in vivo* RNA, please contact Dharmacon Technical Support by phone at 800-235-9880 or 303-604-9499, by email at ts.dharmacon@ge.com, or visit the website at www.gelifesciences.com/dharmacon.

References

1. S.A. Scaringe, F.E. Wincott and M.H. Caruthers. Novel RNA synthesis method using 5´-silyl-2´-orthoester protecting groups. *J. Am. Chem. Soc.* 120, 11820-11821 (1998).
2. S.A. Scaringe, D. Kitchen, R.J. Kaiser and W.S. Marshall. Preparation of 5' Silyl 2' Orthoester Ribonucleosides for Use in Oligoribonucleotide Synthesis. *Curr. Protoc. Nuc. Acid Chem.* 2-10 (2004).