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Combining Solid-Phase Synthesis with Enzymatic Strategies enables High-Yield Synthesis of Capped RNA, long RNA, and circular RNA/DNA for Therapeutic and Diagnostic Applications

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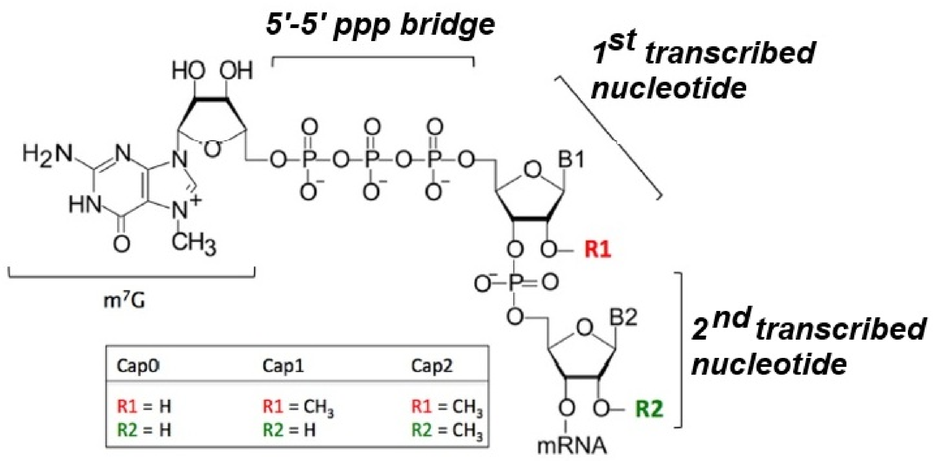
Introduction

RNA capping in humans is essential for mRNA splicing, protection of RNA from 5'-exonucleases in the cytoplasm and targeting of mRNAs to the ribosome. Human RNA guanine-7 methyltransferase (RNMT), Cap Methyltransferase 1 (CMTR1), and Cap Methyltransferase 2 (CMTR2) are RNA methyltransferases involved in RNA capping, play significant roles in the proliferation and differentiation of embryonic stem cells, and have also been implicated in cancer.

A study by Taherian et al. provided a comprehensive understanding of the substrate specificities of human RNA capping methyltransferases, essential for the development of future anticancer therapeutics and assessment of antiviral therapeutics' selectivity. The ability to synthesize highly modified and structurally diverse RNA molecules is crucial for advancing gene editing technologies and nucleic acid-based therapeutics.

Here, we utilize an integrated strategy that combines solid-phase synthesis with enzymatic methods to achieve high-yield production of functional RNA constructs, including 5'-capped RNA (TMG- and m^7G).

RNA Caps



Cap0	Cap1	Cap2
R1 = H R2 = H	R1 = CH_3 R2 = H	R1 = CH_3 R2 = CH_3

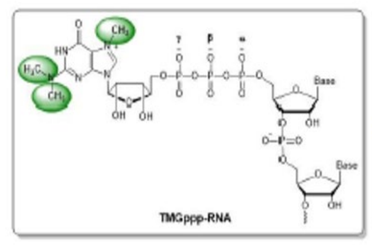
Synthesis Strategy

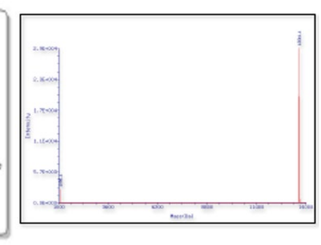
[1] Solid phase synthesis of PRE-oligonucleotides
[2] Purification by HPLC
[3] Quality Control
[4] Addition of functional groups using enzymes
[5] Purification by HPLC
[6] Final Quality Control

Synthesis of modified RNA with a 2,2,7-trimethylguanosine 5'-cap structures (TMG-cap)

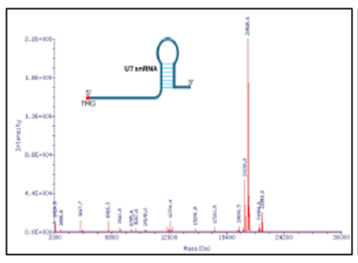
The 2,2,7-trimethylguanosine (TMG) 5' cap is an important modification that enhances RNA stability, facilitates nuclear transport, and promotes translation, particularly in snRNAs and certain viral RNAs.

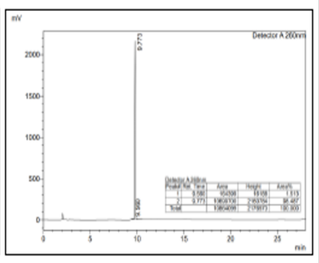
Name	Sequence (5' – 3')	Length	MW
RNA_oligo2	[TMGppp]mAmGmUmUmAGCTGGATTACGGGGTCATTAGTCCAGTACTTTCATAG	42	13,644.68
U7 snRNA	[TMGppp]rCrA rGrUrG rUrUrA rCrArG rCrUrC rUrUrU rUrArG rArArU rUrUrG rUrCrU rArGrU rArGrG rCrUrU rUrCrU rGrGrC rUrUrU rUrArA rCrCrG rGrArA rArGrC rCrCrC rU	63	20,544.96





ESI-MS



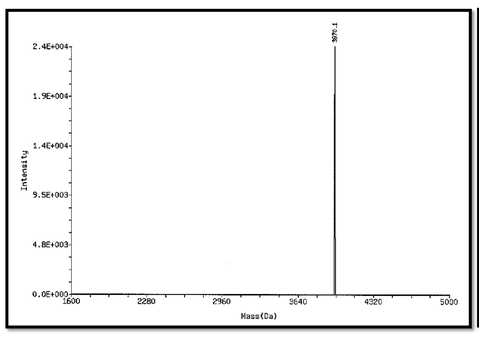


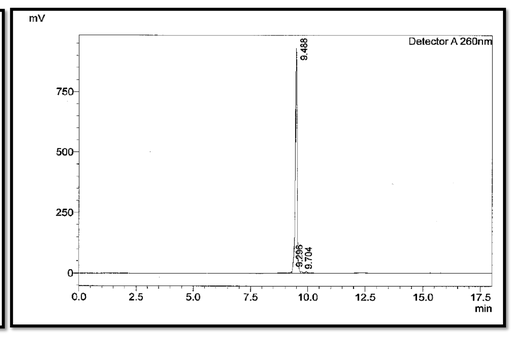
ESI-MS HPLC

The 2,2,7-trimethylguanosine (TMG) cap and capped synthetic U7 snRNA.

Synthesis of modified RNA with a Guanosine 5'-cap structure (Gppp-cap)

Sequence (5' – 3')	Calculate MW
[Gppp][mA]rC rCrCrC rCrCrC rCrC[3-Biotin]	3970.51



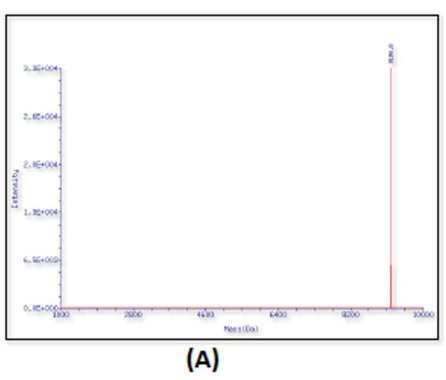


ESI-MS Result (A) and HPLC profile (B) of RNA_oligo1 synthesized using a combined chemical and enzymatic approach.

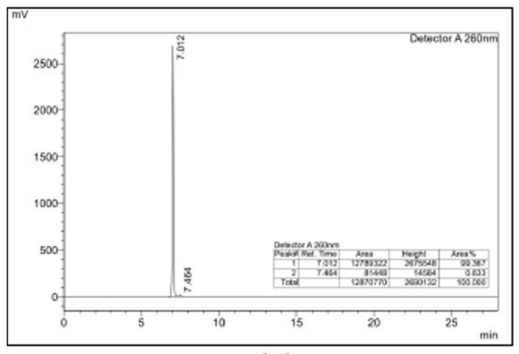
Synthesis of a highly modified RNA with 7-methylguanosine 5' cap structures (m^7G-cap)

Large-scale synthesis of m^7G-capped RNA with high purity for the production of high-quality capped RNAs for diverse downstream applications.

Name	Sequence (5' – 3')	MW
RNA_oligo2	[M7Gppp]rGrArCrUrUrCrGrArCrGrCrUrCrArUrCrGrArGrUrArUrArGrA[AmC6]-[5-6ROX]	9,197.73



(A)



(B)

ESI-MS analysis (A) and HPLC profile (B) of m7GpppG-RNA oligonucleotides synthesized using a combined chemical and enzymatic approach.

Synthesis of modified RNA with a Guanosine 5'-cap structures (Gppp-cap)

In eukaryotes, capping is executed by a series of highly coordinated enzymatic steps, typically recruited by the phosphorylated C-terminal domain (CTD) of RNA Polymerase II:

1.RNA Triphosphatase:

Removes the γ-phosphate from the 5'-triphosphate end of the nascent pre-mRNA, leaving a 5'-diphosphate end ($5'$).

2.RNA Guanylyltransferase:

Transfers a GMP moiety from a GTP molecule to the 5'-diphosphate, forming the unique $5'5-$ triphosphate bridge. This results in an unmethylated $G(5')ppp(5')N$ structure.

3.RNA (Guanine-N7)-Methyltransferase:

Methylates the guanine base at the N7 position, using *S*-adenosylmethionine (SAM) as the methyl donor to yield the definitive m^7G cap.

Depending on subsequent methylations of the adjacent transcribed nucleotides, caps are classified into different variations:

Cap 0: Only the terminal guanine is methylated (m^7GpppN). This is typical in lower eukaryotes like yeast.

Cap 1: The 2'-hydroxy (2) group of the first transcribed ribose nucleotide is additionally methylated by a 2'-O-methyltransferase (m^7GpppN_m). This is the predominant form in higher eukaryotes and serves as a crucial marker to prevent the innate immune system from recognizing the mRNA as foreign.

Cap 2: The 2'-OH groups of both the first and second transcribed nucleotides are methylated ($m^7GpppN_mN_m$).

Conclusion

We show that an integrated synthesis strategy combining solid-phase synthesis with enzymatic modifications enables the production of high-quality, structurally diverse nucleic acids. Our optimized workflow enables the efficient generation of highly modified RNA molecules, including 5'-capped (TMG- and m^7G-) RNAs, ensure enhanced stability and functionality.

By integrating chemical synthesis precision with enzymatic efficiency, our approach overcomes the limitations of traditional methods such as in vitro transcription. This scalable, reproducible, and high-yield synthesis platform facilitates advancements in gene-editing technologies, RNA therapeutics, and molecular diagnostics.

References

Chan SH, Molé CN, Nye D, et al. Biochemical characterization of mRNA capping enzyme from Faustovirus. ***RNA***. 2023;29(11):1803–1817. doi:10.1261/rna.079738.123.

Ignatochkina AV, Iguchi JA, Kore AR, Ho CK. Trypanosome mRNA recapping is triggered by hypermethylation originating from cap 4. ***Nucleic Acids Res***. 2024;52(17):10645–10653. doi:10.1093/nar/gkae614.

Molé CN, Chan SH, et al. Kinetics study reveals interconnected relationships of component activities in trifunctional RNA capping enzymes. ***J Biol Chem***. 2026;302(8):113237.

Podvalnaya N, Bronkhorst AW, Lichtenberger R, et al. piRNA processing by a trimeric Schlafen-domain nuclease. ***Nature***. 2023;622(7982):402–409. doi:10.1038/s41586-023-06588-2.

Taherian F, Mabanglo MF, Hajian T, et al. Defining substrate specificities of human RNA capping methyltransferases through quantitative assessment of independent yet cooperative activities. ***Protein Sci***. 2026;35(1):e70428. doi:10.1002/pro.70428.

Wang J, Chew BLA, Lai Y, et al. Quantifying the RNA cap epitranscriptome reveals novel caps in cellular and viral RNA. ***Nucleic Acids Res***. 2019;47(20):e130. doi:10.1093/nar/gkz751

Wolf EJ, Grünberg S, Dai N, et al. Human RNase 4 improves mRNA sequence characterization by LC–MS/MS. ***Nucleic Acids Res***. 2022;50(18):e106. doi:10.1093/nar/gkac632.