TC RNA Oligonucleotides Synthesis and Application

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Introduction

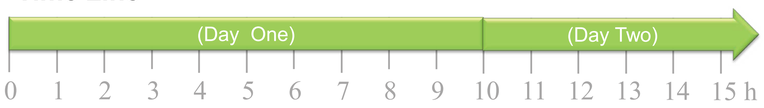
RNA phosphoramidites with a 2'-TC (1,1-dioxo-λ6-thiomorpholine-4-carbothioate) protective group have been introduced into oligoribonucleotide synthesis*) in order to greatly simplify the downstream processing of synthetic RNA and thus to reduce the cost of synthetic RNA. RNA oligomers produced with TC RNA Phosphoramidites can be completely deprotected in one step through a treatment with ethylene diamine, which eliminates any use of highly toxic HF as applied in the conventional RNA synthesis with 2'-TBDMS (tert.-butyldimethylsilyl) protected phosphoramidites. Notably the 2'-TC group introduces comparably less sterical hindrance in coupling reactions than the 2'-TBDMS group thus allowing high coupling efficiencies, short coupling times and the synthesis of RNA longmers (100 bases plus).

The present poster illustrates the advantages of the 2'-TC protected RNA phosphoramidites in the synthesis of oligoribonucleotides with a length of 24, 48 and 106 nucleotides and demonstrates the biological functionality of the resultant products.

* D.J.Dellinger et al., Patent application US2010/0076183A1, J. Am. Chem. Soc. 2011, 133, 11540

Downstream Processing

Time Line



TBDMS Chemistry

Deprot. AMA** | Desilyl. (TREAD) | Cartridge Purif. | QC

TC Chemistry

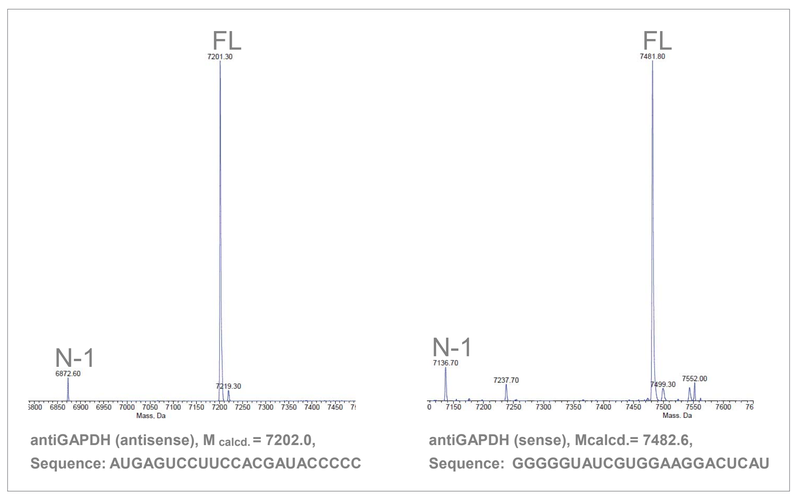
Deprot. EDA* | Cartridge Purif. | QC

* EDA = neat Ethylenediamine

**AMA = Ammonia with Methylamine

TC Chemistry with neat EDA protection enables to shorten post-synthesis work by nearly 50%

Synthesis of TC antiGAPDH siRNA oligos - ESI/MS



antiGAPDH (antisense), M calcd. = 7202.0,
Sequence: AUGAGUCCUUCCACGAUACCCCC

antiGAPDH (sense), Mcalcd.= 7482.6,
Sequence: GGGGGUAUCGUGGAAGGACUCAU

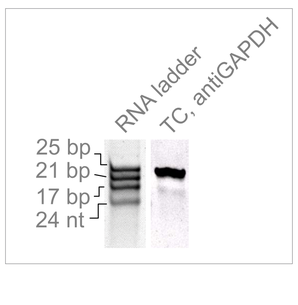
antiGAPDH siRNA

AntiGAPDH siRNA were synthesized; deprotected using neat EDA, and purified using RP-HPLC. Identity of oligonucleotides confirmed by LC/ESI-MS

siRNA	Sequence	Yield	MW_{found}	MW_{calc}
antiGAPDH as	AUGAGUCCUUCCACGAUACCCCC	400nmol	7201.9	7202.4
antiGAPDH s	GGGGGUAUCGUGGAAGGACUCAU	300nmol	7482.1	7482.6

TC antiGAPDH siRNA-QC via native PAGE

The oligonucleotides were annealed, desalted and assayed for residual single-stranded RNA in a native polyacryl amide gel.

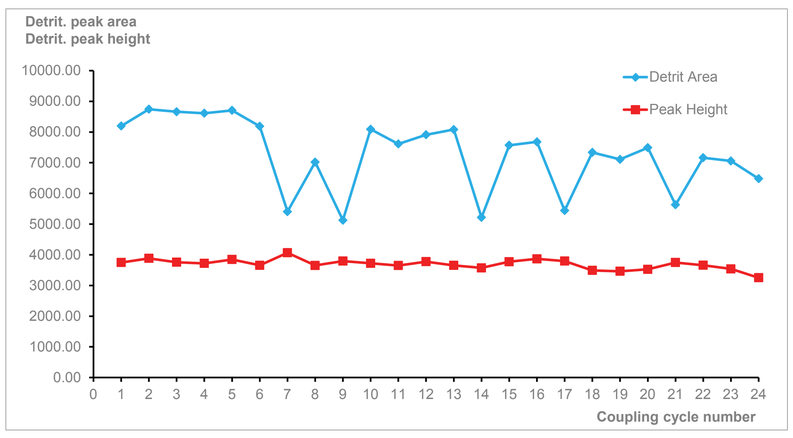


Stability of TC-RNA amidites

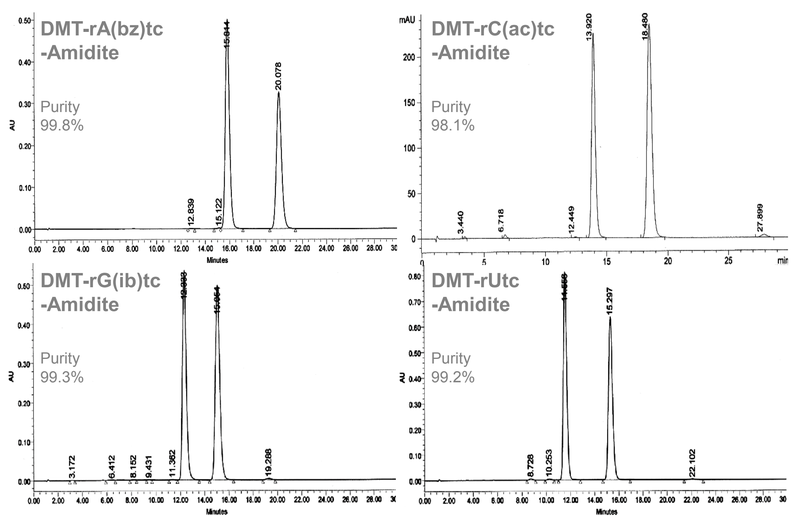
Synthesis with aged (10 d) phosphoramidites and reagents.

Synthesis scale: 1 µmol. Activator 42, Coupling time: 6 min.

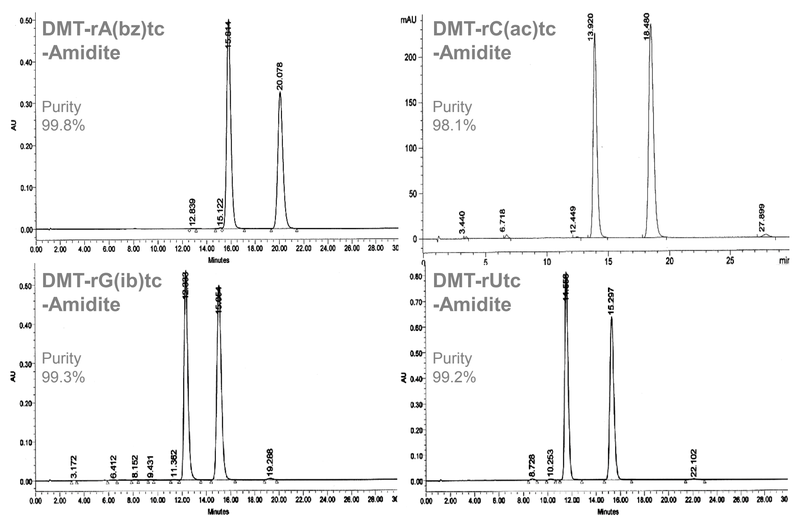
Sequence: UAAGCACGAAGCUCAGAGUCCCCC. Yield (crude): 560 nmol.

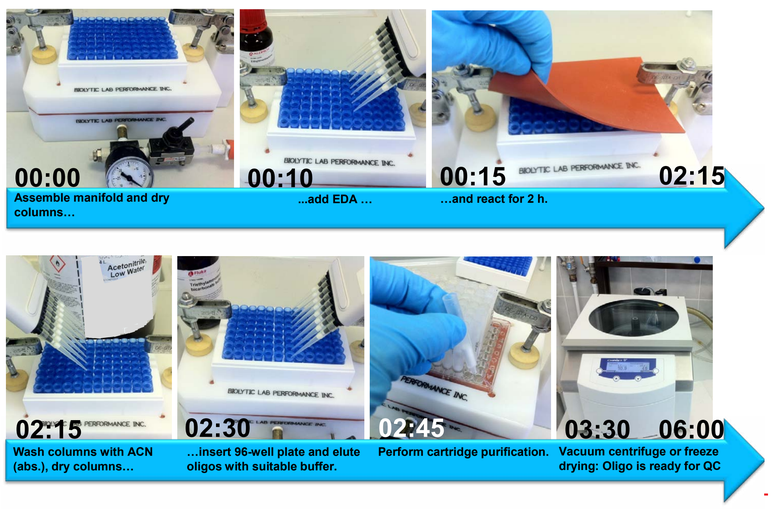


TC Amidites P-NMR Data

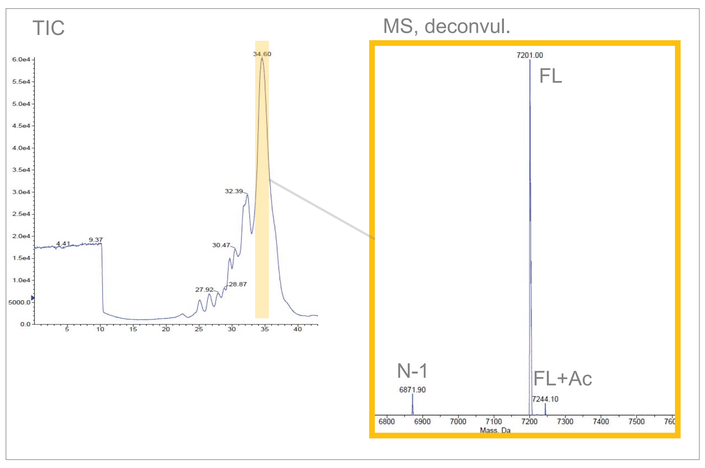


TC Amidite HPLC Data





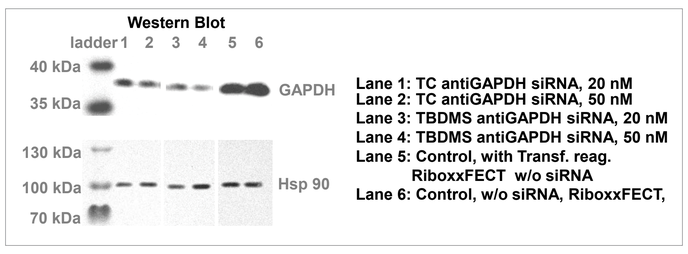
LC/ESI-MS of an 23mer Oligonucleodite Manufactured with TC Amidites (Crude Product)



siRNA Activity in HeLaCells

HeLa cells were cultivated and transfected with TC or TBDMS antiGAPDH siRNA using RiboxxFECT as transfection reagent. HeLa Cells were subsequently incubated for 96 h and assayed for GAPDH and Hsp90 (control) protein levels by western blot. Cells without siRNA and with or without transfection reagent were used as control for siRNA activity.

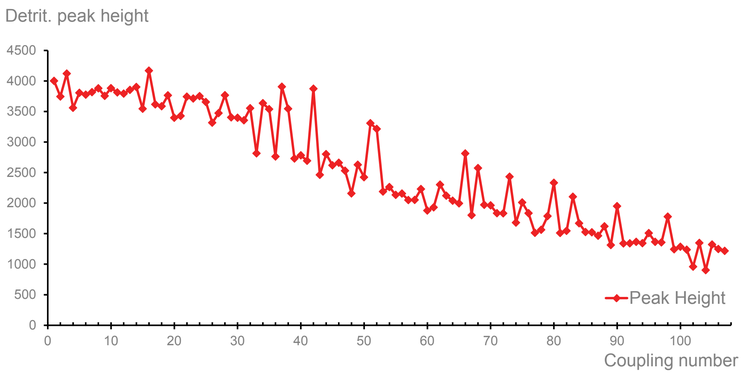
TC and TBDMS siRNA treated HeLa cells reveal reduced GAPDH levels



Lane 1: TC antiGAPDH siRNA, 20 nM
Lane 2: TC antiGAPDH siRNA, 50 nM
Lane 3: TBDMS antiGAPDH siRNA, 20 nM
Lane 4: TBDMS antiGAPDH siRNA, 50 nM
Lane 5: Control, with Transf. reag. RiboxxFECT w/o siRNA
Lane 6: Control, w/o siRNA, RiboxxFECT,

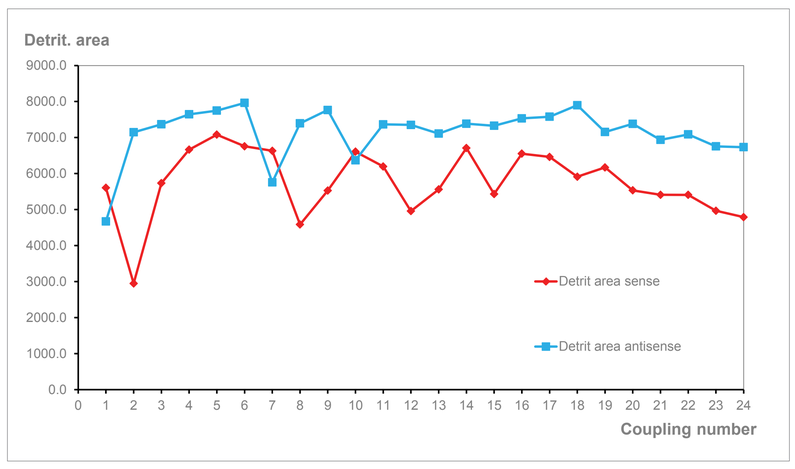
106mer Synthesis Using 2'-TC Purine and 2'-Fluoro Pyrimidine Amidites

Synthesis scale: 1 µmol. *Activator 42; Coupling time* 6 min.
A & G = 2'-TC, C & U = 2'-Fluoro.
Seqence: 5'GGGAGACAAGAAUAAACGCUCAAUGACGUCCUUAGAAUUGCGCAUUCCUCA CACAGGAUCUUUUCGACAGGAGGCUCACAACAGGCCCUCUGCUUCGGUGUCGAAA3'

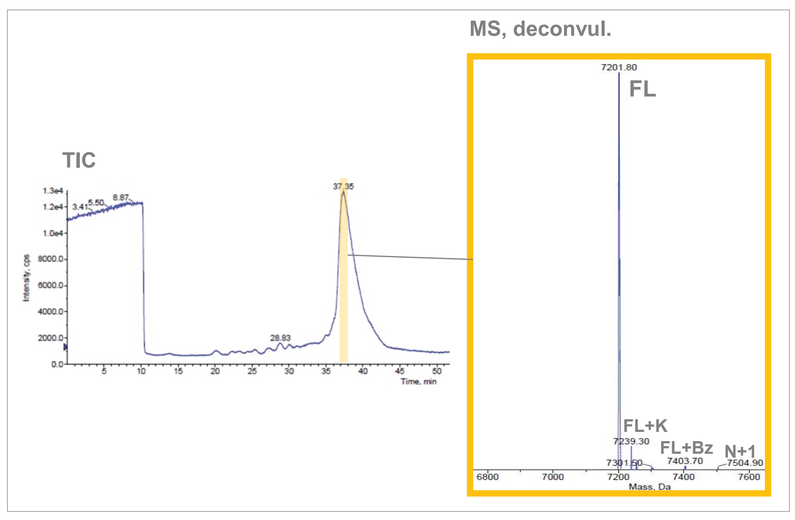


Synthesis of TC antiGAPDH siRNA

Synthesis scale: 1 µmol, Activator 42®, Coupling time: 6 min.

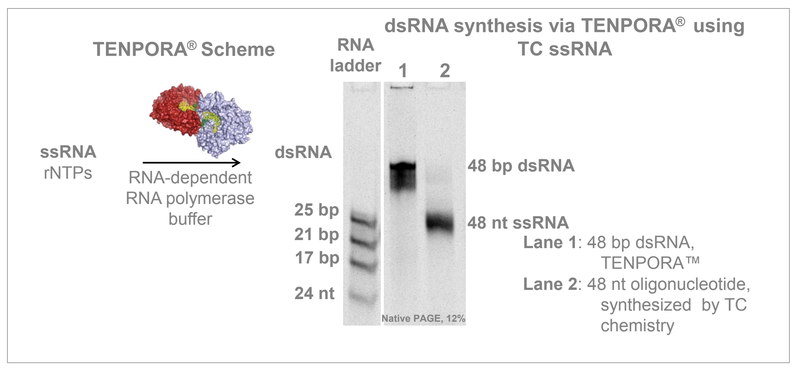


LC/ESI-MS 23mer Oligonucleodite Manufactured with TBDMS Amidites (RP Cartridge-Purified)



RdRP-Catalyzed Synthesis of dsRNA

48bp dsRNA synthesized via Riboxx's propriatory RdRP-based approach (TENPORA®) using TC ssRNA oligo as a template



Lane 1: 48 bp dsRNA, TENPORA™
Lane 2: 48 nt oligonucleotide, synthesized by TC chemistry

RdRP synthesizes the dsRNA in a primer-independent manner using ssRNA as a template. After removal of the RdRP and buffer salts, a perfectly annealed dsRNA oligonucleotide is obtained.

Conclusions

In the present poster it has been demonstrated that TC RNA phosphoramidites:

- Are available in high purity thus enabling very good synthesis results
- Can be conveniently applied to short (siRNA), long (48-mer) and very long (106-mer) oligoribonucleotide synthesis
- Shorten the downstream processing time by > 50% when compared with 2'-TBDMS RNA phosphoramidites
- Simplify the deprotection process to 1 step (EDA-treatment) instead of 2 steps (AMA or ammonia treatment followed by desilylation) and eliminate any use of toxic HF reagents in the process
- Can be applied to produce biologically active synthetic RNA as illustrated for antiGAPDH siRNA and RdRP-catalyzed synthesis of dsRNA
- Result in oligoribonucleotides with at least similar quality as oligomers derived from the conventional 2'-TBDMS RNA phosphoramidies

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