

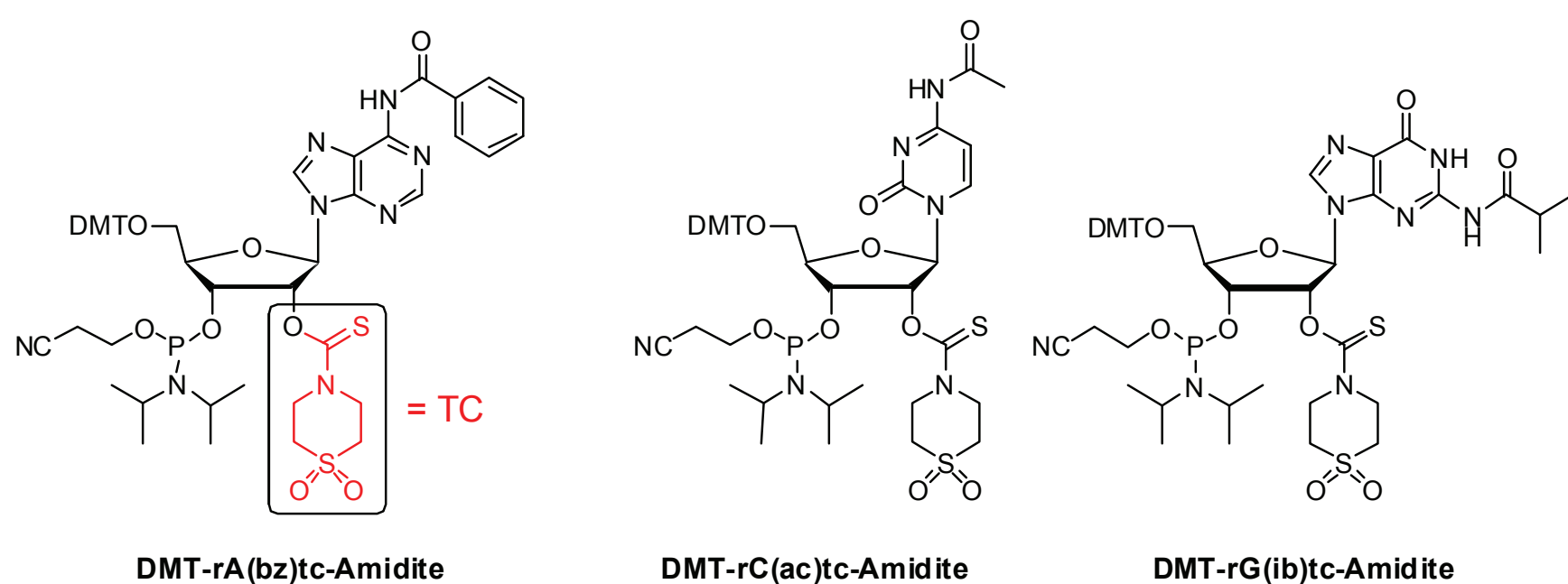
Introduction

RNA phosphoramidites with a 2'-TC (1,1-dioxo-λ⁶-thiomorpholine-4-carbothioate) protective group have been recently introduced into oligoribonucleotide synthesis¹. The novel RNA amidites have very fast coupling kinetics, high coupling efficiency and the resulting RNA oligomers can be completely deprotected in one step. A separate deprotection step for 2'-protective groups with fluoride salts or hydrofluoric acid as it is mandatory for removal of the TBDMS group is not necessary.

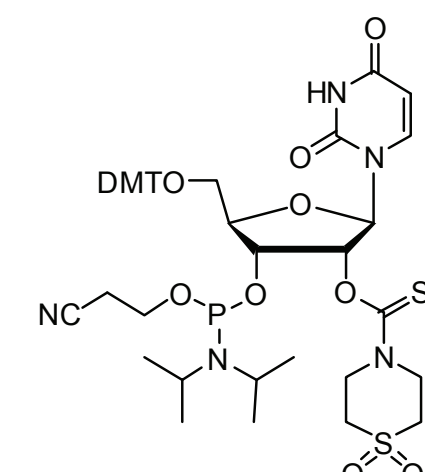
The present poster highlights the properties of 2'-TC protected RNA phosphoramidites with respect to their stability in solution, their solubility, and their coupling efficiency in comparison with TBDMS-protected monomers. The stability of the TC-group against the most common reagents of oligoribonucleotide synthesis and the kinetics of its deprotection with 1,2-ethylenediamine as well as the cleavage of succinate anchor groups and nucleobase protective groups with the latter reagent are also presented.

¹ Patent application US2010/0076183A1

2'-O-TC-RNA Phosphoramidite Structures

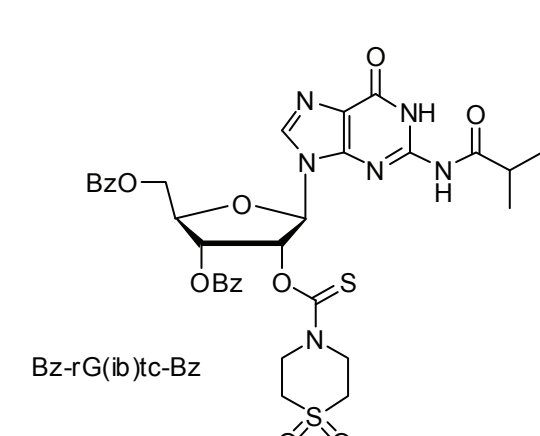


TC = 1,1-dioxo-λ⁶-thiomorpholine-4-carbothioate



Stability of the TC-Group in SPOS Reagents

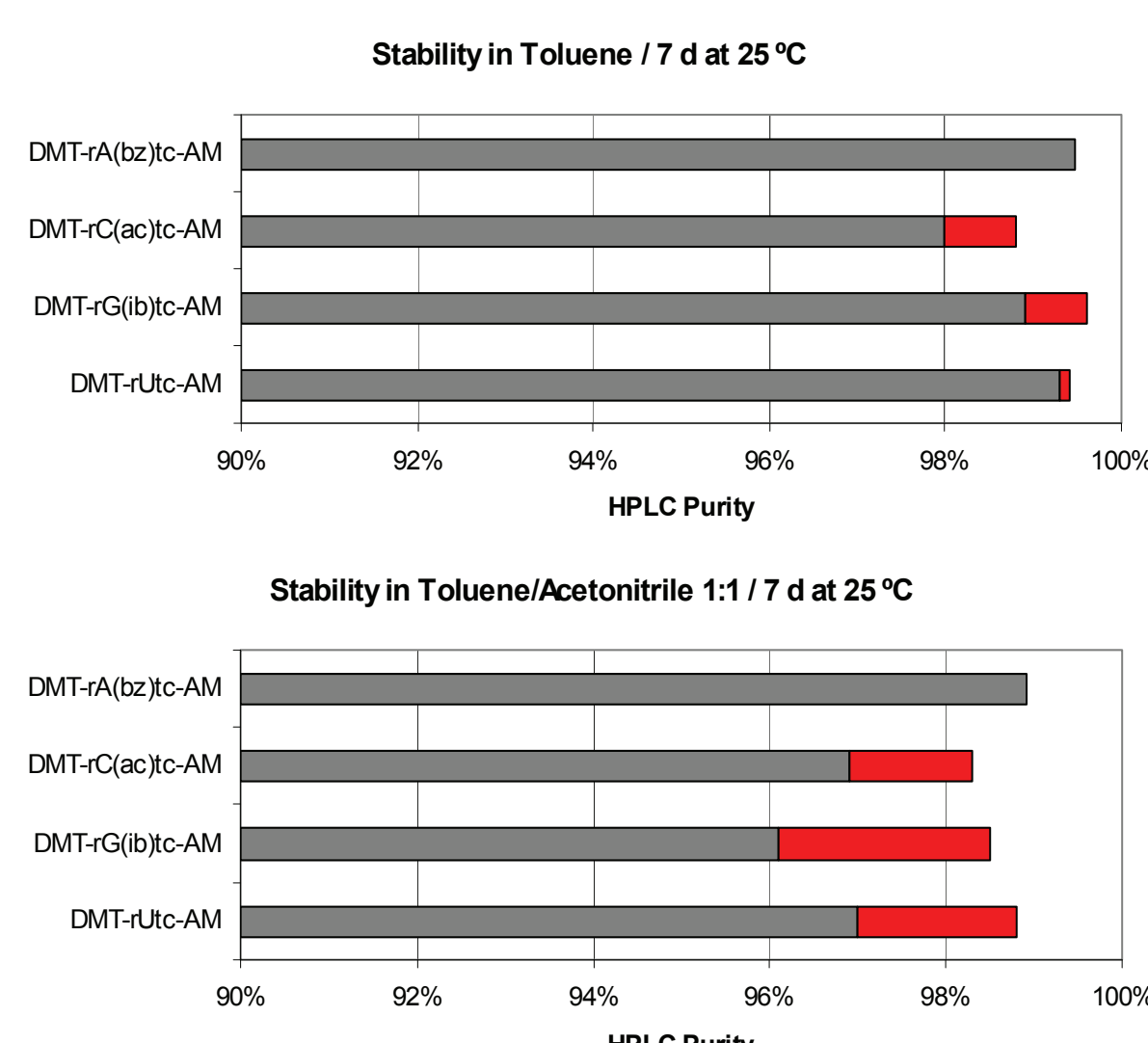
- Preparation of a std solution of 100 mg Bz-rG(ib)tc-Bz in 1 mL acetonitrile
- Sample preparation by dissolving 50 μL of std solution in the respective SPOS reagent to make up 1 mL; conc. = 5 mg/mL
- Storage of samples for 20 h at 25 °C; RP-HPLC analysis



Amidite Stability in Solution

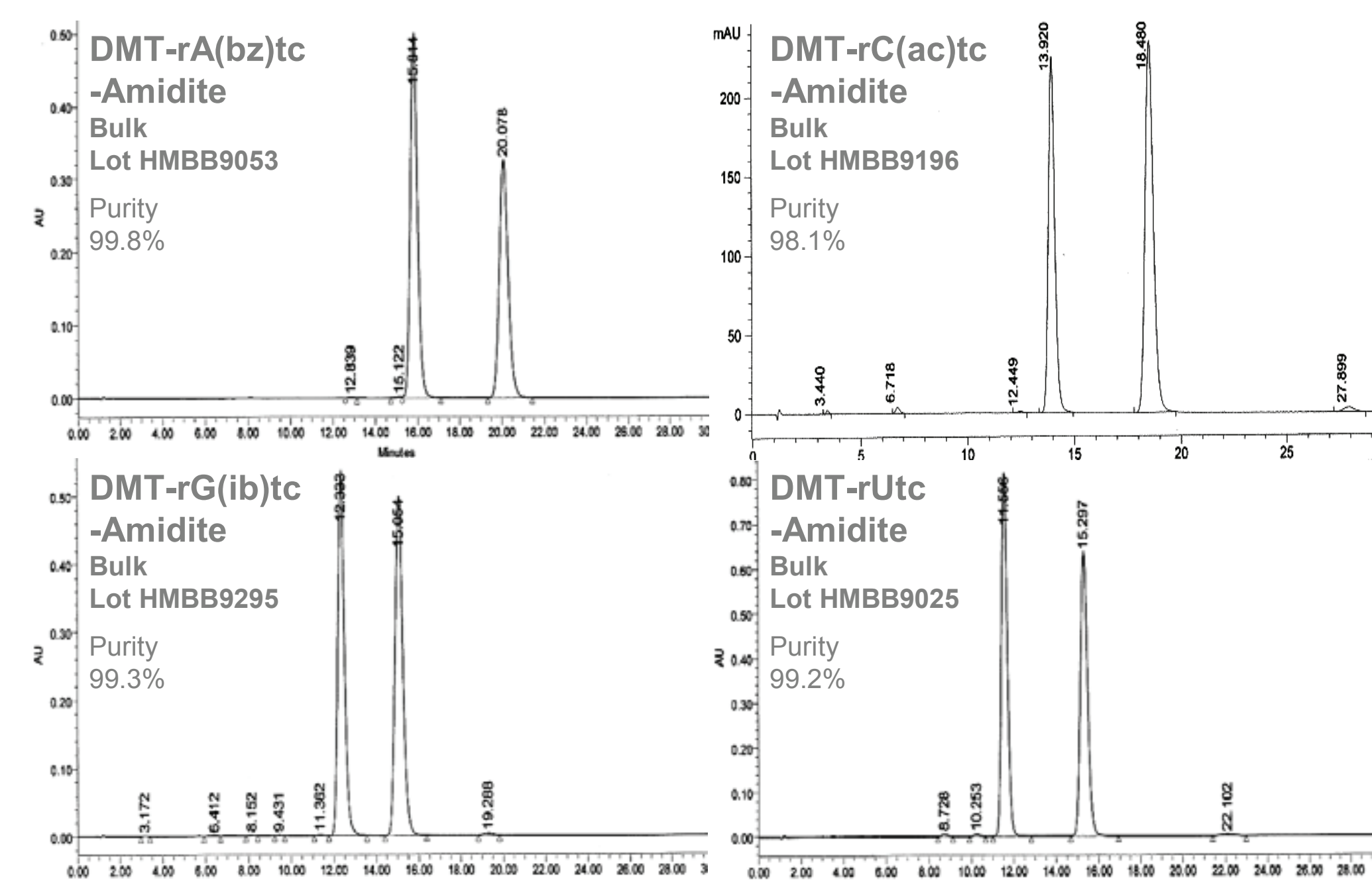
Stability Study

- 5 mg Amidite in 1 mL toluene (top chart)
- 10 mg Amidite in 100 μL toluene/acetonitrile 1:1 (bottom chart)
- Storage at 25 °C for 7 d
- Red: purity t = 0
- Grey: purity t = 7 d



TC-Amidites are stable in solution for up to 7 days at ambient temperature

TC-Amidites HPLC Data



Amidite Solubility

Investigation of solutions of TC monomers in recommended solvents

Monomer	DMT-rA(bz)tc-AM	DMT-rC(ac)tc-AM	DMT-rG(ib)tc-AM	DMT-rUtc-AM
MW	1051,18	965,08	1033,16	924,03
Lot.#	MK 1197	HMBB7331	HMBB7563	HMBB7330
Water content	0,20%	0,16%	0,04%	0,09%
Isomeric ratio (amidite)	55:45	43:57	37:63	18:82

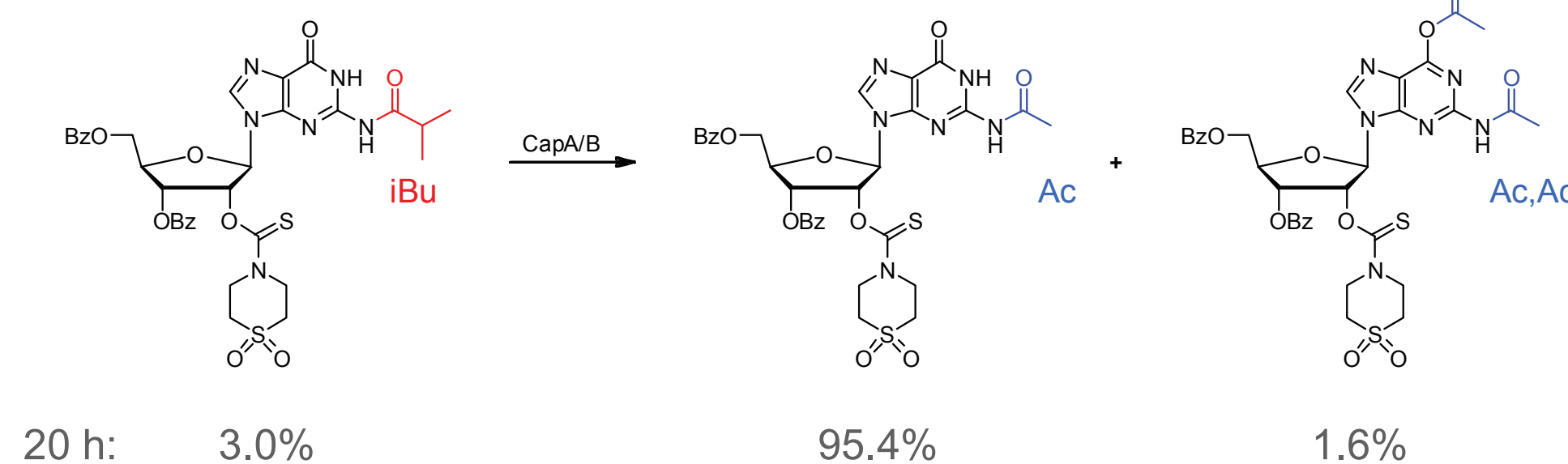
0,1M Amidite solution in toluene/acetonitrile 1:1 (v/v)				
Storage 5 d at 25 °C	clear solution	clear solution	clear solution	clear solution
Storage 5 d at 10 °C	clear solution	clear solution	clear solution	precipitation

0,2M Amidite solution in toluene				
Storage 5 d at 25 °C	clear solution	clear solution	clear solution	clear solution
Storage 5 d at 10 °C	clear solution	clear solution	clear solution	clear solution

Solutions of TC-Amidites stay clear for min. 5 days at ambient temperature

Stability of the TC-Group in SPOS Reagents CapA/B

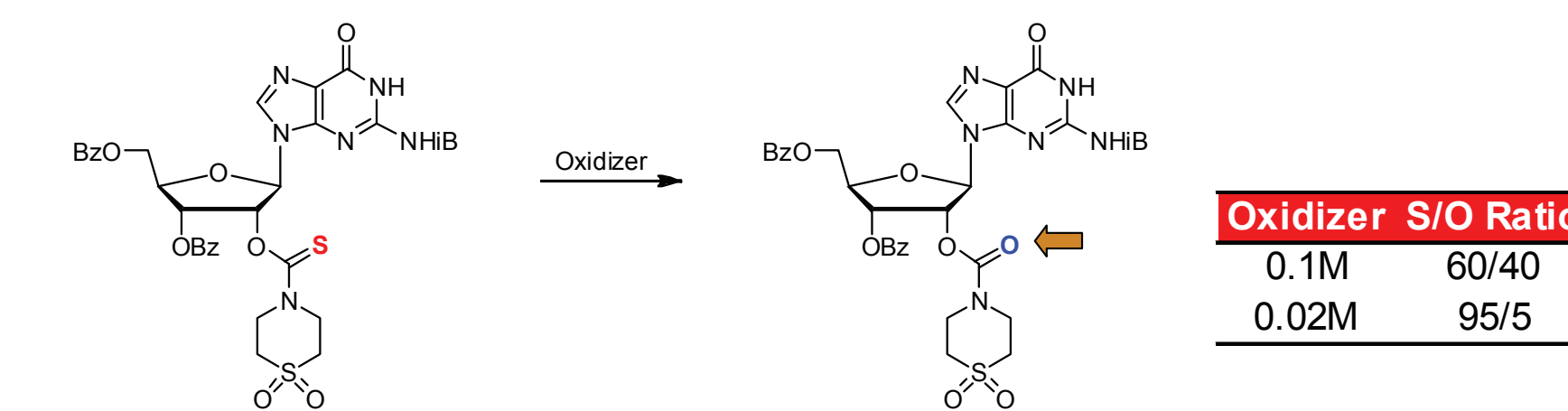
- Treatment of Bz-rG(ib)tc-Bz model compound with CapA/B mix for 20 h at 25 °C
- Analysis via LC-MS



dG-Nucleobase transamidation side reactions occur during capping but are deemed to have no negative impact on SPOS

Stability of the TC-Group in SPOS Reagents Oxidizer

- Treatment of Bz-rG(ib)tc-Bz model compound with oxidizer solutions for 92 h at 25 °C
 - 0.1M Oxidizer: 25.4 g/L iodine in pyridine/water/THF
 - 0.02M Oxidizer: 5.1 g/L iodine in pyridine/water/THF
- Analysis via LC-MS

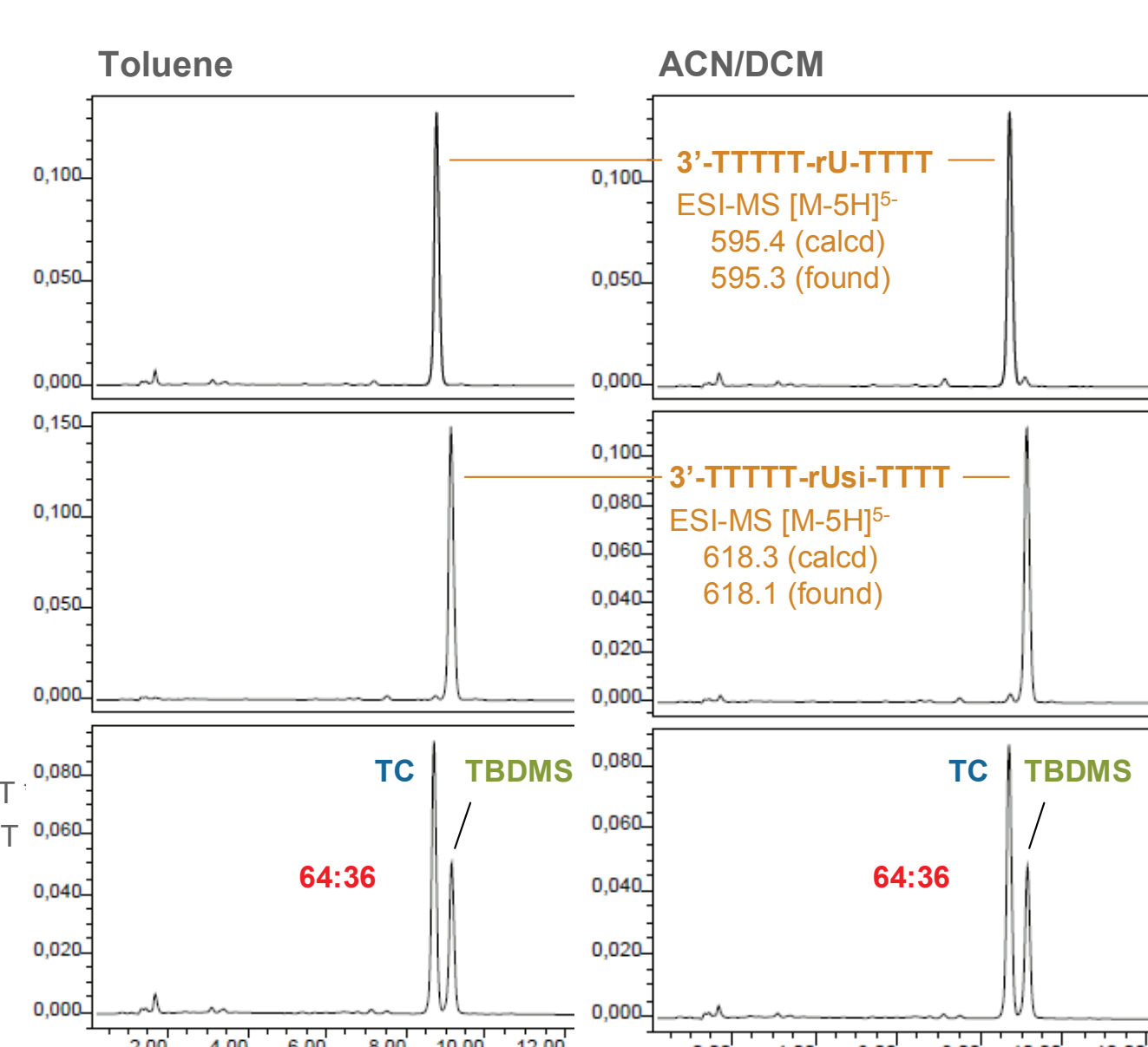


Conversion of thiocarbamate into carbamate with higher iodine conc. Application of 0.02M iodine solution recommended

Coupling Comparison TC vs. TBDMS

Expedite™ Coupling Test

- Sequence: 3'-TTTTT-rU-TTTT
- Monomers
 - DMT-rUtc-AM
 - DMT-rUti-AM
 - Mixture of DMT-rUtc-AM & DMT-rUti-AM, 1:1 molar ratio
- Amidite solutions
 - 0.1M in toluene
 - 0.1M in ACN/DCM 4:1 (v/v)
- RNA coupling step
 - 0.25M Activator 42
 - 5 min Coupling time
 - Modified 0.2 μmol DNA protocol
- Cleavage/deprotection
 - 20% DEA in ACN for 3 min at RT
 - 50% EDA in toluene for 2 h at RT

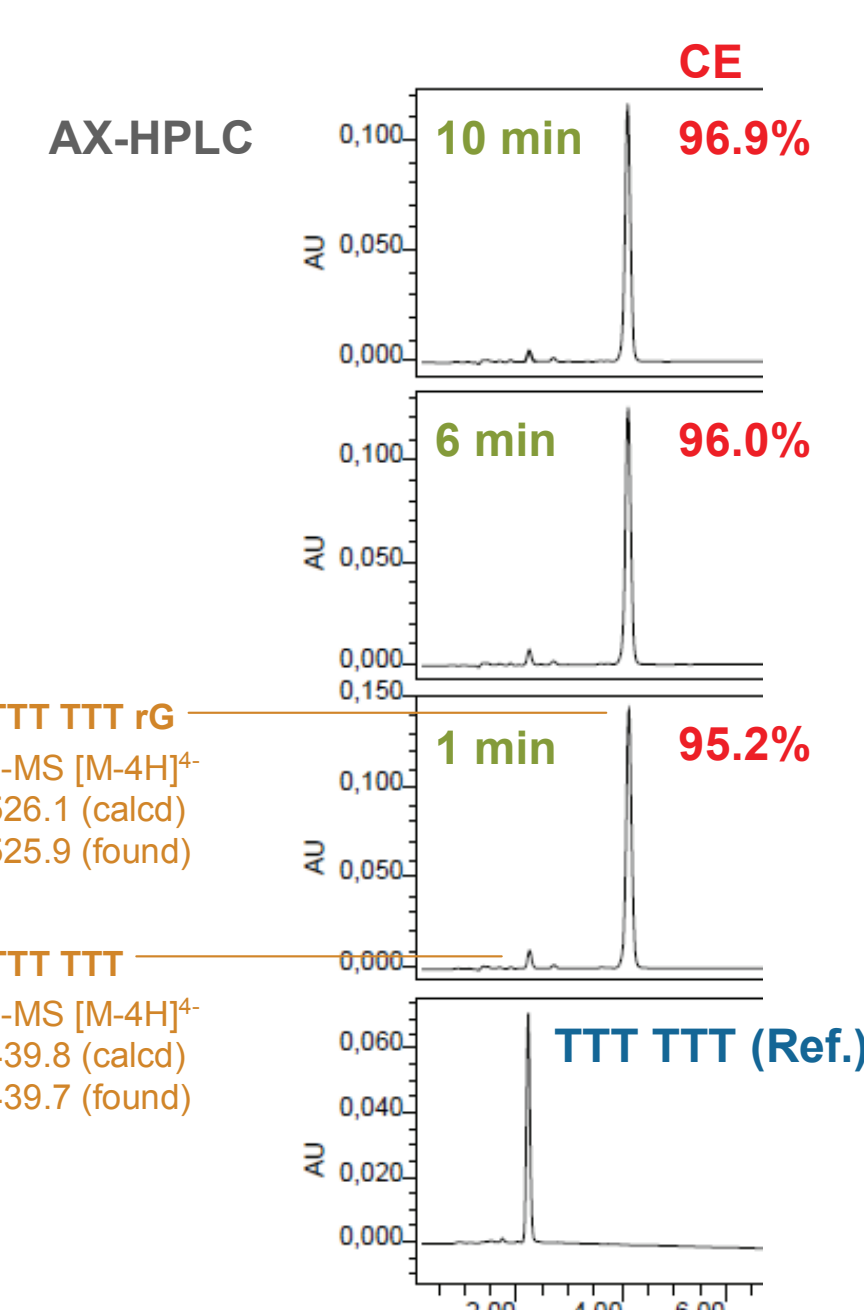


¹ For RNA synthesis with TC monomers DEA treatment is optional

Coupling Time Example 1

Expedite™ Synthesis

- Sequence: 3'-TTT TTT rG
- Monomer: DMT-rG(ib)tc-AM in 0.1M in toluene
- Activator: 0.25M Activator 42
- Synthesis protocol
 - Modified 0.2 μmol DNA
 - Variation of coupling time: 1 - 10 min
- Cleavage/deprotection
 - 20% DEA in ACN for 3 min at RT
 - 50% EDA in toluene for 2 h at RT

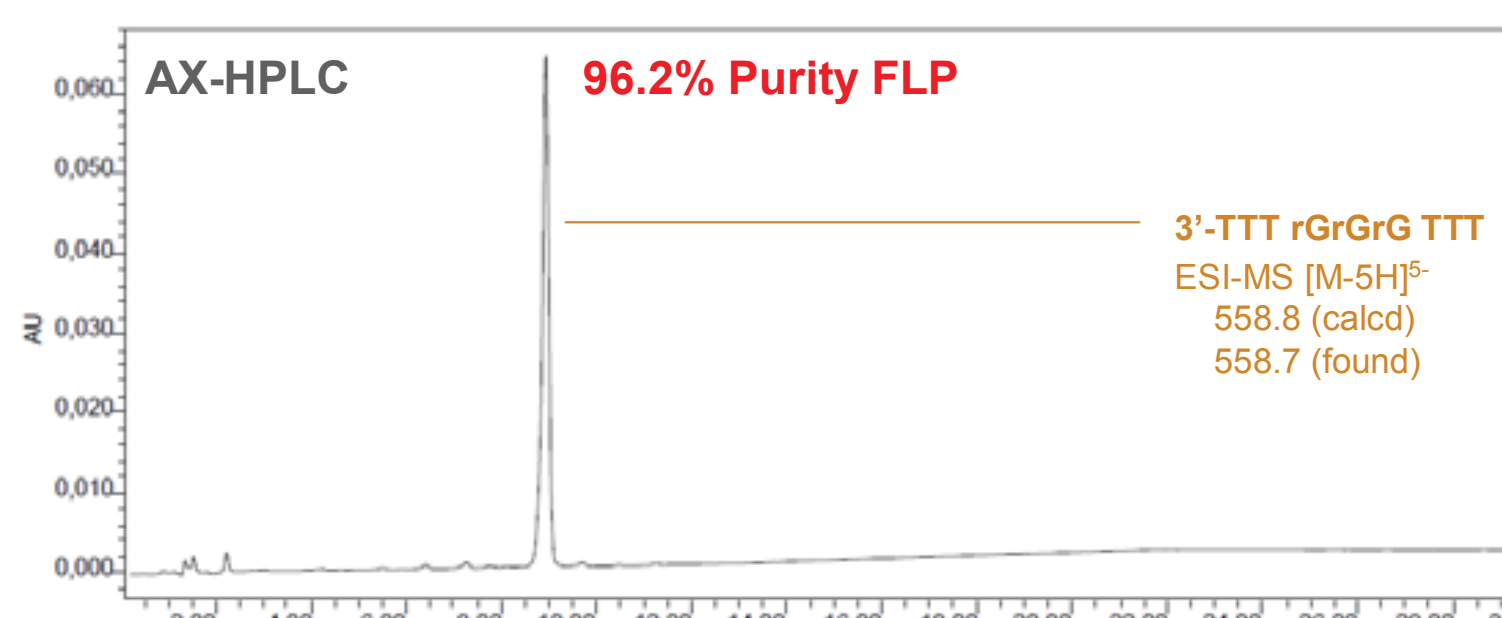


Coupling Time Example 2

Expedite™ Synthesis

- Sequence: 3'-TTT rGrGr TTT
- Monomer: DMT-rG(ib)tc-AM in 0.1M in toluene
- Activator: 0.25M Activator 42

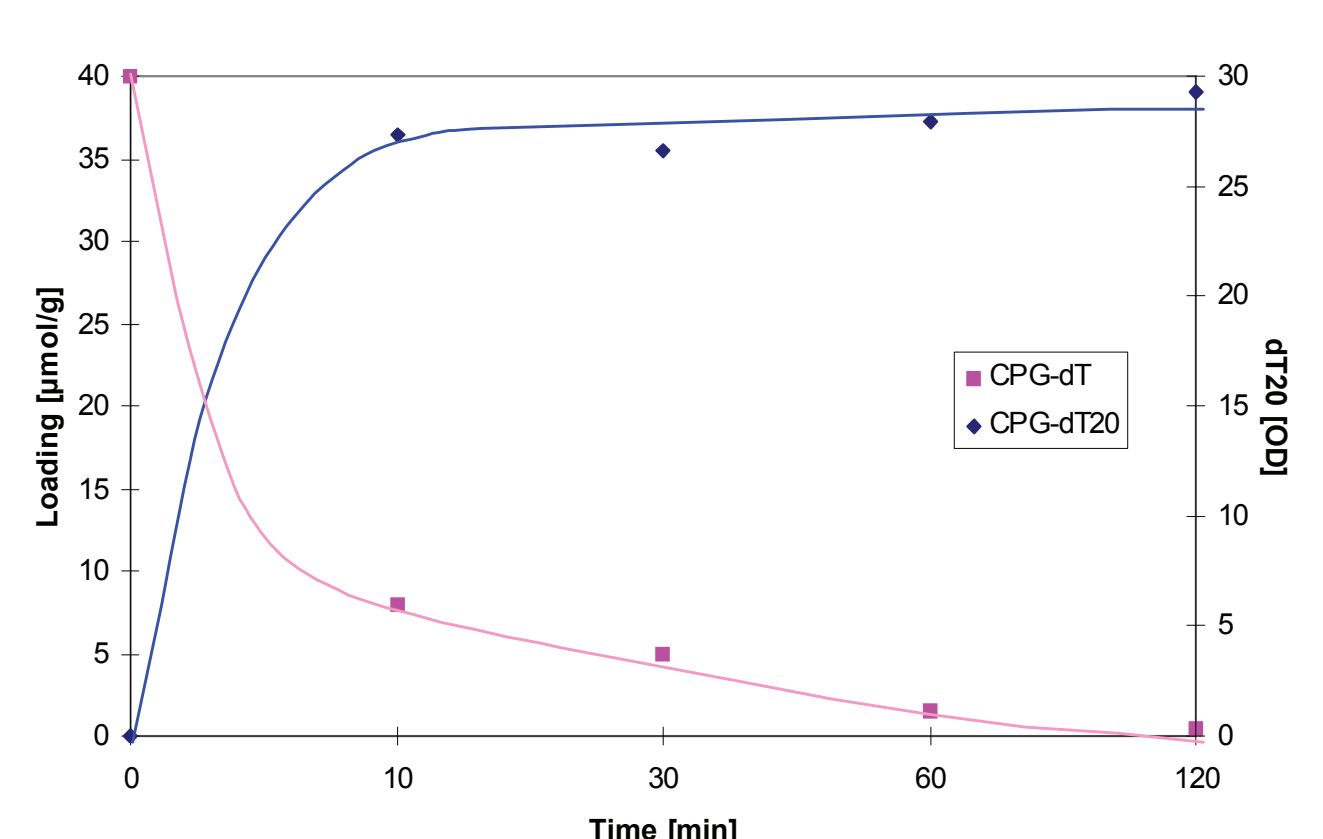
- Synthesis protocol
 - Modified 0.2 μmol DNA
 - Coupling time: 2 min
- Cleavage/deprotection
 - 20% DEA in ACN for 3 min at RT
 - 50% EDA in toluene for 2 h at RT



Cleavage from Support with EDA/Toluene 1:1

1. Cleavage CPG-dT

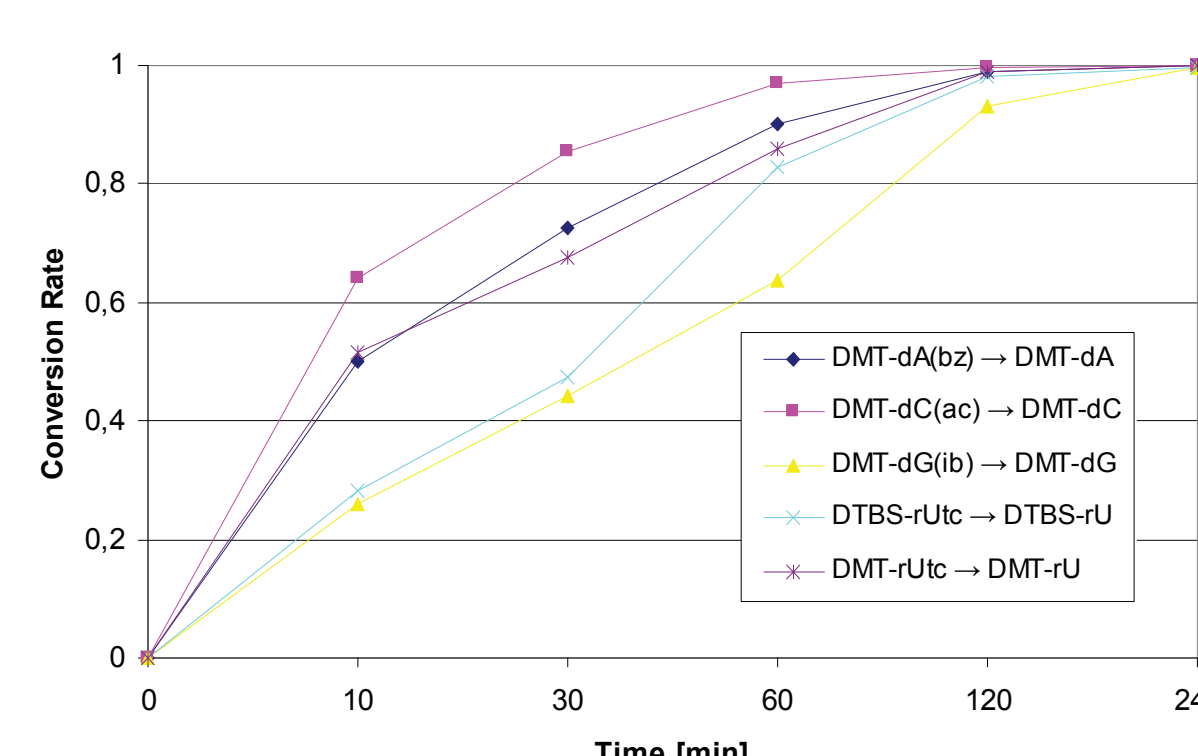
- 10 mg CPG samples
- Cleavage
 - 500 μL EDA/toluene 1:1
 - Ambient temperature
 - Acetonitrile wash
- DMT-Assay
- 2. Cleavage CPG-dT20
 - 10 mg CPG samples
 - Cleavage
 - 500 μL EDA/toluene 1:1
 - Ambient temperature
 - Acetonitrile wash
 - Assay
 - Removal of oligonucleotide from support with water
 - OD determination



Deprotection with EDA/Toluene 1:1

Deprotection Kinetics *)

- Nucleoside samples
 - 5 mg, each
- Deprotection
 - Ethylenediamine/toluene 1:1
 - 500 μL, c = 10 mg/mL
 - Temperature: 25 °C
 - Up to 240 min
- Assay: RP-HPLC



*) Recommended deprotection conditions:
For RNA > 10 bases use neat EDA for 2 h at ambient temp.
For RNA < 10 bases use EDA/toluene 1:1 for 4 h at ambient temp.

TC-Cleavage	Reaction Time [min]	Cleavage
DMT-rUtc	120	98.7%
EDA/Toluol 1:1	60	99.9%

Conclusions

- RNA phosphoramidites with a 2'-TC protective group are available in high purity similar to 2'-TBDMS-protected phosphoramidites
- The TC-amidites exhibit solution stability and solubility for min. 5 days
- The TC-group is stable under oligoribonucleotide synthesis conditions *)
- A coupling time of less than 2 min. is sufficient for TC-amidites
 - The coupling is considerably faster than the coupling of 2'-TBDMS amidites
- The deprotection of 2'-TC groups is complete within 120 min. with 1,2-ethylenediamine as deprotection agent
 - Cleavage from the solid phase support and deprotection of nucleobases can be achieved simultaneously

*) 0.02M iodine oxidizer