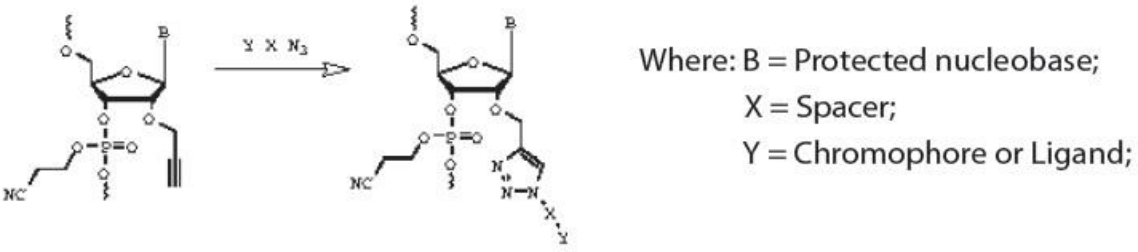
CombiClick, Combinatorial Click Chemistry

2'-O-Propargyl Amidites and Supports
3'-O-Propargyl Amidites and Supports
2'-O-Butyne Amidites and Supports
Azide Reporters

CombiClick™: A New Versatile Method for Oligonucleotide Labeling:

- Click chemistry is a novel method that is developed by the Sharpless group in 2002,[1] and it has became a very versatile tool for incorporating different molecules of interest such as fluorophores, quencher dyes, other reporter molecules and lipophilic ligands into oligonucleotides.
- CombiClick™ is the term coined by ChemGenes for Solid Support Based Combinatorial "Click Chemistry" for Efficient Oligonucleotide Conjugation: This is a versatile method for Oligonucleotide Labeling (Scheme 1).

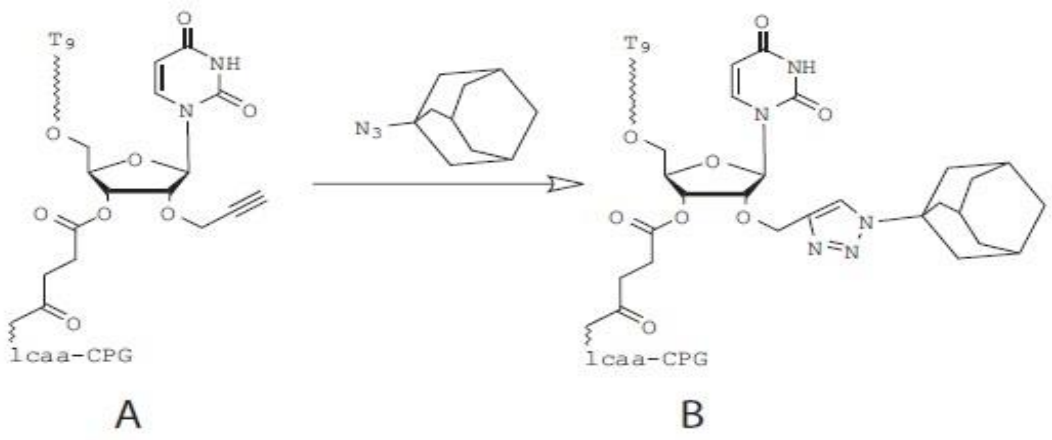


Scheme 1: Schematic illustration of CombiClick™ strategy.

- CombiClick™ technology utilizes a two step process via 2'-*O*-propargyl building block incorporation followed by a "click" attachment of the molecule of interest.
- We have developed a method for efficient attachment of a variety of dyes to the oligonucleotide backbone in nearly quantitative yields (95-98%).
- We have developed over twenty 2'-O-propargyl building blocks and several azido modified chromophores and ligands for CombiClick™ Technology.[2]
- Using ChemGenes CombiClick™ strategy, the synthesis of modified oligonucleotides can be done in a combinatorial fashion with multiple incorporations of molecules of interest internally or at the 5'-, 3'- and 2'- terminus of the oligonucleotide.
- An important advantage of the CombiClick™ approach is that reporter molecule can be attached to the 2'- or 3'- position of ribose ring. This position usually does not interfere with base-pairing and has minimal effect on the hybridization process.

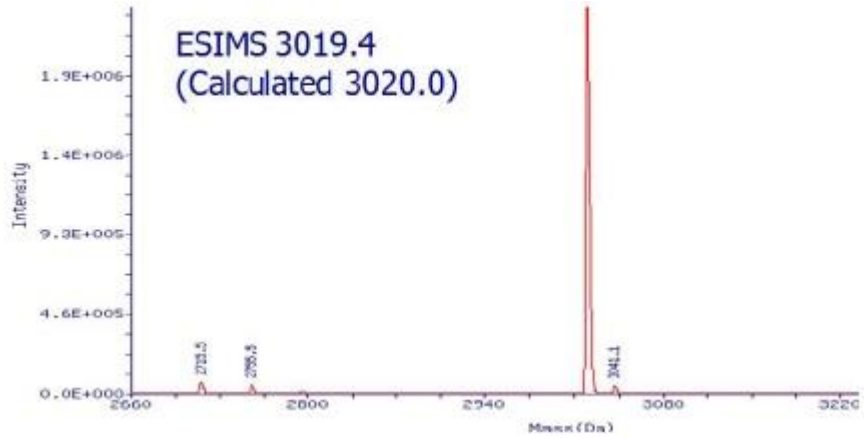
Analytical Data Demonstrates High Efficiency of CombiClick™ Technology:

- 1-Adamantyl azide was employed as a test reagent with 2'-O-propargyl-uridine containing oligonucleotide (T9rU, Scheme 2). Moreover, Adamantyl is used as a lipophilic ligand to improve cellular uptake of therapeutically active oligonucleotides.[3,4]



Scheme 2: CombiClick™ reaction of 2'-O-propargyl-uridine with Adamantyl azide.

- Overnight reaction yielded 2'-adamantyl modified oligonucleotide B quantitatively by ESI mass analysis (Figure 1 and 2).



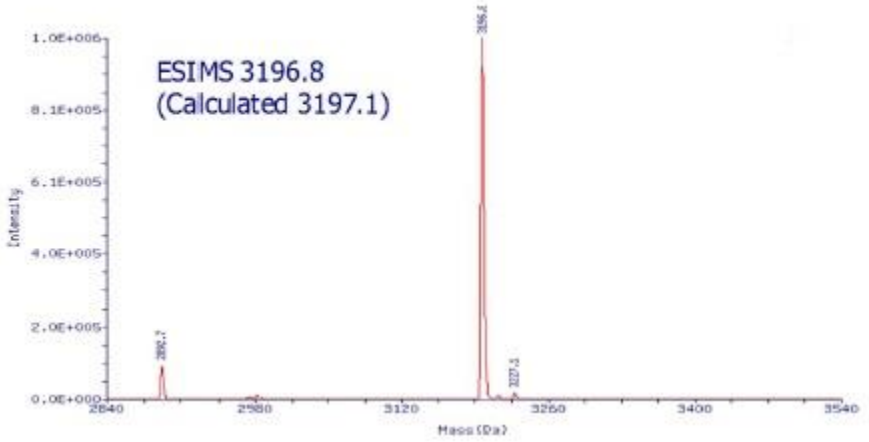


Figure 1: The T9-2'-O-propargyl-rU oligonucleotide before "click" attachment. **Figure 2:** The T9-2'-O-propargyl-rU ODN after attachment of adamantly group.

- Furthermore, decreasing the reaction time from over night to 2 hrs and the molar excess of azide reagent from 10 to 5 did not reduce the yield of conjugate B. This shows the efficiency of our CombiClick Technology.

References:

1. Rostovtsev, V. V.; Green, L.G.; Fokin, V. V.; Sharpless, K. B. *Agnew. Chem. Int. Ed.*, **2002**, *41*, 2596-2599.

2. Raza, S. K.; Srivastava, S. C. 'Synthesis of propargyl modified nucleosides and phosphoramidites and their incorporation into oligos' **WO 1995018139 A1**.

3. Manohoran, M.; Tivel, K. L.; Cook, P. D. *Tet. Lett.* **1995**, *36*, 3651-3654.

4. Rule, G.S.; Frim, J.; Thompson, J. E.; Lepock, J. R.; Kruuv, J. *Cryobiology*, **1978**, *15*, 408-414.