ChemGenes CORPORATION

TOM Amidites[1,2]

(2′-Triisopropylsilyl-oxy methyl amidites)

Highly suitable for Large Scale Therapeutic Grade RNA Synthesis & Long RNA sequences, Aptamers Etc.

Perfected Technology at ChemGenes makes Available Bulk quantities (100 g to kilo gram scale batches) & Affordable Prices

Produced Under GMP Guidelines

The Following superior qualities makes TOM amidites most desirable for Therapeutic oligonucleotide production:

- Highest coupling kinetics and efficiency per step, due to lower steric hindrance compared to conventional 2′-TBDMS RNA monomers. A very fast coupling time of TOM amidites (2-4 minutes for different scale of synthesis), and complete absence of 3′ impurities, either manufactured TOM amidites. No possibility of 2′ to 3′ migration during oligo synthesis or during work up. Results in highest purity oligonucleotiodes.

  The synthesis resembles DNA Synthesis like behavior

- Coupling Efficiency consistently greater than 98% per step.

- Short deprotection time for n- protecting group with a time of 4 hrs -6 hours depending on chain length, and with liberal amount of water.

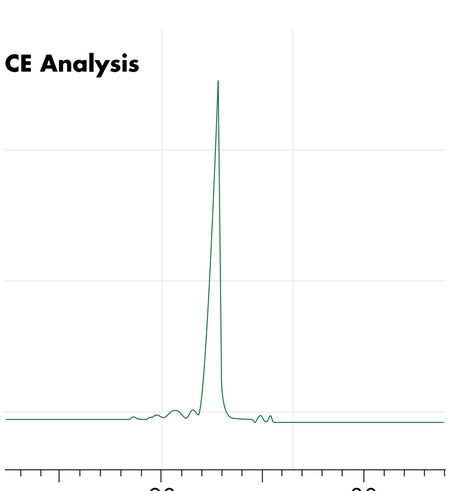
  {Labile Base Protecting groups used are (n-acetyl-A ; n-acetyl-C and n-acetyl –G)
  Easy removal of TOM protecting group is achieved under mild conditions and in significantly excess amount of water without any side effects}

- No formaldehyde adduct formation is detected in the oligonucleotides made by using TOM amidite chemistry, after desilylation step using 1 M TBAF. Single Ion –Exchange purification and ESI/Mass analysis showed no peak corresponding to hydroxymethyl group (CH2 –OH) either as single unit or multiple units, with mass corresponding to 30 Daltons or its multiples (Fig.1a and Fig.1b).

- A comparative study of the quality of oligonucleotides synthesized using TOM amidites and tBDSilyl amidites showed that the RNA oligos synthesized using TOM amidites were found to result in far superior quality after single Ion-Exchange purification by CE (capillary Gel analysis and ESI MS Data).

- There is an overall reduced time involved using TOM amidite chemistry, starting with base deprotection, TOM deprotection and overall ease of purification to obtain high purity.

CE Analysis

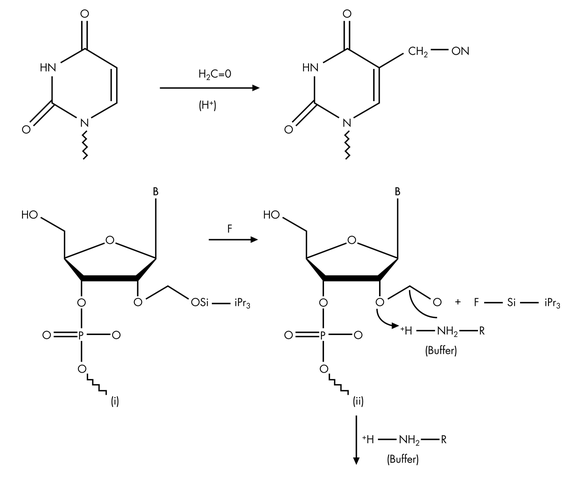


12 Mer RNA @ 15 Micromole

Studies to Confirm Absence of Formaldehyde Adduct formation :

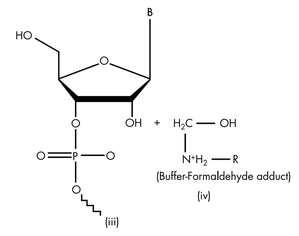
During the loss of silyl and generation of formaldehyde and C-5 formylation or n-formylation of nucleoside bases does not occur due to presence of aq TRIS buffer,Ph 7.4, which captures all the formaldehyde formed and prevents any base modification as analyzed by ESI mass spectral data. None of the Mass spectral data showed any peak corresponding to hydroxymethyl group (CH2 –OH) either as single unit or multiple units, with mass corresponding to 30 or its multiples (fig. 1 a and Fig. 1b). adduct is seen (see the ESI MS data)

ESI/ MS data is presented here to substantiate clean RNA synthesis. No peak corresponding to the formaldehyde adduct (molecular ion + 30) has been detected.



Scheme 1.

Mechanism of TOM cleavage and Scavanging of formaldehyde adduct



It is likely that formaldehyde formed after the cleavage is captured by buffer, which is mildly basic, pH 7.4. Because of acidic nature of formaldehyde addition to pyrimidines (see Scheme below) pH 7.4 is the recommended during desilylation step.

Figure 1a.

Shows correct and clean Mass. No M+30 or multiples of M+30 are seen.

Observed: MW: 3715.3
Target: MW: 3734.5

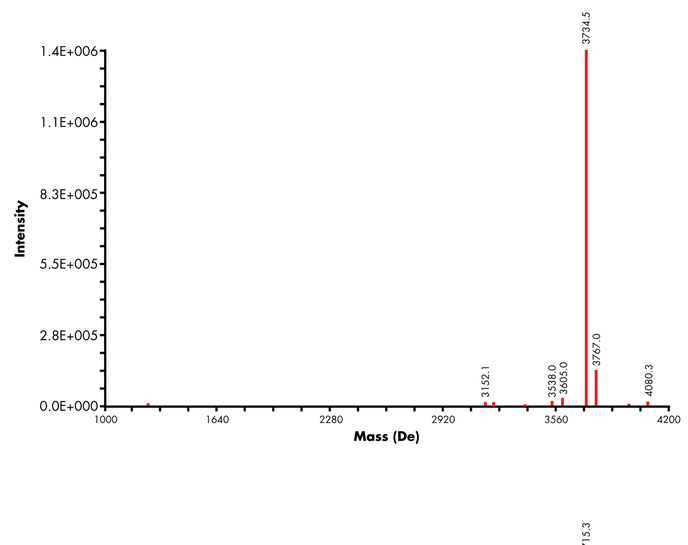
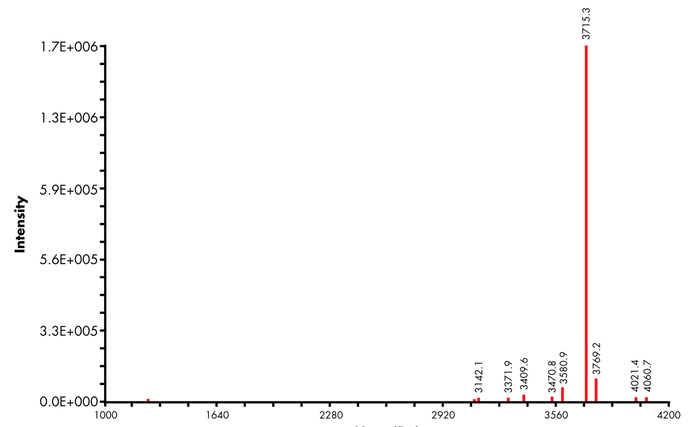


Figure 1b.

Shows correct and clean Mass. No M+30 or multiples of M+30 are seen.

Observed: MW: 3734.5
Target: MW: 3734.5



Notes :

1. Tom Amidites are intellectual property of Qiagen Inc., Germantown, MD and licensed to ChemGenes Corp. for sales in Therapeutics RNA market.

2. Stefan Pitsch, Patrick A. Weiss, Luzi Jenny, Alfred Stutz, and Xiaolin Wu, Helv. Chem. Acta- 3773-95, Vol. 84 (2001).

3. TOM Protecting group chemistry is covered by US PAtent No. 5,986,084. ChemGenes Corp. holds license agreement with Qiagen Inc. for worldwide supply for Therapeutics market.

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